European Journal of Human Genetics

Volume 22 Supplement 1          May 2014                                           www.nature.com/ejhg

European Human Genetics Conference 2014
May 31 - June 3, 2014
Milan, Italy

Abstracts
	nature publishing group npg
European Human Genetics Conference
in conjunction with the
European Meeting on Psychosocial aspects of Genetics

May 31 - June 3, 2014
Milan, Italy

Abstracts
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Future European Human Genetics Conferences

European Human Genetics Conference 2015
Glasgow, United Kingdom
June 6 – 9, 2015

European Human Genetics Conference 2016
Barcelona, Spain
May 21 – 24, 2016
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RASopathies. The other face of RAS signalling dysregulation

M. Tartaglia
Istituto Superiore di Sanità, Rome, Italy.

RAS proteins are small monomeric GTPases that function as molecular switches controlling a major intracellular signaling network that, depending on the cellular context, guides diverse biological functions, including proliferation, cell fate determination, survival, migration, differentiation, and senescence. RAS signaling is switched on upon stimulation by numerous cytokines, hormones, and growth factors, and mediates the appropriate cell response to external stimuli through the regulation of transcription, cytoskeletal rearrangement, and metabolism. Within this network, signal flow through the RAS-RAF-MEK-ERK pathway, the first identified mitogen-associated protein kinase (MAPK) cascade, mediates early and late developmental processes, including determination of morphology, organogenesis, synaptic plasticity and growth. Signaling through the RAS-MAPK cascade is tightly controlled, and its enhanced activation has been known for decades to represent a major event in oncogenesis. Activating somatic RAS gene mutations occur in approximately 30% of human cancers, and upregulation of this signaling pathway can also result from enhanced function of upstream signal transducers or RAS effectors, and inefficient function of feedback mechanisms. Unexpectedly, discoveries derived from a massive disease gene hunting effort have recently established a novel scenario in which the upregulation of this signaling pathway represents one of the most common events affecting developmental processes. These discoveries have also provided us with unpredicted molecular mechanisms converging toward the dysregulation of this signaling network.

PLI.2
Evolution of the HD gene
E. Cattaneo
Department of Bioscience, University of Milan, Milan, Italy.

The Hdh gene arose with no CAGs in Dicyostelium discoideum (Dd), around 800 million-year ago before the protostome-deuterostome divergence (Zuccato, Physiol Rev 2010). The CAG then has appeared in and is unique to the deuterostome branch. Two CAGs are found in Hdh in sea urchin (Strongylocentrotus purpuratus, Sp), the first specie to carry a primitive nervous system, and two CAGs are present in amphioxus (Branchiostoma floridae, Bf), the first specie to exhibit a rudimental hollow nerve tube and cephalization. Four CAG are found in Hdh from the more evolved fishes, amphibians, and birds. The CAG further expands in mammals and reaches its maximum length in humans. A study of 278 normal subjects revealed an increase in grey matter with increasing length of the CAG repeat (Muhlau, PlosOne 2012), indicating that CAG size could influence normal brain structure. Our hypothesis is that the progressive increase in CAG length in the Hdh gene observed during evolution may be implicated in the evolutionary changes that have occurred in the developing and adult nervous system throughout vertebrate phylogeny, with a possible role for the CAG in newly emerging cognitive functions in the mammalian brain. We have now collected the Hdh gene from new specie both in the protostome and deuterostome branch. In addition to further analyze the CAG tract during mammalian evolution we have collected genomic DNA and amplified the CAG tract from non-arthropomorphic and anthropomorphim primates. Our reconstruction of h1l hphl lypgyn supports further that the CAG tract expands during deuterostome evolution and seems to correlate with the appearance and/or evolution of progressively more complex nervous systems.

PLI.3
Genetic engineering of hematopoietic stem cells for the treatment of inherited diseases
L. Kaldini
Milan, Italy.

No abstract received at production date. Check the online database at www.esgh.org/abstracts2014.0.html for possible updates.
An accurate genetic diagnosis is fundamental for pediatric patients with rare genetic disorders to improve disease management, access to resources, and recurrence risk counseling. A diagnosis also provides psychosocial benefits to families. The Finding of Rare Disease Genes (FORGE) Canada project, which sought to identify rare disease genes through whole-exome sequencing (WES), shows that 36% of the solved disorders were secondary to mutations in known genes; 95 known disease genes were identified out of 264 total disorders studied. All patients had undergone standard care genetic testing in Canada and no diagnosis was forthcoming. Thus, for 104 families, WES provided a clinical diagnosis; 24 of these were dominant (most de novo), 68 were autosomal recessive, four were X-linked mutations and one family had two disorders. Although many of the 104 families who received a clinical diagnosis were ascertainment biased because of familial recurrence or consanguineous parents, 91 single affected patients without a family history were also included for WES. This latter subset of the above 264 disorders are representative of what geneticists often see in the clinic and had a diagnostic rate of 43%. Thus, WES would seem to be an efficient and cost-effective means of clinical diagnosis for many patients who are currently undiagnosed. Our findings suggest that patients with genetically heterogeneous disorders or sibling recurrence are the most likely to be diagnosed by WES. Canada is building on the success of these 104 diagnosed families to develop a national framework for clinical exome sequencing.

**PL2.6**

**Genome sequencing identifies major causes of severe intellectual disability**

C. Gilsen1, J. Y. Hehir-Kwa1, D. T. Thung2, M. Van de Vorst3, B. W. M. van Boxt4, M. H. Willemse5, M. Kwint1, J. Jansen1, A. Hoischen1, R. Laird6, R. Klein1, R. Tearle1, T. Bo3, R. Pfundt7, H. G. Interna7, B. B. A. De Vries8, T. Kleefstra9, H. G. Brunner4,5, L. E. L. M. Vissers4,5,10

1Radboud university medical center, Nijmegen, Netherlands, 2Complete Genomics Inc, Mountain View, CA, United States, 3State Key Laboratory of Medical Genetics, Central South University, Changsha, China, 4Maastricht University Medical Centre, Maastricht, Netherlands

Severe intellectual disability (ID) occurs in 0.5% of newborns and is thought to be largely genetic in origin. The extensive genetic heterogeneity of the disorder requires a genome wide detection of all types of genetic variation. Microarray studies and more recently exome sequencing have demonstrated the importance of de novo copy number variations (CNVs) and single nucleotide variations (SNVs) in ID but the majority of cases remains undiagnosed. Here we applied whole genome sequencing (WGS) to 50 patients with severe ID and their unaffected parents. All patients were negative after extensive genetic prescreening, including microarray-based CNV studies and exome sequencing. Notwithstanding this prescreening, de novo SNVs affecting the coding region provided a conclusive genetic cause in 13 patients and a possible cause for another 8 patients. In addition, we identified 7 clinically relevant de novo CNVs as well as one recessively inherited compound heterozygous CNV. These CNVs represented diverse single exon and intronic deletions of known ID genes as well as interchromosomal duplications. Local realignment of sequence reads allowed the mapping of most of these CNVs at single nucleotide resolution and provided positional information for duplicated sequences. These results show that de novo mutations and CNVs affecting the coding region are the major cause of severe ID. Genome sequencing can be applied as a single genetic test to reliably identify and characterize the comprehensive spectrum of genetic variation, providing a genetic diagnosis in the majority of patients with severe ID.

**PL3.1 - Summary**

**Incidental findings in clinical exome and genome sequencing**

Incidental exome and genome sequencing data can be interrogated for clinically relevant variants other than those relevant for a diagnostic request. There are different opinions on the way to deal with these ‘incidental findings’ in the clinic, on the potential benefit and risks to patients, on patient autonomy and on the obligations of clinicians to report these findings. This e-seminar will be debated with representatives from both sides of the Atlantic.

**PL3.1**

**Incidental findings in clinical exome and genome sequencing**

R. Green1,2

1Boston, MA, United States
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In 2013, the American College of Medical Genetics and Genomics (ACMG) issued Recommendations for incidental findings in clinical exome and genome sequencing. The Recommendations were written by a Working Group of 14 medical geneticists, laboratory geneticists, genetic counselors and ethicists who met regularly for 14 months to draft them. The draft Recommendations were presented for commentary in a public forum at the 2012 Annual Meeting of the ACMG, reviewed by 15 additional experts, and reviewed several times by the Board of the ACMG before being issued in March, 2015. The Recommendations...
were recently amended in March, 2014. The recommendations were consensus-based, as empirical data on population penetrance and medical outcomes of identifying incidental variants are not available. The recommendations called for molecular laboratories performing clinical genomic analysis on patients of any age to examine 56 genes associated with 24 actionable conditions for pathogenic variants. The recommendations now suggest that patients be offered an option to decline these tests as a group at the time they are ordered. Several clinical sequencing laboratories in the US have adopted the recommendations and over 1000 reports of incidental findings have been issued. The incidence of the ACMG variants in unselected populations appears to be 2–4%. Additional data from our experimental laboratory of translational genomics on the issue of incidental findings will be presented.

PL3.3 Debate: Active search for clearly pathogenic variants that require direct clinical intervention; When related to late onset disorders, adults only.

M. Kriek
Clinical Geneticist, Department of Clinical Genetics, Department of Human Genetics, Leiden University Medical Center, Leiden, Netherlands.

In 2013 the ‘American College of Medical Genetics and Genomics’ (ACMG) published a list of 56 genes that are proposed specific targets of a search for pathogenic variants in both children and adults (Green et al). These genes are associated with diseases with an indication for immediate medical intervention of the patient or family member. The publication asserts that, in principle, the necessary patient data are available following a NGS procedure and therefore cannot be ignored. For purposes of clarity, the vast majority of these variants are removed during the various filtering stages of the normal trio analysis process and that identification of these variants will require additional actions. During the American Society of Human Genetics meeting 2015, it was argued that consensus on a basic list of clearly pathogenic variants in the relevant genes is essential if this form of diagnosis is to be implemented in genetic diagnosis. A disadvantage of the approach as proposed by the ACMG (analysis of the entire gene, rather than focusing on known pathogenic gene variants) is that variants will be detected that have not been previously reported and/or are of undefined pathogenicity. This could result in considerable distress for the patient and family. To avoid this, only selected variants in the genes nominated by the ACMG will be analysed.

Another point of discussion is whether conditions that manifest later in life should be investigated in children. While the ACMG is in favour, Ploem et al are of a different opinion: ‘When it comes to the unsolicited findings of NGS diagnostics in a young child, the interests of the child prevail over any wishes of the parents (not) to receive certain information.’ (NTVG 2014;158:A6757). This is consistent with ESHG recommendations (EJHG 2013;21:580-4).

‘In case of testing minors, guidelines need to be established as to what unsolicited information should be disclosed in order to balance the autonomy and interests of the child and the parental rights and needs (not) to receive information that may be in the interest of their (future) family’

A solution to these conflicting interests is as follows: where the index patient is a minor, a genetic predisposition for ‘late onset’ diseases for which immediate medical treatment is indicated will only be sought in parents. This is made possible by the setup of trio analysis in which variant lists are available for each parent. Genetic variants that result in clinical manifestations that in require direct intervention during childhood will be analysed in the index patient, even when a minor.

PL3.4 Debate: Whole-genome sequencing in health care: proceed with caution and avoid incidental findings

M. C. Cornel
VU University Medical Center, Amsterdam, Netherlands.

Whole-genome sequencing (WGS) of the human genome has a great potential to identify the genetic component of health problems, and probably, in the near future, at a lower cost than that of the current techniques. Proof of principle regarding the clinical utility of WGS followed by whole genome analysis (WGA) has been reported, especially for rare diseases. The Public and Professional Policy Committee of ESHG developed recommendations on whole genome sequencing for health care (EJHG 2013;21:580–4).

As a first element of the discussion, we have to consider all stakeholders involved: patients looking for a diagnosis, primary care physicians referring patients and following them for many years after the diagnosis, laboratory experts and clinical geneticists, legal and ethical experts. If a new technology is being implemented, a mutual learning process starts. Structures are needed for sharing experiences and establish testing guidelines at local, national and international levels.

When in the clinical setting either targeted sequencing or analysis of genome data is possible, it is preferable to use a targeted approach first in order to avoid unsolicited findings or findings that cannot be interpreted. The ACMG advocates (Genet Med 2013;15:565–74) that there is a potential for the recognition of the occurrence of incidental or secondary findings unrelated to the indication for ordering the sequencing but of medical value for patient care. The ESHG position is that whenever possible, such testing should be targeted to genome regions linked to the indication. Wider testing requires a justification in terms of necessity and proportionality. Adding screening targets to a diagnostic test violates the necessity criterion. Imposing this extra testing upon patients who need an answer to their clinical problem is at odds with respect for autonomy (Science 2013;341:958-9). For instance, the informed consent procedure in WGA must be developed (Hum Mutat. 2013;34:1223-8).

Also in a targeted analysis it is possible to detect an unsolicited genetic variant, indicative of serious health problems (either in the person tested or his/her close relatives) that allow for treatment or prevention. In principle, a health-care professional should report such genetic variants (EJHG 2013;21:580–4).

To prepare the health care professionals for WGS in health care, a sustained effort at genetic education is required at various levels: in primary care to inform and refer people appropriately and in specialized care to counsel or refer patients, and to discuss and interpret genetic test results adequately. Genetic experts should engage in discussing new developments in genetics, and explain the pros and cons of genetic testing and screening in clinical and commercial settings to inform the public and raise public awareness. Enhancing genetic literacy in patients and the lay public will help to involve wider society in this debate.

PL4.1 Gene Targeting into the 21st Century: Mouse Models of Human Diseases from Cancer to Neuropsychiatric Disorders

M. Capocci
Howard Hughes Medical Institute, University of Utah School of Medicine, Salt Lake City, UT, United States.

Gene targeting provides the means for modifying any gene in any desired manner in an intact, living animal, the mouse. This technology permits the evaluation of the function of genes in mammals. Because nearly all biological phenomena are mediated or influenced by the activity of genes, this methodology permits the analysis of the most complex biological processes such as development, learning, normal and aberrant behavior, cancer, immunology and a multitude of congenital human diseases. In the past, gene targeting has been used primarily to generate ‘knockout’ mice, that is mice in which the chosen gene has been disrupted in the germline, such that every cell in the mouse carries the mutations. This methodology permits evaluation of the function of that gene in the intact mouse. However, many, if not most, genes have multiple functions. If one of those functions is critical to the survival of the mouse, then subsequent functions of that gene in the mouse cannot be evaluated by the above means. This problem can be circumvented by generating conditional mutations, that restrict the effects of the mutation temporally, spatially (to defined sets of cells or tissue) or both. In mice, conditional mutagenesis is achieved by combining gene targeting, which is achieved through homologous recombination, with a site-specific recombinase system, such as Cre/loxP or Flp/FRT. Cre and Flp are recombinases that mediate recombination between specific, short DNA sequences, loxP and FRT, respectively. Gene targeting is used to flank your chosen gene with either loxP or FRT sites in the mouse germline. By restricting the production of the Cre or Flp recombinase to either a specific set of cells, a chosen temporal period, or both, within the developing or adult mouse, the gene is only excised from the genome of the mouse, in those selected cells, during that chosen temporal period, or both. I will describe a number of applications of gene targeting and conditional mutagenesis derived in our laboratory that address interesting biological questions from modeling human cancer to neuropsychiatric disorder in the mouse.

PL5.1 Signatures of Mutational Processes in Human Cancer

M. Stratton;
Wellcome Trust Sanger Institute, Hinxton, United Kingdom.

All cancers are caused by somatic mutations. However, the processes underlying the genesis of somatic mutations in human cancer are remarkably poorly understood. Recent large-scale cancer genome sequencing initiatives have provided us with new insights into these mutational processes through the mutational signatures they leave on the cancer genome. In this talk I will review the mutational signatures found across cancer and consider the underlying mutational processes that have been operative.
SYMPOSIA

S01.1 From rare disease to management of common disorders M. Summar; Washington, DC, United States.
No abstract received at production date. Check the online database at www.eshg.org/abstracts2014.0.htm for possible updates.

S01.2 Breast cancer genes: beyond BRCA1 and BRCA2 P. Phamah; Dept. of Oncology, Cambridge, United Kingdom.
No abstract received at production date. Check the online database at www.eshg.org/abstracts2014.0.htm for possible updates.

S01.3 Age-related Macular Degeneration C. Klaver; Rotterdam, Netherlands.
No abstract received at production date. Check the online database at www.eshg.org/abstracts2014.0.htm for possible updates.

S02.1 Variation and genetic control of chromatin in humans B. Deplancce; Lausanne, Switzerland.
Understanding how a genome gives rise to complex phenotypes is one of the key unresolved questions in biology. In this talk, I will present results from our study aiming to examine the mechanistic relationship between genomic and molecular phenotypic variation. Specifically, we profiled three histone modifications (H3K4me1, H3K4me3, H3K27ac), two DNA binding factors (PU.1, RNA polymerase II), and gene expression in lymphoblastoid cell lines from 50 European individuals, reasoning that an integrated analysis of intermediate molecular phenotypes coupled with personal genome information might enable an in-depth characterization of non-coding variation. I will discuss how the resulting data allowed us to measure inter-individual variation in chromatin states, to predict sex-biased regulatory regions, and to map the regulatory architecture behind gene expression variation. As such, I will argue that we were able to provide a comprehensive view of chromatin state variation in a human population and to generate novel molecular insights into the propagation of genetic signals along the complex chain of molecular, regulatory events.

S02.2 Control of gene expression in disease M. Georges; Liège, Belgium.
No abstract received at production date. Check the online database at www.eshg.org/abstracts2014.0.htm for possible updates.

S02.3 Computational challenges in single-cell transcriptomics J. Marioni; EMBL-EBI, Hinxton, United Kingdom.
Recent technical developments have enabled the transcriptomes of hundreds of cells to be assayed in an unbiased manner. These approaches have enabled heterogeneity in gene expression levels across populations of cells to be characterized as well as facilitating the identification of new and potentially physiologically relevant, sub-populations of cells. However, to fully exploit such data and to answer these questions, it is necessary to develop robust computational methods that take account of both technical noise and underlying, potentially confounding, variables such as the cell cycle.

In this presentation I will begin by briefly describing how we used spike-ins to quantify technical noise in single-cell RNA-seq data, thus facilitating identification of genes with more variation in expression levels across cells than expected by chance. Subsequently, I will discuss a computational approach that uses latent variable models to account for potentially confounding factors such as the cell cycle before applying it to study the differentiation of Th2 cells. I will show that accounting for cell-to-cell correlations due to the cell cycle allows identification of otherwise obscured sub-populations of cells that correspond to different stages along the path to fully differentiated Th2 cells. To conclude, I will briefly discuss further applications of single-cell RNA-seq in the context of studying neuronal cell types, including olfactory neurons.

S03.1 Pontocerebellar hypoplasia K. Kutsche; Institute of Human Genetics, University Medical Center Hamburg-Eppendorf, Hamburg, Germany.
Pontocerebellar hypoplasias (PCH) constitute a group of autosomal recessively inherited neurodegenerative disorders with prenatal onset. Classification based on clinical, neuroradiological and neuropathological characteristics divided PCH into eight subtypes; the underlying genetic cause has been identified in six PCH types (PCH1, PCH2, and PCH4-6 and 8). PCH2 represents the most frequent form of PCHs and typical clinical features are respiratory and feeding difficulties at birth, dyskinesia and chorea, severe impairment of cognitive and motor development, progressive microcephaly, and frequent death in late infancy or early childhood. MRI imaging in PCH2 shows brainstem and cerebellar hypoplasia, with the cerebellar hemispheres more affected than the vermis. In patients with PCH2, 4 and 5, biallelic mutations in genes encoding three subunits of the heterotetrameric transfer RNA (tRNA) splicing endonuclease complex, TSEN54, TSEN2, and TSEN34 have been identified. PCH2 shows significant overlapping features with microcephaly with pontine and cerebellar hypoplasia (MCPCCH), generally associated with loss-of-function CASK mutations. The classical MCPCCH phenotype can be found in females who typically have moderate to severe intellectual disability and progressive microcephaly with pontine and cerebellar hypoplasia. Possible findings are ophthalmologic anomalies and sensorineural hearing loss. Males with a CASK mutation have also been identified. The phenotypic spectrum ranges from severe intellectual disability and MCPCCH, or early-infantile epileptic encephalopathy to mild X-linked intellectual disability (XLID) with or without nystagmus. I will present an overview on clinical and genetic data of patients with PCH and MCPCCH and how to discriminate the two conditions. My focus will be on the different CASK mutations in females and males and their associated phenotypes to help understanding genotype-phenotype relationships.

S03.2 The neurobiology of lissencephaly A. Wynshaw-Boris; Cleveland, OH, United States.
No abstract received at production date. Check the online database at www.eshg.org/abstracts2014.0.htm for possible updates.

S03.3 Neuronal migration defects associated with mutations in tubulins and MT-related proteins L. Broix, K. Poirier, Y. Saillour, N. Bahu-Buïsson, J. Chelly; Cochran Institute, INSERM Unit 1016, CNRS –UMR 8104, Paris-Descartes University, Paris, France.
Neuronal migration disorders such as malformations of cortical development are frequent causes of epilepsy and intellectual disability. Disrupted biological and cellular processes such as neuronal progenitors proliferation, neuronal migration, and cortical organization, are traditionally used as a basis for the classification of MCD. Etiologies of these disorders are heterogeneous and genetic studies in humans and mice have identified a spectrum of mutations in genes involved in an array of crucial processes that often disrupt the development of the cerebral cortex. Aside from genes such as ARX, GPR56 and WDR62, the importance of genes encoding cytoskeletal proteins has become evident. For example, mutations in DXC and LS1, both of which encode proteins involved in MT homeostasis, are associated with a large spectrum of neuronal migration disorders. Moreover, MCD associated with mutations in α- or β-tubulin-encoding genes such as TUBA1A, TUBB2B, TUBB3 and TUBB5, have been also described. These tubulin-related cortical dysgenesis are thought to involve a combination of abnormal neuronal proliferation, migration and differentiation. More recently, we reported the association between mutations in TUBG1, DYN1C1H1, KIF2A and KIF5C, and diverse forms of malformations of cortical development with or without microcephaly. We further showed that the mutations in these MTs-related proteins: KIF5C, KIF2A and DYN1C1H1 affect ATP hydrolysis, productive protein folding and microtubule binding, respectively. In addition, we showed by in utero electroporation that in vivo downexpression by shRNA of mouse, Tub3 and Tubg1, as well as expression of Kif2a mutants, interferes with proper neuronal polarization and migration, morphogenesis and intermediate progenitor proliferation. Altogether, these findings together with literature data support the hypothesis that proliferation and migration are genetically and functionally interdependent. Finally, they reinforce the importance of centrosomal and
MT-related proteins in cortical development and strongly suggest that MT-dependent mitotic and post-mitotic processes are major contributors to the pathogenesis of MCD.

**S04.1**

**Disease, networks and epistasis**

C. Weibber; Neurological Disease Genomics, MRC Functional Genomics Unit, Department of Anatomy & Genetics, Oxford University, Oxford, United Kingdom.

I will give an overview of our recent work in identifying the pathways and processes underlying complex disorders, illustrating how different functional genomics resources can each provide novel biological insights into the same phenotype-influencing gene network. The topologies of the identified networks can identify pathway loading (both additive and epistatic) along with the direction in which the pathway is perturbed, thereby inviting drug repurposing. I will also illustrate some of the novel integrative approaches that we have been applying in GWA/exome studies and used to determine the significance of a functionally-clustered genome.

**S04.2**

**Understanding molecular mechanisms of human disease mutations and coding variants through 3D protein networks**

H. Yu; Ithaca, NY, United States.

To better understand the molecular mechanisms and genetic basis of human disease, we combined the massive scale of network systems biology with the supreme resolution of traditional structural biology to generate the first comprehensive atomic-resolution 3D interactome-network comprising 3,398 interactions between 2,890 proteins with structurally-defined interface residues for each interaction. We found that disease mutations are significantly enriched both among interface residues and other non-interface ones within the same domains, contradicting the previous assumption that only a few interface residues are mutation hot spots for disease. We further classified 94,476 disease-associated mutations according to their inheritance modes and found that the widely-accepted “guilt-by-association” principle does not apply to dominant mutations. Furthermore, recessive truncating mutations on the same interface are much more likely to cause the same disease, even if they are close to the N-terminus of the protein, indicating that a significant fraction of truncating mutations can generate functional protein products.

**S04.3**

**From protein networks to disease mechanisms**

R. Sharan; Tel Aviv University, Department of Computer Science, Tel Aviv, Israel.

In recent years, there is a tremendous growth in large scale data on human proteins, their interactions, and their relations to diseases. These allow for the first time a systems-level analysis of the molecular basis of disease. In my talk I will describe several recent works in this direction, aiming to uncover novel disease proteins and their underlying pathways with implications to diagnosis and therapy.

**S05.1**

**Dynamic blastomere behaviour**

R. Pera; Stanford, CA, United States.

No abstract received at production date. Check the online database at www.esgh.org/abstracts2014.0.html for possible updates.

**S05.2**

**24 chromosome copy number analysis for preimplantation genetic screening**

A. H. Handyside; Illumina, Cambridge, United Kingdom.

Chromosome aneuploidy in human gametes and embryos is a major cause of IVF failure and miscarriage and can result in affected live births. To avoid these outcomes and improve implantation and live birth rates, preimplantation genetic screening (PGS) aims to identify any euploid embryos prior to transfer but has been restricted to analysis of a limited number of chromosomes. Over the last 15 years, various technologies have been developed which allow copy number analysis of all 23 pairs of chromosomes, 22 autosomes and the sex chromosomes, or ‘24 chromosome’ copy number analysis in single or small numbers of cells. The pros and cons of these technologies will be reviewed and evaluated for their potential as screening or diagnostic tests when used in combination with oocyte or embryo biopsy at different stages.

**S05.3**

**Preimplantation genetic diagnosis**

T. Voet; Leuven, Belgium.

No abstract received at production date. Check the online database at www.esgh.org/abstracts2014.0.html for possible updates.

**S06.1**

**Risk is More Than a Number: About Risks and Probabilities and People’s Perceptions of Genetic Risks**

D. R. M. Timmermans; 1Department of Public and Occupational Health, EMGO Institute, VU University Medical Center, Amsterdam, Netherlands, 2National Institute for Public Health and the Environment, Bilthoven, Netherlands.

Risk communication is an essential component of genetic counseling. Genetic testing and providing information about genetic predisposition may enable early disease detection, targeted surveillance, and may lead to effective prevention strategies and behavioral change. However, the impact of genetic information on people’s perceptions may be limited. Most people are unfamiliar with probabilistic thinking and find it hard to understand risks. Moreover, risks are experienced differently depending on the characteristics of the risk. A risk is more than a number. It is not only the probability, the quantification of the uncertainty, but also the nature of the risk, the severity of the consequences and the degree of control what matters. People process and evaluate information about potential risks in two different ways: analytical-rational if possible, but always intuitive-affective. The characteristics of the risks as well as the way risk information is processed impact people’s understanding and perception of risks. This perception may be in discordance with the way experts’ perceive genetic risks. In order to enable people to make informed decisions, information about (genetic) risks should not only be adequate but should also be in line with people’s perceptions or mental model. Discrepancies with people’s mental model of genetic risks as well the abstractness of the risk information may hamper people’s informed decision making. In this presentation I will discuss the factors affecting the perception of health risks and genetic risks in particular, the way people understand the risks communicated to them and what this means for risk communication.

**S06.2**

**Risk perception: what could be at stake in multiple genetic testing?**

C. M. Julian-Reynier; Institut Pauli-Calmettes, UMR912 Inserm, Marseille, France.

First the concept of risk perception will be introduced, highlighting its interest for clinical practice or various research fields. We will focus on associated factors and in particular on the potential role of emotions in risk perception. Then the evidence for the relevance of previous findings will be reviewed in the context of genetic risk assessment and genetic test result disclosure such as investigated in clinical genetics/genetic counseling. Different application fields such as cancer genetics will be selected as illustrations of different situational contexts. Finally the way these previous experiences and body of knowledge could help to anticipate the potential consequences of multiple risk information disclosure and to document specific recommendations in the context of the process of multiple genetic testing or next generation sequencing will be discussed.

**S06.3**

**Risk Communication Methods for Helping Patients Understand the Risks and Benefits of Genetic Testing**

A. Fagerlin; University of Michigan, Ann Arbor VA Center for Clinical Management Research, Ann Arbor, MI, United States.

Making decisions about whether to undergo genetic testing or how to use information from genetic tests is exceedingly complex. The complexity is due, in part, to the numerous demands required of patients to understand the information being presented. In this talk, I will discuss methods for improving patients’ understanding of risk and benefits information. Furthermore, I will discuss how different risk communication methods can influence risk perceptions. Finally, I will discuss the role of family history and how that affects patients’ (hypothetical) perception of risk and how patients weigh information about their family history and their risks to make screening decisions.
S07.1 Gene therapy of human genetic diseases with AAV vectors
A. Auricchio; Telethon Institute of Genetics and Medicine (TIGEM), Napoli, Italy.
No abstract received at production date. Check the online database at www.eshg.org/abstracts2014.html for possible updates.

S07.2 Epithelial stem cell in cell and gene therapy
M. De Luca; Dipartimento di Scienze della Vita sede ex-Scienze Biomediche, Modena, Italy.
Adult stem cells are cells with a high capacity for self-renewal that can produce terminally differentiated progeny. Stem cells generate an intermediate population of committed progenitors, often referred to as transit amplifying (TA) cells, that terminally differentiate after a limited number of cell divisions. Human keratinoctye stem cells are clonogenic and are known as holoclones. Human corneal stem cells are segregated in the limbus while limb-derived TA cells form the corneal epithelium. Self-renewal proliferation and differentiation of limbal stem cells are regulated by the ΔNp63 (α, β and γ), C/EBPs δ and β and transcription factors. Cultivated limbal stem cells gene-nate sheets of corneal epithelium suitable for clinical application. We report long-term clinical results obtained in an homogeneous group of 154 patients presenting with corneal opacification and visual loss due to chemical and thermal burn-dependent limbal stem cell deficiency. The corneal epithelium and the visual acuity of these patients have been restored by grafts of autologous cultured limbal keratinoctyes. In post hoc analyses, success was associated with the percentage of p63-bright holocline-forming stem cells in culture. Graft failure was also associated with the type of initial ocular damage and postoperative complications. Mutations in genes encoding the basement membrane component laminin 5 (LAM5) cause junctional epidermolysis bullosa (JEB), a devastating and often fatal skin adhesion disorder. Epithelial stem cells transduced with a retroviral vector expressing the β3 integrin cDNA can generate genetically corrected cultured epitheral grafts able to permanently restore the skin of patients affected by LAM5-β3-deficient JEB. The implication of these results for the gene therapy of different genetic skin diseases will be discussed.

S07.3 Therapeutic targeting of Phosphatidylinositol-3-kinase/AKT/mTOR signalling in segmental overgrowth disorders
R. Semple; University of Cambridge, Metabolic Research Laboratories, Addenbrooke's Hospital, Cambridge, United Kingdom.
The type 1A phosphatidylinositol-3-kinase (PI3K) enzyme complex serves as a signal transducer for a diverse range of hormone and growth factor receptors, and harbours somatic activating mutations in a large number of cancer. We and others have recently established that mosaicism for many of the same mutations underlies a spectrum of disorders of segmental overgrowth, ranging from isolated macrodactyly to catastrophic overgrowth affecting large parts of the body and several tissues, and commonly associated with complex vascular anomalies. Identification of the underlying signalling defect in affected tissues has immediately suggested that pharmacological targeting of the PI3K/AKT/mTOR pathway may offer the first rational, effective therapeutic approach for these disorders. The effect of mTORC or PI3K inhibition in dermal fibroblasts from affected patients will be discussed, as well as therapeutic approaches, as DCM alleles result in functional changes that are the cause of heart failure. However, in patients with other causes of heart failure the picture is clearer, and may lead to approaches to therapy, as DCM alleles result in functional changes that are the cause of heart failure.

S08.1 Demographic inference from identity by descent
I. Pe’er; New York, NY, United States.
No abstract received at production date. Check the online database at www.eshg.org/abstracts2014.html for possible updates.

S08.2 Insights into European genetic history at fine geographic scales using haplotype-based approaches
S. Myers; Dept of Statistics, Oxford University, United Kingdom
Wellcome Trust Centre for Human Genetics, Oxford University, United Kingdom.
Modern genetic datasets are revealing features of our genetic history, and how this has shaped our genomes, in unprecedented detail. Across Europe, multiple invasions and migrations have taken place over the past several millennia, but the possible genetic effects of these and of subsequent regional isolation remain uncharacterised, partly due to the extremely sub-
opposite of the changes seen with HCM alleles: depressed motor function and reduced Ca(2+) sensitivity.

S09.2 Mendelian Randomization
M. V. Holmes; University of Pennsylvania, Philadelphia, PA, United States.

Identifying causal biomarkers is important in the quest to prevent and treat cardiovascular disease. Mendelian randomization is a genetic epidemiological technique that can be used to make causal inference about biomarkers and environmental exposures.

I will talk about recent applications of Mendelian randomization with a focus on identifying potential therapeutic targets and understanding the role of physiological traits and environmental exposures in cardiovascular disease pathogenesis.

S09.3 Genetic testing in the clinical arena, current and future perspectives
P. Charron1,2; 1Dept. of genetics, Pitié-Salpêtrière hospital, AP-HP, Paris, France; 2INSERM UMR1166, Paris, France.

The recent development of molecular genetics in cardiovascular diseases has created a new understanding of their pathogenesis and natural history, and also new possibilities for the diagnosis of these genetic disorders through genetic testing. This has induced new expectations, and new demands, from both families and physicians regarding genetic counselling, DNA testing and application of this knowledge in clinical practice. The integrated use of genetic testing in routine practice developed rapidly in the context of monogenic cardiovascular disorders (such as cardiomyopathies, channelopathies, Marfan syndrome) with significant medical impacts on the management of patients or their families. This refers to diagnostic testing as well as predictive testing, prognostic evaluation and, rarely, prenatal or pre-implantation diagnosis. Encouraging data were also identified in the context of pharmacogenetic interactions (such as Vitamin K antagonists, antplate-let treatment) and multifactorial diseases (such as coronary artery diseases and heart failure).

The clinical utility of genetic testing was acknowledged through the production of international or national guidelines recommending routine genetic testing in monogenic disorders. However, its use in everyday clinical practice has been limited by the cost and complexity of conventional sequencing technologies. Advances in next generation sequencing technology (NGS) have the potential to solve this problem by analyzing substantially larger genomic regions at a lower cost than conventional capillary Sanger sequencing. But they may also pose new challenges. This was already obvious in preliminary studies that compared a number of variants found in cardiovascular diseases with exome or genome data from general population, and questioning the pathogenicity of variants previously considered as disease-causing. We will review the advantages, pitfalls, and clinical utility of NGS in the clinical setting of cardiovascular disorders, especially in the context of monogenic disorders.

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S10.2 Kataegis: a mutation signature identified through whole-genome sequencing of human cancers
S. Nik-Zainal1,2, M. Neuberger3, M. R. Stratton1; 1Wellcome Trust Sanger Institute, Cambridge, United Kingdom; 2East Anglian Medical Genetics Service, Cambridge University Hospitals NHS Foundation Trust, Cambridge, United Kingdom; 3Laboratory of Molecular Biology, Cambridge, United Kingdom.

Cancer is the ultimate disorder of the genome, characterised by not one or two substitutions, indels or copy number aberrations, but hundreds to thousands of acquired mutations that have been accrued through the development of a tumour. The set of mutations observed in a cancer genome is not simply a random accumulation of variants. It is the aggregate outcome of several biological mutational processes comprising an underlying mechanism of DNA damage mitigated by the DNA repair pathways that exist in human cells. Each mutational process will leave its distinctive mark or mutational signature on the cancer genome.

Recently, we set out to extract the mutational signatures characterizing the mutational processes that have been operative in 21 whole-genome sequenced breast cancers. Multiple distinct known and novel substitution, insertion/deletion and rearrangement signatures were unearthed by these analyses. Here, I will describe one particularly intriguing signature of localized regions of dense somatic hypermutation, called kataegis, in which substitutions at CpG base pairs occurring within a distinctive sequence context were found associated with clusters of genomic rearrangements. Using an algorithm developed to allow efficient detection of kataegis, we investigate other whole-genome sequenced cancers to show that this phenomenon is not restricted to breast cancer. These studies harness the full scale of whole-genome sequencing. Furthermore, detailed and considered analyses of genomic data can provide biological insights that would otherwise remain buried.

The mechanism of kataegis however, is unknown. On the basis of similarities in mutation class and sequence context in experimental systems, members of the AID/APOBEC family of cytidine deaminases were implicated. Using whole-genome sequencing approaches in model organisms, the mechanism underlying kataegis is slowly being unraveled.

S10.3 Medulloblastoma links chromothripsis with TP53 mutations
J. O. Korbel; Heidelberg, Germany.

No abstract received at production date. Check the online database at www.eshg.org/abstracts2014.0.html for possible updates.

S11.1 Copy number alterations in skin disorders
X. Zhang; Beijing, China.

No abstract received at production date. Check the online database at www.eshg.org/abstracts2014.0.html for possible updates.

S11.2 Congenital heart disease
B. Keavney; Manchester, United Kingdom.

No abstract received at production date. Check the online database at www.eshg.org/abstracts2014.0.html for possible updates.

S11.3 Copy number variants are a common cause of short stature
C. T. Thié1, A. Reis1, H. Dörr2, A. Rauch1; 1Institute of Human Genetics, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany; 2Department of Pediatrics and Adolescent Medicine, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany; 3Institute of Medical Genetics, University of Zurich, Zurich, Switzerland.

Shortness of stature is one of the most common pediatric concerns and has an incidence of 3% in the general population. In the majority of patients with idiopathic growth deficit the etiology remains elusive in the absence of morphological details. This unknown etiology prevents a sufficient medical care in most cases. As it has been proposed that the growth fundamentally regulated by genetic factors, GWAS found significant evidence for both single nucleotide and copy number polymorphisms associated with height variation in the general population. However, these associations explain only a small fraction of the overall variability of human height.

Based on the early identification of SHOX gene deletions as a common cause
of idiopathic and syndromic (Leri-Weil syndrome) short stature as well as copy number variation (CNV) as a common cause of intellectual disability, the hypothesis of a "rare variant - frequent disease" hypothesis seemed to be feasible for short stature. To address this hypothesis we thoroughly build a study group of more than 400 families with idiopathic short stature and conducted SNP array analysis to demonstrate the presence of CNVs as a common underlying cause of short stature. Molecular karyotyping was performed and CNVs of a minimum size of 50kb scored and compared to healthy controls. Based on this technique we found a significant odds ratio for aberrations above 100 kb only. Due to the number of potential disease causing CNVs a gene-centric analysis comparing known CNVs, gene functions, tissue expression and knock-out phenotypes was necessary. We confirmed that 10 % of the patients had de novo and inherited CNVs in agreement to the segregation of the short stature phenotype in the families. These CNV regions include known microdeletion/duplication loci expanding the phenotypical spectrum of these entities. The pathogenicity of novel loci was substantiated by comparison to available information, especially the overlap with loci of genome wide association for short stature. Our data showed a clear connection between the prenatal onset of short stature as well as the severity of the growth deficit with the likelihood of the identification of causal CNVs. Thus, we confirmed CNVs as a main cause of idiopathic short stature. Further improvement of the array technology as well as the application of CNV identification based on next generation sequencing will lead to more elaborate and detailed view on even smaller CNVs. Application of these methods can help to illuminate the complex heterogeneity of short stature.

S12.1 The Epigenetic Basis of Common Human Disease
A. P. Feinberg
Johns Hopkins University, Baltimore, MD, United States.

Although epigenetic changes in the cancer genome have been known for 3 decades, the role of epigenetics in common human disease generally and its relationship to genetic variation has only recently begun to be explored. We have been developing whole-genome approaches to epigenetic analysis of human disease. Surprising results have been the discovery of CpG island shores and large unmethylated blocks corresponding to nuclear lamina / heterochromatin in syne-modification regions (LOCks), and accounting for the vast majority of epigenetic changes in cancer. We have also been contributing to a new field of epigenetic epidemiology that integrates genetic, epigenetic, and environmental factors, and we are applying these approaches to the study of autoimmune and neuropsychiatric disease. One of the most exciting developments in this recent work is the idea that epigenetic plasticity under genetic control may itself confer a survival advantage in evolution, and may also be important in normal tissue differentiation and response to the environment. In support of this idea, we have identified variably methylated regions (VMRs) in normal development. These same VMRs appear to be targets for disrupted methylation in cancer, pointing to a unifying mechanism underlying cause of short stature. Molecular karyotyping was performed and CNVs of a minimum size of 50kb scored and compared to healthy controls. Based on this technique we found a significant odds ratio for aberrations above 100 kb only. Due to the number of potential disease causing CNVs a gene-centric analysis comparing known CNVs, gene functions, tissue expression and knock-out phenotypes was necessary. We confirmed that 10 % of the patients had de novo and inherited CNVs in agreement to the segregation of the short stature phenotype in the families. These CNV regions include known microdeletion/duplication loci expanding the phenotypical spectrum of these entities. The pathogenicity of novel loci was substantiated by comparison to available information, especially the overlap with loci of genome wide association for short stature. Our data showed a clear connection between the prenatal onset of short stature as well as the severity of the growth deficit with the likelihood of the identification of causal CNVs. Thus, we confirmed CNVs as a main cause of idiopathic short stature. Further improvement of the array technology as well as the application of CNV identification based on next generation sequencing will lead to a more elaborate and detailed view on even smaller CNVs. Application of these methods can help to illuminate the complex heterogeneity of short stature.

S12.2 Intergenerational epigenetic programming in a mouse model of undernutrition
A. C. Ferguson-Smith
University of Cambridge, Department of Genetics, Cambridge, United Kingdom.

Environmental factors during early life are critical for the later metabolic health of the individual and of future progeny. In a mouse model of maternal caloric restriction during pregnancy the metabolic physiology of offspring over two generations is affected, including via paternal transmission to the second generation. Here we explore whether the paternal experience of in utero undernutrition, with an impact on the health of his offspring, is an epigenetically inherited memory transmitted via his sperm methylome.

S12.3 Cancer Genetics and Epigenetics: Two Sides of the Same Coin?
P. A. Jones
Chief Scientific Officer, Van Andel Research Institute, Grand Rapids, MI, United States.

Genetic and epigenetic alterations used to be considered as providing two separate pathways leading to cancer. However, recent whole genome sequencing of a large number of human cancers has led to the realization that many previously unknown mutations occur in genes which regulate the epigenome. These mutations could potentially alter DNA methylation patterns, histone modifications and the positioning of nucleosomes to profoundly alter gene expression in cancer. Mutations in these genes can therefore contribute to cancer just as epigenetic processes can cause mutations in tumor suppressor genes and disable DNA repair enzymes such as MLH1 and MGMT. The cross talk between the genome and the epigenome is the source of a fascinating new area of research which gives us unprecedented opportunities to understand carcinogenesis. Because many of the genes which are mutated are enzymes, these findings may also lead to new avenues for drug discovery. Epigenetic therapies are already a reality and we can expect great progress to be made over the next few years as new targets are identified and tested for their potential new cancer targets.

S13.1 State of the Art of Non-Invasive Prenatal Testing
L. S. Chitty
London, United Kingdom.

Traditionally definitive prenatal diagnosis of genetic and chromosomal disorders has required analysis of fetal tissue obtained by invasive testing (usually chorionic villus sampling or amniocentesis) which carries a small but significant risk of miscarriage. The identification of cell free fetal DNA (cffDNA) in maternal plasma from four weeks gestation offered an alternative source of fetal genetic material for testing based on a simple maternal blood sample, thereby allowing earlier and safer prenatal diagnosis. The majority of cell free DNA in maternal plasma emanates from the mother herself. This high background of maternal cffDNA meant that early application was based on the detection of exclusion of alleles that were inherited from the father, e.g. SRY, or that arose de novo, e.g. aochondroplasia. Fetal sex determination based on analysis of cffDNA is now widely available in Europe in pregnancies at risk of sex-linked disorders, where it is used to direct invasive testing. It is also available clinically in the UK for the definitive diagnosis of a number of conditions including some skeletal dysplasias and Apert syndrome, as well as for the exclusion of the paternal allele in families at risk of cystic fibrosis where parents carry different mutations. Elsewhere in Europe it has been used for the non-invasive diagnosis of other conditions such as Huntington Disease, but invasive testing has been required to confirm the fetal mutation status. The other major application is fetal Rhesus-D typing in RhD negative mothers where it is used both to inform pregnancy management in women with a history of haemolytic disease of the newborn, as well as to direct m unite immunophrophylaxis with anti-D immunoglobulin to those RhD negative mothers carrying an RhD positive baby. With the advent of next generation sequencing (NGS) it has become possible to estimate the proportion of sequence aligning to different chromosomes present in maternal blood and thereby detect fetal aneuploidy. This is now widely available in the private sector with detection rates for trisomies 13, 18 and 21 in excess of 99% with similarly high specificities. However discordant results are regularly reported and this test can only be considered a highly predictive screening test with invasive testing required to confirm positive results. The potential for easy access to non-invasive prenatal diagnosis and testing has raised significant ethical concerns, largely with regard to maintaining informed parental choice, and the need for good health professional and public education. The huge commercial drive to implement NPT for aneuploidy serves to heighten the need for early introduction of educational programmes and careful consideration of strategies required to facilitate appropriate implementation into public health services.

S13.2 Noninvasive prenatal testing creates an opportunity for antenatal treatment of Down syndrome
1Tufts Medical Center, Boston, MA, United States, 2National Institute for Public Health and Environment, Bilthoven, Netherlands, 3Tufts University, Medford, MA, United States.

Noninvasive prenatal testing (NIPT) for Down syndrome (DS) using massively parallel sequencing of maternal plasma DNA facilitates early detection of affected fetuses. NIPT is performed at ~ 12 weeks of gestation it creates a potential 28-week window of opportunity in which to treat the fetus by orally administering small molecules to the mother. Our laboratory is using a 4-phase translational approach that involves human biomaterials (amniocytes and amniotic fluid), a mouse model of DS, and living human fetuses. In phase 1 we compared the transcriptome of fetuses with and without DS by analysis of cell-free RNA in amniotic fluid and showed that oxidative stress was a significant difference in the affected fetuses. In phase 2 we explored differentially-regulated genes in the Connectivity Map to identify candidate FDA-approved therapeutic molecules. In phase 3 drugs that had high efficacy in negating oxidative stress and showed low toxicity were selected for further studies in an animal model. We use the Ts1Cje mouse model of DS because affected males are fertile, yet...
cognition is significantly impaired. We can therefore use wild type females for the treatment experiments, ensuring both a normal intrauterine environment and normal postnatal nurturing behavior. Treated and untreated affected pups and littermate controls are then evaluated using a variety of brain studies that include analyses of gene expression, histology, cellular proliferation and migration, and neurorrhesis. In parallel, in phase 4 we are preparing for a human clinical trial by analyzing fetal brain growth using quantitative fetal magnetic resonance imaging (MRIs). To date, we have identified significant phenotypic differences in Ts1Cje embryos and neonates as endpoints to evaluate therapy. We have also shown encouraging, statistically-significant improvement in some neurobehavioral tests in adult mice. These results suggest that prenatal treatment in DS is an achievable goal.

S13.3 Clinical and social implications of NIPT
K. E. Ormond;
Stanford University School of Medicine, Stanford, CA, United States.
Noninvasive prenatal testing (NIPT) became clinically available in late 2011. Since that time, hundreds of thousands of pregnant women have undergone NIPT. This talk will discuss the social and ethical concerns that preceded the clinical use of NIPT, as well as the data that exists on patient, provider and stakeholder attitudes towards NIPT now that it is in use.

S14.1 Developments in rapid DNA sequencing technology
J. O’Halloran; J. Tyson;
1 Newcastle upon Tyne, United Kingdom, 2QuantaMdx, Newcastle upon Tyne, United Kingdom.
No abstract received at production date. Check the online database at www.eshg.org/abstracts2014.0.html for possible updates.

S14.2 DNA sequencing in neonatal intensive care units
S. Kingsmore;
Kansas City, MO, United States.
No abstract received at production date. Check the online database at www.eshg.org/abstracts2014.0.html for possible updates.

S14.3 Impact of rapid DNA sequencing on diagnostic and public health microbiology
C. U. Köser;
University of Cambridge, Cambridge, United Kingdom.
Whole genome sequencing (WGS) promises to be transformative for the practice of clinical microbiology, and the rapidly falling cost and turnaround time mean that this will become a viable technology in diagnostic and reference laboratories in the near future. The objective of this talk is to provide an overview of a modern diagnostic microbiology laboratory in order to analyse at a very practical level where WGS might be cost-effective compared to current alternatives. I propose that molecular epidemiology performed for outbreak investigations and genotypic antimicrobial susceptibility testing for microbes that are difficult to grow represent the most immediate areas for application of WGS, and discuss the technical and infrastructure requirements for this to be implemented.

S15.1 Signaling networks in the auditory sensory cells unveiled by hereditary deafness
N. Michalski1,2, C. Petit1,2,3,4,5,6,7,8,9,10;
The biochemical study of the components and associated molecular networks of the auditory sensory cells, the hair cells, are strongly impeded by the small number of these cells. In contrast, human and mouse genetic approaches have proven to be powerful to identify such components. The presentation will focus on the hair bundle of the hair cells that operates the mechanoelectrical transduction (MET). This sensory antenna is comprised of several rows of rigid microvilli, known as stereocilia of a few femtoliters in volume, that are organized in a staircase pattern. The MET process relies not only on the MET machinery but also on the proper architectural and biophysical properties of the hair bundle. In addition, any defect that affects the mechanical elements involved in sound-induced oscillation of the hair bundle or its ionic environment necessary to drive hair cell depolarization, also perturbs MET. For each of these contributors to the MET, several key components have been identified by studying inherited deafness forms; they are used as entry points to decipher the involved molecular networks. The presentation will illustrate how the genetic approach led to the discovery of molecules controlling the development, maturation and maintenance of the correct structure of the hair bundle and the MET process. It will illustrate how it enlightens our understanding of the way these proteins form molecular complexes in vivo and how these complexes work and cooperate together. A special emphasis will be put on the networks composed of proteins encoded by the genes responsible for the Usher syndrome, the most frequent cause of hereditary sensorineural deafness associated with retinitic pigmentosa. Usher genes are critically involved in hair bundle development and the MET machinery as well as the functioning of the photoreceptor cells. Recent results based on mouse and human genetics, which provide evidence for a molecular maturation of the auditory MET machinery, will be discussed.

S15.2 Genes and cellular pathway of Fanconi’s anemia
J. Surralles;
Group of Genome Instability and DNA Repair, Department of Genetics and Microbiology, Universitat Autònoma de Barcelona (UAB) and Center for Biomedical Network Research on Rare Diseases (CIBERER), Barcelona, Spain.
Fanconi anemia (FA) is a rare genetic disease characterized by bone marrow failure, malformations, chromosome fragility, hypersensitivity to DNA interstrand-cross-linking (ICL) chemotherapeutics, increased cancer risk, and childhood leukemia. Fanconi anemia is also a major cause of birth defects and solid tumors. The only cure of the hematological disease, which is the major cause of early death, is bone marrow transplantation using an HLA compatible donor. Those families with unavailable donor rely on preimplantation genetic diagnosis with selection of an HLA related embryo and in the future implementation of advanced gene and cell therapies. All these novel therapeutic applications to human health are further complicated by the fact that at least 16 genes, from FANCA to FANQ, are involved in this disease and their products interact in a complex genome stability and tumor suppression network. Notably, 4 out of 16 FA genes (FANCD1/BRG2, FANCN/PALB2, FANQ/BRIP1 and FANCO/Rad51C) are breast cancer susceptibility genes in otherwise unaffected mutation carriers. Therefore, the discovery of novel Fanconi anemia genes is important not only for the affected patients but also for the general population. In this context, I will present data on the discovery of a novel Fanconi anemia gene by whole exome sequencing. Interestingly, this gene (ERCC4 or FANQ) is involved not only in FA but also in xeroderma pigmentosum, a melanoma susceptibility disorder with defective nucleotide excision repair (NER), and in X-Factor progeria. Our findings indicate that depending on the balance between ICL repair and NER, mutations in the same gene lead to two distinct cancer susceptibilities.

S15.3 Analysis of signalling pathways in Tbx1 mutants identifies a novel mechanism in coronary artery morphogenesis
P. J. Scambler; S. Vinet; J. Chappell; J. Sunnabararamham; B. Vernon; T. Mohan; 1UCL Institute of Child Health, London, United Kingdom, 2MRC National Laboratory for Medical Research, London, United Kingdom.
Much of the phenotype of 22q11 deletion syndrome (22q11DS) is secondary to haplinsufficiency of the transcription factor TBR1. Several studies have demonstrated distinct temporal and tissue-specific requirements for Tbx1 during development. Identification of candidate targets has led to new insights into cardiovascular morphogenesis. One such potential target is the signalling protein CXCL12 and its receptor CXCR4. This pathway is involved in collective cell migration, initiating contact inhibition of locomotion (CL (“chase and run”) between NCC and endothelial plaque. We predicted that disruption of this pathway in Tbx1 null mice would lead altered NCC patterning, and therefore conditionally mutated the receptor in cardiac neural crest cells. However, no defects were observed suggesting major species differences in the role of CXCL12-CXCR4 signalling. Rather, we showed that CXCR4 positive endothelial cells were the main target cell population, and that disruption of signalling led to VSDs, great vessel defects, and semilunar valve defects. In addition, lack of CXCL12-CXCR4 signalling led to a major failure in the elaboration of mature coronary arteries from the coronary endocardial cushion. Thus, CXCL12-CXCR4 signalling is required for endothelial cell migration, initiating contact inhibition of locomotion (CL) and NCC patterning. This finding impacts our understanding of the role of CXCL12-CXCR4 signalling in the development of the aortic outflow tract of Tbx1 null embryos may therefore underlie the minor coronary artery dysmorphogenesis seen in these animals. Others have implicated the CXCL12-
Cancer genetic heterogeneity: implications for therapy responsiveness and acquisition of therapy resistance
A. Bardelli
Cancer Institute, Trieste, Italy.
No abstract received at production date. Check the online database at www.eshg.org/astracts2014.0.html for possible updates.

Non-cell autonomous interactions promote sub-clonal heterogeneity
A. Marusyk1, 2, D. Tabanou3, V. Almendro4, P. Altheimer1, 5, N. Zelent6, K. Polyakov7
1 Dana Farber Cancer Institute, Boston, MA, United States; 2Harvard Medical School, Boston, MA, United States.

Cancers arise and progress due to the underlying Darwinian somatic evolution. It has been commonly accepted that biological and clinical behaviors of individual tumors reflect properties of the most abundant cells that have achieved clonal dominance by virtue of carrying the most “advanced” complement of oncogenic driver mutations. However, the recent influx of data from tumor genome sequencing has revealed a remarkable degree of genetic divergence within tumors, including sub-clonal differences in mutational status of key driver genes. This clonal heterogeneity begs obvious questions: i) what are the mechanisms that enable co-existence of genetically distinct populations, and ii) what are the biological consequences of this co-existence? Microenvironmentally constrained tumors represent a particularly interesting scenario: overcoming the bottleneck requires changes in the tumor environment induced by secreted factors, but it is not clear a priori whether the expression of a secreted factor capable of driving tumor outgrowth can provide an autonomous fitness advantage. To address this scenario, we have developed a mouse xenograft model of microenvironmentally-constrained tumors. Using this model, we have interrogated the impact of sub-clonal expression of secreted factors in contexts of either competition against the parental clone or that of polyclonal tumors. We found that tumor progression can be driven non-cell autonomously by a sub-population of cells that is unable to achieve clonal dominance. This non-cell autonomous driving of tumor growth is predicted to stabilize sub-clonal heterogeneity within tumors throughout clinically relevant time frames. This co-existence of biologically distinct sub-populations enables inter-clonal interactions that can affect clinically important phenotypes.

Circulating tumor cells: Detection, biology and clinical implications
K. Pantel
Institute of Tumor Biology, University Cancer Center Hamburg, University Medical Center Hamburg Eppendorf, Hamburg, Germany.

Sensitive methods have been developed to capture circulating tumor cells (CTCs) in the peripheral blood at the single cell level (Pantel et al., Nat Rev Cancer 2008). CTCs are usually detected by immunostaining or RT-PCR assays, and more recently by the EPISPOT assay which measures the number of cells releasing secreted tumor-associated marker proteins. Interestingly, detection of cell-free nucleic acids released by tumor cells into the blood might become an indirect way to detect micrometastatic disease (Schwarzenbach et al, Nat Rev Cancer 2011). At present, most CTC assays rely on epithelial markers and miss CTCs undergoing an epithelial-mesenchymal transition (EMT). New markers such as the actin bundling protein plastin-3 (Yokobori et al., Cancer Res. 2013) are not downregulated during EMT and not expressed in normal blood cells might overcome this important limitation and, therefore, increase the sensitivity of CTC assays. Recently, in vivo capture of CTCs with an antibody-coated wire placed into the peripheral arm vein has become feasible and allows now the “fishing” for CTCs from approx. 1.5 liters of blood within 30 minutes. CTC enumeration and characterization with certified systems provides reliable information on prognosis and may serve as liquid biopsy (Alix-Panabieres & Pantel, Clin. Chem. 2013; Pantel & Alix-Panabieres, Cancer Res. 2013). Interestingly, the subset of EpCAM−/CD44−/CD44+/c-Met+ CTCs obtained from the peripheral blood of breast cancer patients might represent metastasis-initiator cells (Baccelli et al, Nature Biotech. 2013). Moreover, monitoring of CTCs before, during and after systemic therapy (e.g., chemotherapy, hormonal therapy, antibody therapy) might provide unique information for the future clinical management of the individual cancer patient. This information can be used as companion diagnostics to improve the stratification of therapies and to obtain insights into therapy-induced selection of cancer cells.
S18.1

Glyco-lipophobia: association with disorders of glycolipid and glycosylphosphatidylinositol anchor synthesis
H. H. Freeze;
Sanford-Burnham Medical Research Institute, La Jolla, CA, United States.

Thankfully, DNA and protein sequencing technologies revolutionized genetics and proteomics. Sadly, the two other macromolecules, carbohydrates and lipids, missed that technical revolution creating a malady called Glyco-lipophobia. Perhaps its development was predictable based on their molecular complexity, template-independent biosynthesis, and structural analysis without having defined functions. Fortunately, this condition is treatable, and given the discovery of over 100 genetic disorders in these pathways, treatment should not be delayed.

Glycosylation related genes comprise 1-2% of the human genome and those genes often fall into a series of distinct (sometimes overlapping) pathways. Each pathway generates sugar chains (glycans) for typical “clients”. The best-known pathway is N-glycosylation found in secreted proteins, membrane-bound receptors, and signaling molecules. Some glycosylation disorders involve addition of glycans to lipids, not proteins. A few glycolipids are biosynthetic precursors of other pathways, but two define distinct pathways: glycosylphosphatidylinositol (GPI) anchors and glycosphingolipids (GSL). These molecules are often located in lipid rafts. Fifteen distinct disorders disrupt these pathways. All cells contain GPI and GSL, and affected patients display a range of mostly severe phenotypes that can include intellectual disability, seizures, ophthalmologic abnormalities, heart defects, and inflammation. In the last decade many new inborn errors of metabolism have been identified, many by next generation sequencing, that are caused by a deficiency of genes involved in phospholipid metabolism. A complementary technique that is frequently applied to investigate the functional defect in these disorders is lipidomics, which strives to measure and quantify as many lipids as possible by (tandem) mass spectrometry. This technique will be introduced followed by different examples that show that combining next generation sequencing and lipidomics is a synergetic combination that yields novel biomarkers for inborn errors of phospholipid metabolism.

S18.2

Disorders of phospholipids, sphingolipids and fatty acids biosynthesis
F. Mochel;
Paris, France.

No abstract received at production date. Check the online database at www.eshg.org/abstracts2014.0.html for possible updates.

S18.3

Update on lipidomic approaches in disorders affecting complex lipids metabolism: the example of cardiolipin
F. M. Vaz;
Academic Medical Center, Laboratory Genetic Metabolic Disease, Amsterdam, Netherlands.

Lipids are important components of cellular membranes but also function as signal molecules in many cellular processes including apoptosis, autophagy and inflammation. In the last decade many new inborn errors of metabolism have been identified, many by next generation sequencing, that are caused by a deficiency of genes involved in phospholipid metabolism. A complementary technique that is frequently applied to investigate the functional defect in these disorders is lipidomics, which strives to measure and quantify as many lipids as possible by (tandem) mass spectrometry. This technique will be introduced followed by different examples that show that combining next generation sequencing and lipidomics is a synergetic combination that yields novel biomarkers for inborn errors of phospholipid metabolism.

S19.1

Whole genome sequencing of 4000 individuals provides insight into genetic architecture of complex traits
N. Soranzo;
Hinxton, United Kingdom.

No abstract received at production date. Check the online database at www.eshg.org/abstracts2014.0.html for possible updates.

S19.2

Using transcriptome sequencing to understand mechanisms of disease
T. Lappalainen;
New York Genome Center, New York, NY, United States.

Detailed characterization of cellular effects of genetic variants is essential for understanding biological processes that underlie genetic associations to disease. This is a particularly pressing question in the interpretation of personal genomes that necessitates accurate prediction and measurement of genome function. One approach to address this challenge is to combine genomic data to a cellular phenotype, such as the transcriptome measured by RNA-seq. The early population-scale RNA-seq studies such as GENOVA and VADIS are now being complemented by Genotype-Tissue Expression project (GTEx), where we are creating a comprehensive public atlas of genetic effects on the transcriptome across multiple human tissues. These data have been used to characterize regulatory and loss-of-function genetic variants as well as imprinting both at the population and individual level. Common expression quantitative trait loci that associate to gene expression levels provide a powerful tool to understand genetic architecture of regulatory variation and its role in GWAS associations. Furthermore, allele-specific expression driven by regulatory variants and imprinting can impact on penetrance of coding variants. Finally, we systematically analyzed transcriptome effects of protein-coding loss-of-function variants. Measuring nonsense-mediated decay (NMD) triggered by SNPs and indels allowed us to understand and better predict NMD trigger and escape. Importantly, effects of loss-of-function variants appear highly context-dependent with frequent tissue-specific effects and dosage compensation. Altogether, our results demonstrate the power of integrating genome and transcriptome data not only to improve our general understanding of genetic variants, but also as a practical approach in future medical and clinical applications of personal genomics.

S19.3

High resolution genetic analysis to detect variants associated with quantitative traits and diseases in the founder Sardinian population
F. Cucca;
Sassari, Italy.

No abstract received at production date. Check the online database at www.eshg.org/abstracts2014.0.html for possible updates.
EDUCATIONAL SESSIONS

ES1.1 Genetics of familial forms of thrombocytopenia
A. Savoia

Inherited thrombocytopenias (ITs) are a heterogeneous group of rare diseases characterized by bleeding risk sometimes associated with platelet dysfunction or other clinical features. More than twenty different forms have been characterized (Table 1). They account for almost 50% of the IT patients, suggesting that many forms are still unrecognizable. The IT genes play a variety of roles in the complex process of megakaryopoiesis and platelet production though their function is often unclear. In the rare forms of CAMT, CTRUS and TAR, thrombocytopenia is severe because of absence or reduction of megakaryocytes (MKs). Whereas in TAR, the platelet count tends to rise during life, in CAMT and CTRUS the disease progresses to bone marrow failure. Loss of function of MPL, the receptor of thrombopoietin, prevents production of MKs in the bone marrow of the CAMT patients. Less certain is instead the role of HOXA11 and RBM8A in the rare forms of CAMT, CTRUS and TAR, thrombocytopenia is severe because of absence or reduction of megakaryocytes (MKs). Whereas in TAR, the platelet count tends to rise during life, in CAMT and CTRUS the disease progresses to bone marrow failure. Loss of function of MPL, the receptor of thrombopoietin, prevents production of MKs in the bone marrow of the CAMT patients. Less certain is instead the role of HOXA11 and RBM8A in

Other modulators, including FLI1, whose haploinsufficiency due to 11q23-ter deletions determines TCPT or JBS. In case of mutations of GATA1 or FLG, another two hematopoietic transcription factors, thrombocytopenia associates with red cells defects and reduction of alpha-granules. Absence of alpha-granules resulting typical gray appearance of platelets is a pharmacogenic feature of GPs. NBEL2, the causative gene in this form, is likely to function in vesicle trafficking and generation of platelet granules. The largest group of ITs is related to defects of proplatelet formation, a process involving dynamic reorganization of the cytoskeleton, signaling pathways or apoptosis. Regarding the first aspect, mutations in genes encoding for components of the cytoskeleton (MYH9, ACTN1, TUBB3, WAS or FLNA) are likely to interfere with the correct proplatelet extension and platelet release. Of note, FLNA interacts with the C-terminus of GP Ib alpha, one subunit of the von Willebrand factor receptor (GPIb/IX/V). Alterations of GPIb/IX/V cause BSS characterized by mild thrombocytopenia associated with reduction of platelet aggregation. Finally, defects of cytochrome c (CYCS) could be associated with increased apoptosis and premature release of platelets into bone marrow instead of blood stream. This heterogeneity makes the molecular diagnostic testing a complex and time-consuming process. Next generation sequencing strategies will have a strong impact in the diagnosis of the known forms and cloning novel IT genes.

Table 1. Features of inherited thrombocytopenias classified according to possible defective processes of megakaryopoiesis and platelet production.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Abbreviation</th>
<th>OMIN entry</th>
<th>Inheritance</th>
<th>Gene (chromosome localization)</th>
<th>Platelet size</th>
<th>Other features</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Congenital amegakaryocytic thrombocytopenia</td>
<td>CAMT</td>
<td>604498</td>
<td>AR</td>
<td>MPL (1p34)</td>
<td>Normal</td>
<td>Reduced megakaryocyte evolution into bone marrow aplasia</td>
<td>Ballmaier et al. Semin Thromb Hemost 37:673, 2011</td>
</tr>
<tr>
<td>Familial platelet disorder and predisposition to acute myelogenous leukemia</td>
<td>FPD/AML</td>
<td>601399</td>
<td>AD</td>
<td>RUNXI (21q22)</td>
<td>Normal</td>
<td>Increased risk (40%) of leukemia or MDS</td>
<td>Baldini et al. Hum Genet 131:1821, 2012</td>
</tr>
<tr>
<td>ANKRD26-related thrombocytopenia</td>
<td>ANKRD26</td>
<td>188010</td>
<td>AD</td>
<td>ANKRD26 (10p2)</td>
<td>Normal</td>
<td>Increased risk of leukemia or MDS</td>
<td>Norris et al. Blood 117:6673, 2011</td>
</tr>
<tr>
<td>Paris-Trousseau thrombocytopenia with ashen hair syndrome</td>
<td>THPS</td>
<td>188025</td>
<td>AD</td>
<td>Large deletion (11q23-ter)</td>
<td>Large</td>
<td>Cardiac and facial defects, developmental delay +/- other defects</td>
<td>Baldini et al. Hum Genet 131:1821, 2012</td>
</tr>
<tr>
<td>Dyserythropoietic anaemia with thrombocytopenia</td>
<td>GATA1RD</td>
<td>300367</td>
<td>XL</td>
<td>GATA1 (Xp11)</td>
<td>Large</td>
<td>Haemolytic anaemia, possible unbalanced globin chain synthesis, possible congenital erythroproietic poikilocytosis</td>
<td>Baldini et al. Hum Genet 131:1821, 2012</td>
</tr>
<tr>
<td>X-linked thrombocytopenia with thalasssemia</td>
<td>X-linked thrombocytopenia</td>
<td>GATA1RD</td>
<td>314050</td>
<td>XL</td>
<td>GATA1 (Xp11)</td>
<td>Large</td>
<td>Haemolytic anaemia, possible unbalanced globin chain synthesis, possible congenital erythroproietic poikilocytosis</td>
</tr>
<tr>
<td>GFI1B thrombocytopenia</td>
<td>GFI1B</td>
<td>nd</td>
<td>AD</td>
<td>GFI1B (9q34.13)</td>
<td>Large</td>
<td>Reduced megakaryocytes. Platelet count tends to normalize in adult life. Bilateral radial aplasia +/- other malformations</td>
<td>Stevenson et al. J Thromb Haemost 11:2039, 2013</td>
</tr>
<tr>
<td>Thrombocytopenia associated with stiosterolaemia</td>
<td>STSL</td>
<td>210250</td>
<td>AR</td>
<td>ABCG5, ABCG8 (2p21)</td>
<td>Large</td>
<td>Cardiac and facial defects, developmental delay +/- other defects</td>
<td>Baldini et al. Hum Genet 131:1821, 2012</td>
</tr>
<tr>
<td>MYH9-related disease</td>
<td>MYH9RD</td>
<td>155100</td>
<td>AD</td>
<td>MYH9 (22q12-13)</td>
<td>Giant</td>
<td>Reduced megakaryocytes, cataracts, nephropathy or/and deafness</td>
<td>Baldini et al. Br J Haematol 154:161, 2011</td>
</tr>
<tr>
<td>ACTN1-related thrombocytopenia</td>
<td>ACTN1RD</td>
<td>615193</td>
<td>AD</td>
<td>ACTN1 (1q24.1)</td>
<td>Large</td>
<td>Anisocytosis</td>
<td>Kishimoto et al. Am J Hum Genet 92:431, 2013</td>
</tr>
<tr>
<td>FLNA-related thrombocytopenia</td>
<td>FLNA</td>
<td>nd</td>
<td>XL</td>
<td>FLNA (q42)</td>
<td>Small/giant</td>
<td>May be associated with periventricular nodular heterotopia (MIM 300049)</td>
<td>Berruezo et al. Arterioscler Thromb Vasc Biol 33:1592, 2013</td>
</tr>
<tr>
<td>TUBB3-related macrothrombocytopenia</td>
<td>TUBB3</td>
<td>613112</td>
<td>AD</td>
<td>TUBB3 (6p21.3)</td>
<td>Giant</td>
<td>None</td>
<td>Kishimoto et al. Blood 113:458, 2009</td>
</tr>
<tr>
<td>X-linked thrombocytopenia</td>
<td>X-linked thrombocytopenia</td>
<td>XLT</td>
<td>313908</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Monoallelic</td>
<td>nd</td>
<td>AD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ITGA2/ITGB3-related thrombocytopenia</td>
<td>nd</td>
<td>187800</td>
<td>AD</td>
<td>ITGA2B (5q23-q31), ITGB3 (17q21.32)</td>
<td>Large</td>
<td>Platelet anisotropy, possible defects of platelet function</td>
<td>Baldini et al. Hum Genet 131:1821, 2012</td>
</tr>
<tr>
<td>CVC-related thrombocytopenia</td>
<td>CVC</td>
<td>612004</td>
<td>AD</td>
<td>CVC (7p15.3)</td>
<td>Normal</td>
<td>None</td>
<td>Moritos et al. Nat Genet 40:387, 2009</td>
</tr>
</tbody>
</table>

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ES1.2 Diagnosis and management of inherited thrombocytopenias

C. L. Baldini, P. Noris, A. Pecchi
IRCCS Policlinico San Matteo Foundation - University of Pavia, Pavia, Italy.

Until the end of the last century, a few forms of inherited thrombocytopenia (IT) were known and these disorders were considered exceedingly rare. Spontaneous bleeding was considered as a feature common to all patients and as the main clinical consequence of these disorders. Recently, many new forms of ITs have been identified and our view of these disorders has considerably changed. We realized that ITs are less rare than previously thought and that most patients have mild or no spontaneous bleedings. In many cases thrombocytopenia is discovered incidentally during adult life and its genetic origin is missed, exposing patients to the risk of misdiagnosis with immune thrombocytopenia. To avoid this mistake, it is therefore essential that ITs are considered in the differential diagnosis of any thrombocytopenia of unknown origin both in children and adults. Family history, to search for other affected family members, and physical examination, to identify the defects that typically associate with thrombocytopenia in syndromic forms, are useful tools in this respect, although negative results do not exclude the genetic origin of platelet defect. Microscope evaluation of blood films is another key element for differential diagnosis, as morphological anomalies of platelets (increased or reduced size, defects of granules or vacuolization), leukocytes (Döhle-like bodies in neutrophils) or red cells (anisocytosis) are observable in many ITs. Once an IT is suspected, a diagnostic algorithm can guide further investigation toward the specific patient’s disease. It is likely that next generation sequencing techniques will simplify this process in the near future.

A definite diagnosis is essential for proper patient management. In some forms of IT, the major risk for affected subjects does not derive from bleedings, but from the increased propensity to develop additional disorders, as hematological malignancies or kidney failure. In syndromic ITs, associated extra-hematological defects often have a much higher impact on patients’ quality of life than the reduced platelet count. The identification of genotype-phenotype correlations for same disorders made feasible to identify and quantify the risks affecting each patient and to design personalized follow-up protocols.

A definite diagnosis is also required to personalize treatment, as platelet transfusions are no longer the only remedy for ITs (Table 1). Hematopoietic stem cell transplantation can cure ITs and this treatment is HLA-matched donors to reduce the risk of allogeneic immunization.

<table>
<thead>
<tr>
<th>Platelet transfusions</th>
<th>All ITs. To stop bleedings when local measures failed or are not possible</th>
<th>Use HLA-matched donors to reduce the risk of allogeneic immunization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemopoietic Stem Cell Transplantation</td>
<td>Wiskott–Aldrich syndrome - Congenital amegakaryocytic thrombocytopenia</td>
<td>Successfully used also in a few patients with biallelic Bernard Soulier syndrome and life threatening hemorrhages</td>
</tr>
<tr>
<td>Eltrombopag</td>
<td>MYH9-related disease</td>
<td>Used instead of platelet transfusions for preparing patients to surgery. Efficacy in other conditions to be tested</td>
</tr>
<tr>
<td>Splenomegaly</td>
<td>Wiskott–Aldrich syndrome - C-linked thrombocytopenia</td>
<td>It increases platelet count but also the risk of infections</td>
</tr>
<tr>
<td>Desmopressin</td>
<td>All mild forms of IT. To stop bleedings in patients to surgery</td>
<td>Experimental. A test dose is recommended to identify patients who might benefit from this treatment for future bleedings-surgery</td>
</tr>
<tr>
<td>Antifibrinolytic agents</td>
<td>All mild forms of IT. To stop or prevent bleedings, and to prepare patients to surgery</td>
<td>Experimental (anecdotal evidence of efficacy)</td>
</tr>
<tr>
<td>Recombinant factor Vila</td>
<td>Thrombocytopenias. To stop bleedings when all other treatments failed</td>
<td>All inherited. Experimental (anecdotal evidence of efficacy). Risk of thrombosis</td>
</tr>
</tbody>
</table>

ES2.1 Using prediction scores in cardiovascular medicine

S. Ripatti
1) Hiet Institute, University of Helsinki, Helsinki, Finland, 2) Institute for Molecular Medicine Finland (FIMH), Helsinki, Finland, 3) Wellcome Trust Sanger Institute, Hinxton, United Kingdom.

Genome-wide association (GWA) studies have provided hints and pointers towards causes of many common complex diseases and the current wave of large-scale sequencing efforts is likely to refine many of these signals. Using cardiovascular diseases (CVD) as an example, I will describe efforts to turn the association results into risk evaluations and efforts towards developing tools to help in preventive medicine.

Almost two thirds of the incident CVD cases are not identified as high-risk individuals using the currently widely used risk scores, such as the Framingham risk score. Almost 50 genetic loci have been identified for coronary artery disease and even though the effect sizes of individual common variants identified by GWA studies are small, the large number of loci provide an opportunity to use the SNPs jointly in genetic risk scores (GRS). These scores have comparable risk profiles with quantitative risk factors like blood pressure or LDL cholesterol and have a potential to identify high risk individuals missed by the traditional risk factor screens.

Using results from prospective cohorts, I show how the genetic risk scores provide complementary information for prediction over the traditional risk factors and show how GRS help in identifying individuals with high risk. I discuss the potential benefits of targeted interventions using both traditional risk factor and genetic risk score information and show how already using the currently known risk loci provide an opportunity to prevent disease events. Finally, I discuss the need for developing tools to communicate the genetic risk to medical community and citizens, and the need to test the risk scores in clinical settings.

ES2.2 The benefits of using genetic information for design prevention trials

A. Hingorani
London, United Kingdom.

No abstract received at production date. Check the online database at www.eshg.org/abstracts2014.0.html for possible updates.

ES3.1 Novel sequencing approaches in genetic disease research

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Over the past decade the next generation sequencing has led to a revolution in human genetics and in disease gene identification in particular. This revolution was driven by new technologies that allowed new applications. But what’s next?

In this educational session I will address the latest technological developments, for example: What to do with more and less expensive sequencing data? What do longer sequencing reads offer? Can we detect mosaicism reliably? I will also show new applications, for example the benefits of whole genome sequencing, new developments for exome sequencing and also new methods for highly multiplexed targeted re-sequencing.

ES3.2 Single cell genome and transcriptome sequencing

J. Lundeberg
SciLifeLab, KTH- Royal Institute of Technology, Stockholm, Sweden.

Cells in a particular tissue are not identical. Instead, cells that are identical from a genomic point of view may have considerable variation in gene expression profile and protein levels, giving rise to a heterogeneous collection of cells with different behavior and appearance. Even the genomic DNA sequence may vary slightly between neighboring normal cells within a tissue albeit the differences can be significant in adjacent tumor cells. In order to get information from single cells one need to isolate, study, and sometimes culture them, separately. This lecture will describe some of the advancements in genome and transcriptome analysis of single cells using massively parallel DNA sequencing and describe in more detail an approach to spatially identify gene expression in single cells in a tissue sections.
ES5.2 Revertant mosaicism in skin disease
M. F. Jonkman, A. M. G. Pasmooij
University Medical Center Groningen, Groningen, Netherlands.

Revertant mosaicism (RM) refers to the co-existence of cells carrying disease-causing mutations with cells in which the inherited mutation is genetically corrected by a spontaneous post-zygotic event. RM in skin was first reported by us in 1997 in the genetic disorder epidermolysis bullosa (EB), which is characterized by lifelong fragile skin that easily forms blisters and erosions. In a patient with generalized atrophic benign EB, caused by compound heterozygosity at the COL7A1 locus, we found several patchy areas of normal appearing skin and provided molecular proof that the keratinocytes in the clinically unaffected skin were corrected by a gene conversion event, and consequently produced normal type XVII collagen. Mutations in as many as 18 genes can result in EB. Five of these genes have shown to revert: KRT14 encoding keratin 14 in EB simplex, LAMB3 encoding the β3 chain of laminin-332, and COL7A1 encoding type VII collagen in junctional EB, COL7A1 encoding type VII collagen in dystrophic EB, and FERM1 encoding kindlin-1 in Kindler syndrome. RM was also found in other heritable skin diseases: dyskeratosis congenita, and in ichthyosis in confetti (ichthyosis variegata) induced by increased homologous recombination of KRT10. Similar examples of “natural gene therapy” by RM have been described in Bloom syndrome, leukocyte adherence deficiency type 1, Wiskott-Aldrich syndrome, and RAG1-deficient severe combined immunodeficiency. This “natural gene therapy” phenomenon manifests as normal appearing skin areas surrounded by affected skin. Although initially thought to be rare, RM is now considered relatively common in genetic skin diseases. To address the issues relevant to RM, we will discuss the following questions: 1) What is the incidence of RM in heritable skin diseases? 2) What are the repair mechanisms in RM? 3) When do the revertant mutations occur? 4) How do you recognize revertant skin? 5) Do the areas of RM change in size? The answers to these questions allow us to acquire knowledge on these reverted cells, the mechanisms of RM, and utility of the reverted cells to the advantage of the patient. The revertant skin could potentially be used to treat the patient’s own affected skin. Revertant skin cells can be used for transplantation by means of: 1) own skin biopsies, 2) cell suspension, 3) cultured epithelial cells sheet, 4) differentiated pluripotent stem cells reprogrammed as epithelial sheet for skin grafting or as haematopoietic stem cells for infusion. Transplantation of revertant skin biopsies has already successfully been performed in a patient with EB.

ES6.1 Strategies for rare disease gene discovery in the era of next-generation sequencing
K. M. Boycott, F. S. Alarajo;
1Children’s Hospital of Eastern Ontario Research Institute, University of Ottawa, Ottawa, ON, Canada, 2King Faisal Specialist Hospital and Alifial University, Riyadh, Saudi Arabia.

Assigning medically-relevant roles to human genes is at the core of medical genetics as a field. In the area of Mendelian genetics, such assignment should be relatively straightforward since the phenotypic effect of Mendelian genes is usually large and measurable. The recent ability to interrogate the entire genome (or its coding portion) has provided an unprecedented opportunity to rapidly discover disease-causing genes by circumventing historical roadblocks that characterized previous approaches. However, even this cutting-edge tool has its own set of challenges. In this session, the two presenters will share with the audience the lessons they learned from their ongoing effort to unravel the “Mendeliose”, one gene at a time. They draw from their combined experience not only in the successful identification of >120 Mendelian genes, but also, and as importantly, from the many unexpected results they encountered in their research programs. The first presenter will propose an overview of the recent successes and pace of novel gene discovery and discuss the implementation of next-generation sequencing to date, or in combination with automatic positional mapping, to identify autosomal recessive Mendelian genes. The second presenter will continue the same theme by sharing experience with autosomal dominant disorders and their own set of associated challenges. Both presenters will discuss in detail the pitfalls of the various approaches and suggest some helpful strategies. The immediate and critical need to exchange information about rare variants in genes, which is likely to grow in even more importance as we start to identify variants that contribute a decreasing percentage to the overall Mendelian mutation burden, will be emphasized by both presenters. At the end of the session, it is hoped that interested investigators will have acquired basic knowledge on optimal designs of projects aimed to discover novel disease genes.
ES7.1

From Mutations in the Few to Drugs for the Many
M. R. Hayden
Teva Pharmaceutical Industries, Petah-Tikva, Israel.

Black swans have existed in the imaginations of philosophers for thousands of years as a metaphor of extreme outliers and unexpected rare events of large magnitude and consequence. Philosopher’s argued that even though black swans were extremely rare, collectively they had a vastly larger impact than common, regular occurrences. In genetics and drug discovery, the exceptional black swans of rare genetic illnesses can lead to high-impact discoveries beyond the realm of normal expectations. Following “extreme genetics” and “opposite phenotype” strategies, we have made inroads in studying many devastating rare genetic illnesses in order to decipher the basis for common diseases. Investigating the “Opposite Phenotype” of pain in rare patients who are unable to perceive or understand pain, we have developed new drugs that may be able to treat pain in a profound way for the general population. The importance of the rare patient and clinical genetics is crucial in the identification and validation of novel drug targets.

ES7.2

Genetic, cell biological and clinical interrogation of disease-causing CFTR mutations informs strategies for future drug discovery
*The Hospital for Sick Children, Toronto, ON, Canada; **Uppsala University, Uppsala, Sweden, *Hamad Medical Corporation, Doha, Qatar.

Cystic Fibrosis (CF) is a common genetic disease caused by mutations in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene that lead to reduction of CFTR anion channel function on the surface of fluid transporting epithelial tissues. The clinical phenotype is variable but severely affected individuals typically suffer from airway obstruction with recurrent episodes of inflammation and infection as well as pancreatic insufficiency. The need to overcome this gap in knowledge is urgent, particularly given the recent success in developing drugs that target the basic defects caused by CFTR mutation. VX-770 (or Ivacator) has been approved as a drug for patients bearing the relatively rare mutation: p.Gly551Asp mutation. The most common mutation, p.Phe508del, has been studied extensively and found to impair CFTR protein folding during synthesis. Knowledge regarding the molecular defects caused by p.Phe508del has driven development of targeted, interventional compounds, such as VX-809. The development of these drugs highlights the therapeutic relevance of understanding the basic defects caused by CFTR mutation. Approximately 2000 different variants in the CFTR gene have been reported in the CF Mutation Database, yet, the molecular consequences of less than 10% of these variants are understood. In this presentation, our interrogation of a rare variant, c.3700 A>G, will be discussed. This mutation leads to aberrant splicing and the in-frame deletion of six amino acids (p.Ile1234_Arg1239del) in a conserved region of the CFTR protein. The deletion of these residues, caused protein misfolding as in the case of p.Phe508del. These insights led us to test the efficacy of an investigational compound in trials for p.Phe508del and found that, in cell culture, VX-809 partially ameliorated the folding defect of p.Ile1234_Arg1239del-CFTR. Hence, these studies support the rationale for defining the molecular consequences of rare CFTR mutations in guiding decisions regarding future drug discovery efforts.

ES8.1

New Proposals for the Regulation of in vitro Diagnostic Devices (IVDs)
D. E. Barton*, S. Hogarth**;
*National Centre for Medical Genetics, Dublin, Ireland, **School of Medicine & Medical Sciences, University College Dublin, Dublin, Ireland.

The 1998 IVD Directive regulates the market for diagnostic tests within the EU, setting out standards for the design and manufacture of in-vitro diagnostic devices (IVDs) and providing mechanisms for the oversight of these standards. The current system is inflexible, unresponsive and does not do a good job of protecting patients from harm. In September 2012, the European Commission launched a proposal for a complete overhaul of the IVD regulations. While welcoming these proposals as a move towards a flexible, transparent and proportionate system which would bring the EU closer to international best practice, EuroGentest and ESHG have also expressed concerns about a number of provisions which do not provide effective regulation of the burgeoning commercial market for genomic diagnostics, including direct-to-consumer genetic testing. We have made detailed submissions to the Commission, to MEPs and to regulatory bodies to improve the proposed regulation in these areas. Tests manufactured and used within health institution labs are exempt from the current IVD Directive - the so-called “health institution exemption”. EuroGentest has proposed that the health institution exemption should, in future, be restricted to accredited laboratories and should not apply to commercial laboratories. We believe that this provides an appropriate balance of test availability and patient safety. This proposal has been included in the new proposed Regulation. The ENVI Committee at the European Parliament has proposed that the new Regulation should include far-reaching provisions that would govern and restrict the interaction between clinical geneticists and their patients. The ESHG has taken a strong stance against these proposals and has commissioned a legal opinion that demonstrates that they breach the principle of subsidiarity and are beyond the legal competence of the European Union. We will report on progress in these efforts and on our plans for further action.
CONCURRENT SESSIONS

C01.3 mtDNA mutations variously impact mtDNA maintenance throughout the human embryofetal development


The presence of cell-free fetal DNA in the maternal circulation has allowed for the development of methods for non-invasive detection of fetal chromosomal aneuploidies. Non-invasive prenatal testing (NIPT) thus avoids miscarriages due to invasive sampling of fetal material. We developed an innovative, fast, cost efficient workflow and high throughput analysis pipeline. For validation, cell-free DNA of 297 maternal blood samples were collected from women between 9-15 weeks of gestational age who were also undergoing invasive sampling for increased risk of fetal aneuploidy. Whole genome sequencing was performed on cell-free DNA sequencing libraries on a HiSeq2500 using the fast mode 50bp single-ends. In addition to the Z-scores, traditionally the main measure for non-invasive aneuploidy detection, we defined different new parameters which allow for a higher accuracy aneuploidy detection. Moreover, the use of Z-scores across sliding bins enables the distinction to be made between maternal and fetal CNVs. This approach resulted in 100% specificity and sensitivity for trisomy 21 and 18 detection (trisomy 21, n=17; trisomy 18, n=7). This analysis pipeline has been clinically implemented and accredited (ISO15189). An overview of our clinical experience, currently standing at 500 samples, will be provided. In addition to the traditional trisomies, we will present data on other aneuploidies and clinical management thereof.

C01.4 Clinical validation of non-invasive prenatal aneuploidy detection

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Fetal cell-free DNA (cfDNA) in maternal plasma enables screening for fetal aneuploidy using next generation sequencing technologies. We have previously described using DANSR assays for biochemical analysis of chromosomes 13, 18, and 21, combined with the Fetal Trichotomy Optimized Risk for Trisomy Evaluation (FORTÉ™) algorithm to compute the risk of trisomy with high specificity and sensitivity. We have developed additional DANSR assays for the X and Y chromosomes and have applied the FORTÉ algorithm on a two blinded sets of samples with and without sex chromosome aneuploidies (SCA). We report clinical validation results on Harmony™ Prenatal Test’s ability to detect fetal SCA.

609 samples comprised the two sets. All subjects provided informed consent. Matching fetal karyotype results from invasive testing were available for all subjects. Samples were processed as previously described with lab matching fetal karyotype. All samples that passed standard Harmony QC metrics generated a sex chromosome result (100%; 95% CI: 99.4-100%). All were concordant with karyotyping for fetal sex (100%; 95% CI: 99.4-100%).

Directed analysis of cfDNA is accurate for risk assessment of non-mosaic fetal SCA. This is the largest fetal SCA validation study to-date. This demonstrates ability to expand Harmony to genetic conditions besides trisomies 13,18 and 21.

C01.4 Whole-genome single-cell haplotyping, a generic method for preimplantation genetic diagnosis

M. Zanami Esteki1, E. Dimitriadou2, L. Mateiu3, C. Melotte3, N. Van der Aa1, P. Karma1, R. Dau1, J. Cheng1, E. Legius1, X. Morreau1, S. Debrock1, T. D’Hooghe2, P. Verdyck4, M. De Rycke1, K. Sermon5, J. Vermeesch1, T. Voet6;7.

1Centre for Human Genetics, University Hospital Leuven, Department of Human Genetics, 2KU Leuven, Leuven, Belgium, 3Department of Electrical Engineering, ESAT-SISTA, 4Leuven University Fertility Center, University Hospital Gasthuisberg, Leuven, Belgium, 5Centre for Medical Genetics, Universitair Ziekenhuis Brussel, Brussels, Belgium, 6Research group Reproductive and Genetics (REGE), Vrije Universiteit Brussel (VUB), Brussels, Belgium, 7Single-cell Genomics Centre, Welcome Trust Sanger Institute, Hinxton, United Kingdom.

Preimplantation genetic diagnosis (PGD) is the genetic testing of embryos prior to implantation to avoid the transmission of germline genetic disorders or of unbalanced chromosomal rearrangements when a parent is a balanced carrier. Current single-cell PCR or FISH PGD-assays require family specific designs and labor-intensive workflow. Array comparative genomic hybridization (aCGH)-based methods, which are mainly applied for preimplantation genetic screening (PGS) to discern diploid from aneuploid embryos, enable genome-wide aneuploidy detection but do not allow diagnosing single gene disorders. Here, we present a generic method that detects in single blastomeres not only the presence of Mendelian disorders genome wide, but also chromosomal rearrangements and aneuploidies, including their parental origin as well as the meiotic or mitotic nature of chromosomal trisomies. The method interrogates single nucleotide polymorphisms (SNPs), uses a novel computational pipeline for single-cell genome-wide haplotyping and imputation of linked disease variants (siCHLD). Robustly stringent single-cell QC-metrics, a bimodal approach, based on discrete SNP-calls and continuous SNP B-allele fractions, respectively, reconstructs the parental haplotypes of the biopsied single cell. The approach proved accurate on 55 embryos from 12 couples carrying either autosomal dominant, recessive or X-linked Mendelian disorders, or simple or complex translocations. The method allowed diagnosing an embryo for multiple monogenic disorders at once, and, in contrast to current PGD for translocation cases, it enables distinguishing embryos that inherited normal chromosomes from embryos that inherited a balanced configuration of the rearranged derivatives.

The method facilitates genetic selection of embryos, and broadens the range of classic PGD.

C01.5 Scenarios for implementation of non-invasive prenatal testing (NIPT) for Down syndrome in a national health care system


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The implementation of non-invasive prenatal testing (NIPT) of Down syndrome (DS) in national health care systems requires decision-making about the possible restriction of NIPT to high-risk pregnancies, the timing of NIPT, and the combination with other tests. In this study, we combined ethical exploration with a decision-analytic model. We compared three implementation strategies: (1) restriction of NIPT to high-risk pregnancies on the basis of the combined test (CT) risk for DS ≥ 1:200; (2) NIPT for all women at 13 weeks of gestation; (3) NIPT for all women at 10 weeks of gestation. Comparing strategy 1 to strategy 2, 95% fewer women underwent NIPT, and false positive 1st trimester screening results and invasive procedures were reduced by 95% and 37%, respectively. This was at the expense of a decrease in DS detection by 13% before 15 weeks of gestation and 4% throughout the entire pregnancy (3 DS cases/100,000 pregnancies). Comparing strategy 2 to strategy 3, NIPT was avoided in an additional 4% of pregnancies that would have resulted in miscarriage. Furthermore, delaying NIPT to 13 weeks allows for immediate confirmation by amniocentesis, and may be beneficial to women who may feel overloaded with information in 1st trimester. As NIPT currently does not detect all prenatal abnormalities, it should be offered subsequent to or alongside a maulch translucency (NT) measurement. In all cases of fetal anomaly on ultrasound (including NT enlargement), broad genetic testing after invasive procedure should still be performed.

Whole genome sequencing and analysis in prenatal screening: ethical reflection

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Maastricht University, Maastricht, Netherlands.

Non-invasive prenatal testing (NIPT) for fetal abnormalities currently focusses on Down’s syndrome. It is to be expected, however, that it will become possible to broaden the scope of screening with NIPT. As proof of principle, it has been demonstrated that the complete fetal genome can be sequenced using fetal DNA from maternal plasma, potentially allowing prenatal whole genome analysis.

This scenario makes a proactive ethical reflection of utmost importance. Normative issues to be addressed include the following: First, the prerequisite of proportionality. Do the possible benefits of whole genome sequencing and analysis in prenatal screening outweigh the possible harms and disadvantages? Secondly, would it be possible to fulfill the requirement of informed consent, given the wide range of possible outcomes of whole genome prenatal screening? Could so-called generic consent, based on pre-test information about general categories of test outcomes, be accepted as a sound variant of informed consent? And thirdly: if whole genome prenatal screening would result in the birth of children whose genotype has been elucidated prenatally, the child’s right not to know may be violated, i.e. the right of the future, competent, person to decide about predictive testing for later onset diseases. How to handle, then, possible conflicts between future parents’ right to know and future children’s right not to know? This presentation offers a systematic exploration of these issues, aimed at contributing to adequate ethical guidance for whole genome prenatal screening.

A novel variant in the SLC9A9 gene influences disease activity in interferon-beta treated multiple sclerosis patients


1San Raffaele Scientific Institute, Laboratory of genetics of complex neurological disorders, Milan, Italy; 2San Raffaele Hospital, Department of Neurology, Milan, Italy; 3Hospital Purpan, Department of Neurology, Toulouse, France; 4University of Toulouse, Toulouse, France; 5Harvard Medical School, Boston, MA, United States.

Program in Medical and Population Genetics, Broad Institute, Cambridge, MA, United States.

Aim. To identify genetic variants able to predict response to interferon beta (IFNB) therapy in multiple sclerosis (MS) patients treated with IFNB. Rs9828519, achieved genome-wide significance (p=4.43x10^-8), and its effect was implicated in three independent cohorts of American, Italian and French patients (meta-analyzed p=7.78x10^-4). Rs9828519 is intronic of SLC9A9 gene, coding for a sodium/hydrogen exchanger localized in the endosomes, not known to play a role in MS or in IFNB pathways. Within in vitro experiments, we observed an upregulation of SLC9A9 expression after IFNB stimulation in PBMCs of 20 healthy controls (HC, p<0.01x10^-7) which was unrelated to the genotype status. Such upregulation was confirmed also in IFNB-treated MS patients (GSE24427 and GSE26104 experiments: p=3.81x10^-3 and 0.30). No cis-effect of rs9828519 was observed in whole blood of MS patients, nor in PBMCs, CD14 monocyes or CD4+ T lymphocytes collected from HC. For this reason we performed a trans eQTL analysis in 211 CD14+ monocyes isolated from HC followed by pathway analyses using three independent software. All of them enlighten an enrichment of IFNB-related pathways of genes trans-regulated by rs9828519. We observed in the rs9828519-19G sample an up-regulation of genes involved in IFNB signaling, including STAT1, JAK1, STAT5B, but also an impairment of the final effects of IFNB, with an upregulation of osteopontin and a downregulation of IL10. Here we report a new genetic marker that affects the response to IFNB in MS patients supported by experimental evidences of the involvement in the IFNB pathways.

High throughput sequencing in sporadic forms of steroid-resistant nephrotic syndrome: heterogeneous genetic alterations can predict resistance to treatments

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Steroid-resistant nephrotic syndrome (SRNS) is a disorder that results in end-stage renal disease and can be potentially related to a genetic cause, especially when it occurs in children. However, because of genetic heterogeneity, the real burden of genetic alterations in sporadic cases is unknown. In this study, we explored the possibility that genetic alterations of podocyte’s structure may explain lack of response to steroid treatment in children affected by sporadic childhood-onset nephrotic syndrome (NS), not only in a causative manner, but also as critical disease modifiers that influence response to steroid treatment and immunosuppressive agents. We designed a custom sequencing array to target all exons and part of flanking sequences for known podocyte genes responsible for SRNS, that was applied through next generation sequencing to a selected cohort of 50 patients with sporadic NS and variable response to steroid treatment. We identified a genetic cause in 40% of the 19 SRNS patients analysed. Modifier genes were observed in an additional 25% of patients exhibiting resistance to steroid, but nor disease causing neither disease modifier genes were observed in podocyte’s genes of 30 additional children that were sensitive to steroids. Treatment with immunosuppressive agents was effective only among patients that were negative to the test, while none of the patients displaying genetic alterations responded to any immunosuppressive agent.

The results of this study suggest that in children affected by sporadic NS, resistance to steroid treatment may be related to genetic alterations in genes that control structure and function of the podocyte.

Personalized thiopurine dosing based on TPMT genotyping reduces leucopenia occurrence and results in cost-savings in IBD patients; results from a randomized trial in the Netherlands

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More than 20% of inflammatory bowel disease (IBD) patients discontinue thiopurine therapy due to severe adverse drug reactions, among which leucopenia is one of the most serious. Thiopurine S-methyltransferase (TPMT) pharmacogenetic testing can be used to optimize thiopurine safety and efficacy. Nonetheless, its only use is currently on a limited scale. We performed a randomized controlled trial including 769 IBD patients starting on thiopurine treatment. Patients were randomly assigned to standard treatment (control) or pre-treatment screening (intervention) for three common TPMT-variants (TPMT*-2, *3A and *3C); patients heterozygous for a TPMT-variant received 50% of the standard thiopurine dose. We observed an effect on leucopenia occurrence (mean difference in leucocyte count 3.01x10^-7) in the first 5 months after treatment initiation. Cost-effectiveness analysis included complete cases (patients with outcome (EQ-SD) and self-reported costs). Thirty-nine (9.8%) patients in the intervention and 35 pa-
CO2.4 Genome-wide identification and phenotypic validation of loss of function mutations
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National Institutes of Health, Bethesda, MD, United States.

A typical human genome includes numerous apparent loss of function (LOF) variants, which may be attributed to sequencing or alignment errors, carrier states, hypomorphic alleles, gene redundancy, and true LOF alleles for haploinsufficiency-sensitive genes. It is critical for understanding gene function and for predictive medicine to assess the consequences of LOF variants. Prior efforts to understand the genotype-phenotype relationships were limited by ascertainment biases and the limitations of deidentified data. To approach this problem, we characterized the phenotypic consequences of LOF variants in >950 individuals to identify functional haploinsufficiency in known and novel phenotypes. Exomes were generated by targeting and Illumina sequencing. Potential LOF variants were defined as nonsense, frameshift, and essential splice site variants. Analysis was restricted to HGMD genes. Variants withmaf >0.005 in ClinSeq or dbSNP were excluded. We filtered for genes that cause disease in an autosomal dominant pattern by haploinsufficiency. Remaining variants were considered variants of interest. 87 were identified in 112 individuals. 85 of these individuals were evaluated for these disorders. We used stringent criteria for phenotyping and family history to identify variants that were causing disease. Of the evaluated patients, half (38/85) had evidence of disease. The negative cases illustrated issues with gene model errors, mechanisms of alternative translation starts, etc. The positive cases demonstrated the ability to perform predictive medicine and to use filtering methods to identify apparently genetic disorders. These data also point to approaches that could unravel the functional consequences of genes not yet known to cause human phenotypes.

CO2.5 The SickKids Genome Clinic: Developing and evaluating a pediatric model for individualized genomic medicine
M. Meyn1, S. R. Newton1, A. N. Monda2, D. Mertoc1, D. Srivastava1, M. Girdes1, R. Hayeems1, M. Szaego2,3, G. Bader2, R. D. Cohn1,2, J. A. Anderson1, R. Zlotnik-Shaul1,2, M. Girdea1,2, S. Bowdin1,2, N. Monfared1, D. Merico1, D. J. Stavropoulos1,2, M. Genomics Health Alliance, Melbourne, Australia; 1University of Melbourne, Melbourne, Australia; 2Australian Genome Research Facility, Melbourne, Australia; 3Royal Melbourne Hospital, Melbourne, Australia; 4The Commonwealth Scientific and Industrial Research Organisation (CSIRO), Melbourne, Australia; 5The Royal Children’s Hospital, Melbourne, Australia; 6Murdoch Childrens Research Institute, Melbourne, Australia.

Genomic technologies are proving transformative, but ensuring full clinical and research benefit from their application in clinical settings requires planning and collaboration. Working to a five year vision, seven research and healthcare organisations are conducting a demonstration project to test an ambitious, unified approach to meeting their diverse needs. Demonstration projects examine the application of structural innovations such as technology and non-structural innovations like health programs. They provide new insights into the process and value of implementing innovation, thereby informing decision-making about future implementation. Our highly novel, prospective project evaluates the feasibility of whole exome/genome sequencing as a single common assay for germline and somatic conditions, replacing current tests, in clinical and predictive care. Mimicking usual clinical practice, patients with one of five diverse germline or somatic conditions are being offered whole exome sequencing in parallel to routine investigations. Sequence data is generated by multiple diagnostic laboratories. The associations of genes known to cause disease is being validated as a clinical condition using a common analytic pipeline. Data is available for reanalysis and research. Patient and clinician engagement in this clinically-led project is paramount. The demonstration project is being evaluated in terms of feasibility (can the model be built?), requirements (what does it take to build?), and impact (can it make a difference?) across all processes and with feedback from patients, clinicians and scientists supplementing data collection. We will describe the ‘three’ key strengths, weaknesses and barriers identified during collaborative implementation of the demonstration project across the seven organisations.

CO2.6 Collaboration to integrate genomics into clinical care: a demonstration evaluation
C. Galton, A. Torner, I. MacCormick, P. Waring1, S. Forrest, P. Eberst, L. Winship2, T. K. Hambleton3, M. South, A. Sinclair; 1Melbourne Genomics Health Alliance, Melbourne, Australia; 2University of Melbourne, Melbourne, Australia; 3Australian Genome Research Facility, Melbourne, Australia; 4The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia; 5Royal Melbourne Hospital, Melbourne, Australia; 6The Commonwealth Scientific and Industrial Research Organisation (CSIRO), Melbourne, Australia; 7The Royal Children’s Hospital, Melbourne, Australia; 8Murdoch Childrens Research Institute, Melbourne, Australia.

Genomic technologies are proving transformative, but ensuring full clinical and research benefit from their application in clinical settings requires planning and collaboration. Working to a five year vision, seven research and healthcare organisations are conducting a demonstration project to test an ambitious, unified approach to meeting their diverse needs. Demonstration projects examine the application of structural innovations such as technology and non-structural innovations like health programs. They provide new insights into the process and value of implementing innovation, thereby informing decision-making about future implementation. Our highly novel, prospective project evaluates the feasibility of whole exome/genome sequencing as a single common assay for germline and somatic conditions, replacing current tests, in clinical and predictive care. Mimicking usual clinical practice, patients with one of five diverse germline or somatic conditions are being offered whole exome sequencing in parallel to routine investigations. Sequence data is generated by multiple diagnostic laboratories. The associations of genes known to cause disease is being validated as a clinical condition using a common analytic pipeline. Data is available for reanalysis and research. Patient and clinician engagement in this clinically-led project is paramount. The demonstration project is being evaluated in terms of feasibility (can the model be built?), requirements (what does it take to build?), and impact (can it make a difference?) across all processes and with feedback from patients, clinicians and scientists supplementing data collection. We will describe the ‘three’ key strengths, weaknesses and barriers identified during collaborative implementation of the demonstration project across the seven organisations.

CO3.1 Dominant β-catenin mutations cause a recognizable syndrome with intellectual disability, and are associated with learning deficits and structural and functional brain abnormalities in mice
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Introduction: The recent identification of multiple dominant mutations in both humans and mice has enabled us to explore the molecular and cellular basis of beta-catenin function in cognitive impairment. Mutations in human beta-catenin have been identified as causative in a spectrum of neurodevelopmental disorders. Methods and Results: In identifying de novo beta-catenin mutations in patients with intellectual disability and careful characterization of their phenotype, we were able to define a recognizable intellectual disability syndrome. We have collected detailed clinical information of 7 patients with this novel syndrome. In parallel, the characterization of a chemically-mutagenized mouse line displaying features that are similar to these human mutations has enabled us to investigate the consequences of beta-catenin dysfunction through development and into adulthood. The functional analysis, including carrying a Th65SLys substitution in the C-terminal armadillo repeat in beta-catenin, displays a reduced affinity for membrane-associated cadherins. In association with this decreased cadherin interaction, we found that the mutation results in decreased intrahemispheric connections with deficits in dendritic branching, long-term potentiation and cognitive function. Conclusion: For the first time in vivo we showed how dominant mutations in beta-catenin underlie losses in its adhesion-related function leading to severe phenotypes including intellectual disability, childhood hypotonia, progressive spasticity of lower limbs and abnormal craniofacial features in adults.
The 3p25 microdeletion syndrome. The synthesis pathway mutational event. de novo important causes of ID. This analysis provides sufficient evidence that loss of ...ritualised behaviour and autism. feature including an obsessive-compulsive disorder, hand flapping with length discrepancy in two cases. Congenital heart defects, inguinal hernia set ears. Skeletal anomalies were a frequent finding including significant leg tubular nose; long narrow upslanting palpebral fissures and large fleshy low set ears. Skeletal anomalies were a frequent finding including significant leg length discrepancy in two cases. Congenital heart defects, inguinal hernia or hypospadias were all reported. Behavioural problems were a prominent feature including an obsessive-compulsive disorder, hand flapping with ritualised behaviour and autism. SETD5 lies within the critical interval for the 3p25 microdeletion syndrome. The SETD5 mutation individuals have phenotypic similarity to those previously reported with a deletion in 3p25 and thus loss of SETD5 may be sufficient to account for the clinical features observed in this condition. Our findings add to the growing evidence that mutations in methyltransferases that regulate histone modification are important cause of ID. This analysis provides sufficient evidence that loss of function of SETD5 is a relatively frequent cause of ID (0.7%) and occurs as a rare de novo mutation event.

C03.2
De Novo loss of function mutations in SETD5, a novel methyltransferase gene within the 3p25 microdeletion syndrome critical region, cause intellectual disability

C03.3
Genetic heterogeneity in Hyperphosphatasia with Mental Retardation Syndrome due to mutations in PGAP3, a member of the GPI anchor synthesis pathway

C03.4
The significance of small copy number variants in neurodevelopmental disorders

C03.5
Rare large CNVs are associated with intellectual disability, education level, and female fertility in general population

C03.6
Altered neuronal network in iPSC derived cortical neurons from patients with MECP2 duplication syndrome

ABSTRACTS
CONCURRENT SESSIONS
CO4.1 EIF2AK4 mutations cause pulmonary veno-occlusive disease, a recessive form of pulmonary hypertension


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Pulmonary veno-occlusive disease (PVOD) is a rare and devastating cause of pulmonary hypertension that is characterized histologically by widespread fibrous intimal proliferation of septal veins and preseptal venules and is frequently associated with pulmonary capillary dilatation and proliferation. PVOD presents either sporadically or as familial cases. In the French referral center for severe pulmonary hypertension, we have identified 13 PVOD families: 5 with a confirmed diagnosis based on histological studies and 8 with a highly likely diagnosis, based on clinical, functional, and radiological criteria. All PVOD families were characterized by the presence of at least two affected siblings and unaffected parents, suggesting that the disease segregates as a recessive trait. We used a whole-exome sequencing approach and detected recessive mutations in the EIF2AK4 gene that co-segregated with PVOD in all families. The identified mutations are likely to impair EIF2AK4 function and lead to pulmonary vascular remodeling. In conclusion, our study identifies EIF2AK4 as a novel genetic cause of PVOD and highlights the potential of whole-exome sequencing to identify novel genetic causes of rare diseases.
Elevated body mass index (BMI) associates with cardiometabolic traits on observational analysis, yet the underlying causal relationships remain unclear. We conducted Mendelian randomization analyses using 14 SNPs associated with BMI from a recent discovery analysis to investigate the causal role of BMI with cardiometabolic traits. We used eight population-based cohorts, including 34,538 individuals of European ancestry with 4,407 type 2 diabetes (T2D), 6,073 coronary heart disease (CHD) and 3,813 stroke cases. A genetically- elevated one kg/m² increase in BMI resulted in higher levels of fasting glucose, insulin, interleukin-6 and systolic blood pressure but reduced levels of HDL-C and LDL-C (values reported in Table). Apart from LDL-C, all causal estimates were directionally consistent to observational estimates. A genetically-elevated one kg/m² increase in BMI increased odds of T2D but did not affect risk of CHD or stroke. A meta-analysis incorporating published studies with 27,465 CHD events in 219,423 individuals yielded a pooled odds ratio of 1.04 (95%CI: 0.97, 1.12) per 1 kg/m² increase in BMI. In conclusion, we identified causal effects of BMI on several cardiometabolic traits, however whether BMI causally impacts on CHD risk requires further evidence.

Table. Causal estimates for the relationship of BMI with cardiometabolic traits and events.

<table>
<thead>
<tr>
<th>Traits (units)</th>
<th>Studies (Individuals)</th>
<th>Regression coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Metabolic</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting glucose (mmol/l)</td>
<td>6 (20,677)</td>
<td>0.18 (0.12, 0.24)</td>
</tr>
<tr>
<td>Fasting insulin (% difference)</td>
<td>3 (12,758)</td>
<td>0.84 (0.59, 1.10)</td>
</tr>
<tr>
<td><strong>Blood pressure</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>6 (31,637)</td>
<td>0.70 (0.42, 1.16)</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>6 (31,637)</td>
<td>0.28 (0.03, 0.53)</td>
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<tr>
<td><strong>Lipid</strong></td>
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<td></td>
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<tr>
<td>HDL-C (mmol/l)</td>
<td>6 (24,943)</td>
<td>0.02 (-0.03, -0.01)</td>
</tr>
<tr>
<td>LDL-C (mmol/l)</td>
<td>6 (23,364)</td>
<td>0.04 (-0.07, -0.01)</td>
</tr>
<tr>
<td><strong>Inflammation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting insulin (% difference)</td>
<td>6 (24,761)</td>
<td>0.88 (0.61, 1.27)</td>
</tr>
</tbody>
</table>

Myocardial Diseases, Careggi University Hospital, Florence, Italy, 3University of Florence, Dept. of Medical Biochemistry and Molecular Biology, University of Oulu, Oulu, Finland, 4Center for Vascular Anomalies, Div. of Plastic Surgery, Cliniques universitaires St Luc, Brussels, Belgium. Venous anomalies are composed of ectatic veins with irregular smooth muscle walls and are commonly associated with pain. They usually occur as a single lesion without family history (sporadic Venous Malformation, VM). Some sporadic patients have multifocal lesions (Multifocal Sporadic Venous Malformation, MSMV). In the sporadic Blue Rubber Bleb Nevis syndrome (BRBN), patients also have multifocal lesions; pathognomonic are rubbery palmar plantar lesions and those located in the GI-track. In rare cases, venous malformations are multifocal because of autosomal dominant inheritance (Mucocutaneous Venous Malformation, MVM). VMs progressively expand causing deformity, pain and local intravascular coagulopathy. Despite sclerotherapy or excision, lesions often progress or recur.

We have identified activating mutations in the endothelial tyrosine kinase receptor TIE2 in all four forms. VMs are mostly due to a single somatic amino acid change L914F. MSVMs and BRBNs are due to double mutations in somatic and ion-channel genes among HCM patients raises important issues in 7/18 DCM patients (39%) and 5 candidate variants in 3/6 AC patients (13 novel) in desmosomal and ion-channel genes in 14 patients (20%). We identified 48 variants (27 novel) in sarcomeric or associated genes in 20X or more, mean coverage on target of 530X). A mean of 1016 C04.5 VMs refractory to standard-of-care, an mTOR inhibitor diminished pain, in 15 patients. Finally, in our therapeutic pilot study comprising five patients with VMs refractory to standard-of-care, an mTOR inhibitor is able to deter lesion development. Interestingly, an mTOR inhibitor is able to deter lesion development in human VM. Non. The capacity to form lesions clearly resides in mutant endothelial cells, which when injected to immunodeficient mice generate lesions mimicking non. The role of BMI with cardiometabolic traits. We used eight population-based cohorts, including 34,538 individuals of European ancestry with 4,407 type 2 diabetes (T2D), 6,073 coronary heart disease (CHD) and 3,813 stroke cases. A genetically- elevated one kg/m² increase in BMI resulted in higher levels of fasting glucose, insulin, interleukin-6 and systolic blood pressure but reduced levels of HDL-C and LDL-C (values reported in Table). Apart from LDL-C, all causal estimates were directionally consistent to observational estimates. A genetically-elevated one kg/m² increase in BMI increased odds of T2D but did not affect risk of CHD or stroke. A meta-analysis incorporating published studies with 27,465 CHD events in 219,423 individuals yielded a pooled odds ratio of 1.04 (95%CI: 0.97, 1.12) per 1 kg/m² increase in BMI. In conclusion, we identified causal effects of BMI on several cardiometabolic traits, however whether BMI causally impacts on CHD risk requires further evidence.

Table. Causal estimates for the relationship of BMI with cardiometabolic traits and events.

<table>
<thead>
<tr>
<th>Traits (units)</th>
<th>Studies (Individuals)</th>
<th>Regression coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Metabolic</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting glucose (mmol/l)</td>
<td>6 (20,677)</td>
<td>0.18 (0.12, 0.24)</td>
</tr>
<tr>
<td>Fasting insulin (% difference)</td>
<td>3 (12,758)</td>
<td>0.84 (0.59, 1.10)</td>
</tr>
<tr>
<td><strong>Blood pressure</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>6 (31,637)</td>
<td>0.70 (0.42, 1.16)</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>6 (31,637)</td>
<td>0.28 (0.03, 0.53)</td>
</tr>
<tr>
<td><strong>Lipid</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL-C (mmol/l)</td>
<td>6 (24,943)</td>
<td>0.02 (-0.03, -0.01)</td>
</tr>
<tr>
<td>LDL-C (mmol/l)</td>
<td>6 (23,364)</td>
<td>0.04 (-0.07, -0.01)</td>
</tr>
<tr>
<td><strong>Inflammation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting insulin (% difference)</td>
<td>6 (24,761)</td>
<td>0.88 (0.61, 1.27)</td>
</tr>
</tbody>
</table>

Randomization Group, E. E. Schadt 6, F. W. Asselbergs 2, A. P. Reiner 9, B. J. Keating 1; 1McMaster University, Hamilton, ON, Canada, 2Mount Sinai School of Medicine, New York, NY, United States, 3University Medical Center Utrecht, Utrecht, Netherlands, 4Department for Cardiogenetic Research, Utrecht, Netherlands, 5University of Virginia, Charlottesville, VA, United States, 6Fred Hutchinson Cancer Research Center, Seattle, WA, United States.
allele frequency of 1% for this population. Preliminary results of primer extension analyses of RNA from peripheral blood suggest a negative effect of the 34 bp-deletion on the expression level of this allele. Further functional analyses are currently being performed. In conclusion, our results indicate that BIKM is an autosomal recessive condition caused by an unusual mode of inheritance and highlight the importance of analyzing regulatory regions of causative genes.

C05.2 Genetic studies of mosaic birth defects affecting the skin by next-generation DNA sequencing


Epidermocyteacommentary syndromes. These findings highlight the value of next-generation genotyping we identified the c.910C>T transition in a STIM1 protein (STIM1) is a finely tuned endoplasmic reticulum Ca2+ sensor. The effect of the mutation on the structure of STIM1 was investigated by molecular modeling, and its effect on function was explored by calcium homeostasis experiments. We show that STIM1 p.R304W variant may affect the conformation of the inhibitory helix and unlock the inhibitory state of STIM1 molecule. Results obtained from calcium imaging experiments using transfected cells together with the fibroblasts from one affected patient were in agreement with impairment of calcium homeostasis. The p.R304W mutation probably causes a constant Ca2+ release activated Ca2+ channels (ORAC) opening leading to a permanent entry of calcium in many cell types. Our results are in agreement with already published models for STIM1 structure and activation. We conclude that p.R304W mutation in STIM1 may be the cause of the Stormorken syndrome.

C05.4 TashT is a novel mouse model that phenocopies both the variable penetration and male sex-bias of Hirschsprung’s disease

N. Pilon1, K. Bergeron2, J. Courcet1,3, Fédération des Centres Labellisés « Anomalies du Développement » (FeCLAD), Filière des Maladies Rares en Dermatologie (FIMARAD), Société française de Foetopathologie (SOFFOET), L. Faivre1, B. Demene3, P. Vables3.

Neural crest cells (NCC) are progenitors of diverse cell types such as peripheral nervous and glia as well as melanocytes. Via an insertion mutation screen for loci affecting NCC, we identified several mouse lines that combine defects in pigmentation and formation of the enteric nervous system. One of these lines, named TashT, displays an aganglionic megacolon phenotype in a sex-biased manner, with the majority of affected males. This is highly reminiscent of human Hirschsprung’s disease, a neurocrystophaic phenotype with an incidence of 1/5000 newborns and a currently unexplained 4:1 male-to-female bias. We localized the TashT transgene insertion site in a gene desert containing multiple highly conserved elements on chromosome 10. Migration assays as well as time-lapse imaging showed that megacolon is due to defective NCC migration within the gut mesenchyme, a defect generally more severe in males than females. At the molecular level, RNAseq analysis of TashT enteric NCC revealed a remarkable upregulation of many genes encoding secreted proteins and downregulation of several X-linked genes. This analysis also identified the novel gene Faml62b as a strong candidate for being the TashT causative gene. Fam162b is located near the transgene insertion site and reporter gene as well as 3C assays suggest that this TashT overexpressed gene is normally repressed in NCC via long range interactions with some of the highly conserved elements near the transgene insertion site. Altogether, our results demonstrate that the TashT line represents a unique mouse model that will help understand the male sex bias of Hirschsprung’s disease.

C05.5 WNT pathway downregulation and Cornelia de Lange Syndrome

A. Scolieri1, A. Marozzi1,2, J. Courcet1,3, Fédération des Centres Labellisés « Anomalies du Développement » (FeCLAD), Filière des Maladies Rares en Dermatologie (FIMARAD), Société française de Foetopathologie (SOFFOET), L. Faivre1, B. Demene3, P. Vables3.

The cohesin complex is formed from a multi-subunit core and their associated regulatory proteins. Genetic variants within components of the cohesin complex (NIPBL, SMC1A, SMC3, RAD21, PDS5, ESCO2, HDAC8) are believed to be responsible for a spectrum of human syndromes known as “cohesinopathies” that includes Cornelia de Lange Syndrome (CdLS), a multiple malformation syndrome for gene discovery studies due to the challenges of detecting mutations in mosaic birth defects affecting the skin. Neural crest cells (NCC) are progenitors of diverse cell types such as peripheral nerves and glia as well as melanocytes. Via an insertion mutation screen for loci affecting NCC, we identified several mouse lines that combine defects in pigmentation and formation of the enteric nervous system. One of these lines, named TashT, displays an aganglionic megacolon phenotype in a sex-biased manner, with the majority of affected males. This is highly reminiscent of human Hirschsprung’s disease, a neurocrystophaic phenotype with an incidence of 1/5000 newborns and a currently unexplained 4:1 male-to-female bias. We localized the TashT transgene insertion site in a gene desert containing multiple highly conserved elements on chromosome 10. Migration assays as well as time-lapse imaging showed that megacolon is due to defective NCC migration within the gut mesenchyme, a defect generally more severe in males than females. At the molecular level, RNAseq analysis of TashT enteric NCC revealed a remarkable upregulation of many genes encoding secreted proteins and downregulation of several X-linked genes. This analysis also identified the novel gene Faml62b as a strong candidate for being the TashT causative gene. Fam162b is located near the transgene insertion site and reporter gene as well as 3C assays suggest that this TashT overexpressed gene is normally repressed in NCC via long range interactions with some of the highly conserved elements near the transgene insertion site. Altogether, our results demonstrate that the TashT line represents a unique mouse model that will help understand the male sex bias of Hirschsprung’s disease.
Cornelia de Lange syndrome (CdLS) is a highly variable multisystem disorder with a broad phenotypic spectrum. Most typically-affected individuals carry de novo heterozygous loss-of-function mutations in NIPBL. Mutations in other components of the sister chromatid cohesion system, SMCA1, HDAC3, SMCC and RAD21, result in phenotypes that overlap with CdLS but which can be highly atypical. We carried out trio-based exome sequencing in ten unrelated individuals with the diagnosis of atypical CdLS, who had previously been screened for intragenic mutations in the known CdLS genes and for genomic deletions or duplications. Using a likelihood-based approach, we identified nine de novo variants in eight cases, including de novo loss-of-function mutations in NIPBL (c.315delA [p.Glu105Glnfs*20]) and KMT2A (c.3649C>T [p.Glu1216]*) and a missense mutation in NAA10 (c.247C>T [p.Arg83Cys]). Furthermore, de novo, probably damaging missense mutations were identified in Pik3c3, Pdcd6ip, Unc45a, Nup210 and Cel3f. We then re-sequenced these genes in 85 of our mutation-negative CdLS and CdLS-like cases by AmpliSeq® Ion Torrent sequencing, which revealed three additional loss-of-function mutations in KMT2A. We also detected the aforementioned de novo mutation in NAA10 (c.247C>T [p.Arg83Cys]) in a similarly-affected, unrelated individual. Molecular modelling suggests that the p.Arg83Cys conversion is likely to alter binding of NAA10 to acetyl CoA and would result in reduced acetylation activity of the protein. Our results highlight the genetic heterogeneity in atypical CdLS, and they also demonstrate the phenotypic overlap between atypical CdLS and other dysmorphic conditions such as Wiedemann-Steiner syndrome as shown by molecular data.

C06.2 The long non-coding RNA landscape of autoimmune diseases
S. Wijmenga1, C. R. C. M. van der Zee2, L. H. C. van't Veer3, A. M. Cremer3, M. P. J. Kwok4, M. Leleu2, S. G. Mehta9, A. Ross10, F. J. Kaiser2, M. S. Taylor1, D. R. FitzPatrick1; 1Genetics Department, Groningen, Netherlands. We have recently shown that the majority of predisposing autoimmune disease-SNPs are intronic or intergenic and have the potential to be regulatory (Ricacho-Ponce & Wijmenga, 2013) by affecting expression of nearby genes (so-called eQTLs). It has become clear that non-coding RNAs are an important class of regulatory elements. Annotating autoimmune SNPs shows that close to 10% map to long non-coding RNA genes (IncRNAs). Transcriptome analysis across 11 distinct immune cell types (granulocytes, monocytes, NK cells, B-cells, memory-T cells, naive CD4+ and CD8+ T-cells, and four CD4+ T-helper cell populations) revealed that these “autoimmune” IncRNAs are significantly enriched in immune cell types. We also correlated the autoimmune SNP genotypes with expression levels (eQTLs) of both coding genes and IncRNA genes and observed >70% of the autoimmune SNPs to be eQTLs. Interestingly, ~16% of these eQTL SNPs also affect IncRNAs and as high as 6% of these eQTLs are specific to IncRNAs alone. To gain a first understanding on the biological processes in which these “autoimmune” IncRNAs are involved, we performed pathway analysis based on co-expression profiles of IncRNAs and protein-coding genes using 5000 publicly available RNAseq samples. More than 90% of the autoimmune IncRNAs were significantly co-expressed with genes in cis (P < 0.009 to 4.64x10^-92) that are at an average distance of 245 kb. Our results show that IncRNA-eQTLs represent a novel link between non-coding SNPs and the expression of genes, which can be exploited to understand the process of gene-regulation through IncRNAs in more detail.

C06.3 Population Scale Comprehensive Identification and Analysis of Complex Structural Variation Using Nanochannel Array
H. Dai1, A. Hastei1, E. Lam2, W. Andrews3, T. Anantharaman4, A. Pang4, M. Saghbin5, H. Sadowski5, H. VanSteenhouse1, M. Austin1, X. Yang1, T. Dickinson1, Z. Dzakula1, M. Xiao1, P. Rerek1, H. Cao1; 1BioNano Genomics, San Diego, CA, United States, 2Drexel University, Philadelphia, PA, United States, 3University of California - San Francisco, San Francisco, CA, United States. Diseases are known to be associated with large (>1kb) genomic structural variation (SV). A variety of techniques such as karyotyping, FISH and array-CGH have been used for SV analysis. However, population scale comprehensive SV analysis remains impractical—too expensive or incomplete (exemplified by missing inversions and balanced translocations by aCGH methods). More recently, next generation sequencing (NGS) has been shown efficient for discovery of SNPs and small indels. However, complete and accurate SV discovery and analysis is complicated by the fact that variants often span tens to hundreds of kb or are rearranged throughout the genome, difficult to infer from short fragment sequencing. Thus, there is a blind spot in effectively detecting SVs within this range (1 kb ~ 1Mb), referred to as the “dark matter” of the genome, overlooked in the past due to insufficient tools. We demonstrate a new platform technology (Irys) to effectively linearize very long strands of gDNA (100 kb to MbS) through nanochannels to directly visualize SVs and rearrangements preserved within intact and unamplified genomic DNA at single molecule level. De novo genome maps are assembled and hundreds to thousands of SV events are called. Data from individuals and trios will demonstrate this highly comprehensive and cost effective approach, with results validated by multiple orthogonal methods. For the first time, it is now feasible to do large population-based comprehensive structural variation studies using a single platform. This innovation will transform the diagnosis and treatment of diseases resulting from structural variants, particularly cancer.

C06.4 Chromatin loops and CNVs: the complex spatial organization of the 16p11.2 locus
M. Loviglio1, M. Leleu1, N. Gheleda1, E. Migliavacca1, R. Mannik1, J. Beckmann1, S. Jacquesm2, J. Rougemont1, A. Reynaud1; 1Center for Integrative Genomics, Lausanne, Switzerland, 2Swiss Institute of Bioinformatics, Lausanne, Switzerland. 16p11.2 is a highly variable region of the human genome, with 600 kb of BPs and reciprocal deletions and translocations. This region contains several eQTLs, including the so-called eQTLs. However, it is not well understood how these eQTLs are regulated by the underlying genetic and epigenetic factors. We have recently shown that the majority of predisposing autoimmune disease-SNPs are intronic or intergenic and have the potential to be regulatory (Ricacho-Ponce & Wijmenga, 2013) by affecting expression of nearby genes (so-called eQTLs). It has become clear that non-coding RNAs are an important class of regulatory elements. Annotating autoimmune SNPs shows that close to 10% map to long non-coding RNA genes (IncRNAs). Transcriptome analysis across 11 distinct immune cell types (granulocytes, monocytes, NK cells, B-cells, memory-T cells, naive CD4+ and CD8+ T-cells, and four CD4+ T-helper cell populations) revealed that these “autoimmune” IncRNAs are significantly enriched in immune cell types. We also correlated the autoimmune SNP genotypes with expression levels (eQTLs) of both coding genes and IncRNA genes and observed >70% of the autoimmune SNPs to be eQTLs. Interestingly, ~16% of these eQTL SNPs also affect IncRNAs and as high as 6% of these eQTLs are specific to IncRNAs alone. To gain a first understanding on the biological processes in which these “autoimmune” IncRNAs are involved, we performed pathway analysis based on co-expression profiles of IncRNAs and protein-coding genes using 5000 publicly available RNAseq samples. More than 90% of the autoimmune IncRNAs were significantly co-expressed with genes in cis (P < 0.009 to 4.64x10^-92) that are at an average distance of 245 kb. Our results show that IncRNA-eQTLs represent a novel link between non-coding SNPs and the expression of genes, which can be exploited to understand the process of gene-regulation through IncRNAs in more detail.

C06.1 Resolving variants of unknown significance through reanalysis of 4,978 public RNA-seq samples
P. Deelen1, D. V. Zherbina2, M. van der Sijde, K. J. van der Velde, M. de Haan, M. A. Bouwmeester, R. J. Sinke, M. A. Swertz, J. Fu, L. Franke; 1University Medical Center Groningen, Groningen, Netherlands. In recent years, exome sequencing has emerged as a very effective strategy for genome diagnostics. However, the functional significance is unclear for many of the identified variants, hindering clinical interpretation. To improve upon this, we hypothesized that if a variant of unknown significance is affecting gene expression, it is more likely to be pathogenic (similar to what is known for common disease-associated SNPs, Westra et al, Nature Genetics 2013). We therefore analysed publicly available RNA-seq data from 4,978 human samples from European Nucleotide Archive. We developed methodology to QC and harmonize the RNA-seq data and to account for differences in sequencing strategy, tissue differences and other (unknown) confounders. We subsequently called SNPs using GATK and imputed genotypes using Eagle. We assessed genotype quality using 462 samples for which both RNA-seq data and 1000G genotypes are available (Lappalainen et al, Nature 2013) and observed a 97% genotype concordance, indicating that RNA-seq is suitable for genotyping. This enabled us not only to identify effects of common variants on the gene expression levels of 6,005 genes (cis-eQTLs), but also to identify the effects associations of rare variants and gene expression levels assessing allele specific expression (ASE). We observed that many rare variants known to be pathogenic strongly associate with gene expression levels. Since the amount of RNA-seq data that is available in public repositories is growing exponentially, we expect ASE analysis of rare variants will likely become an essential tool to resolve many of unknown significance.
We are currently testing our approach in large-scale projects such as the Undiagnosed Disease Program and CareForRare.

C07.4 Lysoplex: an efficient strategy to study the role of lysosomal-autophagic-endocytic pathway

G. Di Fruscio1,2, A. Schulz2, R. De Cegli2, M. Mutarelli4, M. Savarese4,2, Y. Singhmarwah2, M. Filicamo2, D. Di Bernardo2, S. Banfi1,2, T. Braulick2, V. Nigro2,4, A. Ballabio1,2
1Telethon Institute of Genetic and Medicine, Naples, Italy; 2Dipartimento di Biochimica, Biologia e Patologia Generale, Seconda Università degli Studi di Napoli, Naples, Italy; 3Department of Pediatrics, University Medical Center Hamburg-Eppendorf, Hamburg, Germany; 4Centro di Diagnostica Genetica e Biochimica delle Malattie Metaboliche, Istituto G. Gaslini, Genova, Italy.

Lysosome is a control centre for cellular clearance and energy metabolism: it is responsible for the degradation of the foreign molecules and the endogenous macromolecules. The essential role of lysosomes in phagocytosis and autophagy puts these organelles at the crossroads of several cellular processes. To understand the role of the lysosomal-autophagic-endocytic pathway in health and disease, we have developed Lysoplex, a Next Generation Sequencing-based workflow to sequence at high coverage 1.2786 human exons of 891 genes involved in lysosomal function, autophagy and endocytosis pathway. We designed the enrichment probes using a Haloplex custom platform targeting 99.48% of exons. To validate the methodology, we created a training set of 15 DNA samples belonging to patients affected by 14 different LSDs, where the molecular diagnosis was already known. Using Lysoplex, we were able to detect all the known mutations. Moreover, we used our strategy to identify disease-causing mutations in 50 patients clinically diagnosed as affected by neuronal ceroid lipofuscinoses (NCL). About 50% of samples have causative mutations and a subset of additional variations in other genes not directly correlated with the disease was also identified in each sample. In conclusion, Lysoplex is a cheap and fast NGS targeted platform for the molecular diagnosis of Mendelian LSDs and it is a precious tool to have a complete view of sequence variants in the genes involved in the lysosomal-autophagic pathway. Moreover, it can also be used to identify causative or predisposing mutations in a variety of debilitating human conditions such as common neurodegenerative diseases.

C07.5 Comparing Clinical Exome Sequencing versus Whole Exome Sequencing for monogenic diseases and undiagnosed patients

P. Jost1,2, M. Papik3, K. Steinid3, S. Papuc4, M. Vincent5, L. Gogol6, B. Oneda2, A. Baumer2, A. Rauch2
1Institute of Medical Genetics, University of Zurich, Schlieren-Zurich, Switzerland; 2Institute of Medical Genetics, University of Zurich, Schlieren-Zurich, Switzerland.

Since Whole Exome Sequencing (WES) demonstrated mutations in known disease genes in more than 30% of patients with severe non-specific ID (Rauch et al. 2012) and in 25% of mixed clinical patients (Yang et al. 2013) we investigated the diagnostic yield of NGS of large gene panels including all known disease genes (CNS: “clinical exome sequencing”) versus WES. So far we analyzed WES data of 99 unrelated patients with a variety of neurodevelopmental or congenital anomalies and CES data covering 2761 or 4813 known disease genes from 12 patients. WES revealed pathogenic mutations in known disease genes in a total of 23% of cases, which increased to almost 70% in cases with a precise clinical syndrome diagnosis. Diagnostic yield of CES was 50% in total. Comparison of the average yield of ~135 private non- synonymous variants per case targeting the whole exome versus the ~65 for the 2761 and ~107 for the 4813 gene panel, indicates a more sensitive variant detection on the clinical disease panel. The increased sensitivity may be explained by the high average coverage of over 200x and a 10x coverage in almost 98% of the targeted region. Of 29 diagnosed cases, 41% represented autosomal dominant mutations, 52% were inherited in an autosomal recessive pattern and 7% had X-linked inheritance. In about 14% of WES cases in total, and in 31% of affected sib pairs we detected new possible disease causing candidate genes.

We conclude that CES is highly sensitive especially in cases with a syndromic clinical diagnosis.
C07.4 Setting sequencing thresholds for the use of next generation sequencing as a diagnostic tool
Y. Sun, M. J. V. Hoffer, C. A. L. Ruivenkamp, J. T. den Dunnen, G. W. E. Santen; Leiden University Medical Center, Leiden, Netherlands.

Before replacing gene-by-gene Sanger sequencing with genome-wide NGS, it is essential that diagnostic laboratories investigate the relation between sequencing depth and test characteristics such as false-positive/negative rates, yield and coverage of relevant regions. In addition, capture bias needs to be evaluated when choosing between whole exome (WES) and whole genome sequencing (WGS).

To determine these factors we investigated 9 samples sequenced in diagnostic laboratories using both WES and WGS and 4 samples analysed by SNP-array and deep sequenced by WES. As intellectual disability (ID) is considered the primary indication for diagnostics using WES/WGS, we focused on a panel of ~400 ID genes. We observed no false-negatives in WES versus WGS for the ID gene panel region. In contrast, duplicated regions and regions with many variants in close proximity were problematic, suffering from increased false negative rates (> 1/100).

Subsequently we determined minimal sequencing criteria by applying GATK’s experimental reference confidence score model on subsets of WES data (20-140×106 mapped reads). This revealed that (1) false positive rates are negligible, (2) false negative rates are below 1/1000, (3) the yield of identified variants is >95% with >30×106 mapped reads (~25X average depth), and (4) adequate depth can be reached for >95% of the bases of the ID gene panel with >60×106 mapped reads (~70X average depth) and >99% with >140×106 mapped reads (~160X average depth).

We conclude that the applied methodology allows diagnostic laboratories to make informed decisions on sequencing criteria using few deeply sequenced WES datasets.

C07.5 EuroGenTest guidelines for diagnostic next generation sequencing
G. Matthijs, M. Albers, P. Bauer, A. Carolevich, S. Eck, F. Fenech, C. V. Ruiter, H. Scheffner, E. Siersema, M. Sturm, M. Weiss, H. Veltman, and the Participants to the EuroGenTest workshop on Diagnostic NGS Guidelines; 'Center for Human Genetics, Leuven, Belgium; 'Department of Clinical Genetics, Academic Medical Center (AMC), Amsterdam, Netherlands; 'Institute of Medical Genetics and Applied Genomics, University Hospital Tübingen, Germany; 'Center for Human Genetics and Laboratory Diagnostics, Dr. Klein, Dr. Rust, Martinsried, Germany; 'Department of Human Genetics, Radboud university medical center, Nijmegen, Netherlands; 'VU University Medical Center, Amsterdam, Netherlands.

Next generation sequencing (NGS) is quickly being optimised for use in diagnostics. The expanding challenges at the technical level, in terms of data management and in the interpretation of results. Over the past 2 years, guidelines have been issued by the American, Australian, Dutch and British genetic professional societies. At EuroGenTest, an expert group has been working on compiling, integrating and completing these guidelines. For instance, we believe that defining the ‘diagnostic utility’ of the NGS test is the laboratory’s first duty when preparing to offer diagnostic NGS. Second, we introduce a scoring system for the different NGS assays depending on their quality and comprehensiveness. This is important for patients and clinicians to allow comparison of the diagnostic offer from the different laboratories. It could also be used by the health care system to evaluate and reimburse the tests. This scoring system is new, as it does not feature in any other guideline. As far as ‘reportable range’ is concerned, we propose the use of 3 specific percentages depending on the reference (technical target, coverage of transcript in a gene panel, coverage with reference to the genome) which again allow to compare individual results within runs, between tests and between laboratories. The guidelines propose a template for reporting NGS results as well. Finally, the guidelines also deal with informed consent, unclassified variants and unsolicited findings, again from the laboratory standpoint. Similarly, they define the difference between research and diagnostics, with a practical solution to the ‘duty to recontact’.

C07.6 Clinical exome sequence performance for reporting secondary genetic findings
E. Londin, P. Clarke, M. Spoonier, L. Kricka, P. Fortinot, J. Y. Park; 'Thomas Jefferson University, Philadelphia, PA, United States; 'Children’s Hospital of Philadelphia, Philadelphia, PA, United States; 'Sapienza Università di Roma, Rome, Italy; 'University of Pennsylvania Medical Center, Philadelphia, PA, United States; 'University of Texas Southwestern Medical Center and Children’s Medical Center, Dallas, TX, United States.

The American College of Medical Genetics and Genomics (ACMG) has recommended the reporting of clinically actionable incidental genetic findings in addition to the scope of clinical exome sequencing. Specific exome datasets of 56 specific genes with known clinical importance should be reported. However, this assumes that exome sequencing returns data of sufficient quality using methods that were not validated for clinical use. To address this issue, we surveyed the potential false negative rate of mutations in the 56 ACMG genes. We retrospectively, analyzed forty-four exome datasets from four different laboratories capturing known exome sequence variants in the 56 ACMG genes. These datasets were examined for their ability to detect clinically relevant mutations in the 56 ACMG genes. A total of 17,774 pathogenic nucleotide variants are annotated in the Human Gene Mutation Database (HGMD) for the 56 genes, and data was examined for depth of coverage in the exome datasets. Overall, the four-exome methods had inadequate depth of coverage for accurate base calling ranging from 5.2% to 34.0% of the pathogenic variant position in a single gene in each exome method was missing >40% of the pathogenic variant positions. The worst performing method was predicted to miss >90% of clinically significant variant positions in the genes TMEM43, PCSK9, KCNQ1 and LMNA. The heterogeneous and occasional poor depth of coverage across this set of 56 genes illustrates the opportunity for further innovation in standardizing clinical NGS methods. Implementation of the ACMG incidental findings guideline requires recognition of the substantial possibility of reporting false negative results.
Comprehensive annotation of splice junctions supports pervasive alternative splicing at the BRCA1 locus: a report from the ENIGMA consortium

**C08.2**

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Loss-of-function germline mutations in BRCA1 (MIM #113705) confer markedly increased risk of breast and ovarian cancer. The full-length transcript codifies for a protein involved in DNA repair pathways and cell-cycle checkpoints. Several BRCA1 splicing isoforms have been described in public domain databases, but the physiological role (if any) of BRCA1 alternative splicing remains to be established. An accurate description of naturally occurring alternative splicing at this locus is a prerequisite to understand its biological significance. However, a systematic analysis of alternative splicing at the BRCA1 locus is yet to be conducted. Here, the ENIGMA Network for the Interpretation of Germline Mutant Alleles (ENIGMA) consortium combines RT-PCR, exon scanning, cloning, sequencing, and relative semi-quantification to describe naturally occurring BRCA1 alternative splicing with unprecedented resolution. The study has been conducted in blood related DNA sources, commonly used for clinical splicing assays, as well as in other biologic tissue. We have characterized a total of 63 BRCA1 alternative splicing events, including 35 novel, ranging from 10 splice sites (Δ9,10, Δ9,11, Δ11q, Δ13p, Δ14p) represent a substantial fraction of the full-length expression level (ranging from 5% to 100%). Remarkably, our data indicates that BRCA1 alternative splicing is similar in blood and breast, a finding supporting the clinical relevance of blood-based in vitro splicing assays. Overall, our data suggests an alternative splicing model in which most non-excluded alternative splicing events are randomly combined into individual mRNA molecules to produce hundreds of different BRCA1 isoforms.

Germline mutations in BRCA1 predispose to gastric cancer

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Mutations in the E-cadherin gene, CDH1, account for 40% of hereditary diffuse gastric cancer (HDGC) cases. The genes responsible for the remaining cases of HDGC, as well as other familial gastric cancers (FGC) are currently unknown. We examined a large family with FGC and no CDH1 mutations using whole-exome sequencing and comparative genomic hybridization analyses, and confirmed and extended the involvement of loss-of-function germ-line CDH1 mutations in FGC. The findings of this study provide further evidence that truncating CDH1 mutations are associated with FGC, and that the use of microtubules poisons may be of particular interest in patients with Li-Fraumeni syndrome. This new functional assay should facilitate the identification of new non-genotoxic anti-cancer molecules.
Objective: Germline pathogenic mutations in DNA mismatch repair (MMR) genes and especially in MSH2 and MLH1 predispose to Lynch syndrome (LS). However, many of the found variants are of uncertain significance (VUS), which complicates their risk assessment and calls for functional analyses. Therefore, we investigated the functional impact of the four selected mutations in a set of Italian families with suspected LS. The probands from these families were investigated by sequencing of the coding regions of the genes and by microsatellite instability (MSI) and immunohistochemical (IHC) analyses of MMR proteins on tumor samples. Functional significance of the 9 putative LS predisposing VUS was analyzed in an in situ MMR assay and expression/ stability of the mutated variants evaluated by Western blot analysis (WB).

Results: A total of 14 mutations were identified in 9 patient families. The observed missense mutations in MLH1 and MSH2, affecting protein structure, were found to be pathogenic.

Conclusions: The functional analysis together with clinical, tumor pathological and in silico analysis helped to confirm pathogenicity in 5 of 9 putative Lynch syndrome variants.

C09.4

Exome sequencing of familial Parkinsonism in Scandinavia


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Parkinson’s disease (PD) is a progressive neurodegenerative disease, and the second most prevalent neurologic disorder of the elderly. Although ~1% of patients have a family history of parkinsonism in only a small proportion of such pedigrees have pathogenic mutations been identified to uncover disease susceptibility. The majority of causal and disease-modifying variants remain unknown. We report longitudinal clinical, genealogic and comparative sequence (exome) analyses of affected multi-generational families. Genome alignment, variant annotation and comparative analyses were used to identify shared coding mutations. Initially we have examined concordant/discordant variant sharing within multi-incident pedigrees (n=10), using Mendelian models and maximum parsimony. We subsequently

ly inspected variant segregation with disease and extended these findings to unrelated patients (n=1500) and control subjects (n=1500) of Scandinavian descent. In two families missense mutations in NOVA2 and RPE65 were found to segregate with parkinsonism and were not observed in control subjects of non-Caucasian origin. Both variants were subsequently genotyped in a multi-ethnic case-control series submitted by the GEOPD Consortium (n=5000). Interestingly, two additional patients with familial parkinsonism were identified as RPE65 carriers and no controls. Moreover, sequencing of entire coding region of RPE65 and NOVA2 in multi-ethnic probands with familial parkinsonism (n=100) led us to identify more missense mutations.

Our data provides evidence for two novel genes in the etiology of PD.

C09.3

Genome-wide analysis of microRNA coding genes in bipolar disorder


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Bipolar disorder is a severe and highly heritable disorder of mood with a lifetime prevalence of about 1%. Molecular genetic studies have identified a number of susceptibility genes, with the relevant pathways, however, being still largely unknown.

microRNAs are a class of small non-coding RNAs. Accumulating evidence suggests that microRNAs contribute to basic mechanisms underlying brain development and plasticity thus suggesting their possible involvement in the pathogenesis of several psychiatric disorders, including bipolar disorder.

The aim of the present study was to systematically investigate whether common variants at all known microRNA loci (miRBase release 13.0) contribute to the development of bipolar disorder. We performed gene-based analyses for all microRNAs and +/−20 kb flanking sequences using VEGAS on the largest existing GWAS dataset of bipolar disorder comprising of 9,747 patients and 14,278 controls (Mühlbeyer et al, Nature Commun 2014). In this dataset we combined our data obtained from four European countries, Canada and Australia with the GWAS results of the multinational Psychiatric Genomics Consortium.

Our analysis revealed that 98 of the 609 microRNAs showed nominally significant p values, indicating that bipolar disorder–associated microRNAs are enriched within the known microRNA loci (p<0.006). After correction for multiple testing, nine microRNAs (let-7g, miR-135a, miR-499, miR-581, miR-611, miR-640, miR-644, miR-708, miR-1908) showed a significant association with bipolar disorder. These included microRNAs known to be involved in neural development and synaptic plasticity.

Results from the investigation of the affected target genes and underlying regulatory networks supports these disease mechanisms and also suggests new mechanisms.

C09.4

Imbalance between excitation and inhibition in Neurons derived from MECP2, CDKL5 and FOXG1 iPSCs

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Rett syndrome is due to de novo mutations in MECP2, CDKL5 or FOXG1 genes. MeCP2 and RettG1 are transcriptional regulators; CDKL5 encodes for a
kinase protein involved in multiple cellular processes. In spite of their involvement in the same disease, a functional interaction between the three genes has not been proven and disease mechanism remain elusive. It has been suggested that an excitation/inhibition imbalance might play an important role in Rett pathogenesis. We have used a mouse model and a human model based on patients-specific iPSCs-derived neurons to test the hypothesis that in maturing neurons an initial excess of excitatory synapses might result in excitotoxicity finally leading to loss of excitatory synapses. We have established three iPSC lines for each gene. Quantitative RT-PCR on iPSCs-derived neurons demonstrated that VGLUT1 is over-expressed while GAD1 is down-regulated. Similarly, down-regulation of another inhibitory marker (VGAT) is observed in this model. However, in this model a reversion of excitatory synapses (VGLUT1, Glut1, GluA2, NR1) is observed opposite to iPSCs-derived neurons. Moreover, RNA and protein analysis shows an over-expression of GRID1, a member of the delta family of ionotropic glutamate receptors which induces preferentially inhibitory presynaptic differentiation of cortical neurons. Our data provide evidence in favor of excitotoxicity as an important contributing factor in Rett pathogenesis due to MECP2 mutations and suggest a similar mechanism for CDKL5- and FOXP1-mutated patients. These results confirm the presence of an imbalance between excitation and inhibition thus providing novel insights into Rett pathophysiology.

C09.5
Left/right asymmetry genes are associated with handedness and appear relevant for neurodevelopmental disorders
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Humans display structural and functional asymmetries in brain organization, strikingly manifested through language and handedness. While we understand the biology of body asymmetries, the molecular basis of brain laterality is still unknown. We report a genome-wide association study (GWAS) for a quantitative measure of handedness and dexterity (pegboard), in individuals with dyslexia (n = 728). The most strongly associated variant, rs7182874 (p = 8.68×10⁻⁹), is located in PCSK6, a gene known to activate NODAL, which is required to regulate left/right body axis determination. A novel approach for GWAS pathway analysis, based on gene-set enrichment strategies, showed that left/right asymmetry pathways are associated with handedness in both the dyslexia and a general population (n = 2666) cohorts. In particular, genes involved in corpus callosum development were enriched among the GWAS top hits. Furthermore, different markers at the PCSK6 locus were found to be associated with a measure of handedness in a completely independent study. The dyslexia-specific marker-trait association could be the result of an epistatic effect. Recent findings show that dyslexia candidate genes play a role in cilogenesis, an important developmental process at the basis of left/right structural asymmetries determination. We propose that handedness is a polygenic trait controlled in part by the molecular mechanisms which establish left/right body asymmetry early in development, which in turn influence brain midline development and might be implicated in neurodevelopmental disorders. We are now investigating the molecular mechanisms underlying this association using neuronal stem cell and zebrafish models.

C09.6
Exome sequencing to disclose potential new pathogenic variants in Rett patients without mutations in the known Rett genes
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Mutations in the X-linked gene MECP2 are the main cause of classical Rett syndrome (RTT) in about 25% of patients. Two other major early onset seizures and a small percentage of those with congenital variant forms carry mutations in CDKL5 and FOXP1 genes, respectively. About 5% of classical and 40-50% of atypical RTT patient remain without a molecular diagnosis. Aiming at discovering novel candidate genes, a cohort of 3 RTT patients with both classic and atypical phenotype and unascertained genetic defect was processed on a Human exome data platform by True Seq Exome Enrichment. As most mutations in the known RTT causative genes discovered up to day have a de novo origin, we adopted this model and filtered heterozygous variants, prioritizing those predicted to introduce a frameshift or stop codon or splice or missense changes. Prioritization of the interesting genes took into account their expression in Central Nervous System and/or neuronal function, previous reported involvement in a known genetic disease and the interaction to genes/protein responsible for a resembling phenotype. By this approach we have validated by Sanger sequencing 57 variants corresponding to 38 genes in RTT patients. In 8 cases parental analysis has proved the de novo origin of the SNVs, which occur in GABA receptors, Sodium and Potassium neuronal channels, synaptic vesicular releasing factor and a transcriptional regulator. Our data confirm exome targeted NGS as a promising tool to disclose novel pathogenetic mechanisms leading to RTT.

C10.1
Mutations in X-linked osteoporosis and fractures: unravelling a new bone regulatory pathway
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Osteoporosis with its consequences, i.e., fractures, is major health problem in ageing societies. As osteoporosis is a prevalent disorder, understanding its etiological factors is very important. We recently identified novel pathogenic variants in PL3 (encoding Plastin 3 (PL3), a filamentous-actin bundling protein) as a cause of X-linked osteoporosis and osteoprotic fractures in five Dutch families (van Dijk et al. NEJM 2013;369(16):1529-36). These loss-of-function variants cause decreased bone mineral density and increased risk of fracture in hemizygous young men whereas the clinical picture in heterozygous women ranged from asymptomatic to early-onset osteoporosis. It was highly unexpected that mutations in this gene would cause osteoporosis and fractures as it had never been described as a candidate gene for osteoporosis nor was it known to play a role in bone formation. However, results of in vivo analyses in zebrafish strongly supported a role for PL3 as a bone regulatory protein. Furthermore, a rare variant (rs140121121) in PL3 was found to be associated with a twofold increased fracture risk in elderly female carriers in the normal population indicating genetic variation in PL3 as a novel etiological factor involved in common, multifactorial osteoporosis. However, the exact mechanism by which PL3 causes osteoporosis and fractures is currently under subject of further investigations. Unravelling this new bone regulatory pathway is of great importance for understanding the aetiology of osteoporosis, increasing also the possibilities for prevention, diagnosis and treatment aimed at bone formation.

C10.2
Mutations in plastin 3 cause osteoporosis with fractures. Overexpression of PL3 and other F-actin bundling proteins influence skeletal development in zebrafish and mice
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Osteoporosis affects a large proportion of the human population, particularly women after menopause. Recently we reported that pathogenic loss-of-function variants in the plastin 3 (PL3) gene, localized on Xq23 are causative of familial osteoporosis with fractures. Furthermore, a rare variant in PL3 (rs140121121) associated with a 2-fold increased risk for fractures among elderly heterozygous women in two large cohorts from Rotterdam (van Dijk et al. NEJM, Oct 2013). PL3 is an ubiquitously expressed actin-
bundling protein that largely influences the dynamics of the actin cytoskeleton. We demonstrated that PLS3 mRNA co-injection dose-dependently rescued malformations of the craniofacial muscular-skeletal system, body axis and tail phenotype induced upon plls3 morpholino injection in 3 and 5 dpf controls: G6P transgenic zebrafish. Remarkably, affected patients with PLS3 loss of function mutations only presented a bovine phenotype. The absence of systemic manifestations has led us to hypothesise that other F-actin bundling proteins may compensate for the loss of PLS3 in other tissues. Interestingly, α-Actinin (ACTN) was found to be overexpressed in patients’ fibroblasts, possibly preventing more severe disease manifestations. Indeed co-injection of ACTN1 or ACTN4 mRNA, rescued the muscular-skeletal phenotype induced by plls3 knock-down in fish. Moreover, analysis of fibromas by microcomputed tomography in 3-month-old transgenic mice overexpressing human PLS3 showed significant differences in cortical and trabecular bone structures when compared to control mice. These results strongly indicate that PLS3 is a novel etiologic factor for monogenic and multifactorial osteoporosis and PLS3 and other F-actin bundling proteins are important regulators of bone development and maintenance.

C10.3

XYLT1 mutations in Debuquisois dysplasia type 2

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to cause suture fusion. Interestingly, the 4 identified ZIC1 variants all cluster within 42 nucleotides of the terminal exon; analysis of fibroblast RNA from the first case showed that the mutant transcript escapes nonsense-mediated decay. To gain further insight into the molecular mechanism we are characterising the in vitro activity of the mutant proteins. Furthermore we show that in mouse embryos Zic1 is expressed in the supranotral region at E11.5-E12.5, consistent with a role for ZIC1 at a very early stage of cranial suture biogenesis. Overall these findings confirm ZIC1 as a new disease gene in coronal craniosynostosis, particularly when accompanied by unexplained learning disability.

C11.3

Polygenic risk for ADHD is associated with impaired educational achievement and lower IQ in the general population

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Introduction

High levels of ADHD symptoms during childhood carry risk of worse academic performance and can impact on employment and earnings in adulthood. ADHD is used as a way to compare common risk allele burden for clinical ADHD contribute to the risk of having higher ADHD symptoms in the general population (Martin et al. in press). We have used polygenic score analysis to investigate the contribution of common risk variants for clinical ADHD on educational performance and IQ in the general population.

Methods

Academic performance was assessed using results from Key Stage 3 national examinations (GCSE) for 6,385 children from the Avon Longitudinal Study of Parents and Children (ALSPAC). Polygenic risk scores were calculated for ALSPAC children and their mothers based on the results of an ADHD GWAS (Stergiakouli et al. 2012).

Results

ADHD polygenic scores on the children were associated with worst educational outcomes as represented by both time points and also with lower IQ scores at age 15.5 (see Table). Moreover, ADHD polygenic scores on the mothers were associated with lower IQ in the mothers and worst educational outcomes in the children (see Table).

Discussion

Our results suggest that the same genetic variants that are relevant for an ADHD diagnosis are also implicated in impaired academic performance in the general population and lower IQ score in both children and adults.

C11.4

Polygenic risk score analysis shows shared genetic aetiology between AN and five other psychiatric disorders

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Several modern technologies, such as nuclear magnetic resonance and mass spectrometry platforms in metabolomics, produce high-dimensional phenotypic data on individuals. A first step towards utilizing high-dimensional phenotypes in genetic studies is to understand how their genetic components are related.

Recent algorithmic advances in multivariate linear mixed models have enabled variance component estimation for pairs of traits using population samples of individuals and genome-wide panels of SNPs. However, current methods have not been tailored for situations where hundreds of traits are available on the same set of individuals. For such settings, we introduced an algorithm that efficiently decomposes pairwise polygenic correlations into genetic and environmental components. We illustrate our approach with an application to 105 pairs of metabolic and anthropometric traits measured on up to 14,000 Finnish individuals. For example, we estimate that the observed polygenic correlation (-0.41) between triglycerides (TG) and HDL cholesterol decomposes into an additive genetic correlation (-0.59, s.e. 0.06) and an environmental correlation (-0.36 s.e. 0.02).

We discuss the interpretation of genetic correlations as correlations between locus-wise genetic effects and characterise settings where prior information about genetic correlation increases statistical power to identify pleotropic loci, i.e. loci that contribute to multiple traits.

C11.5

The influence of genotype and phenotype data quality control on SNP based heritability estimates within and across studies

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Currently, many GWAS studies are accompanied by additional analyses to get an estimate of the amount of heritability explained by the GWAS SNPs. Such analyses in unrelated individuals critically depend on the construction of a Genetic Relatedness Matrix (GRM) as implemented for example in the software package GCTA [1]. What sometimes seems a bit surprising is the large variation in the obtained point estimates between studies. Although heritability is a population specific characteristic, these GRM-based heritabilities seem more variable than e.g. heritabilities based on twin data. Using genotype data and a wide range of phenotypes from two large population based studies in the Netherlands (NEDDA: Netherlands Study of Depression and Anxiety and NTR: Netherlands Twin Register). In addition to a set of simulated phenotypes, we examined the effects SNP selection based on GWAS QC, quality control and phenotype transformations. For continuous traits, the results show that the GRM method is sensitive to SNP selection criteria, with - or without imputations, as well as deviation of phenotypes from the assumed normal distribution. For dichotomous traits, additional issues including non-random missingness of genotypes, play a role. Thus, a substantial part of the variation in GCTA heritability estimates may depend on data quality control and the selection of study individuals prior to the analysis.

1GCTA is a tool for genome-wide complex trait analysis by J. Lee SH, Goddard ME, Visscher PM. Am J Hum Genet. 2011 Jan 7;88(1):76-82. PMID 21167468.
Next generation sequencing represents the most efficient technology to identify mutations in RD patients and families. Underrepresented regions were examined by Sanger sequencing. We found mutations in 50 to 60% of retinitis pigmentosa and approximately 80% of syndromic (Bardet-Biedl or Leber congenital amaurosis) cases. Seventy-one novel mutations were identified, among which the genes USH2A, EYS, ABCA4, and RHO were more frequently affected by pathogenic sequence alterations. Occasionally, carried mutations in more than one RD-associated gene suggesting modifier effects of the additional variants. We further found possible dominant novo mutations in sporadic RD cases imposing difficulties for counseling of patients and families. Some sequencing showed similar detection rates in additional research-based cases confirming that both approaches are efficient to identify mutations in known disease genes. In contrast to panel sequencing, exome analysis provided the bases to search for gene candidates for RD.

In conclusion, NGS-based mutation analyses provide reliable and cost-efficient approaches to investigate genetically heterogeneous diseases like RD.
**C12.3 Disclosure of false disease genes - an underestimated potential of targeted and genomewide NGS: The example of MYO1A and deafness type DFNA48**

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MYO1A is considered the gene underlying autosomal dominant non-syndromic hearing loss type DFNA48, based on six missense variants, one small in-frame insertion and one nonsense alteration. By next-generation sequencing of the deafness genes in 50 hearing-impaired patients, we identified three families (including a consanguineous one) that provide strong evidence against a causative role of MYO1A in inherited deafness: Two novel nonsense mutations (p.Tyr740* and p.Arg626*) and a previously described missense mutation were identified not only in the index patients in heterozygous state, but also in unaffected relatives. The hearing deficit in these families was clearly due to mutations in other deafness genes, MYO7A, EVA1 and CIB2, respectively. All but two of the altogether ten MYO1A mutations have been annotated in dBNSP, and population frequencies (dBNSP, 1000 Genomes, Exome Sequencing Project) above 0.1% contradict pathogenicity assuming a dominant model. Moreover, one healthy individual from the consanguineous family was homozygous for the nonsense mutation p.Arg626*, compatible with a previously reported homozygous MYO1A knockout miR lacking any overt pathology. We conclude that MYO1A is dispensable for normal hearing and may even represent a non-essential gene, adding to the list of “eroneous disease genes” that is growing rapidly with increasing availability of data from large-scale sequencing projects. Data from individuals born to consanguineous parents are particularly valuable because they are enriched for homozygous loss-of-function variants, with the potential to mask “false disease genes.” Their identification is urgently needed to improve mutation databases and avoid pitfalls in diagnostics and genetic counseling.

**C12.4 AON intravitreal injections to manipulate splicing in retinal cells**

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**Purpose:** Leber congenital amaurosis (LCA) is the leading cause of hereditary blindness in children. CEP290 encodes a ciliary protein important to photoreceptor connecting cilium assembly and function. The CEP290 intronic c.2991+1655A>G change is the most common LCA-causing mutation (10%). It introduces a cryptic exon in the mRNA encoding a premature termination codon. Recently, we made the proof-of-concept of antisense oligonucleotide (AON)-mediated exon skipping to correct the splicing in patient fibroblasts which recovered ability to ciliate. The purpose of this study was to make the proof-of-concept of exon-skipping in vivo using intravitreal injections of AONs. Methods: AONs were designed to skip mouse Cep290 exon 36. Variable concentrations of 6-FAM-AONs were injected into the vitreous of C57BL/6J mice. Retinal sections and mRNA were prepared during 30 days post-injections to follow i) the distribution of oligonucleotides across cellular layers and ii) exon skipping efficiency. Results: The most efficient AONs identified by in vitro analyses were injected in the vitreous of animals. Excellent correlation between the efficiency of exon skipping and AON injected dose has been demonstrated. Histological analyses revealed a wide distribution of AON in all retinal cell layers at least until 30 dpi. A linear amount decrease of mRNA lacking exon 36 was measured but still detectable at 30 dpi. Conclusion: Here we report that single intravitreal injection of AON allows efficient and persistent exon skipping in retinal cells. Hence, this strategy may be regarded as an attractive alternative to gene replacement therapy for 10% of patients affected with LCA.

**C12.5 Mutations in the tricarboxylic acid cycle enzyme, Aconitase 2, cause either isolated or syndromic optic neuropathy with encephalopathy and cerebellar atrophy**

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Inherited optic neuropathy has been ascribed to mutations in mitochondrial fusion/fission genes such as mitofusin-1, mitofusin-2, and mitochondria-encoded respiratory enzyme genes or nuclear genes of poorly known mitochondrial function. On the other hand, enzymopathies of the tricarboxylic acid cycle (TCA) have been reported to cause severe encephalopathies or isolated retinitis pigmentosa, but no TCA-cycle enzyme deficiency has been hitherto reported in isolated optic neuropathy. Studying a series of five patients with optic atrophy, we found homozgyous or compound heterozygous missense and frameshift mutations in the gene encoding mitochondrial aconitase (ACO2), a TCA-cycle enzyme, catalyzing interconversion of citrate into isocitrate. Retrospective studies using patient-derived cultured skin fibroblasts revealed various degrees of deficiency in ACO2 activity but also in ACO1 cytotoxic activity. Our study shows that autosomal recessive ACO2 mutations can cause either isolated or syndromic optic neuropathy. This observation supports the view that optic atrophy is a hallmark of defective mitochondrial energy supply and that extra-ocular involvement is not related to severity of the enzyme deficiency.

**C12.6 Isovalate foveal hypoplasia with secondary nystagmus and low vision is associated with a homozygous SLC38A8 mutation**

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Foveal hypoplasia, always accompanied by nystagmus, is found as part of the clinical spectrum of various eye disorders such as aniridia, albinism and achromatopsia. However, the molecular basis of isolated autosomal recessive foveal hypoplasia is yet unknown. Individuals of apparently unrelated non-consanguineous Israeli families of Jewish Indian (Mumbai) ancestry presented with isolated foveal hypoplasia associated with congenital nystagmus and reduced visual acuity. Genome-wide homozygosity mapping followed by fine mapping defined a 8.30 Kb disease-associated locus (LOD score 3.5). Whole-exome sequencing identified a single missense mutation in the homozygosity region: c.957T>G, p.(Ile32Ser), in a conserved amino acid within the first predicted transmembrane domain of SLC38A8. The mutation fully segregated with the disease-associated phenotype, demonstrating an ~10% carrier rate in Mumbai Jews. SLC38A8 encodes a putative sodium-dependent L-type amino acid/proton antiporter, which we showed to be expressed solely in the eye. Thus, a homozygous SLC38A8 mutation likely underlies isolated foveal hypoplasia. Intracellular localization of SLC38A8 using confocal microscopy indicated that SLC38A8 resides within the nuclear envelope and in the Golgi apparatus as well. The sub-localization of SLC38A8 is different than that of other SLC8 proteins, as all other members of this family are cytoplasmic transmembrane proteins. This may suggest that SLC38A8 has a different function altogether, and may not be an amino acid transporter as previously predicted. The precise role of SLC38A8 in normal eye development and function, and the molecular mechanisms through which its mutation causes foveal hypoplasia are yet to be elucidated.

**C13.1 Stratified cancer screening in Europe using genomic information: conclusions and recommendations from the COGS project**

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As part of the Collaborative Oncological Gene-environment Study (COGS), we investigated using genomic and other information to estimate individuals’ risk of developing cancer and offering different screening and other preventive interventions according to the results. We concluded that genetic testing may soon be used in risk-stratified screening for breast and prostate cancer and that policy-makers should prepare for this. Stratified screening could produce high rates of diagnosis and early treat-
ment, while sparing lower risk, disease-free people the risks and inconvenience of screening, and reducing costs. Achieving this with genomic information is attractive and increasingly feasible.

We will describe how this approach can be implemented, including how the screening offer could be made, how risk would be estimated, the age at which this could occur and the potential use of genetic data for other purposes. We will describe how management might differ depending on individuals’ risk, communication of results and follow-up arrangements, and the different issues raised by modification of an existing screening programme (breast cancer) and the establishment of a new one (prostate cancer).

These issues will need careful handling to ensure outcomes are optimal and harm minimised. Although we do not think the evidence is yet adequate to support risk-stratified screening for breast and prostate cancer, we believe that point will soon be reached. Further research is needed into impact, utility, cost-effectiveness, acceptability and ethical, legal and social implications. A critical factor may be whether targeting resources according to risk is seen as acceptable by the entire screening population.

C13.2 Expanding access to genetic counseling for hereditary breast and ovarian cancer with telephone delivery: A cluster randomized noninferiority trial

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Geographic barriers limit access to in-person cancer genetic services. We conducted a cluster randomized trial with high-risk women to test the equivalence and noninferiority of BRCA1/2 telephone counseling to remote in-person counseling. 988 women, 25–74 years of age, with a personal or family history of breast and/or ovarian cancer enrolled. Participants were randomized by family unit to either telephone or in-person counseling. Assessments were done 1 week following pre-test and post-counseling, and at 6 months. Cluster bootstrap methods were used to estimate the 95% confidence interval (CI) for the difference between test uptake proportions. Between-group intervention differences in psychosocial outcomes were estimated using linear models together with one-sided 9.5% CI. Telephone BRCA1/2 genetic counseling fulfilled the criteria for noninferiority to in-person remote counseling with regard to anxiety, cancer-specific psychological distress, and quality of life measures, as well as assessments of the information, interpersonal sensitivity and partnership building of genetic counselors for both urban and rural dwellers. BRCA1/2 testing uptake was lower following telephone (21.8%) than in-person pre-test counseling (31.8%) (CI=3.9%–16.3%). However, in-person counseling had higher average cost per participant counseled than telephone counseling ($654 vs. $278). The higher uptake of testing in the rural (35.1%) compared to the urban (25.2%) subgroups suggests that the genetic screening interests of rural populations may be underserved by existing health care systems. BRCA1/2 telephone counseling appears to be safe and as effective as in-person counseling with regard to minimizing adverse psychological reactions and delivering patient-centered communication for both rural and urban dwellers.

C13.3 New approaches to bridge the gap between genetics research and primary health care in Ireland

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The translation of research findings on rare disorders from the laboratory into hospital practice can be challenging. Translation into primary healthcare is even more problematic due to lack of funding, infrastructure and communication with primary care staff. Through funding specific for dissemination, we have been able to engage with healthcare professionals (HCPs) using a multi-faceted approach. We developed a microsite (http://www.ucd.ie/medicine/rarediseases/), animation videos, guidelines handbook, Bioinformatics course and four nationwide educational seminars. The animation videos were designed for HCPs to help explain inheritance patterns and to educate about consanguinity. These graphical educational tools are being used by HCPs to overcome poor literacy in some families. We liaised with HCPs to develop a guidelines handbook to help with the management of common genetic disorders at a local level. We produced a series of common clinical scenarios which HCPs might encounter to help with addressing the relative risk and genetic testing procedures. The two-day Clinical Bioinformatics course aimed to update our laboratory staff to enable translation of our research findings into our local diagnostic laboratory. Our educational seminars covered topics such as carrier testing, intellectual disability, consanguinity, rare disease research and the role of advocacy. Discussions with seminar attendees suggest that there is a significant knowledge deficit when it comes to genetics in healthcare. However, encouraging HCPs voiced a genuine interest in genetics knowledge about genetics and its application to their practice. We plan to continue our efforts and develop e-learning tools to support the integration of genetics into mainstream medicine.

C13.4 Unanticipated results in whole exome study: we’re still a lot to learn


Mutations in SCNSA are associated with Brugada syndrome, LQTS, atrial fibrillation and other conditions with risk of sudden cardiac death. Evidence-based guidelines exist to significantly improve or avert the mortality and morbidity associated with these conditions. As such the ACMG included SCNSA on its list of incidental findings which it recommends should be returned to all whole-exome sequencing (WES) results. Given the controversial challenges, we have introduced the concept of the NCGENES whole exome sequencing study, these challenges are being explored in a cohort of ~500 patients. Incidental, medically actionable findings are reported and this is emphasized during the pre-test counseling. We present here our experience with incidental finding of SCNSA mutations detected in 8 of 145 (5.5%) participants. Each variant, previously reported as pathogenic, was independently reviewed and discussed with members of the molecular analysis team to reach consensus regarding interpretation of its pathogenicity. Upon review we determined there was convincing evidence for only one to be considered pathogenic and reported. The remaining were not reported, though future information may change the assessment of these interpretations.

C13.5 The stepping stone approach towards the Genetics Clinic of the Future

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Routine application of genomics in the clinic faces many cross-cutting, controversial challenges. To map them, we have introduced the concept of the Genetics Clinic of the Future (GCoF). Moreover we have developed a stepping stone approach to meet these challenges, comprising of field laboratory tasks (the ‘stepping stones’) that address controversial, multi-dimensional
and interdependent challenges. Here we present three field laboratories, each of which are rooted in one of the main areas of the GCOF. Initially, we considered the effect of new phenomena like genomics and data exchange and health data management on the organisation of the ‘server room’. Finally, we explored the possibilities for involvement of patients and non-patients in the ‘living room’ of the GCOF. Each laboratory established focuses, constructive interdisciplinary collaborations around ‘radically interdisciplinary’ topics. Based on the ‘stepping stones’ initiatives we set the route towards the GCOF: researchers from the natural and social sciences, medical professionals, healthcare managers, industry representatives, policy makers, patients and citizens collaboratively define the design principles of genome data infrastructures as genomic technologies mature and become embedded in routine diagnostic procedures and health management systems.

C13.6 Teaching Genomic Medicine to Physicians - this is our responsibility as medical geneticists

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Background: Due to new discoveries in genomic medicine, and the transition of genetic knowledge from research laboratories into clinical practice, an increasing number of medical societies incorporate recommendations regarding genomic tests and therapeutics into routine clinical guidelines. Primary care practitioners have inadequate knowledge and skills in medical genetics and many are unaware of the technical, ethical, legal and psychosocial implications of genetic testing. Methods: We initiated a “genomic education” program for the purpose of providing physicians from different medical fields advanced knowledge in genomic medicine. We emphasized the main take-home messages for physicians, defined as: risk calculation in various genetic diseases, recognition of the mode of inheritance from the pedigree, guidelines for decision-making on which molecular tests to use, interpretation of test results and their clinical implications. Results: To date, 82 physicians have participated in 6 courses of our “genomic education” program, which included lectures, workshops and guided tours in genetic laboratories. In the “pre-course” examination the average score was 56% (range 20%-80%), whereas in the “post-course” examination it was 79% (range 40%-100%). The average improvement in score as a result of the course was 21% (range 0%-80%). The physicians who participated in our program reported a very high level of satisfaction from the theoretical and practical knowledge they acquired as well as the concept of a one-week update (course). A one-week “genomic education” program is an effective strategy to update primary care physicians regarding the advances in Genomic Medicine in order to improve their care of patients.

C14.1 Insights into the genetic architecture of anthropometric traits using whole genome sequence data

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Body weight and fat distribution measures are associated with increased risk of cardio metabolic disease. As part of the UK10K study, we have investigated the genetic architecture of anthropometric traits in 3,538 individuals with 6.5x whole genome sequence (WGS) data from the ALSPAC and TwinsUK cohorts. Variants discovered through WGS, along with those from the 1000 Genomes Project (1KGP), were imputed into additional individuals from the ALSPAC and TwinsUK cohorts with GWAS data (total sample size 9,979). We investigated association between anthropometric traits and ~15,000 samples, 43 out of 66 novel signals for BMI have the same direction of effect, respectively. We estimated the improvement in genome-wide signal captured relative to those present in HapMap 2, HapMap 3 or 1KGP. We find no appreciable increase in variance explained as density increases, suggesting that the contribution of variants with MAF<0.01 are likely to be well-captured by existing GWAS implementation. Larger sample sizes will be required to refine these estimates.

C14.2 Genome of the Netherlands imputation identifies seven new loci for quantitative ECG traits in meta-analysis of 30,000 samples


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Genome-wide association studies (GWAS) of quantitative electrocardiographic (ECG) traits in large consortia identified over 50 loci associated with QRS, RR, QT and PR intervals, with even more loci discovered in ongoing HapMap-based meta-analyses in larger sample sizes. We hypothesized that imputation from sequencing-based reference panels may help in identifying new loci, as the contribution of lower-frequency variation has not yet been extensively characterized. In the current study, we meta-analyzed GWAS results from 30,000 samples on the QRS, RR, QT and PR intervals after imputing 19 million SNPs from the Genome of the Netherlands (GoNL) reference panel (998 unique haplotypes). This approach proved successful; in addition to many known loci, we identified seven novel locus-trait associations. Of the seven novel loci, three were for PR, three were for QT and one was for QRS. Two were novel trait locus combinations for loci previously observed for other ECG traits. Several others are involved in ion handling crucial to cardiac electrophysiology. The dense coverage of the genome (~seven times more SNPs than HapMap-based studies) also enabled us to study known loci in much finer detail. In conclusion, we show that larger reference panels with more accurate haplotype estimates and denser SNP data enable us to find new loci and fine-map previously known ones.

C14.3 Genome-wide association analysis identifies a new gene involved in salt perception and liking

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Salt perception and genetic variation in taste receptors may be important determinants of individual differences in salt intake, which in turn represents a risk factor for the development of hypertension and cardiovascular diseases. In recent years genetic variation in taste perception has been better known for bitter, sweet and umami taste, while little is known on the genetic bases of human salt perception. To investigate this area, salt taste responses were collected on ~900 healthy adults coming from 6 different small villages from North Eastern Italy. NaCl taste intensity was assessed with the labelled magnitude scale (LMS) using a concentration of 1M and the log10 of the intensity ratings were used for the analysis. Genotyping data were imputed to the 1000G SNP set and used to perform a GWAS of salt perception including sex and age as covariates. A significant association with rs547916 SNP (p-value=5.6x10-08), closely located to the KCNA5 gene, was detected. A replication analysis was carried out on an American cohort confirming the association with salt liking phenotype (p-value=0.002). KCNA5 encodes a member of potassium channel voltage-gated; the related subfamily and belongs to the delayed rectifier K+ (DRK) channels that in the mammalian taste system play a central role in specific taste transduction pathways. A possible implication of this finding is that KCNA5 plays a role in salt perception and liking.  The selective role of KCNA5 in salt perception and liking is possible implications for cardiovascular diseases.

C14.4 ImmunoSeq: Discovery of novel rare variants implicated in autoimmune and inflammatory diseases by targeting regulatory regions in immune cells

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C14.5 Exome array analysis in >30,000 Europeans establishes a functional role for G6PC2 and identifies novel coding variants influencing glycemic traits
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To identify coding variants associated with fasting glucose (FG) and fasting insulin (FI) levels, we analysed exome array data in up to 33,407 non-diabetic individuals of European ancestry. We identified multiple glucose-lowering coding variants in G6PC2, which resides in an established FG gene-gene association study (GWAS) locus. Conditional single-variant association analysis established the presence of two coding variants at exome-wide significance (P<5x10^{-8}) in this gene, one common (P_{GWAS} = 7.1x10^{-10}, 0.8% MAF, p.H177Y) and one rare (P_{GWAS} = 1.3x10^{-10}, 0.3% MAF, p.V219L). These data establish a functional role for G6PC2 and suggest a previously unknown impact of rare and variant coding variants in an diabetes susceptibility gene.

C14.6 Transethnic association study of IBD identifies novel risk loci and shows pervasive sharing of genetic risk factors across populations
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There are now over 163 loci associated with inflammatory bowel disease (IBD) susceptibility in European populations, though little is known about their contribution to disease risk in non-European populations. Using the custom Immunochip array, we genotyped 9,846 individuals of East Asian (Japan, South Korea, Hong Kong, China and UK individuals of East Asian descent), Indian and Indo-European (Indian) descent. Combining these with the existing cohort of 81,248 individuals of European descent (Jostins et al. Nature 2012, we identified 14 novel IBD susceptibility loci at genome-wide significance (P<5x10^{-8}). The majority of IBD risk loci were shared between both Europeans and non-Europeans, and differences in the amount of variance explained in disease liability at each locus were driven by a combination of differences in allele frequencies and effect sizes. For instance, the variance in Crohn's disease (CD) liability explained by four independent risk variants at TNFSF15 was 19 times greater in East Asians than Europeans, a reflection of the risk-increasing alleles being more common and having larger odds ratios in East Asians. Aggregation of all SNPs assayed on the Immunochip revealed significant genetic correlation (0.4-0.85) for IBD between European and non-European populations. Together, this study increases the total number of IBD susceptibility loci to 177 and demonstrates the pervasive sharing of genetic risk factors across populations, though there exists heterogeneity in the variance explained per locus.

C15.1 BCAP31 mutations cause a new X-linked syndrome with deafness, small stature, central hypomyelination and disorganization of the Golgi apparatus
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BAP31 is one of the most abundant proteins of the membrane of the endoplasmic reticulum (ER). It is a chaperone protein involved in several pathways, including ER-associated protein degradation, export of proteins from the ER to the Golgi, and programmed cell death. BAP31 is encoded by the BCAP31 gene located in Xq28. It is highly expressed in neurons. We identified loss of function mutations in BAP31 in seven individuals from three different families and one missense mutation in another fourth family. These people suffer from intellectual and motor disability, dystonia and sensorineural hearing loss with abnormal white matter. The association of these clinical signs define a new X-linked syndrome. In primary fibroblasts from patients, we found that the absence of BCAP31 changed the morphology of the ER and disorganized the Golgi apparatus in a significant proportion of cells. We demonstrated that the constitutive deficiency of BCAP31 did not activate the response to unfolded proteins (UPR) nor triggered cell death. Rather, our data suggest that the absence of BAP31 affects ER and Golgi apparatus metabolism and that BAP31 plays an important role in ER-Golgi exchange. These results provide a molecular basis for a new Mendelian syndrome and connect intracellular protein trafficking with a severe congenital neurological disorder.

C15.2 Mutations in KPTN Cause Macrophage, Neurodevelopmental Delay, and Seizures
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The proper development of neuronal circuits during neurodevelopment depends on a coordinated and intricate series of molecular and cellular cues and responses. Although the cortical actin cytoskeleton is known to play a key role in neurorhogenesis, relatively little is known about the specific molecules important for this process. We identified nine affected individuals from four Ohio Amish families affected by an autosomal recessive, variable form of neurodevelopmental delay. The most consistent features were global developmental delay, macrocephaly, anxiety, and some features suggestive of a pervasive developmental disorder. In addition, a primary seizure disorder was described in three cases. Using a combination of autozygosity mapping, linkage

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analysis and whole-exome sequencing, we demonstrated that two founder mutations in KRT15, encoding kaptin, can result in this phenotype. Our immunofluorescence analyses in primary neuronal cell cultures showed that endogenous and GFP-tagged kaptin associate with dynamic actin cytoskeletal structures and that this association is disrupted by the two mutations (p.Ala113Glu and p.Val78Leu) affecting the potential role of CTNND2 in neuron motility, we were particularly interested in assessing defects in neuronal migration in vivo. Knockdown of one of two zebrafish CTNND2 orthologs expressing GFP under the islet promoter (isl:GFP) in knockdown embryos showed the presence of misplaced isl:GFP cells between the telencephalon and diencephalon indicative of defects in migration. This suggests that defective migration of subpopulations of neuronal cells due to loss of CTNND2 could be part of the underlying mechanism behind the cognitive dysfunction in the patients.

C15.5 Loss of CTNNB2 is associated with borderline intellectual dysfunction in humans and neuronal migration defects in zebrafish

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Cytogenetically visible chromosomal translocations are a unique resource as they can pinpoint strong effect genes. Here we report a mother and daughter with borderline intelligence and learning problems within the dyslexia spectrum and two apparently balanced reciprocal translocations; t(1;18)(p22;q24). This suggests that defective migration of subpopulations of neuronal cells due to loss of CTNND2 could be part of the underlying mechanism behind the cognitive dysfunction in the patients.

C15.6 Novel (ovario)leukodystrophy related to AARS2 mutations

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Purpose: This study was focused on patients with a leukoencephalopathy of unknown cause with the aim to define a novel, homogeneous phenotype suggestive of a common genetic defect, based on clinical and MRI findings, and to identify the causal genetic defect, shared by patients with this phenotype.

Methods: Independent exome sequencing studies were performed in two unrelated patients with a novel leukoencephalopathy. MRI findings in these patients were compared to MRS in a database of unclassified leukoencephalopathies. Eleven additional patients with similar MRI abnormalities were selected. Clinical and MRI findings were investigated.
C16.1
A congenital disorder of glycosylation, with lymphopenia, neutropenia, and skeletal dysplasia, caused by mutations in the gene encoding phosphoglucomutase 3 (PGM3).


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Phosphoglucomutase 3 (PGM3) catalyzes the conversion of GlNAc-6-P into Glnac-1-P, during the synthesis of UDP-GlNAc, a sugar nucleotide critical to multiple glycosylation pathways. PGM3-defects have earlier been associated with hematopoietic deficiency in a mouse model. We identified three unrelated patients with recurrent infections, congenital leukopenia including lymphopenia, neutropenia, and skeletal dysplasia, with severe skeletal anomalies and intellectual disability. Whole exome sequencing revealed novel, deleterious mutations in the PGM3 gene in all three subjects, delineating a new type of craniosynostosis syndrome.”

C16.3
Homologous FIBP truncating mutation in a new multiple congenital anomalies syndrome with overgrowth, macrocephaly, Iris coloboma, and learning disabilities

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Fibroblast growth factors (FGFs) pathways determine crucial cellular roles in proliferation, migration, and survival by various extracellular and intracellular modes of action. Acidic FGF extracellular action usually consists of binding multiple forms of cell surface receptors, leading to the activation of cytoplasmic tyrosine-kinase pathways but it also enters into the nucleus to stimulate DNA synthesis. The acidic fibroblast growth factor intracellular binding protein (FIBP) is a FGFl binding protein widely expressed in human tissues, but its function remains unknown in FGF signaling. We performed exome sequencing in a 22-year-old man, born from consanguineous parents, and presenting with an undefined phenotype associating overgrowth, marfanoid habitus, macrocephaly, facial dysmorphism, large thumbs, bilateral iris coloboma, ventricular septal defect, mitral valve prolap, venous insufficiency, moderate scoliosis, and learning disabilities. A homozygous nonsense mutation (p.Gln218*) was identified and was absent in the patient’s unaffected siblings. Whole exome sequencing of the patient’s kidneys, consistent with the patient’s clinical features. Additional functional results argue for the pathogenicity of the mutation in the phenotype: 1) FIBP cDNA was undetectable in patient’s fibroblasts; 2) the intracellular FGF pathway was deregulated with increased FGF-1 protein level. 3) The XTT test and Id67 labeling exhibited a higher proliferation capacity in patient’s fibroblasts.”
Hidden mutations in Cornelia de Lange Syndrome (CdLS) - Limitations of Sanger sequencing in molecular diagnostics

C16.4

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CdLS is a clinically and genetically heterogeneous developmental disorder. Patients are characterized by distinct facial features, growth retardation and cognitive delay. Whereas half of the patients show mutations in the NIPBL gene, mutations in SMC1A, SMCS, RAD21 or HDAC8 account for additional 10%. Interestingly, recent data using DNA from buccal mucosa (BM) tissue could identify a high proportion of mosaic mutations in NIPBL, which were not detected in DNA from blood samples. Here we report on two unrelated patients with characteristic CdLS phenotype, who were mutation negative by Sanger sequencing analyzing DNA from blood and BM samples. Subsequent Ion Torrent panel sequencing on DNA of BM using a custom made Ion AmpliSeq enrichment that includes the five known CdLS genes beside eleven functionally-associated candidate genes, could detect a mosaic nonsense (17%) and a mosaic missense mutation (13%) in NIPBL. Interestingly, Sanger sequencing on DNA of BM, especially when analyzing patients with mosaic mutations in NIPBL could detect both mutations in DNA from BM, fibroblasts and urine samples but did not detect these mutations in DNA from blood. Additional Sanger sequencing approaches using DNA from all four tissues could only detect both mutations in fibroblast DNA. In summary, our data strongly support a high frequency of mosaic NIPBL mutations in CdLS-patients. More importantly, it also shows the limitations of current sequencing approaches in diagnostics, even when using DNA of BM as recently suggested. Thus we recommend the use of high coverage sequencing techniques on DNA of BM, especially when analyzing patients with characteristic CdLS-phenotypes and negative Sanger sequencing results.

C16.5

RNA Polymerase II activity is affected at the promoter regions in SMC1A-mutated Cornelia de Lange Syndrome cells

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Mutations in genes encoding cohesin subunits or regulators, namely NIPBL, SMC1A, SMCS, HDAC8 and RAD21, have been linked to Cornelia de Lange syndrome (CdLS). It has been hypothesized that the dysregulation of gene expression by chromatin remodeling likely represents the underlying pathogenesis of CdLS, however the exact mechanism by which this is effected is unknown. To gain a better understanding of this process we investigated whether the gene transcription machinery was somehow affected by SMC1A mutations. We used chromatin immunoprecipitation coupled with massively parallel DNA sequencing (ChIP-seq) to identify genomic regions co-occupied by cohesin, NIPBL and RNA pol II in normal human lymphoblastoid cells. Genes co-localizing cohesin, NIPBL and RNA pol II have been compared to gene expression data from CdLS cell lines. Finally, we investigated the recruitment of RNA pol II onto genes differentially expressed in CdLS mutated cells. Our results indicate that SMC1A mutations reduce the recruitment of Pol II at promoter regions and also affect the activity of the Pol II elongating form. These findings highlight the pivotal role of cohesin in transcriptional regulation and the effect on Pol II occupancy may explain the typical gene dysregulation observed in CdLS cell lines.

C16.6

In silico and functional characterization of KMT2D/MLL2 missense mutations as causative in Kabuki syndrome

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Kabuki syndrome (KS) is a multiple congenital malformation syndrome characterized by facial features, skeletal anomalies, dermatoglyphic abnormalities, mental retardation, and postnatal growth deficiency. Heterozygous mutations in the KMT2D gene are detected in >90% of Kabuki patients. KMT2D gene encodes a H3K4 histone methyltransferase that plays important role in the epigenetic control of active chromatin states modulating the expression of genes essential for embryogenesis and development. A subset of KS individuals was recently identified with mutations in the chromatin modifier KDM6A. We performed a mutational screening on 303 Kabuki patients by direct sequencing, MLPA, and qPCR, detecting 133 patients with KMT2D mutations and four with KDM6A mutations. Among the KMT2D mutations we identified 46 missense variants across the entire length of the KMT2D gene, 16 were inherited from an apparently asymptomatic parent. Aim of this study is to ascertain the pathogenicity of KMT2D missense mutations through an integrative analysis of bioinformatics tools and bio chemical and cellular assays. We used an innovative in silico approach that combines computational analysis and a deep domain search through homology modeling protocols to predict functional/structural effect of KMT2D missense variants and identify and confirm potential pathogenic mutations. Due to the huge size of KMT2D gene, we devised a strategy where minigenes carrying KMT2D missense variants were generated. We evaluated their potential pathogenicity effects by measuring KMT2D enzymatic activity and expression level of KMT2D known target genes. This work should offer a valuable support to estimate the real deleterious effect of KMT2D missense variants, an issue in diagnostic counseling.

C17.1

A dominant mutation in CHCHD10 causes neurodegenerative disorder with mitochondrial DNA instability

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Mutations in OPA1 and MFN2, two genes encoding membrane proteins involved in mitochondrial dynamics, are responsible for mitochondrial DNA (mtDNA) instability disorder with ‘Autosomal Dominant Optic Atrophy (ADOA) plus’ phenotype. We report a large family with a late-onset complex phenotype including motor neuron disease, cerebellar ataxia, cognitive decline and myopathy. Muscle biopsy showed ragged-red and COX negative fibres with combined respiratory chain deficiency and abnormal assembly of complex V. The multiple mitochondrial DNA (mtDNA) deletions found in skeletal muscle revealed a mtDNA instability disorder. By whole-exome sequencing (WES), we identified a missense mutation (c.176C>T, p Ser59Leu) in the CHCHD10 gene that encodes a coiled-coil helix coiled-coil helix protein, whose function was unknown. We show that CHCHD10 is a mitochondrial protein located in the intermembrane space and enriched at cristae junctions. Patient fibroblasts carrying the CHCHD10 mutation present with a respiratory chain deficiency and a fragmentation of the mitochondrial network. Furthermore, we show that overexpression of CHCHD10S59L triggers mitochondrial fragmentation in HeLa cells, thus confirming the deleterious effect of this mutant on mitochondrial morphology and network. DRP1-K38A, which is resistant to fission, did not modify the mitochondrial fragmentation observed in cells expressing CHCHD10S59L, suggesting that the CHCHD10 mutant leads to impaired fusion activity. This work, suggesting that CHCHD10 plays a role in mitochondrial fusion and/or in maintenance of cristae morphology, highlights the critical role of mitochondrial dynamics in terms of human disease and mitochondrial genome stability.

C17.2

Decoding Mitochondrial Disorders using Exome Sequencing

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Mitochondrial disorders are a heterogeneous group of diseases characterized by a wide spectrum of clinical manifestations, in terms of human disease and mitochondrial genome stability. The multiple mitochondrial DNA (mtDNA) deletions found in skeletal muscle revealed a mtDNA instability disorder. By whole-exome sequencing (WES), we identified a missense mutation (c.176C>T, pSer59Leu) in the CHCHD10 gene that encodes a coiled-coil helix coiled-coil helix protein, whose function was unknown. We show that CHCHD10 is a mitochondrial protein located in the intermembrane space and enriched at cristae junctions. Patient fibroblasts carrying the CHCHD10 mutation present with a respiratory chain deficiency and a fragmentation of the mitochondrial network. Furthermore, we show that overexpression of CHCHD10S59L triggers mitochondrial fragmentation in HeLa cells, thus confirming the deleterious effect of this mutant on mitochondrial morphology and network. DRP1-K38A, which is resistant to fission, did not modify the mitochondrial fragmentation observed in cells expressing CHCHD10S59L, suggesting that the CHCHD10 mutant leads to impaired fusion activity. This work, suggesting that CHCHD10 plays a role in mitochondrial fusion and/or in maintenance of cristae morphology, highlights the critical role of mitochondrial dynamics in terms of human disease and mitochondrial genome stability.
nes previously not associated with mitochondrial disorders. Mutations in the majority of genes are rare and could be identified due to loss-of-function alleles in evolutionary conserved genes such as *MGM1*, the first exonuclease involved in mitochondrial replication (Kornblum et al., Nat. Genet. 2013). Mitochondrial phenotypes are more frequent, with *ACAD9* being the most common finding with more than 15 cases, providing statistical evidence for the association with isolated respiratory chain complex deficiency. More difficult to identify are missense mutations in genes coding orphan proteins such as FBXL4, a protein with unknown function associated with reduced mitochondrial protein content. Additional diagnostic challenges are patients with recessive mutations in more than one gene resulting in a compound heterozygous phenotype

Evolving topics are RNA modifying enzymes (EL2C, MTO1 and GTPBP3) and RNA synthetases, both involved in the translation of mitochondrial proteins as well as cofactor metabolism defects. The latter offers rational therapeutic options as for example riboflavin supplementation in the case of mutations in the riboflavin transporter SLCS2A1.

In summary, the genetically heterogeneous group of mitochondrial disorders is an example par excellence for the application of genome wide sequencing.

**C17.3** Lentiviral vector based hematopoietic stem cell gene therapy mediates sustained expression of functional thymidine phosphorylase in mitochondrial neurogastrointestinal encephalopathy mouse model

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**MNGIE** is an autosomal recessive disease caused by deficiency of the enzyme thymidine phosphorylase (TP), resulting in systemic accumulation of nucleosides thymidine (Tdp) and deoxyuridine (dUrD), and mtDNA deletions, depletion and dysfunction. We aimed to use *ex vivo* lentiviral vector (LV) hematopoietic stem cell (HSC) gene therapy to deliver human TP enzyme in *Tdp-/- Upp-/-* double knockout mice, a model for MNGIE disease. To that end, LV transduced TP-/Upp-/ HSCs containing the native cDNA sequence (TP) or codon optimized (TPco) driven by the phosphoglycerate kinase (PGK) or the spleen focus forming virus (SVFV) promoter were transplanted in sublethally irradiated TP-/Upp-/ mice. Wild type mice had detectable but very low levels of enzyme activity in blood cell fractions (0.07 ± 0.03nmoles/h/mg, N=4), 1 month post transplantation, enzyme activities increased at least 90-fold in LV recipient mice (LV-TP and LV-TPco = 150±4 and 96±4 nmoles/h/mg respectively, N=4), with a 400-fold increase observed in recipients of LV-SFFV-TPco (450±5, N=4), resulting in reduction of Thd and dUrd in plasma respectively, N=4), with a 400-fold increase observed in recipients of LV-SFFV-TPco (450±5, N=4), 1 month post transplantation, enzyme activities increased at least 90-fold in LV recipient mice (LV-TP and LV-TPco = 150±4 and 96±4 nmoles/h/mg respectively, N=4), with a 400-fold increase observed in recipients of LV-SFFV-TPco (450±5, N=4), resulting in reduction of Thd and dUrd in plasma and urine. TP activity was detectable in brain of gene therapy-treated mice 14 months after treatment (average of 1.2-fold in LV-TP and LV-TPco compared to wild type and 36-fold for LV-SFFV-TPco), which significantly reduced the nucleoside levels. High molecular chimerism (76.5±8.2% donor chimerism to wild type and 36-fold for LV-SFFV-TPco), which significantly reduced -

Before next-generation sequencing, genetic testing was based on selection of a gene (or small number of genes) in which mutations had been identified in patients with recognisable, discrete phenotypes. For neonatal diabetes this included a transient subtype and syndromes such as Wolcott-Rallison. In this study we investigated the impact of testing all known genetic causes of neonatal diabetes.

We studied 1020 patients from 79 countries with diabetes diagnosed <6 months of age. Mutations were identified by targeted next-generation sequencing of 21 genes, Sanger sequencing or 6q24 methylation analysis. A pathogenic mutation was identified in 840 patients (82%). The most common cause was a potassium channel mutation (n=390) most of these patients achieved improved glycaemic control after transfer from insulin to sulfonylureas. The median age at referral decreased from 271 weeks in 2004 to 18 weeks in 2013. Patients with a genetic diagnosis of Wolcott-Rallison syndrome referred <12 months of age were therefore more likely to have isolated diabetes at referral (85% vs 33% 12 months) and develop additional features (e.g., liver failure) after testing. A genetic diagnosis also predicts diabetes remission: 88/94 patients (94%) referred <3 months received a genetic diagnosis of transient neonatal diabetes before remission.

Comprehensive genetic analysis identified causal mutations in >80% of cases. More patients are now referred at presentation with isolated diabetes and the genetic result predicts clinical course and development of features. This represents a new paradigm for clinical care with genetic diagnosis preceding the development of clinical features and guiding clinical management.
growth, bone density and survival. On these bases, we conducted in March 2014 a phase II lonafarnib trial in HGPS.

C18.3 Comprehensive NGS based diagnostics in over 1000 patients with epileptic disorders

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Purpose: Epileptic disorders have a highly heterogeneous background and a strong genetic contribution. Knowing the underlying molecular defect can be very valuable for diagnosis, guiding treatment and estimating recurrence risks. For this purpose we have developed a comprehensive diagnostic panel.

Method: 461 relevant genes were selected from the literature, and subdivided into subpanels according to their phenotypes. Following customized target enrichment, NGS was performed, followed by bioinformatic analysis. Variants with a global minor allele frequency <5% were selected for evaluation and identified mutations were validated using Sanger sequencing.

Results: In our study, over 1000 patients with epileptic disorders were analyzed. 20% of patients had pathogenic mutation(s) and 29% were inconclusive, partly being suspected pathogenic but unknown variants strongly due to non-segregation of identified variants. 51% of the cases remained unsolved. We observed rare variants in 203 different genes, with SCN1A, SCN2A, CACNA1A, MECP2 and KCNT1 being mutated most frequently. Across the cohort, 78 genes were identified as causative only once, emphasizing the advantage of diagnostic panels for very rare conditions.

Conclusion: We have developed a highly reliable and cost-efficient diagnostic NGS panel to analyze the genetic basis of epilepsies. We detected mutations in patients with clear and unspecific epilepsies, as well as in patients suffering from very rare conditions. This enables better understanding of genotype-phenotype correlations, and gives new insights into complex modes of inheritance such as combinatorial effects of variants.

C18.4 Planar cell polarity gene mutations contribute to the etiology of human Neural Tube Defects

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Neural tube defects (NTDs) are congenital malformations affecting 1 in 1,000 births. The most common forms of NTDs are anencephaly and myelomeningocele, which result from the failure of fusion in the cranial and spinal regions of the neural tube, respectively. Population and family studies indicate a complex etiology to NTDs involving environmental and genetic factors that remain undetermined. Animal models have strongly implicated the small GTPases of the RhoA family and JNK that upon activation lead to neuronal differentiation and growth, bone density and survival. On these bases, we conducted in March 2014 a phase II lonafarnib trial in HGPS.

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Neural tube defects (NTDs) are congenital malformations affecting 1 in 1,000 births. The most common forms of NTDs are anencephaly and myelomeningocele, which result from the failure of fusion in the cranial and spinal regions of the neural tube, respectively. Population and family studies indicate a complex etiology to NTDs involving environmental and genetic factors that remain undetermined. Animal models have strongly implicated the small GTPases of the RhoA family and JNK that upon activation lead to neuronal differentiation and growth, bone density and survival. On these bases, we conducted in March 2014 a phase II lonafarnib trial in HGPS.

C18.3 Comprehensive NGS based diagnostics in over 1000 patients with epileptic disorders

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Purpose: Epileptic disorders have a highly heterogeneous background and a strong genetic contribution. Knowing the underlying molecular defect can be very valuable for diagnosis, guiding treatment and estimating recurrence risks. For this purpose we have developed a comprehensive diagnostic panel.

Method: 461 relevant genes were selected from the literature, and subdivided into subpanels according to their phenotypes. Following customized target enrichment, NGS was performed, followed by bioinformatic analysis. Variants with a global minor allele frequency <5% were selected for evaluation and identified mutations were validated using Sanger sequencing.

Results: In our study, over 1000 patients with epileptic disorders were analyzed. 20% of patients had pathogenic mutation(s) and 29% were inconclusive, partly being suspected pathogenic but unknown variants strongly due to non-segregation of identified variants. 51% of the cases remained unsolved. We observed rare variants in 203 different genes, with SCN1A, SCN2A, CACNA1A, MECP2 and KCNT1 being mutated most frequently. Across the cohort, 78 genes were identified as causative only once, emphasizing the advantage of diagnostic panels for very rare conditions.

Conclusion: We have developed a highly reliable and cost-efficient diagnostic NGS panel to analyze the genetic basis of epilepsies. We detected mutations in patients with clear and unspecific epilepsies, as well as in patients suffering from very rare conditions. This enables better understanding of genotype-phenotype correlations, and gives new insights into complex modes of inheritance such as combinatorial effects of variants.
Clinical exome sequencing for cerebellar ataxia and spastic paraplegia reveals novel gene-disease associations and uncovers unanticipated rare disorders


Clinical exome sequencing is an unbiased test that is used for straight-forward causative mutation detection, or for the discovery of novel (allelic) gene - disease associations. Often, it leads to broader disease spectra, or reverse phenotype. We here describe the use of clinical exome sequencing for a group of 76 probands with ataxia or spastic paraplegia. Since these patients have had extensive testing (4 genes on average) before inclusion, mutations were not anticipated in the „common“ genes. In a two-tier analysis, variants in known disease genes were analysed first, followed by analysis of the „full“ exome data set if no causative mutations were identified. Subsequently, segregation analyses, enzyme tests or reverse phenotype testing have confirmed or excluded the pathogenicity of most variants. In those 76 patients, we have thus detected causative mutations in 16, and likely-causative in another 9, all in the known disease genes. Some of the identified diseases are very rare or even the second case described. Furthermore, another 8 putatively-causative mutations were detected in genes outside the known disease genes, and await confirmation (functional tests or other family members with mutations in those genes). Some of these latter discoveries concern dominant or allelic disorders, whereas others may be novel. In conclusion, exome sequencing for these neurological disorders has resulted in a molecular diagnosis in one-third of the patients, and has provided a candidate gene identification in approximately 1/10. More importantly, it has also provided some patients with a molecular diagnosis that was both unanticipated and otherwise not revealed.

C18.6

WES detects disease causing SNVs and CNVs in Primary immunodeficiencies


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Endogenous hypercortisolism, referred to as Cushing’s syndrome, is associated with excess morbidity and mortality. Corticotropin-independent Cushing’s syndrome is caused by tumors or hyperplasia of the adrenal cortex.

We performed exome sequencing of ten cortisol-producing adenomas and matched control tissue to identify somatic mutations and evaluated recurrent mutations in candidate genes in adenomas of additional 171 patients. We further performed genome-wide copy number analysis in 35 patients with cortisol-secreting bilateral hyperplasias. We studied the effects of these genetic defects both clinically and in vitro.

Exome sequencing revealed somatic mutations in the PRKACA gene, which encodes the catalytic subunit of cAMP-dependent protein kinase (PKA), in 8 of 10 adenomas. Overall, PRKACA somatic mutations were identified in a total of 22 of 59 adenomas (37%) from patients with overt Cushing’s syndrome; these mutations were not detectable in patients with subclinical hypercortisolism (n=40) or in other adrenal tumors (n=82). Among 35 patients with bilateral cortisol-producing hyperplasia, 5 carried germline copy number gain of the chromosome 19 region, including the PRKACA gene. In vitro studies demonstrated impaired inhibition of both PKA catalytic subunit mutants by the PKA regulatory subunit, while cells from patients with germline chromosomal gains showed increased protein levels of the PKA catalytic subunit; in both instances, baseline PKA activity was increased. This study links genetic alterations of the catalytic subunit of PKA to human disease. Germline duplications or deletions of this gene result in bilateral adrenal hyperplasia, whereas somatic PRKACA gain-of-function mutations lead to unilateral cortisol-producing adenoma.

C19.2

A mutation in SEC61A1 causes autosomal dominant interstitial kidney disorder associated with anemia and growth retardation

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Autosomal dominant tubulo-interstitial kidney disease refers to a group of disorders characterized by progressive loss of kidney function and the eventual need for dialysis and kidney transplantation. While mutations in the UMOD, MUC1, and REN genes have been identified as the primary cause of this disorder, there remain a number of families in which the genetic cause has not been identified. Here we report a three-generation family with autosomal dominant tubulo-interstitial kidney disease associated with congenital anemia and intratertiary growth retardation. Ultrasound examinations revealed small dysplastic kidneys without cysts, and a kidney biopsy revealed tubular atrophy with secondary glomerular sclerosis. We performed whole-genome-wide linkage analysis and identified a candidate region with a maximum LOD-score of 2.7 on chromosome 3q. Combining whole exome sequencing data with our critical interval, we identified a C553A-G variant causing a p.Thr185Ala change in SEC61A1. SEC61A1 encodes the alpha-subunit of the SEC61 complex, responsible for the translocation of proteins across the endoplasmic reticulum membrane. Suppression of se-
Progressive familial intrahepatic cholestasis (PFIC) is an autosomal recessive disorder manifesting as early-onset cholestatic liver disease. In 1998 mutations in three genes encoding membrane transporters were identified. However, 30% of patients remain without genetic diagnosis. Combined targeted resequencing (TRS) and whole-exome sequencing were used to analyse a cohort of 33 children, from 29 families, with chronic cholestatic liver disease and no mutations in known PFIC genes. Most were from consanguineous families. Homozygous mutations in TJP2 were identified in 12 individuals of 8 families; all were predicted to abolish translation. TJP2 encodes a cytosolic component of cell-cell junctional structures, linking integral membrane proteins such as claudins with actin filaments. Immunohistochemistry and western blotting of liver tissue from these patients showed a complete absence of TJP2 protein. Claudin-1 was not localised, suggesting that mutation of TJP2 results in a loss of function. Taken together, our genetic findings and the functional studies support SEC61A1 as a causal gene for a novel, dominant syndromic form of progressive chronic kidney disease with tubular atrophy.
mutation was inherited from one of the parents. In most cases heterozygo-
ties had less severe disease than the double heterozygotes. In two cases muta-
tions involved the COLA5 and COLA6 genes, whereas in the remaining 4,
mutations involved the COLA3 and COLA4 genes. Mutation pathogenicity was
uncertain on the basis of the following criteria: non polymorphic mis-
sense mutations in key aminoads, as Glycine in the collagen GLY-X-Y triple
helical domain, or truncating mutations. In conclusion, results from new
technologies showed that some cases of ATS may segregate according to a
digenic inheritance model. This model has previously been shown in other
diseases, such as retinopathies, cardiomyopathies or intellectual disability.
Our results are of interest both from a scientific point of view and for genetic
counselling. Clinical geneticists should be familiar with more complex mod-
els of inheritance, which could alter the recurrence risk.

C20.1 Single cell allele-specific expression (ASE) in Down syndrome and common aneuploidies
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Trisomy 21 is a model disorder of altered gene expression. Several studies have
addressed the transcriptome differences between normal and affected individuals,
however all of these studies suffer from the presence of noise due to gene expression variation among different individuals. We have previ-
ously used a pair of monzygotic twins discordant for trisomy 21 in order to study
the global dysregulation of gene expression (Nature in press 2014). The majority of previous studies focused on aneuploidies were conducted on
cultured cell populations or tissues, but studies focusing on gene and alle-
lic expression behavior at the single cell level are lacking. In this study we
explore the allele specific expression in Trisomy 21 using transcriptome
studies in single cells. We have used 40 normal cells and 48 trisomic cells from
the fibroblasts of the monzygotic twins discordant for trisomy 21 and compared
the ASE (allele specific expression), and their transcriptional metrics in these two cell groups. We observed a pattern of biased or monoalle-
lie expression for the majority of the genes across single cells. These results
will be presented and discussed. In addition a series of samples from mosaic
trisomy 21, trisomy 13 and trisomy 18 are in different stages of investigation.
These studies in single cells will provide a fundamental understanding of the
gene expression dysregulation and allele specific expression in aneu-
ploidies.

C20.2 Distinct properties of de novo mutations from whole genome sequencing of 50 parent-patient trios
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De novo germline mutations create the genetic variability that is the driving
force of species evolution. However, they also can cause sporadic genetic
diseases if they affect critical genomic regions. Whole Genome Sequencing
(WGS) of parent-offspring trios allows us for the first time to study the re-
sult of mutational processes in a single generation in full detail. Here we
report on the rate and pattern of de novo mutations based on WGS of 50
trios at high (90x median) coverage. We identified an average of 58 de novo mutations (DNM) per trio (range 32-84), 2,883 DNMs in total. By using the segregation of informative SNPs we determined the parental origin for 678 DNMs, and found a paternal/mat-
ernal ratio of 4 to 1 (79%/21%), consistent with previous data. Using the
parental origin of these DNMs we found a significant correlation between the
paternal age and the number of paternal DNMs (Spearman’s p=0.006) as well as a suggestive results for the correlation between paternal age and the
number of maternal DNMs (p=0.08).

We find that DNMs do not occur completely random in the genome, but
are spatially clustered within individuals (observed=28 (0.9%), expected=13
(0.004%), p-value=10-16) and have a sequence context that is enriched for
CpGs (observed=482 (20.6%), expected=50 (2.6%), p-value=10-16). In conclu-
sion, our study provides insight into the parental origin and distribution of
de novo mutations throughout the human genome.

C20.3 Study of the regulatory landscape of SHOX in 503 LWD and ISS cases uncover a key role of the upstream cis-regulatory element CNE-3
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Fragile X syndrome (FXS), the leading cause of inherited intellectual disa-
tability, is caused by the absence of the FMR1 protein due to expansion over 200
repeats of the CGG tract at the 5’ UTR of the FMR1 gene and subsequent

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DNA methylation. We have already characterized rare individuals of normal intelligence with CGG expansion over 200 repeats without DNA methylation (unmethylated full mutation, UFM), which have relatively normal transcription and translation, and represent the status of FXS cell lines prior to gene silencing. We have compared the three types of cell lines (normal control WT, FXS and UFM fibroblasts) with a proteomic approach in order to demonstrate possible differences that might clarify the mechanisms through which the rare UFM cells remain unmethylated and transcriptionally active. Protein extracts were compared by LC-ESI ITQ Orbitrap MS/MS analysis after mono-dimensional SDS-PAGE and tryptic digestion. Interrogation of the dataset for differential protein expression shows that some metabolic pathways are deregulated in UFM cells when compared to FXS cells. Among these pathways, mitochondrial metabolism (oxidative stress) is of particular interest considering its role in neurodegenerative diseases (like FXS) and particularly in epigenetic regulation. The deregulated proteins, specifically mitochondrial SOD (SOD2), were validated by Western blot. The interaction of target mRNAs with FMRP was assessed by RNA immunoprecipitation. Preliminary data suggest that mitochondrial metabolism is likely to have a role in DNA hypomethylation of UFM cell lines. Supported by Telethon Onlus, FRAZA Foundation and Italian Association for fragile X syndrome.

C2.0.6 RNA-DNA Differences in Endoplasmic Reticulum Stress Response
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The endoplasmic reticulum (ER) is an organelle where proteins are synthesized and modified. ER stress occurs when there is an excess of misfolded proteins, and if the stress persists, then apoptosis is induced. Here we study RNA editing as a part of the ER stress response and how RNA editing modulates the cellular response to promote cell survival. We sequenced the RNA and DNA of B-cells from 10 individuals before and following 2 and 8 hours of tunicamycin treatment to induce ER stress. By comparing the RNA and DNA sequences, we identified 15,823 A-to-G editing sites in 1,523 genes as targets of RNA editing by ADAR (Adenosine Deaminase Acting on RNA). Among these sites, 314 showed significant changes in editing level following ER stress (p-value<0.01; ANOVA). These sites are found in genes known to be involved in ER stress response: RNA processing protein transport and apoptosis. For example, sites found in WXAPI (X-linked Inhibitor of Apoptosis) show a 3-fold increase in editing level. Furthermore, ADAR knock-down increases apoptosis following ER stress, suggesting that RNA editing is induced to promote cell survival. The effect of ER stress on editing level is not limited to canonical ADAR editing, as we also detected other types of differences between the RNA and DNA sequences whose levels change following tunicamycin treatment. In this presentation, we will describe RNA sequence modification as a critical step in ER stress response and cell survival.

C2.1.3 Mutations in POGU1, encoding protein O-glucosyltransferase 1, cause autosomal dominant DDD (Dowling-Degos disease)
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Recently, mutations in POFUT1, encoding protein O-fucosyltransferase 1, have been linked to autosomal dominant DDD, an inherited skin disease characterized by hyperpigmented macules and slow growing hair. A recent report in Science described mutations in other RASopathy genes. Multiple defects were documented in patients with DDD. Mitochondrial dysfunction and cardiac defects are common, but differently distributed compared to patients with mutations in other RASopathy genes. Multiple defects were documented in 51% of cases. Overall, Mazzanti syndrome represents a recognizable condition within the spectrum of RASopathies, with a single recurrent mutation, p.S2G, accounting for the vast majority of cases.

Heterozigous germline mutations in A2ML1 are associated with a disorder clinically related to Noonan syndrome
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Noonan-like syndrome with loose anagen hair (Mazzanti syndrome) (MIM 607721) is caused by an invariant mutation in the SHOC2 gene (c.4A>G, p.S2G). The disorder is characterized by facial features resembling Noonan syndrome, short stature, GH deficiency, developmental delay, congenital cardiac defects, and a distinctive hair phenotype characterized by easily pluckable, slow growing hair in anagen phase. We report on a large cohort of patients with molecularly confirmed diagnosis from an international research collaboration. In all patients, the c.4A>G change was invariably identified. Clinical data from a total of 89 patients (25 previously published) was available. The ages ranged from 0.08 - 39 years (median at 9.0 years). Short stature and an intellectual disability were present in most of the patients, and were more common than in patients with PTPN11, SOS1 or RAF1 mutations. Proven growth hormone deficiency was diagnosed in 42% of patients with known test results. SHOC2 mutations positive individuals also have a recognizable facial phenotype. Heart defects were common, but differently distributed compared to patients with mutations in other RASopathy genes. Multiple defects were documented in 51% of cases. Overall, Mazzanti syndrome represents a recognizable condition within the spectrum of RASopathies, with a single recurrent mutation, p.S2G, accounting for the vast majority of cases.
a de novo mutation, p.(Arg802His), in A2ML1 which encodes the secreted protease inhibitor Alpha-2-Macroglobulin-Like-1. Subsequent resequencing of A2ML1 in 155 cases with a clinical diagnosis of NS led to the identification of additional mutations in two families, p.(Arg802Leu) and p.(Arg802Leu). Functional studies showed that these mutations in A2ML1 in zebrafish, showed NS-like developmental defects, including a broad head, blunted face and cardiac malformations. Using the crystal structure of A2M, which is highly homologous to A2ML1, we identified the intramolecular interaction partner of Arg802. Mutation of this residue, Glu906, induced similar developmental defects in zebrafish, strengthening our conclusion that mutations in A2ML1 cause a disorder clinically related to NS. This is the first report of the importance of conserved factors in NS and RASopathies in general, providing new leads for better understanding of the molecular basis of this family of developmental diseases.

**C21.4**

A mutation in PK3 with a dual molecular effect deregulates the RAS/ MAPK pathway and drives an X-linked syndrome phenotype


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RASopathies, a family of disorders characterised by cardiac defects, deficiency in immunity, high body mass index, growth retardation and mental retardation, are caused by constitutional dysregulation of the RAS signalling predominantly through the RAS-RAF-MEK-ERK cascade. We report on two germline mutations in RAS, a small monomorphic GTPase controlling cell adhesion, spreading and migration, underlying a variable phenotype with features partially overlapping Noonan syndrome, the most common RASopathy. We also document that somatic RAS mutations can occur in juvenile myelomonocytic leukaemia, a childhood myeloproliferative/myelodysplastic disease caused by upregulated RAS signalling, defining an atypical form of this haematological disorder rapidly progressing to acute myeloid leukaemia. Two of the three identified mutations affected known oncogenic hotspots of RAS genes, and conferred variably enhanced RAS function and stimulus-dependent MAPK activation. Expression of a RAS mutation homologous to A2ML1 in an enhanced RAS signalling, and gendered protruding vulva, a phenotype previously linked to a RASopathy-causing SHOC2 mutant. These findings establish a functional link between RAS and RAS signalling, and reveal an unrepredicted role of enhanced RAS function in human disease.

**C21.6**

A New Mouse Model for Costello Syndrome

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Costello Syndrome (CS) is a distinctive rare multisystem disorder comprising characteristic prematurely increased growth retardation, coarse facial features, redundant skin with deep palmar, plantar creases and papiloma of later onset. CS patients present also laxity of small joints, tight Achilles tendons, cardiac malformations, and developmental delay. The primary cause of CS was associated to the germline activation of H-Ras oncogene, with a common missense mutation G12S in 80% of the patients. Here we describe the generation and the characterization of a genetically engineered mouse model of CS, by introducing the oncogenic G12S mutation by homologous recombination into the mouse Ras gene. The effect of the H-Ras G12S mutation was evaluated on behavioral, visual, metabolic, cardiac and histological traits in young adult animals. The behavioral analysis revealed that H-Ras G12S mutant males displayed reduced locomotor activity, accompanied by altered muscle strength and impaired conditioned avoidance performance. In addition, the cardiac exploration revealed that H-Ras G12S mutants exhibit a hypertrophic phenotype combined with tachycardia. In conclusion, the H-Ras G12S mutant mice showed a polysyndromic phenotype reproducing some of the CS features observed in patients. The future study of the here-described CS mouse model should have a significant impact on our understanding of CS disease. The use of H-Ras G12S mutant mice as a CS mouse model opens up new fields of investigation to better understand the pathophysiology of the disease and to evaluate drugs dedicated to the reduction of the disease associated symptoms.
probability for predicting rates of diagnostic findings. We primed and validated the model using published variant frequencies and population carrier frequencies. Monte Carlo simulation was used to predict population carrier frequencies as a function of numbers of potentially deleterious genetic variants in sampled individuals.

Results: The model correctly calculates observed rates of IFs and genetic carriers. Changing the model's parameters shows that even minor changes in diagnostic criteria or sequencing accuracy causes large variation in rates of diagnostic findings.

Conclusion: Our model correctly explains observed rates of diagnostic findings. Key drivers of rates include diagnostic criteria, variant frequency, disease penetrance, and sequencing and bioinformatics accuracy. Rates of IFs are relatively insensitive to even large increases in the number of conditions included, but rates of genetic carriers are very sensitive to the number of conditions tested. These findings have great relevance to recommended practice and policy.

C22.2 Defending the child’s right to an open future concerning genetic information
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There has been a discussion regarding the ethical acceptability of genetic testing of children for years, resulting in the majority view that minors should only be tested for early onset disorders where treatment or preventive options exist. Two principles underlie this consensus: first, the beneficence-based best interest standard that urges physicians to test for clinically relevant and actionable results. Second, the child’s right to an open future principle that urges physicians not to test for adult onset disorders and carrier status, in order to preserve the child’s future autonomy right to make its own decisions, also concerning the possible obtainment of genetic information.

The emergence of next generation sequencing (NGS) technology seems to challenge the previous consensus. There now is a growing list of commentators and examples from practice showing disagreement on whether conditions that do not have immediate consequences for the health of the child should be disclosed to parents. The American College of Medical Genetics and Genomics for example recently proposed to relinquish the current distinction between pediatric and adult genetic testing policy, thereby abandoning the child’s right to an open future, while the American Academy of Pediatrics maintains the previous consensus.

In this paper we explain the normative rationale that underlies the current debate on a child-versus family centred genetic testing policy and argue that the right to an open future should remain a leading ethical principle in pediatric genomics.

C22.3 Implementation of a duty-to-recontact system in molecular and clinical genetics: perspectives from professionals and patients
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Advances in DNA-diagnostic techniques and NGS are leading to more diagnoses, but also to interpretation problems. Many findings cannot be interpreted yet, but may prove medically relevant in the future. Currently, there is no moral or legal obligation to recontact former patients. Many geneticists nevertheless consider it desirable to recontact patients when important new information arises. The UMCG (Groningen, NL) is investigating whether to develop a recontacting system in clinical genetics and how one could be implemented. We explored how professionals and patients from our university hospital feel on these two issues. We organised a focus group discussion with 12 professionals (clinicians, clinical geneticists and laboratory staff) and two group discussions with 3 and 5 patients, respectively, in which we discussed the desirability and requirements for successfully implementing recontacting. Both professionals and patients agreed that recontacting is desirable and implementation could be successful if the following requirements are met: (1) provide a guideline describing the responsibilities of molecular geneticists, genetic counsellors, and patients. This would also specify what information requires recontacting, which information should be given priority, and how recontacting should take place; (2) availability of a lab-based databank containing up-to-date genetic information and the possibility to automatically match new genetic information, e.g. on former variants of unspecified significance, with patients/groups for which the information is important; (3) patient’s preferences regarding recontacting (e.g. what type of information and which recontacting medium to use); (4) development of e-health devices facilitating automatic recontacting.

C22.4 International views on sharing incidental findings from whole genome research
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Whilst genome-wide sequencing in a research setting may be used to explore the genetic basis of a phenotype it also offers the chance to opportunistically screen for additional results unrelated to the research project but relevant to the participants’ future medical health (termed ‘incidental findings’, IFs). There is a wealth of medical and ethics literature supporting the feedback of IFs, yet there are limited empirical work offering a voice from both professional and public stakeholders directly affected by this. A cross-sectional, web-based survey investigated the attitudes of 6944 individuals from across 91 countries towards searching for and sharing IFs. Participants included 4961 members of the public, 533 genetic health professionals, 943 non-genetic health professionals and 607 genomic researchers.

Eighty percent of participants believed that IFs from sequencing studies should be made available to research participants if they want them. Treatability and perceived usefulness of the data were important with 98% personally interested in learning about life-threatening conditions that were preventable. However, only 31% of participants thought genomic researchers should actively search for IFs that were not relevant to their research study. Genetic health professionals were the most likely to take this view (OR = 3.09, CI 2.23-4.28, P < 0.0001). This may be due to their appreciation of the complexities involved in translating genomic data in the clinic. Participants felt that genomic researchers should be able to focus on their research question without being forced to actively search for IFs, potentially at the expense of their study.

C22.5 Newborn screenings and whole genome sequencing: the real need of a genuine public involvement
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In the last decade newborn-screening programs and whole genome sequencing are making their way in clinical practice. The question we focus on is whether the intrinsic persistence in time of genetic characters (monitored in newborn-screening programs), once combined with the rules governing bio-banks which store those information and the natural lifelong evolution of personal individuality (from infancy to old age), creates an apparently inextricable set of problems.

In this paper we have the ambition simply to draw a list of them in order to open up a space for a public interdisciplinarity discussion about new arising challenges that need to be framed and faced by public policies. Some examples are:

- The complex and not yet unraveled relationship between the gradual evolution of the interested person and epigenetic developments.
- Regulations of parents’ opting in/out screening programs and the chances of modifying their choices during the child’s life.
- The involvement of relatives and future siblings.
- The (im)possibility of a real opt out during the phase of recontact.
- Difficulties arising from the organizational rules of biobanking in relation to the described long lasting process of consent.
- Privacy issues deriving both from the stable relationship between person and collected data (impossible anonymusness) and the possibility of control over informational flows.
- We maintain it is necessary to foster a real engagement of community in genuine discussion on a) benefits of a universal system of genetic monitoring and b) ethical and legal implications for personal rights.

C22.6 Recent Developments in the Regulation of Direct-to-Consumer Genetic Testing in Europe
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In recent years direct-to-consumer (DTC) genetic testing has provoked a lot of debates and lead to various statements and opinions of professional societies, medical associations and governmental bodies. In October 2013 the European Parliament voted a Regulation on In Vitro Diagnostic Medical Devices. This document is waiting for approval by the Council of the European Union which might still provide amendments to the text. When approved, this new Regulation is expected to introduce important regulatory changes in the field of medical devices, and especially in the field of genetic testing. In this presentation, we aim to present and evaluate the major impact of
this Regulation on the field of DTC genetic testing. First, adopting the current proposal will mean that genetic tests, including both health-related and lifestyle tests, will be subjected to a pre-market assessment by independent notified bodies. Second, according to the risk class they fall into, IVD devices will have to comply with revised requirements for clinical evidence. Third, the proposal requires appropriate genetic counseling for all genetic tests and classifies them as "prescription only". Finally, DTC advertising of devices classed as prescription only will be banned. Full implementation of these provisions would greatly impact DTC genetic testing companies as the present offer and advertising would no longer be tolerated. This work aims to clarify the proposed amendments and contribute to the ongoing discussion regarding the desirable degree of genetic testing regulation and the appropriate balance between promoting innovation and securing consumers' safety by using legal tools.
Two unusual unbalanced X chromosome rearrangements: a case report

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The human X chromosome is characterized by genomic instability and rearrangements, associated with various X-linked disorders such as moderate intellectual disability, muscular dystrophies or reduced fertility. In the laboratory of Medical Genetics that operates as a department of IVF clinic, we are preferentially focusing on fertility connected scope. Here we show two women presenting amenorrhea associated with de-novo X chromosome aberration. First, we report on the case of a 23-year-old woman presenting phenotype close to isochromosome i(X)q with short size and primary oligo-amenorrhea. Classical cytogenetic observation revealed a de novo duplication in the Xq22.2-qter region. Further analysis by FISH, CGH and mBAND methods, de novo duplication in the Xq22.2-qter region was also discovered. As the second case, we describe 30-year-old woman who was referred to our IVF clinic because of infertility and amenorrhea. At the age of 18 years, she was placed on oral contraceptive pills for cycle activation. Now she has been experiencing amenorrhea again and she has been unsuccessful to become pregnant. The cytogenetic analysis revealed de novo deletion in the Xq22.1-qter region. Further analysis by FISH, CGH and mBAND methods led to the revelation of Xp22.1-qter duplication. We have had contented ourselves only with the classical cytogenetic analysis, both cases could be underestimated as simple X chromosome deletions. However, our detailed observations led us to suspect that a complex aberration may be present. These two cases show that conscientious cytogenetic analysis at 550 ISCN bands level in combination with other molecular methods is essential for accurate diagnosis.
P01.007-S
Preimplantation genetic screening of copy number variations (CNVs) using oligonucleotide-based array comparative genomic hybridization. P. Kuglik1, D. Paravalo1, J. Smetana1, V. Vallow1, A. Mikulevska1, R. Gallovi1, V. Habinuk1, L. Stkhova1, S. Machac1, M. Koudela1. 1Department of Genetics and Molecular Biology, Institute of Experimental Biology, Faculty of Science, Brno, Czech Republic, 2Department of Medical Genetics, University Hospital, Brno, Czech Republic, 3Reprofit International Ltd, Brno, Czech Republic.

Chromosome aneuploidy is the most prevalent genetic abnormality in human embryos and represents the leading genetic cause of miscarriages. In view of this fact, diagnosis of embryos for chromosome abnormalities using array-CGH, i.e. preimplantation genetic screening (PGS), is suitable way to improve outcomes in patients undergoing in vitro fertilization (IVF). In our work we analyzed the whole genome profiles from trophectoderm cells in 23 patients and 9 healthy donors (DEM). Overall, we evaluated 118 embryos using oligonucleotide-based CytoSure Single Cell Aneuploidy Array 8x15K. The copy number abnormalities (CNAs) were found in 23.7% of all embryos. While incidence of CNAs in DEM embryos was 11.8% of samples, CNAs in patient’s embryos occurred in 28.6% of samples. Aneuploidies of chromosomes were observed in 26.7%, segmental imbalances were proved in 6.8% of embryos. In patient’s cohort, we found overall 35 different CNAs. The most common aneuploidies were trisomy 21 and 13 (both 8.3%), whereas the most frequent losses of genetic material were monosomy 8 (12.5%) and 18 (8.3%). Two patients had embryos with complex aneuploidy. Segmental CNAs were found in 5q, 8p, 8q, 14q and 16p. In DEM embryos, we observed two structural CNAs (gain 13g: loss 7p) and 2 cases with aneuploidies (trisomy 19, monosomy 18). This study shows that oligonucleotide array is novel progressive tool for sensitive genome-wide analysis of chromosome instability and opens the route towards high-resolution preimplantation screening which improves selection of embryos and implantation rates. Supported by OP VK CZ.1.07/2.40.00/31.0155 and CZ.1.07/2.3.00/20.0183.

P01.008-M
Copy Number Variation (CNV) in azoospermic males. K. L. Eiklid1, I. Holm2, K. Woagner Birkeland1; 1Department of medical genetics, Oslo University Hospital, Oslo, Norway, 2Department of medical genetics, Oslo University Hospital, Oslo, Norway, 3Oslo and Akershus University College of Applied Sciences, Oslo, Norway.

We tested 95 males from the biobank at Department of Medical Genetics, Oslo University Hospital for Copy Number Variations (CNV). All males were azoospermic and referred for infertility testing, but no further information was known. They had normal karyotype, and no deletion in AZF region on chromosome Y nor mutations in the CFTG gene. As controls we used 94 males from our biobank, who had fathered a child and did not have the aberration found in their child on aCGH. The samples were run on Agilent technology array comparative genome hybridization with 180K and 400K resolution according to the manufacturers procedure. 23 patients were run on 180K aCGH against a single sample control, 35 against a multisample control, and 37 patients on a 400K array with multisample control. We found totally 1863 CNVs in the patient group (1507 patients) and 973 (103 patients) in the control group. For patients and controls we run with same resolution (180K) we found 9.2 and 10.3 CNVs per (19.6/patient) and 973 (10.3/patient) in the control group. For patients and with multisample control. We found totally 1863 CNVs in the patient group, 35 against a multisample control, and 37 patients on a 400K array for aCGH. 23 patients were run on 180K aCGH against a single sample control, 35 against a multisample control, and 37 patients on a 400K array with multisample control. We found totally 1863 CNVs in the patient group (1507 patients) and 973 (103 patients) in the control group. For patients and controls we run with same resolution (180K) we found 9.2 and 10.3 CNVs per (19.6/patient) and 973 (10.3/patient) in the control group.

CONCLUSION: Our data is the largest study of its kind on azoospermic males. This study suggests that there are CNVs affecting fertility in azoospermic males in a significant number. Further studies with larger patient populations are needed to confirm these findings.

P01.011-S

Conradi-Hunermann-Happle (CDPX2) syndrome is a rare X-linked dominant skeletal dysplasia. It is usually lethal in males, while affected females show wide clinical heterogeneity. Mutations have been reported in EBX which is involved in cholesterol biosynthesis. To date, severe prenatal occurrence has been reported in only 6 females. In order to characterize this severe phenotype, we analyzed the 8 prenatal female cases of severe EBX mutations listed in France. The mean age at prenatal diagnosis was 22 weeks of gestation. The ultrasound features included mainly bone abnormalities: shortening (7/8) and bowing of the long bones (4/8), stippled epiphysial cartilage (5/8) and irregular aspect of the nuchis (5/8). The pregnancy was terminated in 6/8 cases. Fe- tal examination revealed ichthyosis in all cases and skeletal X-rays showed constant epiphysial stippling, with frequent asymmetrically shortened long bones and bowing. All cases appeared de novo except for two fetuses with moderately affected mothers, who presented ichthyosis and short stature, and one case of germinal mosaicism. In order to explain the high intrafamilial clinical heterogeneity, the X-inactivation pattern was studied in one familial case. Skewed X-inactivation was found in the mother’s lymphocytes and peripheral blood, as well as in the informative male fetus. The study showed that the wild allele was mainly expressed in the female fetus. Skewed X-inactivation was found in the different fetal tissues affected. X-inactivation was found in the different fetal tissues affected. X-inactivation was found in the different fetal tissues affected.
PO01.012-M
Fetal Fraction estimate in twin pregnancies using directed cell-free DNA analysis
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Objective: To estimate fetal fraction (FF) in monozygotic and dizygotic twin pregnancies. Methods: Maternal plasma samples were obtained from 35 monochorionic twin pregnancies with male fetuses (monozygotic) and 35 dichorionic pregnancies discordant for fetal sex (dizygotic) at 11-13 weeks’ gestation. Cell-free DNA was extracted and chromosomese-selective sequencing with digital analysis of selected regions (DANSR TM ) was carried out. The fetal-fractio

PO01.013-S
Prenatal Diagnosis of Aneuploidy by Cell Free Fetal DNA in Maternal Plasma
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This study examined the methylation difference in AIRE and RASSF1A between maternal and fetal DNA, and the implication of this difference in the identification of fetal cells in maternal plasma and in prenatal diagnosis of trisomy 21. Maternal plasma and amniotic fluid samples were collected from 30 singleton pregnancies. Methylation-sensitive restriction enzymes in digestin of differential maternal-fetal DNA methylation followed by fluorescent quantitative PCR (MSRE + PCR) were employed to detect trisomy 21. Diagnosis of trisomy 21 was established according to the ratio of fetal-specific AIRE to RASSF1A that are hypermethylated in maternal plasma and are not digested with methylation sensitive restriction enzymes. All of the results were approved with karyotype results. Based on the data from 22 euploidy pregnancies, the 95% reference interval of the fetal AIRE/RASSF1A ratio in maternal plasma was 0.33-1.77, which was taken as the reference value for determining the numbers of fetal chromosome 21 in 30 pregnancies. Firstly, 18 from 22 euploidy pregnancies were detected euploid correctly and 4 cases incorrectly so the early sensitivity rate was 81.81% (18/22). But by later, 18 from 22 euploidy pregnancies were detected euploid correctly and 4 cases incorrectly so the early sensitivity rate was 81.81% (18/22). By repeating the test with better digestion, the four cases made correct results so the final sensitivity rate was 100% (22/22). All of the eight trisomy 21 pregnancies were diagnosed with this method correctly so the specificity of this method was 100%. Also with performing STR-Typing and checking fetus on chromosomes X, 13, 18, 21 and by STR markers kit (AmpFlSTR Identifiler PCR Amplification Kit). The result of the analysis proved tetragametic origin of the trisomy. Our program underwent three cycles of IVF with own oocytes and ICSI. The third cycle was successful ET of 2 embryos was performed and normal preg

PO01.014-M
Non-invasive Examination of Trisomy (NEXT) Study: Directed-cell-free DNA analysis versus 1st trimester combined screening for Trisomy 21 risk assessment in a large Routine pregnancy population
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Non-invasive prenatal testing (NIPT) with cell-free DNA (cfDNA) is highly accurate for fetal trisomy evaluation in high-risk pregnancies. Routine pregnancy population NIPT performance has not been evaluated in a large prospective study. Our objective was to compare NIPT with directed cfDNA analysis to first trimester combined screening (FTS) for trisomy 21 risk assessment in a general pregnancy population. This prospective multi-center blinded cohort study compared HarmonyTM or Prenatal Test, a directed cfDNA test, with FTS using first trimester PAPP-A, hCG and fetal nuchal translucency measurement. Women with a singleton fetus presenting in the first trimester for routine prenatal screening for fetal aneuploidy were eligible. Participants had both FTS and Harmony. FTS results were provided as part of routine care. Participants and care providers were blinded to Harmony results, calculated as probability scores. Pregnancies were followed for newborn outcomes. Invasive test results or neonatal phenotype were karyotype confirmation in cases of suspected aneuploidy, whereas FTS results for trisomy 21 identification were reported to an independent data coordinating center. Primary outcome was comparison of the area under the ROC curve for trisomy 21 test performance of the Harmony and FTS. 18,955 women were enrolled across 36 centers in USA, Canada and Europe from March 2012 to April 2013. The mean maternal age was 30.6 (18-52) years. The mean gestational age was 12.4 (10-14) weeks. Follow-up is complete. Study results will be presented. Implications for use of NIPT for trisomy 21 risk assessment in the general pregnancy population will be discussed.

PO01.015-S
A case report of a high level 46,XX/46,XY true chimerism without any clinical effect in a healthy female who gave birth to healthy twins after IVF
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Chimerism is a rare event in humans when two or more genetically distinct cell lines occur in an individual. It is mostly connected with ovo-testicular disorder of sexual development. We now present a case of high level 46,XX/46,XY chimerism in a healthy woman who was karyotyped prior to IVF. The karyotype of the proband was that of a normal woman with an unambiguously female external and internal genitalia. The XX/XY cell lines were detected in peripheral lymphocytes, buccal mucosa, urine sediment and also in all other subsequently biopsied tissues: skin, both ovaries, testes, bone marrow and peritoneum and endometrium. The male cell line was detected by FISH technique (CEP X/Y Satellite II DNA probes) in all samples at a proportion of 20-50%. The presence of SRY was confirmed using PCR. The chimerism was confirmed by STR multiplexes on chromosomes X, 13, 18, 21 and by STR markers kit (AmpFLSTR IDentifiler PCR Amplification Kit). The result of the analysis proved tetragametic origin of the chimerism. Our proband underwent three cycles of IVF with own oocytes and ICSI. The third cycle was successful ET of 2 embryos was performed and after normal preg

PO01.016-M
Cell culture conditions of chorionic villous samples do not modify the genomic imprinting pattern at locus 11p15.5
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Methylation of the CpG islands is a common epigenetic marker of gene repression. Monolallelic and parent-specific DNA methylation pattern at the Imprinting Control Region 1 (ICR1) and 2 (ICR2) regulates the expression of IGF2/H19 and KCNQ1/DKCN1A domains at the imprinted locus 11p15.5. The ICR1 and ICR2 methylation state are common in Beckwith-Wiedemann (BWS) and Silver-Russell (SRS) syndromes and a robust molecular investigation is crucial to support phenotypic evidences, particularly in prenatal diagnosis for BWS. Since it is known that cell culture conditions could per se modify the epigenetic signature of the cells, we aimed to compare ICR1 and ICR2 methylation profile in fresh chorionic villous samples (CVS) with the corresponding cell cultures (CVC) to verify whether methylation at ICRs is stable after cell culture. By pyrosequencing we analyzed 9 CVS and their relative CVC from healthy pregnancies that underwent prenatal
P01.019-S
Prenatal diagnosis: chromosomal microarray in fetuses with increased nuchal translucency
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Chromosomal microarray has significant advantages over standard metaphase karyotyping for detecting large chromosomal imbalances and alterations smaller than 10 Mb in size. The method has become an important diagnostic instrument in the prenatal setting for pregnancies with abnormal ultrasound findings. In our clinical setting (The Central Region, Denmark), more than 90% of pregnant women receive combined 1st trimester screening and 2nd trimester anomaly scan via the public health care system. Since January 2013 we have used a two-tiered approach for invasive diagnostic amniocentesis with a small nuchal translucency > 3.5 mm (99% positive predictive) in 1st trimester pregnancies. This consists of a fast prenatal analysis for common aneuploidies by QF-PCR followed by chromosomal microarray on samples with normal QF-PCR test results. The analysis methods used are the Eucli- gene GST®R kit (GenProbe) and comparative genomic hybridization-based microarrays (SurePrint G3 Human GM microarray 180K, Agilent). DNA was extracted directly from chorionic villus samples using the Maxwell® system.

We demonstrate the usefulness of microarray testing in this clinical setting based on our laboratory experiences.

P01.020-M
Clubfoot as an indication to perform array-cgh on prenatal diagnosis even when isolated?
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We report on the first pregnancy of a 31-year-old healthy female. At 13+2 weeks of gestation, a combined test was carried out, which showed a calculated risk for trisomy 21 of 1:38 (NT 1.8 mm, free β-hCG 1.52 MoM and PAPP-A 0.22 MoM). The patient decided to undergo chorionic villus sampling which showed a normal fetal karyotype (46,XY). At 17+6 weeks of gestation, an ultrasound showed isolated bilateral clubfoot, no further genetic testing was offered. At 19+6 weeks of gestation the ultrasound confirmed the isolated bilateral talipes equinovarus and at 23+5 weeks of gestation hypoplasia and dilated hyperechogenic bowel was also noted: array-CGH testing on fetal DNA was offered and the patient accepted. The array-CGH analysis showed a de novo 3.4 Mb deletion on 5q31.1 (131825068-135229731).

This case might suggest that clubfoot, which is generally considered a mainly isolated congenital defect and for which no further genetic testing is offered in prenatal diagnosis, might be the first indication to perform array-CGH on foetal DNA.

P01.021-S
CNV and Aneuploidy Detection by Ion Semiconductor Sequencing

Ion Torrent™ semiconductor sequencing, combined with Ion AmpliSeq technology, provides simultaneous identification of copy number variants (CNVs), single nucleotide variants (SNVs), and small insertions and deletions (indels) from a research sample by means of a single integrated workflow. 100% of assayed CNV regions (n=34) were detected using a reference set of 31 samples with known chromosomal aberrations. Low-pass whole-genome sequencing data, with approximately 0.01x read coverage, allowed the rapid ≤10 hour analysis of aneuploides from research samples with extremely low initial input DNA amounts—even from a single cell. Using a control set of 10 samples with known chromosomal aberrations, 100% of the copy number changes were found, ranging from gains or losses of whole chromosomes to subchromosomal alterations tens of megabases (Mb) in size. The Ion PGM™ System minimizes the high cost and complexity of next-generation sequencing and, with Ion Reporter™ Software, facilitates user-defined CNV and aneuploidy detection, with three sensitivity options so that copy number analysis workflows can be tuned to achieve desired levels of sensitivity and specificity.

P01.022-M
Fetal intrauterine hemorrhage and cataract: think COL4A1
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The COL4A1 gene encodes the alpha 1 chain of type IV collagen, a crucial component of nearly all basement membranes. Mutations in COL4A1 were first associated with cerebral microangiopathy and familial porencephaly and have later been implicated in a clinicopathologic broad-spectrum affecting the brain, eyes, kidneys and muscles. Recently, COL4A1 mutations have also been identified prenatally in fetuses with intracranial hemorrhage (ICH). We report two additional prenatal cases of COL4A1 mutations in fetuses with ICH and cataract.

Case 1: Fetal ultrasound examination (US) at 23 weeks’ gestation (WG) showed left cataract, left ventriculomegaly and hyperechogenic lesion of basal ganglia. Fetal magnetic resonance imaging (MRI) at 32 WG confirmed the subependymal hemorrhage affecting the left hemisphere.

Case 2: Fetal US examination at 31 WG showed hyperechogenic lesion in left hemisphere with thalamic echogenicity and bilateral cataract. Fetal MRI at 32 WG showed a left-sided periventricular parenchymal hemorrhage and mild ventriculomegaly.

In both these cases, the involvement of COL4A1 was evoked because congenital cataracts had been previously reported in association with ICH in pediatric cases.

The sequencing of COL4A1 performed on fetal DNA after termination of pregnancy, evidenced two heterozygous novel missense mutations c.2317G>A (p.Gly773Arg) and c.3005G>A (p.Gly1002Asp) in fetuses 1 and 2 respectively.

The two cases reported here show that the COL4A1 mutation should be envisaged in fetuses with prenatal ICH especially in the presence of less abnormalities at US examination. Molecular confirmation of a COL4A1 mutation may have important implications for the outcome of the pregnancy and for genetic counseling.

P01.023-S
Validation And Clinical Application Of A Next-Generation Sequencing (NGS)-Based Protocol For 24-Chromosome Aneuploidy Screening Of Embryos
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The rapid development of next-generation sequencing (NGS) technologies has generated an increasing interest in determining whether NGS could be reliably used for preimplantation genetic screening (PGS), i.e. for comprehensive aneuploidy screening of human embryos produced from patients undergoing in-vitro fertilization treatments, with the purpose of identifying and selecting for transfer euploid embryos.

We performed a large validation study to determine the accuracy of a NGS-based 24-chromosomes aneuploidy screening protocol. NGS ability to accurately identify aneuploidy was assessed in three steps: 1) a blind evaluation of karyotypically-defined chromosomally abnormal single cells; 2) a retrospective blinded assessment of 244 embryos previously analyzed by array-comparative genomic hybridization (aCGH); 3) a prospective trial involving a parallel evaluation of 192 blastocysts, from 55 clinical PGS cycles, with both NGS and aCGH techniques.

The NGS method was robust, with 454/454 (100%) samples yielding results. Aneuploidy diagnoses were fully concordant with those obtained using aCGH technique. NGS was also able to detect chromosomal mosaicism in 54/54 (100%) of mosaic embryos assessed. Clinical application of the NGS
protocol revealed 76 (39.6%) euploid blastocysts. Following transfer of 50 embryos, 30 women had a sustained pregnancy (63.8%) clinical pregnancy rate (ET); 64.0% implantation rate).

This is the first study reporting extensive validation and clinical application of NGS for PGD allowing identification and transfer of euploid embryos resulting in healthy pregnancies. Evidence of accuracy demonstrates that NGS represents a reliable high-throughput methodology for comprehensive apheresis screening, capable of detecting whole chromosome aneuploidies and segmental changes in embryos, with the potential to revolutionize preimplantation diagnosis.

P01.025-S

A new case of Apert’s syndrome detected prenatally in Romania

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Apert’s syndrome (Acrocephalopolysyndactyly) is a rare congenital condition characterized by primary craniosynostosis, mid face malformations and symmetrical syndactyly of the hands and feet. The incidence of Apert’s syndrome (autoimmune disease) at 1/100,000 live births and is inherited in an autosomal dominant fashion, but sporadic cases are also frequent. Case Report: A 32-year-old, grávida 4, para 0 woman was referred for fetal evaluation at 22 weeks of gestation because of digital abnormalities in the fetus. A prenatal ultrasound at 22 weeks of gestation revealed frontal bossing, low set ears, depressed nasal bridge, digital fusion, and bilateral syndactyly of the hands and feet. Amniocentesis was performed and the karyotype and the molecular test was done. A DNA testing for the FGR2 gene was immediately performed using uncultured amniocytes, which revealed a heterozygous (P253R) mutation in the FGR2 gene. The karyotype was normal, 46,XY. The woman decided to discontinue the pregnancy, and a male baby was delivered with frontal bossing, midface hypoplasia and bilateral syndactyly of the hands (mittenhands) and feet. Conclusions: A molecular analysis of FGR2 using uncultured amniocytes was useful for rapid confirmation of Apert syndrome at prenatal diagnosis. The baby was the first case with Apert’s syndrome confirmed by genetic testing in Romania.

P01.026-M


In the 2010-2014 period 689 chorionic villus sampling (CVS) were successfully performed. Q-PCR and a complete chromosome examination (karyotyping) was a standard examination schema for all samples. Abnormal results were found in 184 samples (26.9 %). Totally 165 (99.7 %) cases out of these 184 pathological results has been announced by Q-PCR (most frequently an autosomal aneuploidy) prior to karyotyping. This is in contrast to 19 abnormal findings detected only due to classic chromosomal examination (9 balanced aberrations, 9 mosaics of both autosomes and gonosomes, 1 unbalanced aberration). In the same period of time 102 CVS (with normal karyotype and Q-PCR results) were evaluated by SNP-array (Illumina). Well-defined pathogenic microdeletions or microduplications were detected in 7 CVS (6.8 %) indicated from following reasons: increased nuchal translucency (5 cases), heart defect (1 case) and anal and esophageal atresia (1 case).

In our study, an examination of CVS based on Q-PCR method only would let 19 abnormal findings (2.5 %) undetected out of which only 10 (1.4 %) are defined as pathological (9 mosaics of both autosomes and gonosomes, 1 unbalanced aberration). In all 10 cases we suppose that these aberrations would be detected by SNP-array evaluation. In addition to it, re-examination of CVSS with normal karyotypes and Q-PCR results by SNP-array allowed us to detect another 7 submicroscopic pathological abnormalities. Based on these data we consider the classic karyotype examination to be still an invaluable part of the CVS testing procedure.

P01.027-S

Invasive prenatal diagnosis in bizaika: a 20-year experience

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Objective: To analyze trends in the number of invasive procedures and the incidence of chromosomal abnormalities over a 20-year single institution experience.

Methods: Data of 16,894 invasive prenatal procedures performed between 1993 and 2013 in Bizkaia were retrospectively reviewed, with particular emphasis on indications and number and type of abnormal results. Results: Traditionally, maternal age has been the main referral reason for prenatal testing (69.3%) but during the last 3 years, prenatal screening has become the most important clinical indication, decreasing the number of invasive procedures performed. Related to karyotype analysis, chromosomal abnormalities were detected in 348 out of the 16,894 (2.55%) cytogenetic studies. Among chromosome aneuploidies (1.55%), the most frequent ones were classical autosomal aneuploidies (1.09%): Trisomy 21, 18 and 13 were diagnosed in 115 (0.94%), 23 (0.07%) and 9 (0.06%) cases respectively. Sex chromosome neuploidies were found in 59 cases (0.46%), being Klenefelter Syndrome the most common sex aneuploidy diagnosed (0.16%). Balanced rearrangements corresponded to 0.67% of the structural abnormalities while unbalanced rearrangements were found in 16 cases (0.15%). Conclusion: Nowadays, positive prenatal screening has progressively replaced advanced maternal age as the main referral reason for amniocentesis. Our study based in 16,894 amniocenteses contributes to establish a standard reference incidence of chromosomal abnormalities in pregnancies. Our data also confirm the karyotype as a reliable method for detecting complex chromosomal abnormalities, providing an important basis for prenatal counseling and for prenatal screening policy in the national strategy.

P01.028-M

Observations following commercial implementation of a cell-free DNA (cfDNA) and single-nucleotide polymorphism (SNP)-based non-invasive prenatal aneuploidy test (NIPT)

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Objective: To describe the clinical observations following implementation of a SNP-based non-invasive prenatal aneuploidy test in more than 28,000 pregnant women.

Methods: 28,709 consecutive cases reported since March 2013 for NIPT. Isolated cfDNA was amplified via multiplex PCR targeting 19,488 SNPs covering chromosomes 13, 18, 21, X, and Y. Sequencing data was analyzed the NATUS algorithm. Reports were issued describing risk for trisomy 21, trisomy 18, trisomy 13, and Monosomy X, and in a subset of cases triploidy, sex chromosomes trisomies and fetal sex. All reports included fetal cfDNA fraction. Follow-up information on samples receiving a high-risk result for trisomy 21, trisomy 18, trisomy 13, or Monosomy X was collected from providers. Results: 510 (1.8%) cases received a high-risk NIPT result for any of the four main indications (325 trisomy 21, 83 trisomy 18, 41 trisomy 13, 61 Monosomy X). Of the 28,709 cases, 49% were under 35 years of age, 17,529 low-risk results and 358 high-risk results were reported to centers participating in follow-up efforts. A response to requests for follow-up information was received for 17,302 cases (99.3%). In total, 14,742 (85.7%) cases completed follow-up, including 10,543 (70.6%) where a positive result was confirmed (115 (0.8%) trisomy 21, 90 (0.6%) trisomy 18, 66 (0.4%) trisomy 13, and 12 (0.1%) Monosomy X). Two (0.01%) trisomy 21 negative results were voluntarily reported.

Conclusions: Performance of this SNP-based approach in a clinical setting, when adopted by a large and diverse population and distribution base, appears consistent with previously reported validation performance characteristics.

P01.029-S

Non-invasive prenatal testing of fetal aneuploidies and follow-up

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In the Netherlands the Minister of Health has licensed noninvasive prenatal trisomy testing (NIPT) as from April 1st 2014, for women with a risk above 1:200 based on the first trimester combined test. In anticipation, we validated the SOLID WildFire on 154 blood samples obtained from pregnant women opting for invasive testing. We show a false negative result in a trisomy 18 case: a growth retarded fetus, small placenta, and maternal BMI of 29 may have resulted in a low fetal fraction. We also present a case of a super-
ESX1 mRNA expression was previously investigated by our group in testicular biopsies of infertile men and correlated to the presence of residual spermatogenesis in Non Obstructive Azoospermic (NOA) patients. Here, we further deepen this issue investigating by real-time PCR ESX1 expression in both testicular fragments (TF) and seminal fluids (SF) of 78 NOA men. The aim was to verify whether a positive ESX1 expression in TF or even in SF was predictive of a successfully sperm recovery at TESE/microTESE. Concerning TF, ESX1 mRNA levels: 1) significantly decrease with the increasing of spermatogenesis defect, as classified by histology; 2) are higher in dilated tubules, likely containing spermatozoa, compared to thin ones. In addition, the presence of a positive or negative testicular expression of ESX1 strongly correlates (p<0.0001) with positive or negative sperm recovery at surgery. Regarding SF, ESX1 mRNA expression was detected in the ejaculate of 44/56 azoospermic men, at lower levels than normospermic men (p=0.05). No significant differences were found in ESX1 expression levels among samples with different degree of spermatogenic failure, based on histological classification. Regarding spermatozoa recovery and ESX1 expression in SF, the two variables were concordant in 33/56 (59%) of cases. The overall data confirm a strong correlation between a positive ESX1 presence and residual spermatogenesis in testes of NOA patients, indicating a role of ESX1 as predictive spermatogenesis molecular marker. We can speculate that in SF discrepancies between ESX1 expression (+) and sperm retrieval (-) could be attributed to limitations in surgery techniques for sperm recovery.

The importance of fetal pathology. Regional assessment report for the years 2010 to 2012

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The Languedoc-Roussillon region in the South of France has a population of about 2 800 000. Over the period extending from 2010 to 2012 the number of births in this region was 91 715. Over the 3-year period 910 requests for termination of pregnancy (TOP) were authorized. 9.2% of these TOPs were done for serious congenital anomalies. 50% of the TOPs were performed for serious malformation syndromes without a precise genetic cause determined during the prenatal period. In order to characterise fetal anomalies, maternalities in the region are encouraged to send fetuses to the Fetal Pathology Unit for genetic investigations and an autopsy. Over the 3-year-period 985 fetuses were received for expertise either following accidental fetal demise or TOP (n=436). In 30% of cases fetal pathology showed additional anomalies (33%), skeletal anomalies (30%), heart defects (18%) and urinary tract malformations (14%). Fetal pathology clarifies fetal anomalies, determines whether a particular genetic syndrome is involved and reveals chromosome anomalies undiagnosed before TOP. This information is important for families (genetic counselling) and for the physicians who managed the interrupted pregnancy or decided on the TOP.

Analysis of FMR1 and FMR2 genes in women with primary ovarian insufficiency from the Basque Country

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Primary ovarian insufficiency (POI) is an ovarian dysfunction defined as irregular menses and elevated gonadotrophin levels before or at the age of 40 years. Several genes have been reported as having significance in POI but the FMR1 (intermediate and premutation alleles) is one of the most important genes associated with it. The FMR1 gene has also been related with the development of this condition. A group of 68 women with POI and 47 control women from the Basque Country has been analysed. Considering the FMR1 gene, the number of women carrying at least one allele with >35 CGG
repeats (intermediate and premutation alleles) was statistically higher in patients (26.47% vs. 0%). The patient group was divided into three categories according to their ovarian condition. Among patients with amenorrhea and elevated FSH levels, the frequency of alleles between 35 and 54 CGG was statistically higher than in controls (15% vs. 0%). This frequency is also statistically higher among women with irregular menses and elevated FSH levels (11.11% vs. 0%). Regarding the FMR2 gene, small alleles with fewer than 11 repeats have been associated with premature ovarian failure. The frequency of these alleles in this study is not statistically different (3.68% vs. 3.19%).

The data suggest that carrying more than 35 CGG repeats in the FMR1 gene might be related with the development of P01. However, the FMR2 gene has not a clear association with this ovarian dysfunction.

P01.035-S
Folicule stimulating hormone receptor gene alterations and ovarian response to gonadotropins in Iranian infertile women
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Diminished Ovarian Reserve(DOR) and Ovarian Hyper Stimulation Syndrome(OHSS) are infertility disorders in which women’s ovaries don’t have proper response to gonadotropins. Folicule stimulating hormone(FSH) has a critical role in the maturation of the ovarian follicles from the antral to the graffian stage. FSH will start a signaling cascade in the granulosa cells after sitting on its receptor(FSHR). Alteration of this receptor may change follicle maturation and therefore result in improper response to gonadotropins. We investigated the association of FSHR receptor gene alteration in DOR and OHSS patients. The presence of PALA665Thr, Ser690Ala, Ala307Thr and Mut.Val341Ala were analyzed in a case control study. 31 Iranian DOR and 34 Iranian OHSS patients were selected as the case group. 30 Iranian fertile women were enrolled as the control group. The patients DNA were extracted from their peripheral blood and amplified by relevant primers. For determining allelic variant status all PCR products were analyzed by Sequencing. The results were unexpected; the homozygous Ser690 and Ala307 variants seem to be significantly associated with OHSS. The FSHR PALA665Thr genotype frequency was similar in all patients and controls. The number of oocytes retrieved was comparable between patients with different FSHR genotype. Although data are accumulating with evidence suggesting that the ovarian response to gonadotropins is mediated by different genetic mechanisms, as in some previous studies homozoguity for Ser690 was significantly associated with DOR, the optimal biomarkers and the efficacy of the tests still remain to be evaluated.

P01.036-M
New molecular approaches for the detection of free fetal DNA
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One of the major effort in medical genetics is the development of non-invasive prenatal diagnosis, aimed to replace current invasive methodologies. It is well known that the circulating free fetal cells may be employed as a new alternative source of DNA for prenatal diagnosis, in order to avoid the risks associated to invasive diagnosis. The cell free fetal DNA (cfDNA) in maternal plasma or serum allows the development of non-invasive method for the detection of genetic, chromosomal and molecular markers in the maternal plasma. cfDNA can be even applied to determine fetal sex and the risk for X-linked genetic disorders. To date, several techniques, such as PCR, Real Time PCR, Next Generation Sequencing, have been employed for cfDNA analysis. Here we propose a new molecular technology (Flexpar® HY System), based on Real Time PCR for fetal sex determination. The Flexpar® technology takes advantage of the specific interaction between two modified nucleotides and the reduction in fluorescence. The qPCR approach allows the simultaneous quantification and detection of fetal sex in pregnant women. Peripheral blood samples were obtained from 50 pregnant women at the 12th to 14th gestational week and the cfDNA was isolated from maternal plasma. Determination of fetal sex of all samples demonstrated a concordance of 100% with the results obtained by traditional method (karyotyping). We also observed, as expected, that cfDNA increases together with gestational age. The experimental data demonstrate that non-invasive and molecular methodologies are able to determine the fetal sex and quantify the cfDNA.

P01.037-S
FSHB -211 G/T polymorphism affects hormonal levels and sperm parameters
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FSHB gene transcription is the rate-limiting step for FSH production. The FSHB -211 G/T single-nucleotide polymorphism (SNP) is the only genetic variant which has a major effect on serum FSH concentrations in men. The aim of this study was to evaluate the effects of this FSHB SNP on male infertility. The SNP was analyzed in 83 men with oligoasthenozoospermia/azoospermia (group 1) and in 82 normozoospermic controls (group 2). Genotyping of SNP was performed by Real-time PCR with TaqMan Genotyping Assay. The FSHB genotypes frequencies were: 74.4% (GG), 22.7% (GT) and 2.9% (TT). The distribution frequency of heterozygous and homozygous T carriers was significantly different between the two genotype groups: 65.9% of GT heterozygotes and 100% of TT homozygotes were found in group 1 (Z-test, p=0.0057). The T allele frequency was statistically different in the two groups: 18.6% and 7.9% in group 1 and 2, respectively (Z-test, p=0.007). Moreover, the FSH serum levels were differently distributed with a trend from the highest values in the GG genotype to the lowest levels in the TT genotype (Kruskall-Wallis test: p=0.037). The T allele was associated with significant declining levels of LH, testosterone (GG+GT vs. TT and GG vs. TT (p<0.05, ANOVA) and sperm concentration (p<0.05, Median test). These results corroborate the observation that the highly conserved promoter regions of the FSHB gene have a regulatory function on the transcription of this gene and demonstrate convincingly that the promoter variant may be involved to the modulation of testicular functions both in healthy and infertile men.

P01.038-M
Noninvasive prenatal diagnosis of Huntington disease in the Netherlands: a validation study
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Huntington disease (HD) is a progressive neurodegenerative disorder, that presents with motor symptoms, cognitive impairment and psychiatric disturbances. HD is caused by the expansion of an unstable polymorphic trinucleotide (CAG) repeat in exon 1 of the HTT gene. Our facility is the only laboratory for diagnostics of HD in the Netherlands. About one third of the requests for molecular prenatal diagnostics we receive are for HD. However, molecular testing is performed on fetal DNA derived from invasive procedures. Therefore, there is a request for alternative, less invasive methods. Noninvasive prenatal diagnosis (NIPD) using total cell-free DNA (cfDNA) from maternal plasma for the detection of paternally inherited mutations in the fetus is achievable for a range of genetic disorders. In this study, we have explored the use of NIPD for HD. We have compared our methods to previously described methods and subsequently optimized and validated them.

Since 2010, n=17 couples have been included in this validation study, resulting in maternal blood samples from n=22 pregnancies. Using a combination of PCR and fragment analysis, paternally inherited fetal CAG repeat length was determined using total cfDNA. All results were confirmed on genomic DNA derived from chorionic villi. The full range of fetal CAG repeats tested in this cohort was 15-70 repeats.

We show that paternally inherited repeats in the intermediate and affected range can be detected in a large background of maternal cfDNA. In addition, ins and outs of detecting trinucleotide repeats in fragmented cfDNA from maternal plasma will be discussed.

P01.039-S
Perinatal hypophosphatasia: A case of extreme intrafamilial variability
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Hypophosphatasia (HPP) is a clinically diverse condition characterized by defective bone and/or teeth mineralization in the presence of low activity of serum and bone alkaline phosphatase. Perinatal lethal and infantile forms of HPP have autosomal recessive mode of inheritance with prenatal ultrasound diagnosis often showing extreme skeletal hypomineralization that often results in neonatal death. Intrafamilial variability for autosomal recessive conditions is rare and HPP is not an exception; however, occasionally unexplained variations have been noted among affected siblings. We report a family with extreme variability in the perinatal presentation of HPP. The couple had two pregnancies affected with HPP confirmed by DNA analysis showing compound heterozygous mutations in the ALPL gene. Fetal ultrasound findings showed short long bones, fractures and demineralization of the skull at 16 weeks gestation. The second affected pregnancy had normal skeleton and normal growth of long bones at both 16.5 and 19.5 weeks gestation ultrasound. Both pregnancies were terminated in the second trimester. Fetal autopsy on the first fetus confirmed the prenatal ultrasound findings while external examination and x-rays on the second fetus showed few significant findings of perinatal HPP. This case emphasizes that in HPP prenatal diagnosis should rely on molecular analysis rather than ultrasound and suggests that other genetic or prenatal environmental factors can significantly modify the phenotype even in known lethal skeletal dysplasias.

P01.040-M Additional Genetic Testing in case of Increased Nuchal Translucency and normal Karyotype
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In fetuses with increased nuchal translucency (NT ≥ 3 mm), after exclusion fet al chromosomal anomaly, more than 100 different developmental/gene tic syndromes are reported, most of them are unique and difficult to diagnose before birth. At the same time the awareness of prospective parents needs to be reduced and exclusion of at least some genetic disorders may be helpful. Our study group consisted of 51 fetuses with increased NT and normal karyotype (analyzed at Tartu University Hospital in 2012-2013). For genetic testing the arrayed primer extension system was used, allowing to examine different genetic diseases with same test: Noonan syndrome (NS) (PTPN11, SOS1, KRAS, RAF, MEK1); Smith-Lemli-Opitz syndrome (SLOS); congenital adrenal hyperplasia (CAH); and spinal muscular atrophy (SMA). Of 51 tested cases, in one case parental testing is ongoing; and in literature the mutation p.Pro655Leu in SOS1 was inherited from healthy parent at least in two cases (in one case parental testing is ongoing) and in literature the mutation is proposed to be as polymorphism. No SMA or SLOS cases or carriers were found among our study group. In conclusion: only in two cases (4%) the test gave valuable information for family; in three cases this test gave concern and uncertainty about fetus’ health. Therefore it is questionable whether this particular test would be the best choice for additional testing.

P01.041-S Two fetal cases with interstitial deletion of chromosome 13 due to maternal germline mosaicism
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De novo structural chromosome aberrations are usually interpreted as a result of a random event during parental gametogenesis and the recurrence risk varies, therefore it is estimated to be very low. We report on a case in which the same supposedly de novo deletion occurred in a second pregnancy. The participant was referred to our institution for fetal karyotyping in her first pregnancy due to a positive first-trimester screening test. Karyotyping after amniocentesis at 17 weeks gestation showed an interstitial deletion of chromosome 13 (46,XX,i(13)(q22q32)). To define the exact deleted region an array-CGH was performed, indicating one copy of 13q11.1,g32.1, 15.9 Mb in range. The ultrasound examination at 23 weeks of gestation showed cerebellar hypoplasia, severe ventriculomegaly as well as associated facial features – hypertelorism, midface hypoplasia and micrognathia. The pregnancy was terminated. Both parents were found to have normal karyotypes. In the second pregnancy, chromosomal analysis of the fetus showed an identical interstitial deletion of chromosome 13 as the one found in the first pregnancy.

Microsatellite analysis of genomic DNA extracted from tissues of both fetuses as well as from the blood of both parents revealed loss of heterozygosity for two markers located inside the deleted region. Missing alleles originated from the mother. The results of molecular and cytogenetic studies strongly suggest that the maternal germlinal mosaicism could be the cause for the increased risk of recurrence in described case. The possibility of germline mosaicism is of particular concern when counselling the parents of a child with a de novo chromosomal abnormality.

Klinefelter Syndrome (KS) is the most common chromosome disorder in men (47,XXY), exhibiting marked phenotypic variation, frequent hypogonadism and increased mortality. It is unclear to what extent the genetic impact of the supernumerary X-chromosome contributes to the pathology. EXAKT (Epigenetics, X-chromosomal features and Clinical Applications in Klinefelter syndrome Trial) involves 132 KS men and their parents assessing a wide range of clinical parameters in comparison to male and female controls (n=50 each) in relation to genetic investigations. The objective was to elucidate gene expression patterns in KS and whether these would be related to inherent pathologies. Gene-expression was substantially disturbed in KS vs. both control groups. The differential expression of 36 not only X-chromosomal genes puts these phenotypical males into a genetic framework located between men and women with normal karyotypes. A range of these genes has previously been attributed to gender-specific modulations of immune responses. The KS cohort exhibited increased insulin resistance/inflammatory status, a procoagulatory state, higher waist circumference, dyslipidemia and an altered cardiac rhythmogenic setting. The extent of clinical dyshomostasis was associated with the expression of dysregulated genes. Paternal origin of the supernumerary X-chromosome was an additional confounder regarding insulin resistance and cardiac phenotype.

In KS patients, the supernumerary X-chromosome contributes to a number of pathologies by altering gene expression patterns: insulin resistance, dyslipidemia, enhanced inflammation markers as well as altered cardiac rhythmogenic setting are involved; this was observable independently from testosterone substitution treatment which may have attenuated responses in KS. Funding IZKF Münster CRA03/09; DFG WI2723/4-1

Klinefelter Syndrome testicular gene expression profile by a whole transcriptome approach
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Klinefelter Syndrome (KS) is the most common sexual chromosome abnormality (47,XXY) and represents the first genetic cause of male infertility. The mechanisms leading to KS testis degeneration are still unclear and no therapy is so far available for affected patients. The present study is aimed to unravel information about molecules playing a key role in the disruption of the spermatogenesis. Gene expression profiles analysis of KS azoospermic testis versus normal testis, could provide useful information about the molecular basis of the alteration of the spermatogenesis. Transcriptome analysis was performed carrying out gene expression profile by a whole genome microarray approach on testis biopsies obtained from 6 azoospermic non-mosaic KS men and from 3 controls, for a total of 12 experiments. T-test and False Discovery Rate were used to evaluate differentially expressed genes. Identification of transcripts were analysed by Ingenuity Pathways Analysis software to disclose genes biological functions. Data analysis revealed the differentially up- and down-expression, in KS testis versus the control ones, of 656 and 247 genes related to Endocrine system development and function, Lipid metabolism, Reproductive disease, Free radical scavenging, and Cell death.

ESHG 2014 | MILAN, ITALY | WWW.ESHG.ORG
The prevalence of luteinizing hormone beta-subunit gene polymorphisms in Czech population and patients with ovarian hyperstimulation syndrome

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There are two common polymorphisms in luteinizing hormone beta-subunit (LHβ) gene, which form the variant LHβ (v-LHβ). These polymorphisms change the amino acid sequence (Trp8Arg and Ile15Thr) of the protein. The hormone with v-LHβ (Arg8/Thr15) was shown to have higher bioactivity (tested in vitro) but shorter lifetime in vivo. The presence of v-LHβ is associated with reduced woman’s fertility, menstrual disorders and abortions. Female IVF patients with at least one v-LHβ allele tend to be hypo-responder to controlled ovarian hyperstimulation. Thus, we propose that women with v-LHβ would be in a lower risk to develop an ovarian hyperstimulation syndrome (OHSS).

In our study, 102 fertile male-controls, 149 fertile female-controls and 58 patients affected by OHSS type III-V were analyzed. The genotype was determined by restriction fragment length polymorphism (RFLP). The prevalence of v-LHβ allele was 10,1% in female controls, 14,7% in male controls and 6,0% in OHSS patients.

There was no statistical difference between Czech control men and women in genotype or allelic frequencies (P=0,22; P=0,12, respectively). The Czech population frequency of v-LHβ is closest to the prevalence from Iceland, Italy and The Netherlands.

There was no difference between female controls and OHSS patients in genotype or allelic frequencies (P=0,41; P=0,25, respectively). The protective effect of the v-LHβ from OHSS was not confirmed, but this needs to be verified in a larger cohort.

Supported by grants IGA NT13770-4/2012, project for conceptual development of research organization 0064203 and OPKK CZ.2.16/3.1.00/24022.

P.01.047-S Genetic basis of male infertility: Belorussian Centrum of Reproductive Medicine data.

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Genetic factors play an important role in male infertility etiology. We present the data of spermagetic impairment infertile couples referred to ART Results. 601 mentally normal men with azoospermia/severe oligozoospermia were examined using cytogenetic and molecular methods. Klinefelter’s syndrome (KS, 47,XXY) was found in 11 (1,8%) patients. Different AZF regions Yq microdeletions (DelAZF) were detected in 34 (5,6%) patients. AZFc deletion calculated for 29 cases (85,3%) with a chance of reproductive failure in testicular biopsy (TESE) and performing ICSI: spermatozoa were received in 15 cases (51,7%) for following cryopreservation. Two AZFb and 3 AZFc deletion’s deletions’ patients showed sperm absence in testicular tissue. 2 men with complete AZFa+b+c region’s deletion didn’t undergo TESE/ICSI. 16 (2,6%) CFTR gene mutation’s heterozygote carriers were identified: d508del (10 cases), CFTR2.3del (3), 2186insA (2), 1677delT (1). Preimplantation genotyping: Carrier partners carrier of CFTR gene mutations have 25% risk of affected outcome, DelAZFc male - 100% deletion’s transmission risk for sons. We recommended donorsperm using or adoption for KS and DelAZFb; DelAZFb+c; DelAZFa+b+c’s patients ARTs: 12 DelIAZFc patients underwent IVF+ICSI procedures. 5 couples (41,6%) got pregnancy: 3 pregnancies resulted in healthy girl delivery, two - ongoing (12 and 22 weeks gestation). Preimplantation testing was performed for 2 couples with CFTR gene mutations heterozygote’s status for both partners. Two pregnancies were obtained: one is developing successfully, other resulted in miscarriage. Conclusion. KS/Yq microdeletions are specific for a severe spermatogenic failure. Genetic testing permits to establish the origin of azoospermia, proagnos, ART strategy’s selection and to avoid unnecessary treatment.

P.01.048-S X chromosome-linked CNVs in male infertility: discovery of duplication load and recurrent deletions with potential clinical relevance

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The X-chromosome genetic content is predicted to be important in spermatogenesis but its role in male infertility remains unknown. We investigated whether X-linked copy number variations (CNVs) might have clinical significance in idiopathic infertile men. We previously detected a c-GHA a number of CNVs including 16 patient-specific gains and 3 recurrent deletions on Xq27.3-q28 (CNV07) and prevalently (CNV64, CNV09) found in infertile patients. Among the 14 gains, we selected 5 for further qPCR analyses on a larger study population including 276 idiopathic infertile patients and 327 normozoospermic controls. The difference in duplication load was statistically different (p=1.65x10-4). PAR1-linked DUP1A displayed the highest...
frequency (1.44% of patients) and we hypothesize it may cause spermato- genic failure by disturbing meiotic XY pairing or by affecting the correct regulation of a gene potentially influencing spermatogenesis (PPPR3B). Concerning the 3 deletions, 627 patients and 628 controls were tested for each deletion, except for CNV64 and CNV67. Our investigation further indicates an as- sociation between X-linked CNV burden and spermatogenic impairment and identifies the first X chromosome-linked recurrent deletion and duplication with clinical significance.

P01.049-S
A novel m.9588G>A missense mutation in the mitochondrial COIII gene as a cause of low sperm motility in asthenozoospermic Tunisian infertile men
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ABSTRACTS POSTERS

Decrease of meiotic cohesins with maternal age in human oocytes
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Oligoasthenoteratospermia (OAT) is a condition characterized by the pres- ence of sperm with abnormal morphology. The causes of OAT are unknown in most cases. Routine genetic examination of patients with nonsyndromic male infertility (Y-chromosome microdeletions and CFTR mutations) does not cover this phenotype. Given the hundreds of genes identified as essential for male fertility in animal models it is probable that substantial fraction of cases with genetically caused OAT remains undetected. Our study aims for mutation screening of selected candidate genes which were linked to OAT in animal models.

We selected candidate genes whose mutation in model organism interfering with spermatogenesis and was demonstrated like OAT. We selected CAP23A, CDC42, CDC14B, CNTR0B, CSNK2A2, G0PC, HOOK1, HRB, OA32, ODF3, RIMBP3 and SPATA16. We performed genealogy, karyotyping and mutation analysis of CFTR gene and Y-chromosome microdeletions. Control group are men with normozoospermia. PCR amplified exons of the selected genes were sequenced using GS Junior next generation sequencer.

To date we accumulated 131 cases and 88 controls. Sequencing is completed for 41 patients and 8 controls. We found 6 persons with chromosomal abnormal- ities and 2 with Y-chromosome microdeletion. We detected 115 known sequence variants and 18 novel variants (in CDC42, CNTR0B, HOOK1 and RIM- BPS3). The clinical significance of the novel variants is uncertain, however, we identified two likely damaging frameshift variants (in CNTR0B and RIMBPS3). The study is supported by Ministry of Health of the Czech Republic grant No. NT/12269-5

P01.050-M
New candidate genes for oligoasthenoteratospermia as a potential cause of human male infertility
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Aneuploidy of fetal chromosomes is one of the causes of pregnancy loss or congenital birth defects. It is known that the frequency of oocyte aneuploidy increases with maternal age in humans. Recent data have highlighted the contribution of cohesin complex in the correct segregation of meiotic chromo- somes. In mammalian oocytes, cohesion is established during the fetal stage and meiosis-specific cohesin subunits are not replenished after birth. This raises the possibility that the long meiotic arrest of oocytes facilitates the deterioration of cohesion leading to the age-related increase in aneuploidy. We here examined the cohesin levels in dictyate oocytes from different age groups. Samples were obtained from the ovarian tissues of 8 women (age range: 19-49 years) and also from 2- and 10-month-old female mice. Ovarian tissue sections were immunostained using cohesion antibodies and the cohesin levels were determined by immunofluorescence. The levels of the meiosis-specific cohesin subunits, REC8 and SMCB1, were found to be decreased in women aged 40 and over compared with those aged around 20. An age-related decrease in meiotic cohesins was also evident in mice. Interestingly, SMC1A, the mitotic counterpart of SMCB1, was readily detect- able in human oocytes but only barely in mice. The mitotic cohesion levels of mice slightly increased with age. Theseresults suggest that, mitotic and meiotic cohesins may act in a coordinate manner in humans to maintain the levels of this protein over a sustained period. The decreased meiotic cohesin subunit levels with age impairs sister chromatid cohesion leading to the age-related increase in aneuploidy. Wene here examined the cohesion levels in dictyate oocytes from different age groups. Samples were obtained from the ovarian tissues of 8 women (age range: 19-49 years) and also from 2- and 10-month-old female mice. Ovarian tissue sections were immunostained using cohesion antibodies and the cohesin levels were determined by immunofluorescence. The levels of the meiosis-specific cohesin subunits, REC8 and SMCB1, were found to be decreased in women aged 40 and over compared with those aged around 20. An age-related decrease in meiotic cohesins was also evident in mice. Interestingly, SMC1A, the mitotic counterpart of SMCB1, was readily detect- able in human oocytes but only barely in mice. The mitotic cohesion levels of mice slightly increased with age. These results suggest that, mitotic and meiotic cohesins may act in a coordinate manner in humans to maintain the levels of this protein over a sustained period. The decreased meiotic cohesin subunit levels with age impairs sister chromatid cohesion leading to the age-related increase in aneuploidy.
miRNAs in heart development, measure miRNA concentration and expression in maternal blood. Peripheral blood samples were collected from 53 women, 27 of whom had healthy and 26 had fetuses with congenital heart defects. Blood samples were centrifuged and miRNA was extracted from plasma. MiRNA concentration was calculated by Nanodrop spectrophotometer. By using Gene Ontology and miRBase databases we searched for those miRNAs which can be detected in plasma and are associated with chromosomal and congenital heart defects. These criteria were fulfilled by let-7c miRNA of chromosome 21. qFqPCR was carried out to validate the miRNAs expression.

There was no significant difference between the miRNA concentrations in the two groups (6.05 ng/µl vs 5.36 ng/µl). We found significant differences in the let-7c concentrations between the control and patient group (1.12±3.19 ng/µl vs 0.0047±0.0038 ng/µl; p<0.0001).

Fetal-derived miRNAs are part of the free nucleic acids found in maternal plasma, expression studies reveals new opportunities for congenital heart defect research and diagnosis. According to our study let-7c seems to be a potential biomarker for fetal CHD.

**POI.055-S**

**Overexpression of mir-21 and mir-221 in preeclamptic placentas**

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Preeclampsia is a multisystem disorder with partial genetic and immunological etiology; the apoptosis is thought to play a role in the development of preeclampsia. The microRNAs are small noncoding molecules involved in regulation of cellular processes including apoptosis. Here we designed a 96-well reaction plate for the analysis of 21 microRNAs in duplicates which are known to be involved in the apoptosis regulation and are expressed in placenta - pro-apoptotic mir-1, let-7c, let-7g, mir-200c, mir-143, mir-205, mir-122, mir-403-3p, mir-449, mir-708, mir-149, mir-204,mir-133, anti-apoptotic -mir-214, mir-221, and mir-222, and miRNAs with both the anti-apoptotic and apoptotic targets mir29a and mir29c. The normalization was performed against RNU4. The miRNAs were extracted from placental tissues obtained after delivery from preeclamptic women and healthy controls. After stem-loop primer reverse transcription in one tube, the samples were analyzed on the plates and evaluated by deltadelta Ct algorithm. The aberrantly expressed miRNAs were validated by TaqMan MicroRNA Assay by relative quantitation with the standard curves. The analysis of 21miRcorNAs revealed overexpression of mir-212, mir-21, mir-221, mir-29a, mir-29c and mir-449. The validation was performed on 13 additional preeclamptic placental samples collected after delivery and 6 placental samples from normal delivery; a significant overexpression of mir-21 and mir-221 with p=0.0055 (CI95% 25.2 to 123.47) and p=0.0047 (CI95% 12.16 to 57.20) was observed, respectively. Although the validation on larger number of samples is necessary, the analysis of miRNA deregulation can help in the identification deregulated signaling in preeclamptic placenta for detection of new biomarkers.

**POI.056-M**

**Analysis of miRNAs expression profile in human placenta from pregnancies complicated by preeclampsia**

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Preeclampsia is a main cause of maternal and neonatal mortality and morbidity. However, the origin of this disease is rather obscure so far. MicroRNAs (miRNAs) are small, non-coding RNA molecules that are involved in gene expression and are involved in the regulation of a wide variety of biological processes. Moreover, they are considered as potential biomarkers in many pathologies. However, the results of many studies are inconsistent, which may be due to the heterogeneity of the studied populations. Here we aimed to investigate the miRNA expression patterns in human placenta from pregnancies complicated by preeclampsia.

Peripheral blood samples were collected from 53 women, 27 of whom had healthy and 26 had fetuses with congenital heart defects. Blood samples were centrifuged and miRNA was extracted from plasma. MiRNA concentration was calculated by Nanodrop spectrophotometer. By using Gene Ontology and miRBase databases we searched for those miRNAs which can be detected in plasma and are associated with chromosomal and congenital heart defects. These criteria were fulfilled by let-7c miRNA of chromosome 21. qFqPCR was carried out to validate the miRNAs expression.

There was no significant difference between the miRNA concentrations in the two groups (6.05 ng/µl vs 5.36 ng/µl). We found significant differences in the let-7c concentrations between the control and patient group (1.12±3.19 ng/µl vs 0.0047±0.0038 ng/µl; p<0.0001).

Fetal-derived miRNAs are part of the free nucleic acids found in maternal plasma, expression studies reveals new opportunities for congenital heart defect research and diagnosis. According to our study let-7c seems to be a potential biomarker for fetal CHD.

**POI.057-S**

**miRNA as potential universal fetal marker in NIPT**

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Introduction: Circulating miRNA molecules are currently studied as potential diagnostic markers in many physiological and pathophysiological conditions such as oncological diseases, heart diseases and pregnancy. The aim of our study was to determine if circulating miRNAs can be used as potential universal fetal marker in NIPT.

Methods: Circulating miRNAs were isolated from plasma of pregnant women and miRNA samples were divided into two groups depending on fetal gender. Firstly, expression levels of 91 miRNAs were established in three female and three male samples by two-step RT-qPCR. Five miRNAs (miR-378, miR-320a, miR-320b, miR-20b, miR-616-5p) whose expression levels significantly differ between two groups were chosen for further analyses. Subsequently, expression levels of five selected miRNAs were evaluated in 14 female and 15 male samples by two-step RT-qPCR. Differences between groups were tested using Student t-test with p<0.05 was considered as statistically significant.

Results: After the first part of this study where panel of 91 miRNAs were investigated, five miRNAs significantly differ in their expression levels between two sample groups. However, there were no significant differences in expression levels of five selected miRNAs between sample groups, when expression levels of these miRNAs were determined in a greater number of samples.

Conclusion: Our study showed, that none of evaluated miRNA molecules in this work is not suitable as the universal fetal marker. However, since our work addressed only limited number of miRNA molecules, for determination if circulating miRNAs can be used as universal fetal marker, further studies are needed.

**POI.058-M**

**Arthrogryposis multiplex congenita caused by compound heterozygosity to Ashkenazi founder mutation and a novel splice mutation in NEB gene**

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Background: Nemaline myopathy (NM) is a heterogeneous disorder. A homozygote 2502bp deletion in NEB - had been detected in 1:40,000 Ashkenazi Jews (AJ). Most cases have congenital hypotonicity that might progress, but arthrogryposis is relatively rare.

Patients: Three affected siblings with arthrogryposis and an increased nuchal fold / cystic hygroma in two, were detected at 11-15 weeks of gestation. Parents were AJ with non-contributory family history.

Methods and Results: Histo-pathology with electron microscopy of muscle samples suggested NM on two siblings. DNA sequencing confirmed compound heterozygosity to the known Ashkenazi mutation (maternal) and splice mutation c.9619-2A>G (paternal) predicted to disrupt the intron 66 splice acceptor site.

Discussion: The splice site mutation revealed in our patients, has been previously detected in two unrelated affected AJ with clinical stigmata of arthrogryposis (Ludtke et al ICHG 2011; Yonath et al. Prenatal Diagnosis, 2012). The detection of this mutation in several AJ families suggests it is either hot spot site or a new AJ founder mutation. Further studies should be performed to validate its carrier frequency among this population. Also, the severe stigmata in affected patients harboring this splice mutation, suggests its critical role in disease pathogenesis. In line with this, when arthrogryposis detected prenatally, screening of the NEB should be considered.

**POI.059-S**

**Severe lower limb skeletal deformities caused by a novel interstitial chromosomal translocation t(5;5)(q26.2;p14): clinical characterization of affected members with neural tube defect as a common clinical feature**

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A 26-years-old woman was referred for prenatal diagnostics because of aggravated obstetric anamnesis. Her first child (male) was born at 34 weeks of gestation via C/D due to polyhydramnios and hypotrophy. Hypotonia, dysmorphic facial features and multiple congenital malformations were observed, including hypertelorism, epicanthal folds, micrognathia, hypoplastic ears, congenital heart defect, optic nerve dysplasia, microopenis, hypoplasia of the scrotum, and suspected spina bifida occulta. The newborn died at age of 1 month. According genealogy, proband’s mother had a miscarriage at the 14th week of gestation and stillborn at 26 weeks of gestation.
with uncertain gender and myelomeningocele. During this pregnancy at the 27th week of gestation ultrasound scan showed polyhydramnios and multiple congenital anomalies in the fetus. In the 31st week of gestation the fetus died in the uterus. Autopsy findings were published in 2015. The conditions of the fetus were brain anomalies, holoprosencephaly, microphthalmia, myelomeningocele, choroid plexus cyst, and spine bifida. Subtelomeric FISH was performed to the proband and balanced translocation ish t(3;5)(pter->pter t(3;5)(pter->pter t(3;5)(pter->pter) was detected. Karyotype was 46,XXt(3;5) (q26.2;p14).

The clinical phenotype of affected family members is similar and could result from the combination of dup(3q) and del(5p) syndromes. Only few cases of 3;5 translocations have been reported to date. Detailed clinical description of affected family members brings new data for the characterization of this rearrangement.

P01.060-M
The Effect of Radiofrequency Waves on Pregnant Mice in association with Genes involved in Neuronal Migration
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The advancements in technology have improved human life in numerous ways, and concerns have been raised regarding health and safety issues in parallel with the spread of these developments. One of those concerns has been focused on radiofrequency waves because of common usage in daily life, such as mobile phones. The aim of this study is to evaluate change in expression levels of 17 genes (Dcx,Tuba1a, Ywhae, Arx, Reln, Large, Flna), which are involved in neuronal migration following the exposure of radiofrequency waves. A total of 16 mice were included in the study. They were divided into two groups as study and control. Each group consisted of 6 female and 2 male mice. Study group was exposed to 0.725 W/kg SAR value for 12 hours per day continuously by establishing an exposure system throughout pregnancy. A total of 29 offspring in the study group and 12 in the control group were achieved. Gene expression analyses were performed by using real time RT-PCR. Significantly increased expression levels were found in 5 (Arx, Dcx, Large, Reln, Ywhae) out of 7 genes in the study group. In conclusion exposure to radiofrequency waves in pregnancy may cause significant changes in the gene expressions involved in neuronal migration, which may also be related with brain anomalies.

P01.061-S
Confined placental mosaicism in a case of false positive NIPT result for trisomy 18
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Non-invasive prenatal testing (NIPT) of cell-free fetal DNA (cfDNA) for fetal aneuploidy risk assessment has been shown to be both highly sensitive and highly specific. False positive rates can be as low as 0.1%. However, discrepancies between positive NIPT result and fetal karyotype on chorionic villus sampling or amniocentesis may occur. The source of aneuploidy may be due to maternal mosaicism, maternal malignancy, true fetal mosaicism, a demised co-twin, an anembryonic sac, confined placental mosaicism. We present a case of a 38-year-old woman, pregnant in 16 gestational weeks, referred to our Unit for amniocentesis due to positive NIPT for trisomy 18. The ultrasound scans were unremarkable. DNA was extracted from uncultivated amniocytes, amplified with commercial QF-PCR kit Aneufast (Molgentix SL, Barcelona, Spain) and analyzed on ABI 3130xl. Karyotyping was performed on cultured amniocytes using standard protocol. QF-PCR and cytogenetic analysis showed normal results, trisomy 18 was excluded.

The pregnancy was still ongoing, with no pathological findings on routine ultrasound scans. Cytogenetic and molecular-genetic analyses are to be done after delivery. Since fetal cell-free DNA mostly originates from invading trophoblast cells, this false-positive result may be due to mosaicism confined to the placenta (CPM). CPM occurs in 2% of the viable pregnancies and before the introduction of NIPT was most often detected by direct analysis of chorionic villus sampling. Our case illustrates that follow-up with diagnostic testing of chorionic villus sampling and/or amniotic fluid for abnormal NIPT results should be performed and that pre- and posttest counseling is important.

P01.062-M
Preliminary experience with the management of positive results of non invasive prenatal testing (NIPT) in a reference centre
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During the past year techniques enabling non-invasive prenatal testing for the most common trisomies have become available clinically. An increasing number of women have been offered the test in connection with their first trimester screening, regardless of their level of risk. Pregnancies with a positive test are often referred to our Centre for genetic counseling and invasive procedures as recommended. To this end, we have created a flow-chart to assist these patients who are not receiving enough information at time of sampling. Clinical aspects of the test, its limits and reasons for false positives are discussed and a guaranteed follow-up is scheduled to collect data at time of birth. Between July 2013 and January 2014 46 patients with positive NIPT (2 trisomy 21, 1 trisomy 18, 1 monosomy X, 1 47,XXY, 1 triple X) were referred. All patients underwent an amniotic fluid procedure and only one case of trisomy 21, which had fetal anomalies at ultrasound, was confirmed. Normal results were obtained in the other 5. Two term placentas have been studied, the others being ongoing pregnancies. Since false positive cases of chromosome aneuploidy after NIPT may have different underlying biological causes (the majority, however, are due to placental mosaicism) we believe that the cytogenetic analysis of term placentas should be acquired in all cases in order to increase our knowledge about the relationship between cfDNA and the fetal genome. Genetic counselling should always be carried out before and after testing to reduce misinterpretation of test validity.

P01.063-S
Non-invasive prenatal detection of a mosaic trisomy 16
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The presence of cell free fetal DNA in the maternal circulation has allowed for the development of methods, which allow for non-invasive detection of fetal chromosomal aneuploidies. Our laboratory offers non-invasive prenatal testing (NIPT) with an innovative pipeline that utilizes all of the information provided by shallow depth whole genome sequencing to identify possible aneuploidies of all chromosomes. We have encountered one case, referred because of high trisomy 21 risk (1/14), with unusually high Z-score (5.7) for the chromosome 16 indicating the presence of a trisomy 16. Follow-up with direct FISH and array analysis of amniotic fluid cells showed a low-level mosaicism (between 7 and 20%). The finding of low level mosaicism is contrasting with the high abnormality which suggests the presence of trisomy 16 in the majority of the cells. This discrepancy could be explained by confined placental mosaicism and, if so, should be taken into account in subsequent counseling. Whereas the gynecologist suggested interruption, the family decided to continue the pregnancy with ultrasonic monitoring of the fetal development following clinical genetic counseling.

P01.064-M
Non-invasive detection of fetal deletions or duplications by low coverage massively parallel sequencing
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To report the performance of fetal chromosomal deletions/duplications detection by massively parallel sequencing (MPS) of fetal maternal DNA, we recruited 1,324 participants and performed the MPS test in a double-blind study. The peripheral blood of each participant was obtained before invasive sampling at 10-28 weeks’ gestation with a median of 21 weeks. Plasma DNA was extracted and sequencing. About 0.08 fold sequencing data per sample was generated and the bioinformatics analysis was performed using PCAPs algorithm. Deletions / duplications, ranged from 3.07 Mb to 26.98 MB, were suspected in 17 of the 1,324 samples, of which 16 were consistent with the results of fetal karyotyping / aCGH. In one case the suspected abnormality was not confirmed by karyotyping, representing a false positive case. No false negative case was observed in the remaining 1,307 low-risk samples. The sensitivity and specificity for detection of fetal chromosomal deletions / duplications were 100% and 99.92%, respectively. Our study demonstrated the MPS-based test is feasible and of high sensitivity and specificity in detecting fetal chromosomal deletions / duplications.
Noninvasive prenatal KEL genotyping using TaqMan Real time PCR and by capillary electrophoresis minisequencing


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Introduction: There are two reasons for establishing a methodology for noninvasive determination of KEL genotypes in early pregnancy. To identify fetuses which are at risk of hemolytic disease of fetus and newborn by alloimmunized pregnant women and to prevent alloimmunization during pregnancy. Aim: KEL noninvasive determination of fetal genotype from cfDNA in plasma KEL negative pregnant women using TaqMan assay and minisequencing (SNAPshot). Project is supported by IGA MZ CR: NT12-225. Material and methods: 1) TaqMan assay involving region of KEL (K/k) polymorphism and region of AMELY gene as an internal control was tested in DNA samples from leukocytes of K/ K, K/ k and k/ k. 2) SNAPshot: Determination of sensitivity threshold KEL calibration was performed using a dilution series. It was tested 141 samples of cfDNA from maternal plasma in the first trimester. Results: 1) TaqMan assay: KEL genotypes determined from leukocyte DNA were distinguishable, but there was fluorescence background. So there wasn’t able to exactly distinguish between positive fluorescence background and fetal DNA admixture. 2) SNAPshot: On the basis dilution series it was possible to detect less than 0.78% admixture of K allele corresponding DNA concentration of 0.04 ng /μL. Seven fetuses with allele K were found in 124 samples (5.7%). This corresponds to approximately 4.5 % of the population frequency. It wasn’t possible to determine KEL genotype at 8 fetal samples due to KEL maternal heterozygous genotype. Conclusion: SNAPshot assay is more suitable to distinguish fetal KEL genotype, than TaqMan assay.
We report prenatal diagnosis of a fetus with partial trisomy of 4q31-qter and partial monosomy of 22q13.3-qter. A 35-year-old woman was referred for prenatal diagnosis at 28 weeks gestation because of fetal hydrothorax and polyhydramnios detected by ultrasound. Amniocentesis was performed and routine G-band analysis of cultured amnioocytes showed additional material of unidentifiable origin at the long arm of one chromosome 22. Ka- rotype was 46,XX,der(22)add(22)q13.3:. Further molecular cytogenetic characterization of the additional genetic material was carried using FISH. Six commercial FISH were used: two centromeric chromosome probes (D4Z41) and 14/22(D14Z1/D22Z1); two whole chromosome paints 4 and 22; two locus specific probes 22q13.3 (SHANK3) and 22q11.2(22S); two subtelomeric probes 4q26(26) and 4q45(223). The monoso- monosomal material was found to be derived from chromosome 4 novo and fetal karotype was redesigned as 46,XX,der(22)q13.3:. A girl was delivered at 38 weeks of gestation with a birth weight of 3000g and AS 10/10. She has mild dysmorphic features including dolichocephaly, microretrognathia, small skin folds on ears, without other anomalies. At the age of one year she shows slight developmental delay especially in motor skills development. Reported children with distal partial trisomy 4q have the wide phenotypic variability which could be related to the location of the breakpoints and associated monosomies of the other chromosomal parts. Partial trisomies of 4q31 to 4qter in general were associated with only mild phenotypic anomalies like slight (mental) retardation and/or dysmorphism which is consistent with our case. Further follow up is necessary especially because of monosomy 22q13.3-qter.

P01.073-S
Prenatal diagnosis of UPD using genomic SNP array in a foetus with ultrasound abnormalities.

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We report the case of a 35-year-old gravid a, para 0 referred at 18 weeks of pregnancy (wg) because of several fetal malformations. First trimester scree ning test showed an increased risk for trisomy 21 with a nuchal translucency of 4.1 mm; the fetal karotype was 46, XX. Ultrasound scanning at 17 wg showed increased fetal biometry (>95°). small thorax, flaccid abdomen wall, umbilical, genitalia. At 19 wg the patient optioned for pregnancy termination and the fetal autopsy showed cleft palate, micrognathia, edematous short neck with a prominent occipital region, low-set ears, ambiguous resembling male genitalia with hypospadia and short limbs with joint contractures. The Fetal real-timecnograms showed bell-shaped thorax with coat-hanger SNP ar ray analysis was performed on DNA from fetal tissue. Data analysis revealed two regions of runs of homozgosity in 14q: the first one is a subcentrome ric region of about 10 Mb, the second is a subcentromeric region of about 9 Mb. Trip analysis performed by two computational tools (UPDtool and SNPtrio) for detection and classification of uniparental isodisomy (UPD) confirmed paternal uniparental iso/heterodisomy of chromosome 14. In particular, data revealed a region of isodisomy in 1q41.2-1q22 from the extra position 20.2 Mb to 30.4 Mb, a region of heterodisomy in 14q12-2q32.2 from 30.9 Mb to 79.7 Mb, and a terminal region of isodisomy from genomic position 98 Mb to telomere. Results was validated by the analysis of microsatellite markers across chromosome 14. This result may contribute to prenatal identification of similarly affected patients.

P01.075-S
Reporting the outcome of molecular PGDs from a single laboratory in Iran.

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Reporting results of preimplantation genetic diagnosis (PGD) for single gene disorders, gender typing, HLA matching and chromosomal aneuploidies from 2009. We mainly multiplied nested PCR containing several short tandem repeats (STR) and sometimes gene fragments for sequencing. PGD were mainly offered for families with common genetic disorder in Iran specially beta-thalassemia, HLA matching, PKU gene, sex typing, some X linked and Y linked aneuploidies and Hemophilia A. In total we have performed PGD for 29 ca ses (total of 158 blastomers). For females with advanced age only few (1-4) eggs could be retrieved. In total only 46 blastomers could be implanted. Our results shows that in total 3 blastomers were tested for F8 linked STRs, 5 for PKU (BH4, PTS gene), 8 for PKU (PAH gene), 8 for Down syndrome (GJB2 gene), 8 for Epidermolysis Bollusa and HLA matching, 20 for β-Thalassemia, 94 for aneuploidies and finally gender selection was done on 132 blastomers. Some of these figures belong to two or three types of simultaneous testing.
This selection is currently mainly performed by array comparative genomic hybridization (aCGH) on blastomers. Current methodology does not take into account the phase of the cell cycle, despite the variable copy number status of different genomic regions in S phase. To evaluate the accuracy of single cell array CGH, different cell cycle phases from three cell lines derived from patients with β-thalassaemia who was born on February 2014 and another twin hemophilia A pregnant at 8 weeks.

P01.076-M
Pre-case haplotyping of the two monogenic diseases in one family in Gennet: Marfan syndrome and SMA
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Genetic pre-case haplotyping (PGH) is an important constituent of the pre-implantation genetic diagnosis (PGD). In our center we have performed haplotyping studies for 86 different monogenic diseases of all types - X-linked, autosomal recessive, autosomal dominant, translocation and small deletions. The list of diagnosis for which we can offer PGD is being continuously extended. PGD is available for a large number of monogenic disorders. For most couples requiring PGD, common pre-case haplotyping analysis of disease-associated haplotype is derived from family anamnesis. We are presenting a case in which affected haplotype will be determined by standard PGH analysis. In this family 2 affected haplotypes were detected which are likely to be disease causing - Marfan syndrome - OMIM # 154700 and AR disease - Spinal Muscular Atrophy(SMA) - OMIM # 253300. During the first PGD cycle in the year 2013 (11.2013): 11 cumulus-oocyte complexes (COCs) were retrieved. 9 blastomeres were biopsied. All tested embryos were analysed.2 embryos were affected of SMA, 3 embryos were affected of Marfan syndrome.2 embryos were diagnosed as monochromy of chr5 carrying high risk paternal haplotype SMN1 gene. 2 embryos did not show PCR-amplification. The embryos were not re-analysed because they didn’t show optimal development. In this first PGD cycle no embryos were transferred. While there was no transfer in this case during the first PGD cycle, PGD still increases the chances of families at risk of transmitting serious genetic disorders to plan a healthy offspring.

P01.077-S
Targeted capture and massively parallel sequencing for PGD of monogenic diseases
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PGD is an important option for known carriers of monogenic diseases to deliver healthy babies. Polymerase chain reaction (PCR) is the most widely used method in PGD for monogenic disorders. However, in order to establish a robust PCR based PGD protocol, extensive preclinical experiments are needed to ascertain the efficiency and reliability of the procedure, which is a labor-intensive, time-consuming and costly process. Many clinical applications based on targeted massively parallel sequencing (MPS) have been reported. In this study, we explored the applicability of targeted MPS for PGD of monogenic disease. Isolated single lymphocytes were used to evaluate the genotyping accuracy of target-MPS at single-cell level at first. Then two families carrying mutation of the HBB causing β-thalassamia were tested using targeted MPS. Pedigree haplotype analysis was applied in the following analysis and double-blind design was used in the study. Over hundred of informative SNPs flanking both sides of the disease causing mutation were detected for each embryonic haplotype. The average interval between informative SNPs and gene mutation was within 10 kb. The final result was 100% consistent with the diagnosis provided by the reference laboratory, and the whole test was finished within a week. The new targeted MPS based method will make the PGD for monogenic disease more accurate, fast and more affordable, providing benefit to many patients requiring PGD for monogenic disease.

P01.078-M
Single cell segmental aneuploidy detection is compromised by S phase
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Carriers of balanced translocations are at high risk for unbalanced gametes which can result in recurrent miscarriages or birth defects. Preimplantation genetic diagnosis (PGD) is often offered to select balanced embryos. This selection is currently mainly performed by array comparative genomic hybridization (aCGH) on blastomers. Current methodology does not take into account the phase of the cell cycle, despite the variable copy number status of different genomic regions in S phase. To evaluate the accuracy of single cell array CGH, different cell cycle phases from three cell lines derived from patients with β-thalassaemia born on December 2013, a carrier boy for β-thalassemia carriers of balanced translocations are at high risk for unbalanced gamete more accurate, fast and more affordable, providing benefit to many patients requiring PGD for monogenic disease.

P01.079-S
Placental growth factor (PIGF) levels for preeclampsia prediction
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The aim of the study was to verify the possibility to predict preeclampsia (PE) after the 20th week of gestation by reliable cut-off levels based on PGF analysis at the time of pregnancy termination within weeks 24-41. Control PIGF levels for weeks 9-19 were derived from 800 cases; 100 PE sera comprised: 17 - PE weeks 24-29, 24 (30-33), 34 (34-37) and 25 (38-41). PIGF was examined by DelphiXpress Tm platform (PerkinElmer). Reliable PIGF control levels (3, 5, 25, 50, 75 and 95 percentiles) were established for weeks 24-19. For 1st trimester PE risk determination commercial software was used, for weeks 14-19 levels ≤ 3 or 5 percentiles were applied. Inverse relationship of decreased PIGF levels to the onset of PE and its clinical impact was documented. PIGF median level increased during pregnancy is significantly retarded. Levels of PIGF for PE (weeks 24-29) correspond to medians of the 11th week, PE (weeks 30-33) to week 15 and intermedi- ate/late PE to week 17. PIGF cut off levels for 100%/95% detection rate were success for PE weeks 24-29,50-30, 34-37, 38-41 are 61.6/46.2, 267.8/19.5, 262.3/180.3, 168.3/146.0 (all pg/ml). Combination of 1st trimester PE screening with software prediction of early intermediate and late PE with 2nd/3rd trimester PIGF examination could improve the complex care not only for PE, but also for other adverse risks of HELLP syndrome, SGA, pre- term delivery and IUGR. Increased detection of PE and perinatal morbidity and mortality. Our prior genome-wide transcriptomic analysis at the time of pregnancy termination within weeks 24-41. Conclusions: PIGF can be a potential marker for PE detection.
phism. This result demonstrates the high informative value of the integrative approach in studies of the genetic components of preeclampsia and show that allelic variations of the differentially expressed genes in placental tissue are associated with preeclampsia in different ethnic groups. Nevertheless, the clinical significance of these findings remains to be determined. This work was supported by the Russian Foundation for Basic Research (grant №14-04-01467).

P01.081-S

**Molecular understanding of the FMR1 premutation and Fragile X-associated Premature Ovarian Failure**

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CGG repeats expansion (55-200 units, premutation range) in the 5'UTR of FMR1 gene (Xq27.23) is associated with Fragile-X Tremor / Ataxia Syndrome and Premature Ovarian Failure (POF). FMR1 premutation represents the most significant single gene variant associated with POF; however, no studies are available to evaluate its pathogenic effect in the ovary context. As for FTTRE, an RNA-mediated toxic gain of function could be hypothesized for FX-POF. Human granulosa cells were transfected with plasmid containing 76CGG repeat elements in the 5'-UTR of EGFP reporter under the control of CMV immediate early promoter (CMV-76CGG-EGFP), or with CMV-EGFP as control. By RNA immunoprecipitation, the interaction of 76CGG for rCGG-Repeat Binding Proteins (rCGG-RBPs), previously identified, was tested. 76CGG solely immobilize precipitate with heat shock proteins, some hallmarks seen in proteins involved in paraplegias formation such as SNORD and HnRNPs, suggesting that in vivo premutated FMR1 mRNA differs from the wild type in making RNA-protein complexes. In this regard in premutated granulosa cells a deregulation of structural components paraplegias was observed suggesting an alteration of splicing. Furthermore an “heat shock like” response associated with a reduced cell viability in human ovary granulosa cells expressing expanded CGG mRNA was observed, indicating a toxic role of premutated mRNA itself in the ovaries. This work is supported by Telethon grant GGP009126.

P01.082-M

**DNA copy number variations in women with premature ovarian insufficiency**

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The cause of premature ovarian insufficiency (POI) remains unknown in many cases. In recent years many studies have focused on the genetic factors of POI. Conventional karyotyping analyses have identified several X chromosome regions that harbour genes critically required for normal functioning of the ovaries. Due to rise of DNA microarray technology, it has been shown that in addition to X chromosome rearrangements, POI is associated with aberrations in autosomal chromosomes. Single-nucleotide polymorphism (SNP) arrays are useful in identifying single genes and genome regions responsible for the onset of POI. The present study included 700 women with idiopathic POI with the cessation of ovarian function before 40 years of age. Women with a history of gynaecological surgery, cancer treatment and gestational diabetes were excluded. Genomic DNA samples were provided by Tartu University Hospital and Estonian Genome Centre and analysed for copy number variations (CNV) and runs of heterozygosity (ROH) using high-resolution SNP arrays. The majority of detected CNVs fall within the common polymorphic regions, while others have clinical significance and harbour previously reported and novel POI candidate regions and genes. Identified aberrations include a 24Mb Xp22.3-p21.3 hemizygous deletion and a novel microdeletion in 15q21.3. In addition to CNV, numerous ROH regions were detected, which may possibly contain homoygous mutations in POI associated genes. DNA microarrays are a suitable tool for evaluating genomic imbalances in POI women when compared to the conventional cytogenetic methods. The present study provides novel data on associations between the genomic variants and aberrations and POI phenotype.

P01.083-S

**Prenascan in Gennet, non-invasive prenatal test trisomy 13, 18, 21 with the possibility of sex determination and determining defects in sex chromosomes X and Y.**

The method is based on the evaluation of fragment’s relative abundance of cell-free DNA (cfDNA) in maternal plasma. The fetal fraction of the total free DNA is 5-25 % according to the stage of pregnancy. Extraction of cfDNA exploits the fact that cfDNA is significantly smaller than the maternal DNA, with fragments approximately 200bp in size. cfDNA subsequently subjects to whole-genome sequencing using next-generation sequencing platform based on the semiconductor sequencing technology. Examination method Prenascan is permissible between 10 and 21 weeks of pregnancy, only after genetic counseling. Diagnostic sensitivity of the method is about 96 % to detect the tested trisomy. The specificity of the test exceeds 99.7 %. Efficiency of the test is 99.9 % for trisomy 21, 98.9 % for trisomy 18 and 98.7 % for trisomy 13. The main limitation of the test is low concentration of cfDNA in maternal plasma. Test results can be distorted by foreign DNA if the mother received transfusion or transplantation of stem cells. From October 2012 to December 2013 1263 patients were tested. Positivity was found in 22.2 %, false positivity in 4 % of cases. False-positive patients were tested by amniocentesis or chorionic villus sampling with normal results.

P01.084-M

**A unique ring chromosome 20 in a fetus with a complex heart malformation**

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A 31-year old woman was referred at 20+6 weeks gestation because of abnormalities at the standard anomaly scan. Advanced ultrasound showed a double outlet right ventricle with transposition of the great arteries and a hypoplastic left ventricle. Furthermore a mandibular retrognathia was noticed. Aneuploidy of Chromosome 20 was confirmed by FISH. In addition the pregnancy was terminated at the parents request and external post-mortem examination revealed mild dysmorphic facial features including mild micrognatia and low set ears. SNP-array analysis showed a duplication deletion rearrangement in the long arm of chromosome 20. The interstitial duplication was 20.5 Mb and the adjacent deletion was 240 kb in size. Karyotyping revealed a ring chromosome 20 in all analysed cell. Chromosome in situ hybridisation analysis demonstrated that the duplication was inverted. Parental chromosomes were normal indicating that this unbalanced rearrangement occurred de novo. Only a few cases with a ring chromosome with telomere deletion and an additional inverted duplication have been described so far. These inv dup del chromosomes are the result of an asymmetrical breakage of a dicentric chromosome that has been formed to stabilize a broken chromosome 20. The ring formation represents a new mechanism, in addition to telomere capture, through which inv dup del chromosomes can stabilize. In conclusion this study presents a prenatal case with a ring chromosome 20, which presented with ultrasonicological anomalies initially.

P01.085-S

**A 3 years worldwide experience with Prenatal BACs-on-BeadsTM for trisomy diagnosis in over 9.500 pregnancies**


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Chromosomal microarrays are the gold standard in prenatal cases with ultrasounds abnormalities normal karyotype. In low-risk pregnancies their use is still controversial because of their diagnostic yield in relation to other diagnostic tests. Conventional karyotyping presented with ultrasonographic anomalies initially. In addition to telomere capture, through which inv dup del chromosomes can stabilize. In conclusion this study presents a prenatal case with a ring chromosome 20, which presented with ultrasonicological anomalies initially.
mosome abnormalities not covered by PNBoBs™ but detectable by karyotype. In 0.3% of cases PNBoBs™was reflected by FISH for further characterization. Overall abnormal results (PNBoBs™+karyotype) were observed in 9.1% of cases; a microdeletion/microduplication was retrieved in 0.7% of cases (n=69); the majority of them (68.1%) involved Di George syndrome critical region (deletion=32; duplication=15). 23 cryptic imbalances were found in low risk pregnancy leading to an additional diagnostic yield of about 1/246 in the low-risk pregnancies; 41 were detected in high risk pregnancies providing a 1.8% of additional detection rate (1/54). We will present incidence of cryptic imbalances stratified by indication for prenatal diagnosis that was surprisingly higher than expected basing on theoretical incidence (1/1700).

Detection of 7p14 deletion in a fetus with aortic coarctation: genetic and fetal morphological data

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We present the case of a fetus with aortic coarctation suspected by ultrasound at 26+6 gw, in which an interstitial deletion 7p12.3p14.2 was identified on amniotic fluid. The deleted region, confirmed by FISH and aCGH analysis, spanned in the range chr7:36,671,630-46,039,765bp, and involved several genes, as GL13, ccm2, GCK, tmdc3.

Decipher db shows at least 5 subjects with a microdeletion involving this region, all with delayed psychomotor development, dysmorphisms and abnormalities of the extremities. One of the subjects also presented cardiac phenotype.
The literature also reports some cases of del(7)(p14) and Greig Cephalopolysyndactyly, an autosomal dominant disorder associated with GLI3 aploinsufficiency, characterized by a distinct combination of craniofacial, foot and hand malformations. D’ligio et al reported a deletion 7p14 with cardiac anomalies (non-compacted myocardium, VSD, ASD and aortic valve dysplasia). Five other subjects, with cytogenetically visible deletions 7p14, had more severe findings as microcephaly, dysmorphism and mental retardation.

After several genetic counseling sessions with the equipe of the prenatal center, the parents decided for ToP.

Fetal autopsy identified cranio-facial dysmorphisms, a complex CHD with mitral valve prolapse, accessory spleen and horseshoe kidney.

This case underlines that ultrasound, genetic testing (including aCGH) and genetic counselling should be included in the protocol of pregnancies with fetal malformations, once again reinforcing the necessity for the different specialists involved in prenatal diagnosis to cooperate in the identification of a complex fetal phenotype, to offer a prognosis that may help parents in their pregnancy options.

Prenatal and Preimplantation Genetic Diagnosis for inherited cardiac diseases in the Netherlands: an overview

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Inherited cardiac diseases are associated with an increased risk of sudden cardiac death (SCD). Common hallmark is the variable disease expression and incomplete penetrance. Therefore the phenotype varies widely between and within families. Hence PND and PGD provide carriers of a mutation that causes an inherited cardiac disease the possibility of having a child without the familial gene mutation and reduce the risk of SCD in their children. The advice from the National Board for PGD indications is to be hesitant with PND in inherited cardiac diseases due to reduced penetrance and variable expression of the phenotype.

In this study, we evaluated the number of PND and referrals for PGD for inherited cardiac diseases in the Netherlands in the past 15 years. PND was performed for three different inherited cardiac diseases: Hypertrophic cardiomyopathy (HCM) (n=2), Dilated cardiomyopathy (DCM) (n=1) and Long QT syndrome (LQTS) (n=1). Couples came for PND intake for HCM (n=9), Arrhythmicogenic right ventricular cardiomyopathy (ARVC) (n=3), DCM (n=9), NCMM (n=1), LQTS (n=2). Brugada syndrome (n=2), and idiopathic VF (n=3). After intensive counseling one couple (DCM) choose to continue the PGD procedure. Another couple (idiopathic VF) started the procedure, but withdrew after the genetic test was made, because of ethical considerations. The other couples choose to fulfill their child wish by other means. The families opting for PND or PGD for inherited cardiac diseases have experienced a severe phenotype or a severe family history of SCD. Nevertheless, the number of patients continuing with PND and PGD is low.

High resolution chromosomal microarrays in prenatal diagnosis significantly increase diagnostic power


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Objective: The objective of this study was to determine for the first time the reliability and the diagnostic power of high-resolution microarray testing in routine prenatal diagnostics. Methods: We applied high-resolution chromosomal microarray testing in 464 cytogenetically normal prenatal samples with any indication for invasive testing.

Results: High-resolution testing revealed a diagnostic yield of 6.9% and 1.6% in cases of fetal ultrasound anomalies and cases of advance maternal age (AMA), respectively, which is similar to previous studies using low-resolution microarrays. In 3 (0.6%) additional cases with indication AMA an aberration in susceptibility risk loci was detected. Moreover, one case (0.2%) showed an X-linked aberration in a female fetus, a finding relevant for future family planning. We found the rate of cases, in which the parents had to be tested for interpretation of unreported copy number variants (3.7%), and the rate of remaining variants of unknown significance (0.4%) acceptably low. Of note, these findings did not cause termination of pregnancy after expert genetic counseling. The 0.4% rate of confined placental mosaicism was similar to that observed by conventional karyotyping and notably involved a case of placental microdeletion.

Conclusion: We conclude that high-resolution prenatal microarray testing is a reliable technique that increases diagnostic yield by at least 17.3% when compared with conventional karyotyping, without an increase in the frequency of variants of uncertain significance.

Noninvasive detection of a balanced fetal translocation from maternal plasma

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Noninvasive prenatal testing based on massively parallel sequencing (MPS) of circulating cell free DNA (cfDNA) from pregnant plasma offers a powerful tool for detecting fetal chromosomal aneuploidies and other copy number variations; however, copy neutral structural rearrangements have proven challenging. We aimed to detect and characterize a balanced fetal specific translocation event by sequencing cfDNA from maternal plasma.

Simulations were used to develop an algorithm which leverages base incremental changes in mapping characteristics of cfDNA to identify paired end reads potentially harboring structural rearrangements. We then applied this methodology on high coverage 100bp paired end data from cfDNA isolated from a 30 year-old pregnant donor carrying a fetus with a balanced translocation. Our algorithm identified the known translocation (p.1.21e-8) and discounted the likelihood of others, enabling the base specific localization of the breakpoints. Furthermore, while no evidence of chromothripsis existed, we identified a 6bp deletion present within der(8) which is absent from the der(11) reciprocal rearrangement after de novo assembly of 766 chimeric reads. Overall, we have demonstrated here the first proof of concept study detecting and characterizing a balanced fetal specific translocation by sequencing cfDNA from maternal plasma.
Identification of rare CNVs involving genes acting in oocyte maturation and differentiation in a cohort of patients affected by Primary Ovarian Insufficiency

P01.091-S

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Primary Ovarian Insufficiency (POI) is a heterogeneous group of disorders with an incidence of 1:10,000 women by age 20, 1:100 by age 30 and 1:10 by age 40. Despite the identification of numerous candidate genes in POI women, the genetic origin has been clarified only in about 20% of the patients. The patients showing the most severe phenotype, characterized by the absence of pubertal development and primary amenorrhea (PA) and 46,XX ovarian dysgenesis, are indeed rare but the search for genetic variations in this extreme phenotype may be more effective in identifying novel pathogenic mechanisms.

To unveil new POI causative genes we searched for rare high-penetration CNVs involving genes essential for ovarian function in a cohort of 46,XX patients affected by POI. Forty-three patients were processed by high resolution array-CGH. The remaining 98 CNVs were reported to date in healthy subjects according to the Database of Genomic Variants (DGV), and 11 CNVs already reported in DGV but relevant to gene content, for a total of 109 CNVs. Several of these genomic alterations include genes implicated in: meiotic resumption, oogonia maintenance, first-polar-body extrusion, DNA repair, β1cicle adhesion/migration regulation, actin remodeling, cholesterol endocytosis, Ca2+ homeostasis. Once characterized by other molecular and bioinformatics approaches, the results of this study are promising to expand the knowledge about the molecular pathways involved in POI pathogenesis and probably provide the basis for a more accurate genetic diagnosis of POI patients.

Conflict resolving QF-PCR and karyotyping due to structural aberration of the Y-chromosome

P01.092-M

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Quantitative fluorescent polymerase chain reaction (QF-PCR) is an accurate and efficient technique for rapid prenatal diagnosis of trisomy 13, 18 and 21 and aneuploidies of the sex chromosomes. Discordant results between QF-PCR and karyotyping have occasionally been reported, mostly due to mosaicism. A 20-year old female underwent chorionic villus sampling at 13 weeks’ gestation because of an increased risk of a chromosomal aberration after first trimester screening. Ultrasound investigation demonstrated no abnormalities. The results from the QF-PCR analysis were interpreted as a possible mosaic 45,XX/46,XY because of the low contribution of the Y chromosome. Conventional karyotyping of the LTC demonstrated a non-mosaic 45,XX. Since no abnormal findings were detected on ultrasound, a confined placental mosaicism was suggested as a possible explanation for the discordant findings. A subsequent amniocentesis revealed a normal male genotype with normal karyotyping, on the contrary, demonstrated a mosaic pattern with 45,X and a structural rearranged Y chromosome, probably an i(Yp). Additional FISH confirmed the presence of an isochromosome of the short arm of the Y chromosome. The case presented here demonstrates that caution should be taken when conflicting results are observed in CVS - even if a normal profile is obtained with QF-PCR in a follow-up amnioncensis. This case further illustrate that mosaicism for an abnormal cell line can result in a normal QF-PCR profile.

Persistent Elevated Population-based Rates of Congenital Malformations (CM) and Elevated Whole Body Counts (WBC) of 137Cs among Pregnant Women in the Polisia Region of the Rivne Province in Ukraine

P01.093-S

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Repeated implantation failure (RIF) is the main problem after using assisted reproductive techniques (ART). The main causes of RIF as a multifactorial problem include decrease in endometrial receptivity, defects of embryo or combinational. Successful embryo implantation depends on trophoblast proliferation, migration and invasion to the endometrium, all associated with vascular endothelial growth factor (VEGF) as the major protein in stimulating of angiogenesis. This study aimed to determine the association between VEGF+405G/C polymorphism and RIF in infertile women. The patients group included 74 women with >3 RIF and the control group consisted of 149 healthy fertile women. Genotypes and allele frequencies of VEGF+405G/C polymorphism were determined by PCR-RFLP method and verified by Sanger sequencing. The frequencies of GG genotype was significantly higher in patients than controls (p<0.001). GG genotype frequency was higher in controls (p<0.001). The frequency of GC genotype did not show any difference between groups. Considering the wild allele was more frequent in controls while frequency of G as the mutant allele was higher in patients (p<0.001). The VEGF+405G/C genotype may influence embryo implantation and lead to

The VEGF+405 G/G Genotype may Influence Embryo Implantation in ART

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VEGF+405G/G Genotype may Influence Embryo Implantation in ART

P01.094-M

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Recurrent miscarriage (RM) is one of the important problems of modern reproductive medicine. RM affects approximately 1–5% of all couples trying to conceive. Various factors have been identified that influence miscarriage, including parental chromosomal abnormalities, endocrine dysfunction, and others. However, 50% of cases fail to reveal an identifiable cause and are therefore classified as idiopathic.

We investigated the association of polymorphisms in oxidative stress-related genes with idiopathic RM. 331 idiopathic RM patients and 197 controls were genotyped for ABRC1, COMT, GPX4, and OGG1 genes. Cumulative gene risk score analysis demonstrated that more than three gene alleles in the genes ABRC1, COMT, GPX4, and OGG1 were associated with idiopathic recurrent miscarriage $P = 1.2 \times 10^{-3}$, OR $= 1.97$, 95% CI 1.31–2.97. In silico data analysis by GeneMANIA revealed genetic, physical, pathway, and coexpression networks for these four genes. The current study shows that cumulative effects of genetic variability in oxidative stress-related genes may play a role in the recurrent miscarriage with no known etiology.

**P01.098-M**

Results of conventional karyotyping and 5 thrombophilic gene mutations in a Turkish population with recurrent pregnancy loss

**Y. Özen, H. García, H. Tastar, S. Uslu, E. Gönçü, D. Eker; Trakya University, Edirne, Turkey.**

Recurrent pregnancy loss (RPL) is the spontaneous loss of clinically established intra-uterine pregnancy before the fetus has reached viability. Here we present the cytogenetic data of couples and prevalence of 5 thrombophilic gene mutations among 175 women referred with RPL to our center.

Chromosome analyses were performed in 179 couples with two or more consecutive miscarriages before 24 weeks' gestation between 19.10.2011-31.12.2013 using G-banding, MTHFR c.677C>T and c.1298A>C, Factor II c.20110G>A, Factor V (Leiden) c.1691G>A and plasminogen activator inhibitor-1 (PAI-1) 4G/5G genotypes were determined using SNP primers designed by the manufacturer (NLM Diagnostics, Italy). Melting curve analysis has been performed with labeled probes using Real-Time PCR method (Qiagen, RotorGene).

Table 1 shows the details of the cytogenetic findings of couples with RPL. The frequencies of studied 5 thrombophilic gene mutations in women referred with RPL were summarized in Table 2. The prevalence of parental chromosomal aberrations was greater in our study (8.4%) than in most studies in the literature, which quote a 3%–5% prevalence. Comparing our results of 5 thrombophilic gene mutations with other studies we suggest considering the mutations in FV Leiden, Prothrombin 20210A, MTHFR c.677C>T, Factor II c.20110G>A, Factor V (Leiden) c.1691G>A and PAI-1 4G/5G genotypes in ART candidates. Since this is the second report on association of these polymorphisms with RIF, further studies in different ethnic populations require for determining this association.

**P01.095-S** Gene-gene interactions and the risk of recurrent miscarriages in EG-VEGF and its receptor genes

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Endocrine gland-derived vascular endothelial growth factor (EG-VEGF) and its receptor genes (PKR1 and PKR2) play an important role in human early pregnancy. Our previous study showed that PKR1 and PKR2 polymorphisms are associated with recurrent miscarriages (RM). This study was conducted to find EG-VEGF PKR1 and PKR2 variants in the coding regions of idiopathic RPL patients and further evaluate gene-gene interactions in 3 genes. Two hundred and ninety one blood samples from 142 RPL women and 149 controls were nucleotide sequenced in the coding regions of EG-VEGF, PKR1 and PKR2. Gene-gene interaction was evaluated in 3 gene variants using multifactor dimensionality reduction (MDR) method. One each nonsynonymous variant of 3 genes were identified, and PKR1 (c.379G>A) and PKR2 (c.331T>A) were significantly associated with idiopathic RM (p=0.006 and p=0.002, respectively). Genetic interactions were founded not only between PKR1 (c.379G>A) and PKR2 (c.331T>A), but also among EG-VEGF (V67I), PKR1 (c.379G>A) and PKR2 (c.331T>A) (p=0.01 and p=0.01, respectively). Women carried low-risk genotypes reduced 77% risk of experiencing miscarriages between PKR1 (I379V) and PKR2 (V331M), but also among EG-VEGF (V67I), PKR1 (I379V) and PKR2 (V331M) (p=0.01 and p=0.01, respectively). Women carried low-risk genotypes reduced 77% risk of experiencing miscarriages compared with those carried high-risk genotypes. The present study corroborates the clinical relevance of the EG-VEGF system in human early pregnancy, and provides evidence for the gene-gene interactions of EG-VEGF and PKR variants.

**P01.096-M** Study of chromosomal alterations and polymorphisms of MTHFR, Factor V and Prothrombin genes in patients with recurrent miscarriage referred to Royan Institute

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Introduction: Recurrent miscarriage (RM) is defined as two or more consecutive pregnancy losses before 20 weeks of gestation which is an important clinical problem, with an incidence of 1%–3% among couples wishing to conceive. Various factors have been identified that influence miscarriage, including parental chromosomal abnormalities, endocrine dysfunction, and others. However 50% of cases fail to reveal an identifiable cause and are therefore classified as idiopathic.

We investigated the association of polymorphisms in oxidative stress-related genes with idiopathic RM. 331 idiopathic RM patients and 197 controls were genotyped for ABRC1 rs1045642, CYP11A1 rs1804943 and rs4649693, COMT rs4680, CAT rs17880664, GCLC rs17883901, GPX4 rs713041, NFR2 rs6721961, SOD2 rs48480, and OGG1 rs1052131. A protective effect of COMT rs4680-G allele on RM was shown in individual SNP analysis: $P = 0.0016$, OR $= 0.47$, 95% CI 0.29–0.75. The multi-factor dimensionality reduction (MDR) approach revealed gene-gene interactions for ABRC1, COMT, GPX4, and OGG1 genes. Cumulative gene risk score analysis demonstrated that more than three gene alleles in the genes ABRC1, COMT, GPX4, and OGG1 were associated with idiopathic recurrent miscarriage $P = 1.2 \times 10^{-3}$, OR $= 1.97$, 95% CI 1.31–2.97. In silico data for Genome analysis revealed significant, physical, pathway, and coexpression networks for these four genes. The current study shows that cumulative effects of genetic variability in oxidative stress-related genes may play a role in the recurrent miscarriage with no known etiology.

**P01.097-S** Polymorphisms in oxidative stress related genes and recurrent miscarriage

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Recurrent miscarriage (RM) is one of the important problems of modern reproductive medicine. RM affects approximately 1–5% of all couples trying to conceive. Various factors have been identified that influence miscarriage, including parental chromosomal abnormalities, endocrine dysfunction, and others. However 50% of cases fail to reveal an identifiable cause and are therefore classified as idiopathic.

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The role of HLA genes in complex immunogenetic predispositions for idiopathic recurrent pregnancy loss

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Influence of HLA-system on reproductive losses is considered from the standpoint of search for specific HLA genes, the similarity of spousal HLA-antigens, or the study of modulating properties of HLA-system in the gene network complex. Aim. To analyze the distribution of allelic polymorphism of the HLA-DRB1, DQA1, DQB1 genes and HLA-G 14-bp insertion/deletion polymorphism in married couples with RPL. Results. Complex analysis of distribution and frequency of allelic variants of genes HLA-DRB1, HLA-DQA1 and HLA-DQB1 was conducted for 200 married couples with recurrent pregnancy loss of undefined genesis. It was determined that allele DRB1*0301 is an allele-aggressor in the group of women with RPL, and possessing this allele presents three-fold increased risk of idiopathic pregnancy loss for a woman (OR = 3.4; 95% CI: 1.0–11.1). The results demonstrated probable significant increase in frequency of genotype +14 bp/+14 bp of HLA-G 14-bp insertion/deletion polymorphism (p < 0.05) in women with RPL against the control group. The study demonstrated over two-fold increase of the risk of pregnancy loss for partner-carriers of homozgyous genotype by allele of insertion/+14 bp 3′ UTR region of HLA-A gene (OR = 2.65; CI: 1.95–7.06; 6-6.68). It was determined that the increase in total homology of spousals by 50% and more in allelic polymorphism in loci HLA-DRB1, HLA-DQA1 and HLA-DQB1 presents twelve-fold increased risk of idiopathic pregnancy loss for a woman (OR = 12.28; CI:1.63-100.27).Conclusions. Changes in the major hystocompatibility complex genes can cause the failure of female reproductive function and lead to the early fetal loss.

Association study of miR-196a2 rs11614913 polymorphism with risk of idiopathic recurrent pregnancy loss in Iranian women

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Introduction: Recurrent Pregnancy Loss (RPL) is defined as the occurrence of two or more consecutive pregnancy loss prior to 20th week of gestation. There are several leading causes of RPL, including uterine anatomical defects, genetic factors, infectious, immunological, environmental and blood dyscrasias. However, despite in a large number of cases no cause has been identified and is classified as idiopathic. Recent studies have implicated miRNAs in endometriosis, preeclampsia, infertility and RPL. Therefore, the aim of the present study was to investigate the association of miR-196a2C>T (rs11614913) with RPL.

Methods: We conducted a case-control study of 185 Iranian women: 85 patients with at least two unexplained consecutive pregnancy losses and 100 healthy controls with at least one live birth and no history of pregnancy loss. Patients with recurrent pregnancy losses due to anatomic, hormonal, chromosomal, infectious, autoimmune, or thrombotic causes were excluded from the study group. Genotyping was performed using tetra-primer amplification and reverse mutation system PCR (T-ARMS-PCR). Results: Significant difference in distribution of miR-196a2 rs11614913 genotypes was observed in RPL patients in comparison to controls, with P-value of 0.04 and odds ratio equal to 2.69 (95% CI: 1.03-7.03). Conclusion: We provide evidence for association between genetic variation in miR-196a2 and recurrent pregnancy loss. Further studies will be required to validate the significance of the studied genetic variation in diverse ethnic populations.
Sex chromosome classification by cell free DNA analysis of maternal plasma in a general obstetrical population


Objectives: To examine the performance of non invasive prenatal testing (NIPT) by sequencing maternal plasma cell-free DNA for fetal sex chromosome classification in a general obstetrical population. Study Design: Blood samples were prospectively collected from pregnant women at 21 US sites in the Comparison of Aneuploidy Risk Evaluation (CARE) study (clinicaltrials.gov NCT01663350). Patients undergoing serum screening for fetal aneuploidy were followed to birth. Sex chromosome status was classified blindly by NIPT and compared to clinical outcome based on newborn physical examination, or karyotype if performed. Sensitivity, specificity and exact 95% confidence intervals based on the Clopper-Pearson method were calculated.

Results: Sex chromosome classification by NIPT and outcomes were available for 1,948 subjects. 1,935 (99.3%) were classified as XX or XY by NIPT. Sensitivity and specificity for predicting female were 97.7% (CI: 96.6-98.6) and 99.2% (CI: 98.4-99.6), respectively, and 99.2% (CI: 98.4-99.6) and 97.7% (CI: 96.6-98.5) for predicting male. Two fetuses classified XY by NIPT had ambiguous genitalia at birth; one showed mosaic karyotype 45,XX/46,XY. NIPT classified 13 (0.67%) samples with sex chromosome aneuploidy (SCA). Nine with Monosomy X (MX) - all bearing normal-appearing female infants; one had prenatal karyotype 46,XX. Three cases classified XX by NIPT appeared female at birth and one classified XY appeared male.

Conclusion: Results demonstrate that NIPT has excellent performance in the general obstetrical population for sex chromosome classification. Maternal contribution or co-twin demise may explain discordance. NIPT is useful in cases of ambiguous genitalia, pregnancies at-risk for sex-linked disorder, or when SCA is not readily detectable by ultrasound/newborn examination.

The XY female: prenatal discrepancy between phenotype and genotype in bichorial diamniotic twin, what’s the differential diagnosis in a general obstetrical twin? E. Pompili; Medical Genetics Unit, St Oronzo Malpighi Hospital, University of Bologna, BOLOGNA, Italy.

Sexual differentiation depends upon a series of complex events. A female healthy pregnant woman underwent a routine prenatal ultrasonographic examination at 12 weeks’ gestation that showed a bichorial diamniotic twin pregnancy with female sex fetuses. Upon personal desire of the mother, amniocentesis was performed at 15+1 weeks of gestation. Ultrasonographic examination at the time of the procedure confirmed, in both fetuses, normal fetal anatomy with female external genitalia. However, amniotic fluid karyotype analysis was discordant with the female ultrasonographic sex revealing a 46,XY male karyotype in both of them. Fetal re-examination was performed with 3D and 4D ultrasound for fetal sex, and a female genitalia was confirmed again. One hypothesis was the search for the presence of mutation on SRY gene on chromosome Yp11.3, causing 46,XY complete gonadal dysgenesis or XY sex reversal. The analysis has revealed no mutation in the analyzed gene. The other hypothesis was the analysis of androgen receptor (AR) gene. The molecular analysis showed a single nucleotide deletion in codon 766, that resulted in a frame-shift mutation in the steroid binding domain of the androgen receptor P766fsX. As expected, the carrier mother had both normal and mutant AR genes. After genetic counseling the couple decided upon voluntary termination of the pregnancy at the 20th week of gestation. The autopsy examination of both fetuses confirmed the external female genitalia and revealed the presence of placental hypoplastic uterus and abdominal testes that confirmed by histopathological examination.

Prenatal detection of a case with Simpson-Golabi-Behmel syndrome as a consequence of GP53 and GP45 gene duplications

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We describe the case of Simpson-Golabi-Behmel syndrome (SGBS) discovered prenatally. Fetal scan in 23rd week of pregnancy identified male fetus with macrosomia, macrocephalia, dilatation of 3rd and 4th ventricle, hypercholesterogenic gut, agenesis of corpus callosum, cystic dilatated interhemispheric fissura, macromesencephaly and liver and kidney polyhydramnion. Aneucripnta was performed. Molecular karyotyping using ISCA 4x180K arrays revealed two intersticial microduplications on Xq26.2 in size of 574 kb and 115Kb. A larger microduplications encompassed four exons of OMIM genes, including whole GP4C4 gene. A smaller microduplication was located within the GP3C2 gene and embrace exon 6 and 7 of the longest transcript of this gene.

We hypothesized that these duplications were inherited from the mother. Persistance with this SGBS are frequently affected by embrogenic tumors of kidney. Also the mother had Wilms tumor in her childhood. The pregnancy was terminated because of ruptured amniotic membrane and amniotic leakage. An autopsy of the infant confirmed organomegaly seen on ultrasound. In addition, a ventricular septal defect, a complete agenesis of the corpus callosum, cerebellar hypoplasia were observed. Distinct facial features were also present, including hypertelorism, short nose with broad nasal bridge, macrostomia and macroglossia, nail hypoplasia, and an extra rib. GPC3 and GPC4 are the two genes in which mutations are known to cause SGBS. There are only few reports on duplications of GP3C gene and one report of duplication of CP4C4 gene causing SGBS in the literature. Mostly deletions and mutations of GP3C gene are described. In our presentation role of two genes involved in SGBS is discussed.

The correlation between transcript expression levels of nuclear encoded and mitochondrial encoded genes in single human oocytes during oocyte maturation

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Introduction: Impairment of human oocyte maturation during oocyte maturation is a cause of infertility in infertile women. Therefore, Oocyte maturation is important in successful reproductive outcome of assisted reproductive technologies (ART). Mitochondria, which are the most organelle in the oocytes, have in important role during oocyte maturation. Little is known about mitochondrial genomes during oocyte maturation. This aim was to identify the correlation between transcript expression levels of nuclear encodred and mitochondrial encoded genes during oocyte maturation.

Conclusion: The correlation between the relative expression levels (P>0.05), whereas there was significant correlation between the relative expression levels of nuclear (TFAM, NRF1) and mitochondrial (MT-CO1) genes and the nuclear respiratory factor 1 (NRF1) as well as the mitochondrial transcription factor A (TFAM) genes, which using by single-cell real-time PCR during human oocyte maturation. 27 consenting women aged 21-35 years, with male factors were selected for ovarian stimulation procedures.

Results: at the germinal vesicle (GV) stage oocytes, no significant correlation between the relative expression levels (P>0.05) whereas there was significant correlation between the relative expression levels of nuclear (TFAM,NRF1) and mitochondrial (MT-CO1) encoded genes at the stages of metaphase I (MI) and metaphase II (MII) stages of oocytes (P<0.05).

Conclusion: Human oocyte maturation is associated with the increased correlation between transcript expression levels of nuclear (TFAM, NRF1) and mitochondrial (MT-CO1) encoded genes. Thus, any defect correlation between transcript expression levels of nuclear mitochondrial genes leads to impaired developmental oocyte competence.

Prenatal molecular diagnosis of skeletal dysplasias - a single center experience


Skeletal dysplasias are a large, heterogeneous group of conditions involving the formation and growth of bone. Some skeletal dysplasias are associated with additional abnormalities in other organ systems.

Prenatal diagnosis relies primarily on fetal ultrasound findings, but molecular analysis is used to confirm the presumptive diagnosis and to determine the recurrence risk.
The diagnosis of a substantial number of the most frequent skeletal dysplasias can be confirmed in a short period of time by molecular genetic analysis of the involved genes (e.g. thanatophoric dysplasia, diastrophic dysplasia, campomelic dysplasia, Ellis-van Creveld syndrome or hypophosphatasia). We have analyzed a total of 300 cases diagnosed previously by an expert team of experienced sonographers and human geneticists. The study was carried out at a single tertiary center during the last 20 years. We demonstrate clinical findings and molecular genetic data and construct work-ups for the diagnosis of “difficult” cases (e.g. short rib-polydactyly syndromes, Filamin B associated skeletal dysplasia). Molecular genetic testing was conducted in 186 cases. We were able to establish a final diagnosis in 118 cases - which is equivalent to a detection rate of 65%. Some cases are exemplified with clinical, radiological, pathological and molecular genetic data. We want to point out the importance of molecular genetic diagnosis for confirming the clinical diagnosis of skeletal dysplasias and providing exact information for genetic counselling.

P01.109-S
Susceptibility loci for neurodevelopmental disorders - genetic counseling and pregnancy management

Objectives: SNP genomic array may detect susceptibility loci for neurodevelopmental disorders (SL) with possibly an increased but unquantified phenotypical risk. This study evaluates the effect of releasing the SL to pregnancy management. Psychological aspects will be reported separately.

Method: Every patient received pre-test counseling with “all pathogenic results will be communicated.”

The post-test genetic counseling concentrated on the phenotype of the particular SL, its incidence in the normal and affected postnatal population and the difference between postnatal and prenatal ascertainment. Targeted parental array testing was offered. Extensive ultrasound (US) examination was offered when the SL (postnatally ascertained) was associated with physical abnormalities. Psychological help was available in all cases if needed.

Results: In 36 cases out of 2108 ongoing pregnancies a SL was found. 2. For patients with fetal US abnormalities the SL was usually considered as less important. The US abnormalities were the reason for TOP. 3. The inherited nature of SL seemed reassuring.

Conclusions: In 5.5% (2/36) of the SL cases the pregnancy was terminated due to the presence of the SL. Further follow up of the families and their children is needed to evaluate the significance of prenatal diagnosed SL, to offer early intervention when neurodevelopmental disorders emerge and to evaluate the psychological impact of prenatally discovered SL.

P01.110-M
Origin of multilocus methylation defects of imprinted genes in first-trimester spontaneous abortions
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Genomic imprinting is one of the most important epigenetic mechanisms of fetal growth regulation. Our studies demonstrated that multilocus methylation defects (MLMD) at imprinted genes may be responsible for pathology of early embryonic development. The aim of the present research was analysis of origin of MLMD in first-trimester spontaneous abortions (SA). Chorionic villus samples (CVS) and extraembryonic mesoderm (EM) were collected from 217 SA from women who underwent abortion procedures. Induced abortion (IA) with normal karyotype (n = 60) were investigated as a control group. The DNA methylation patterns of 7 imprinted genes were analyzed in both tissues indicates about mistake reprogramming in the primordial germ cells during gametogenesis. Whereas tissue-specific compartmentalisation of epimutations allows suggesting independent sporadic epigenetic events in various embryonic germ layers after its divergence. No epimutations were found in the 60 IA samples. Somatic MLMD in SA occurred more frequently than germlinal epimutations (129 epimutations per 1808 alleles vs. 32/904; p<0.001). Multiple somatic epimutations were found more frequently in the EM (52/904; 31/904) than in the CVS (32/904; 14/904; p<0.05). Multiple hypomethylation in both tissues occurred more frequently than hypermethylation (96/1808; 33/1808; p<0.001). Thus, abnormalities of imprinting maintenance in the EM cells derived from epiblast progenitors are associated with fetal loss in first-trimester.

P01.111-S
Gonadal mosaicism for structural autosomal rearrangements with non-centromeric breaks: data from 76 carriers suggests male-specific selection against abnormal cell lines and association of clinical manifestation with a high proportion of abnormal cells
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Mosaicism for chromosomal structural rearrangements (Rea) is rare. There is much to learn about the timing and mechanisms of Rea formation and maintenance, and clinical manifestation. The question as to whether the proportion of abnormal cells in cultured blood is significant remains open. A recent study showed a strong female preponderance among carriers of mosaicism for Rea (Kovaleva NV. AJMG,136A:401-13: Objectives: (i) Comparative analysis of the male/female ratio in carriers of gonadal mosaicism (GM) for balanced and unbalanced Rea, (ii) comparative analysis of the proportion of cells with unbalanced Rea in blood cultures of asymptomatic and affected carriers. Method: Review of mosaicism for normal line/ structural Rea cases of known sex identified from the literature. Results: (1) Among carriers of GM for balanced Rea (all asymptomatic) there was a typical male predominance, 18M/15F, unlike the strong female predominance among carriers of GM for unbalanced Rea (both asymptomatic and affected), 9M/34F p<0.001. (2) Only one of eight male carriers with poor reproductive history was reported to have sterility, the others had partners with spontaneous/habitual abortion. (3) Seven of eight (88%) affected carriers of unbalanced Rea displayed a high proportion (≥50%) of abnormal cells compared to 2/27 (8%) in asymptomatic carriers, p<0.01. Conclusions: A strong female prevalence among carriers of GM for unbalanced Rea suggests male-specific selection against abnormal cells rather than impairment of male gametogenesis. A high proportion of abnormal cells detected in cultured T-Lymphocytes is associated with clinical manifestation of chromosomal imbalance.

P01.112-M
Parental subfertility is not associated with an increased risk of a de novo mutation or microdeletion in the offspring

Background Children born after in vitro fertilisation (IVF) with or without intracytoplasmic sperm injection (ICSI) are at increased risk for congenital anomalies. Recent publications suggest that the underlying cause of parental subfertility is mainly responsible for this risk increase, rather than the IVF/ICSI procedures, but it is unclear how. Our study aimed to identify whether offspring of subfertile couples are at increased risk for a de novo mutation or microdeletion.

Materials and methods Data were used from the birth defects registry Eu-roc: Northern Netherlands. We included malformed foetuses and children born between 1997-2010 (N=5709). Of those, 5249 were born to fertile parents and 61 (1.1%) had a monogenic mutation and 61 (1.1%) had a de novo chromosomal microdeletion. Parental subfertility was not associated with de novo mutations (OR 0.88, 95%CI 0.47-1.63) or microdeletions (OR 1.04, 95%CI 0.41-2.60). Subgroup analyses showed that IVF or ICSI alone were not associated with a de novo event either. Adjustment for paternal and maternal age did not change the results.

Results From the 5709 malformed cases, 156 (2.7%) had a monogenic condition resulting from a de novo mutation and 61 (1.1%) had a de novo chromosomal microdeletion. Parental subfertility was not associated with de novo mutations (OR 0.88, 95%CI 0.47-1.63) or microdeletions (OR 1.04, 95%CI 0.41-2.60). Subgroup analyses showed that IVF or ICSI alone were not associated with a de novo event either. Adjustment for paternal and maternal age did not change the results.

Conclusion Parental subfertility, IVF and ICSI are not associated with de novo mutations or microdeletions in offspring. These results suggest that the previously established increased risk of congenital anomalies after IVF/ICSI is not explained by an increase in de novo events.
P01.113-S
Prenatal detection of TAR syndrome
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Thrombocytopenia-absent radius (TAR) syndrome is a rare genetic disorder that is characterized by the absence of the radius bone in each forearm and a markedly reduced platelet count that results in life-threatening bleeding episodes (thrombocytopenia).

Rare proximal microdeletions of 1q21.1 are found in the majority of patients but are also found in unaffected parents. Recently it was shown that TAR syndrome is caused by the compound inheritance of a low-frequency non-coding SNP and a rare null allele in RBM8A.

We present a molecular and ultrasound and pathological findings in a case of TAR syndrome diagnosed prenatally. In the first pregnancy (2010), ultrasound examination at 22 weeks of gestation revealed bilateral absence of the radials, pregnancy was terminated. A 1q21.1 microdeletion including HFE2, PEX11B and CD160 genes was found using MLPA. Both parents were analyzed in order to determine the parental origin of the deletion, which was subsequently identified in the healthy mother. The second pregnancy (2012) ended in miscarriage. The third pregnancy (2013) with the same ultrasound findings, was terminated. Then the Sanger sequencing was used to analyze the DNA sequence of the region spanning the 5′UTR and the first intron of the RBM8A gene in the fetuses (2010, 2013) and parents. Genotyping of the sequence demonstrated the presence of compound heterozygote for the 5′UTR (rs139428292) and intronic (rs201779890) SNPs in the father. Both fetuses had compound inheritance of an RBM8A SNP (rs201779890 G>C) and a deletion in the 1q21.1 region. Now is the family in the PGD process.
This confirms a strong collaboration between geneticists and gynecologists specialized in ultrasonography and further analysis of discordant results.

P01.118-M

Performance of in-house non-invasive aneuploidy test using benchtop next generation sequencing system

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Since the discovery of cell-free fetal DNA in 1997 by Dennis Y.M Lo, the main goal was formulated - a reliable method for non-invasive fetal aneuploidy detection. The arrival of next generation sequencing technologies finally gave scientists a proper tool to reach this goal. Today, so called large next generation sequencing instruments are used for the so called non-invasive prenatal aneuploidy testing. Few years ago, so called benchtop next generation sequencers were introduced, which allowed researchers worldwide to start adopting wide range of novel methods benefiting from next generation sequencing technology. In our study, we generally considered not to be sufficiently parallel for non-invasive prenatal ploidy test.

In our study, we used one of benchtop next generation sequencers, the MiSeq by Illumina, to test its ability to detect aneuploidy. Plasma DNA from pregnant was isolated, fragment libraries were prepared and sequenced in multiplex setup. Data was analysed using in-house pipeline. A set of 20 samples (average read count 2 870 322 with SD 603 708) high risk group pregnancies with confirmed euploid karyotype was used for training of our analysis pipeline. Analysis of testing set including 5 samples with T21 karyotype resulted in 100% specificity and 100% sensitivity. Based on our results we believe that performance of benchtop next generation sequencers is sufficient for non-invasive prenatal aneuploidy testing. In addition, the inherently higher flexibility of benchtop systems could be of benefit for short turnaround and low sample throughput demands. This research was supported by ERDF grant with ITMS 26240220067.

P01.119-S

12 Mbp chromosomal gain on 7p detected prenatally without major dysmorphic features

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A case of a 25-year-old woman, with a history of two pregnancy losses, presented with a weight of 2340g (P38), length 46.0cm, OFC 31.7cm and AP length 9.2cm. A chromosome analysis was performed. The mother showed the same duplication on chromosome 7p which was inherited from her mother. A karyotype of the fetus was 46,XY, +7, +3, +12, with the chromosome 7p segment duplication of about 12 Mbp. The centromere was not detected. Microarray analysis of placenta DNA gave a positive result for the 12 Mbp segmental duplication. The parents decided to terminate the pregnancy and foetal autopsy was required. Several polymorphic variants were described in human chromosome 15 including increased amounts of short arm heterochromatin (ph+), interpreted as a normal polymorphism. In the majority of cases partial trisomy 6q results from a balanced chromosomal rearrangement in one of the parents, usually of maternal origin. There have also been rare cases in which partial trisomy 6q has appeared from spontaneous (de novo) errors very early in embryonic development. The authors compared the cytogenetic and the foetal autopsy findings with those described in the literature. New every case of a rare chromosome alteration should be reported in order to establish a genotype/phenotype correlation, improving risk evaluation and genetic counseling.

P01.121-S

Assessment of chromosomal changes in trophoderm cells in relation to maternal age

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Unbalanced chromosomal changes in embryos are one of the main causes of low human fecundity and with their incidence presumably increasing in the future. A proper risk assessment of maternal age in the aim to assess the frequency of whole chromosomal and segmental changes in relation to maternal age, we evaluated 224 trophoderm samples by aCGH (for details see table below). Our results showed a statistically significant increase in embryos with abnormal chromosomal results with advancing maternal age. Overall, 539 chromosomal changes involving similarly all chromosomes, with slightly higher frequency on chromosome 15, 16, 21, 22 were described. The frequencies of losses and gains were not significantly different. The rising complexity of changes with higher maternal age was caused predominantly by increasing numbers of whole chromosomal abnormalities whereas frequency of segmental changes was shown to be independent on maternal age.

<table>
<thead>
<tr>
<th>Group (according to maternal age)</th>
<th>Mean No of whole chromosomal changes per abnormal embryo</th>
<th>Mean No of segmental changes per abnormal embryo</th>
<th>Mean No of whole chromosomal changes per abnormal embryo</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) ≤ 32y</td>
<td>30/60 (50%)</td>
<td>45/83 (54%)</td>
<td>30/60 (50%)</td>
</tr>
<tr>
<td>(2) &gt; 32y</td>
<td>63/94 (67%)</td>
<td>60/221 (36%)</td>
<td>141/221 (64%)</td>
</tr>
<tr>
<td>(3) ≥ 40y</td>
<td>55/70 (79%)</td>
<td>49/235 (21%)</td>
<td>186/235 (79%)</td>
</tr>
</tbody>
</table>

* (2) vs (1) P 0,04
* (2) vs (1) P 0,006
** (3) vs (1) P 0,00008
** (3) vs (1) P 0,0000005

P01.122-M

Our contribution to rescue line hypothesis for a case of Turner syndrome

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Turner syndrome (45,X) is the only viable monosomy in humans, about 1% of recognized concepts with this karyotype survive to live birth. Ultrasound examination in 12th week of gestation disclosed hygroma colli cysticum with initial signs of hydrops. The NT was 6.2 mm. The QP-PCR analyses from na-
tive CVS was 46,XY. The long-term CVS cultivation revealed 45,XY as well as QF-PCR and FISH. The same results were found in post mortem examination of muscle cells cultures. These findings suggest that in trisomy21 embryonic tissue, the embryonic cells have a higher risk of complications during pregnancy (OR 3.26; 95% CI 1.07, 9.91; p=0.032).

Comprising the allele frequencies of VKORC1 gene rs9926231 across different populations revealed that its are relatively close in Moldovan populations to those reported for Caucasians and Turkish. The allele frequencies of VKORC1 gene rs9934438 are statistically significant different from data available on NCBI-site, NCP, MAssSource.

Conclusion: VKORC1 gene rs9934438 is a statistical associated with recurrent pregnancy loss and increasing the risk of complications during pregnancy.

P01.126-M

The application of post-light semiconductor-based next-generation sequencing in clinical cases of preimplantation aneuploidy screening (PGS) with fresh embryo transfer

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Screening of all chromosomes is now a gold standard in PGD. Although NGS techniques are today best method of choice they require more than 24 hours to perform, consequently blastocyst vitrification is needed. 8 couples with the average maternal age of 34.4 was referred to PGS procedure from 08/2013. All together 28 blastomeres were biopsied. The short duration of the procedure allowed fresh embryo transfer without need of blastocyst vitrification. 7 out of 8 cases resulted in pregnancy in first cycle giving pregnancy rate of 87.5%. 3.5 blastomeres on average per cycle were biopsied, resulted in 1.25 blastomeres on average per cycle with no aneuploidy detected, 1.5% of embryos were euploid. We not only used cutting-edge technology in the field of PGD but we further and designed and perfomed in clinical IVF-PGD procedure innovative protocol adjusted to single blastomere biopsy and fresh transfer. The additional benefit is the cost 3 times lower in comparison to aCGH. Developing PLS-NGS technology require use of the latest chemistry and software updates to improve analysis quality. The highest standards and stringency in quality system of results is required to avoid diagnostic failure. New technology of PLS-NGS possess strong research potential allowing for generation of large amount of data in scale of hours. Our innovative single-plot short-time protocol require only single blastomere biopsy and is adjusted to fresh embryo transfer. We put efforts in increase of reproduction success rate by increase implantation and decrease miscarriages rates and this aspect need to be followed up.

P01.127-S

Analysis of the AZF region of Y chromosome in Slovak men with azoospermia

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Y chromosome microdeletions are the most common genetic cause of male infertility and screening for these microdeletions in azoospermic men is now standard practice. Analysis of the Y chromosome in men with azoospermia or severe oligozoospermia has resulted in the identification of three regions in the euchromatic part of the long arm of the human Y chromosome (Yq11) that are frequently deleted in men with otherwise unexplained spermatogenenic failure. PCR analysis of microdeletions in the AZFa, AZFb and AZFc regions of the Y chromosome was used Deyser AZF set. For all markers in one multiplex PCR reaction fluorescently labeled primers were used. For automated visualization and identification of the STS markers we used genetic analyzer 3500xl (Applied Biosystems). We reported 9 cases of deletions in the AZF region (7.3%). We have recorded particular types of deletions in each region AZFa,b,c but also a complete deletion of the whole AZF region. The most frequent was microdeletion(s) in the AZFr region. In Slovak azoosperemic patients the percentage of microdeletions in the AZF region is low, but their detection is important for subsequent therapeutic procedures. This work was supported by grants APVV-0716-10 and ITMS 26220120041.

P01.128-M

Indisputable double paternity in twins caused by superfecundation

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Introduction: Paternity tests enable to establish the biological relationship between a child and his alleged father. Today, this test is based on DNA fingerprinting using mainly short tandem repeats (STRs) markers. Indeed, each
individual receives half of his genetic heritage from his biological mother and the other half from his biological father. Rare cases of twin pregnancy were induced by fertilization from two different parents. In this case, we speak about a "superfecundation". This situation is exceptionally confirmed.

Here we report on genetically confirmed superfecundation in the context of paternity test by DNA fingerprinting.

Observation: In the context of paternity, we performed a genetic study of 4 members: a mother, her 2 twin infants, and a supposed father. This study involved the analysis of 15 STR markers by "PowerPlex 16 System" kit. Results were analyzed by Genotyper® v 3.7.

Results: Genetic analyses were performed under the same technical conditions and showed that one of the twins shares the same alleles with the alleged father, while the other infant has different alleles in 11 of the 15 STRs studied. Therefore, despite that these two children are twins, their biological fathers are different.

Discussion and Conclusion: The superfecundation is a rare and special obstetric situation. It is secondary to the fertilization of two eggs from the mother by two sperm from each of a different father. In this study, we have confirmed this situation by molecular genetics tools. However, their clinical and biological implications remain unknown.

**P02.01-S**

**IL-8 is associated with age-related macular degeneration in Italian samples**

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Age-related macular degeneration (AMD) is a macular degenerative disease, representing one of the main socio-economical health issues for the elderly population worldwide. The increasing prevalence of AMD is related to progressive aging of the population and affects more than one million Italian people. AMD presents a multifactorial etiology with several risk factors (age, cigarette smoking, diet).

Genes in the complement pathway (CFH) and a chromosome 10 region (ARMS2) showed to be the most involved loci in the disease. Several studies confirmed the crucial role of inflammation and angiogenesis in AMD pathogenesis and progression. This data a screening of IL8 gene has been proposed (4q12-q13) to evaluate the association of AMD with rs227306 (C/T), that is an intronic SNP in the IL8 gene. The results demonstrated a strong association of T allele (p = 4.15*10^-5, OR = 1.39, 95% CI = 1.19-1.62) in a cohort composed of 721 cases and 660 controls, suggesting the sequencing of the entire IL8 gene. Sequencing analysis revealed two haplotypes associated with AMD development (A-F-T-E, p = 2.08*10^-9, OR = 1.68, 95% CI = 1.43-1.97 and T-C-C-A, p = 7.07*10^-11, OR = 0.60, 95% CI = 0.51-0.70).

It is notably that in human coronary atherosclerosis, IL-8 is an important mediator of inflammation and angiogenesis and may contribute to plaque formation via its anti-angiogenic features. In addition to inflammation and angiogenesis, IL-8 is also associated with AMD development (A-T-T-T , p = 2.08*10^-9, OR = 1.68, 95% CI = 1.43-1.97 and T-C-C-A, p = 7.07*10^-11, OR = 0.60, 95% CI = 0.51-0.70).

In our diagnostic laboratory we have used NGS to analyze the 6 OCA, OA1 and HPS1 genes. This was efficient in terms of both finding mutations and rapidity. We are designing a larger panel including all syndromic albinism genes.

The extensive analysis of all albinism genes will allow us to better characterize gene interactions in this disease that may not be purely monogenic.

**P02.02-M**

**Molecular investigations in patients with oculocutaneous albinism**

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Oculocutaneous albinism is characterized by both ocular and skin and hair features. In addition to TYR, OCA2, TYRP1, SLC45A2 (OCA6) and HPS1 (OCA7) were recently discovered. Ocular (OAI) and syndromic albinism are also known. When studying 400 patients, we found 36% OCA1, 25% OCA2, 2% OCA3, 11% OCA4, 1.25% OCA6, 0% OCA7, 6% OAI and 1% HPS1. Deletions represent 5.6% of the anomalies. In two patients we found a complex rearrangement of OCA2 that comprised the deletion of exons 2 to 19, followed by the reinsertion, after reshuffling, of most of the deleted segment in intron 1 of the gene. 17.5% of patients remain without molecular diagnosis. A first hypothesis is that mutations are located in the introns or regulatory regions of the genes. We undertook the sequencing of the entirety of the OCA1-4 genes to search for alterations not only affecting the exons. A second hypothesis is that new OCA genes remain to be discovered. We have sequenced 11 patients.

In our diagnostic laboratory we have used NGS to analyze the 6 OCA, OA1 and HPS1 genes. This was efficient in terms of both finding mutations and rapidity. We are designing a larger panel including all syndromic albinism genes.

**P02.03-S**

**Two novel (p.E930X and p.C2278X) mutations in ALMS1 leading to Alström Syndrome**

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Alström syndrome (AS, OMIM #208300) is an extremely rare, autosomal recessive disorder with a significantly shorter life expectancy. It is mainly characterized by sensorineural hearing loss, early onset retinal dystrophy, and obesity. Other reported features include recurrent pulmonary infections, progressive renal and hepatic dysfunction, cardiomyopathy, short stature, and endocrinological features. AS is caused by mutations in the ALMS1 gene, which encodes a protein of unknown function that localizes to the basal bodies of cilia, playing a role in intracellular trafficking. Here we report two AS cases born to unrelated healthy parents. The diagnosis of AS was performed accordingly to the criteria previously defined by Marshall et al. (2007). We ascertainment two families with seemingly AS. Molecular analysis was performed by direct sequencing of the known hot spots in the ALMS1 gene (exons 8, 10 and 16) according to the mutational load described in the literature. We identified a homozygous mutation (c.2788G>T, p.E930X) in patient 1 and a homozygous mutation (c.6834A>G, p.C2278X) in patient 2 that co-segregate with the disease phenotype in family 2. Both mutations cause a truncated protein and had not been previously described in literature. We show that direct sequencing of exons 8, 10 and 16 of the ALMS1 gene could represent a useful tool for molecular diagnosis of AS, whilst the mutations described here may contribute to extend the mutational spectrum in AS.
for autosomal recessive pattern; AUNA1 (DIAPH3 gene) for autosomal dominant; and AUNX1 for X-linked. Connexin 26 mutations were also reported in subjects with AN. The main goal of the study was to investigate genetic mutations in patients with clinical diagnosis of AN, to verify its importance and involvement in the etiology of AN in Brazilian patients. Clinical information and genetic evaluation of 39 patients were analyzed. We investigated the most common causes of genetic hearing loss, including pathogenic variants in the GJB2 gene, deletions in the GJB6 gene and m.1555A>G mutation in the MTRNR1 gene. Additionally, direct sequencing is performing for mutation screening of OTOF gene. The common Spanish p.Q829X mutation in the otoferlin gene was not detected in our cohort. The c.53delG mutation in the GJB2 gene was found in two patients in homozygous genotypes. However, it is not established if pathogenic variants in connexin 26 could be involved with AN or if the otocoustic emissions that were recorded from these subjects only represent the residual activity of few outer hair cells that still alive. Further investigation is needed to clarify the link between GJB2 mutations and AN. The study of AN genetic basis is extremely important to improve the diagnosis, management, therapy and genetic counseling of the affected subjects.

P02.06-M

Targeted high-throughput sequencing for mutation detection in Bardet-Biedl Italian patients

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Bardet-Biedl syndrome (BBS) is characterized by trunca obesity, polydactyly, hypogonadism, development delay, learning disabilities, progressive retinopathy, renal disease and susceptibility to diabetes mellitus. Although BBS is mainly transmitted in an autosomal recessive manner, few families exhibit a tri-allelic mode of inheritance. To date, 16 different BBS genes (BBS1-BBS16) are known and BBS1 and BBS10 show the highest mutation frequency in BBS patients. Six unrelated patients evaluated by standard ophthalmologic examination and with a clinical diagnosis of BBS were analyzed by targeted re-sequencing of 130 retinopathies-related genes on HiScanSQ Illumina platform (mean coverage 500x). Bioinformatic analysis identified a mean of 1100 sequence variants per sample. Filtering pipeline (exonic function, frequency, prediction and inheritance model) leads to distil a mean of 10 candidate variants per sample. The candidate variants were independently validated by Sanger sequencing and segregation analysis was performed. We identified two known causative deletions in BBS9 (c.1877_1880del) and BBS7 (c.712_715del) genes and a novel frameshift deletion in BBS10 (c.1677_1678insA and c.1220T>C); one patient present a homozygous variant in BBS2 gene (c.209A>G) and one novel heterozygous variant (c.538C>T) in BBS3 gene. The last patient has two variants in BBS12 gene both in homozygous status [c.1044del (novel) and c.61T>C (described)]. NGS approach has enabled us to detect variants in BBS genes. Although for described variants and ins/del frameshift the variants the genotype-phenotype correlation is clear, in order to clarify tri-allelic variants' role in BBS-ontset, further association studies are required.

P02.07-S

Neuro-developmental genes may underlie human congenital general anosmia

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We report a boy affected by bilateral sensorineural deafness and palmo-plantar hyperkeratosis with hearing loss. The pedigree suggests an autosomal dominant pattern of inheritance with variable expressivity. Molecular analysis of GJB2 was negative and GJB6 deletions were also excluded. GJB6 encodes connexin 30, which is highly expressed in the inner ear and in keratinocytes. Therefore, we sequenced the entire coding region of this gene in the proband and identified the c.175 G>A mutation, causing a substitution of glycine 59 with an arginine residue. The same missense mutation had been previously identified in a patient with an arginine residue. The same missense mutation had been previously identified in a patient with a tri-allelic pattern of inheritance. Both FGR1 and SEMA3A are implicated in Kallmann’s syndrome, where GnRH neuronal migration developmental abnormalities typically lead to syndromic anosmia, associated with sterility. Our patients are unusual in having isolated anosmia. All mutations are currently under further scrutiny. The results suggest that deciphering GGA pathogenic variants might shed light on the embryonic development of the olfactory neuronal system.
Identification of the causative mutations in patients affected by autosomal recessive non syndromic deafness (DFNB forms), is demanding due to genetic heterogeneity. After the exclusion of GJB2 mutations and other mutations previously reported in Tunisian deaf patients, we performed whole exome sequencing in patients affected with severe to profound deafness, from four unrelated consanguineous Tunisian families. Four biallelic mutations were identified in three different genes, i.e. a nonsense mutation, c.208C>T (p.R70X), in LRTOMT, a missense mutation, c.5417T>C (p.L1806P), and two splice site mutations, c.7395+3G>A, in MYO15A, c.2260+2T>A, in TMC1. We thereby provide evidence that whole exome sequencing is a powerful, cost-effective screening tool to identify mutations causing recessive deafness in consanguineous families.

P02.13-S
Diagnosing heterogeneous disorders such as hereditary hearing loss with targeted Next-Generation-Sequencing: a model for categorization and evaluation of the pathogenicity of sequencing variants
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Routine DNA diagnostics of hereditary hearing loss (HL) is complicated considerably by the genetic and phenotypic heterogeneity of the disease. Currently, only 10-20% of the patients receive a clear genetic diagnosis, because only two genes are analysed commonly. We developed a Next-Generation Sequencing (NGS) panel for HL consisting of 79 known genes for all nonsyndromic deafness (DFN forms) and 57 known genes for autosomal recessive HL. Ten patients with autosomal dominant nonsyndromic HL were analysed using this panel. Target enrichment and sequencing was performed using the Illumina TrueSeq Custom Enrichment and HiSeq2000. In total 307 variants were identified in the 124 different patients. In order to deal with the large amount of variants, a classification system was set up to assign the most plausible disease causing variant to each patient. Variants with a MAF below 0.003 were classified according to the variant type predictions based on different prediction programs, in five groups ranging from definitely pathogenic (class 5) to not pathogenic/no clinical significance (class 1) at all. Approximately 3.7% of the patients had variants in two or more groups. In addition there was one patient with two variants in class four. The current diagnostic setting is likely to be less reliable than generally assumed. In addition, our study shows that a straightforward, reliable and a validated classification system is necessary to use targeted resequencing in the diagnostics of hearing loss in the future.

P02.14-M
Molecular diagnosis of Italian patients affected by hereditary Retinal Dystrophies, using targeted high-throughput sequencing
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Hereditary Retinal Dystrophies (RD) represent one of the most frequent genetic causes of blindness in the Western world. Because of their high clinical and genetic heterogeneity, an exhaustive classification of RD is difficult. The progressive forms may have an onset in early childhood, as Leber congenital amaurosis, or later in life, as Retinitis Pigmentosa (RP). There are also described many syndromic forms, as Usher syndrome (USH), and Bardet-Biedl syndrome (BBS).

Identification of the causative mutations in patients affected by RD requires a reliable and cost-effective screening tool to identify the causative gene of the disease. In this study, we performed whole exome sequencing on 115 retinopathies-related genes targeted for Italian patients using HiScanSQ Illumina platform (mean coverage 500X). We obtained a detection rate of 100% for BBS patients, 80% for USH patients and 69% for RP patients. The candidate variants were independently validated using Sanger sequencing and segregation analysis was performed. We also identified 3 large deletions in the genes EYS, RPGR and USH2A, using method CONTRA and confirmed by MLPA.

This study confirms that NGS-based mutation analyses are reliable and cost-effective screening tools to identify the causative gene of the disease.
efficient approaches in molecular diagnosis of genetically heterogeneous diseases like RD. NGS allows also a considerable reduction of costs and an early intervention for diseases for which there are available appropriate therapeutic strategies.

P02.15-S Identification of three novel homozygous variants in the GJB2 gene in Mexican patients with hereditary sensorineural hearing loss M. Mirna1, M. Rivero-Vega1, F. Loceza-Becerra1, L. Gonzalez-Huerta1, H. Urueta-Cuellar1, P. Berruecos2, S. Cuevas-Covarrubias3;

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Background: Hereditary sensorineural hearing impairment (HSNI) is a genetically heterogeneous disorder worldwide. Mutations in the GJB2 gene are a frequent cause of hereditary SNHL. Individuals that are homozygous for the GJB2 gene mutations manifest a wide spectrum of clinical data that ranges from moderate to profound SNHL; this suggests the participation of genotypic and environmental factors in the phenotypic expression Objective: To describe three novel homozygous mutations in the GJB2 gene with HSNI. Materials and methods: Three subjects with prelingual HSNI were included in the study. Genomic DNA was extracted by conventional methods and all exons of GJB2 gene were analyzed through PCR and the DNA was sequenced on an ABI 3730XL automated sequencer. Results: DNA sequencing analysis showed three novel homozygous mutation in the GJB2 gene that corresponded to p.V84M/p.F31I/p.W44X; p.V84M/p.F31I/p.R32S and p.E477X/p.R32S/p.S194R. Parents were tested for this molecular defect and for the presence of the novel homozygous state that allows to confirm the recessive inheritance pattern. These mutations were searching in 100 normal controls to discard a possible polymorphism. Conclusion: We describe three novel varieties of homozygous mutations in patients with HSNI. All patients presented profound hearing loss with no other anomalies. These data show that the genesis of HSNI is complex and that the genetic defects are greater than expected.

P02.16-M Regional distribution of the GJB2/GJB6 gene mutations in Mexican population with hereditary hearing impairment F. Loceza-Becerra1, J. Refugio-Rivera1, M. Martinez-Saucedo1, L. Gonzalez-Huerta1, H. Urueta-Cuellar1, P. Berruecos2, S. Cuevas-Covarrubias3;

1, Genetica, Facultad de Medicina, Hospital General de Mexico, Universidad Nacional Autonoma de Mexico, Mexico D.F., Mexico, 2, Audologia y Foniatría, Facultad de Medicina, Hospital General de Mexico, Universidad Nacional Autonoma de Mexico, Mexico D.F., Mexico.

Background: Hereditary sensorineural hearing impairment (SNHL) is a genetically heterogeneous disorder worldwide. Mutations in the GJB2 gene are a frequent cause of hereditary SNHL. There is a prevalence of certain mutations in various populations which suggests that specific mutations may be influenced by ethnic background. Objective: To analyze the prevalence of GJB2, GJB6 mutations in several geographic areas of Mexico in patients with hereditary SNHL. Materials and methods: One hundred and forty Mexican unrelated propositi with prelingual SNHL were included in the study. All patients had three previous generations born in Mexico and belonged to no specific ethnic group. Analyses of the GJB2 and GJB6 genes and mt.A1555G were performed in all subjects. Results: Twenty three homozygous mutations, 57 heterozygous mutations, 1 double heterozygous (GJB2/GJB6) and 59 wild-type genotypes in the GJB2 gene were observed. Three patients had the homozygous del35 mutation whereas 26 patients were heterozygous for this gene defect. Only one patient with the GJB6 gene deletion was present (it includes the double heterozygous GJB2/GJB6). The mt.A1555G mutation was not detected. Conclusion: We found a great variety of mutations depending on the analyzed region in patients with SNHL: 57.86% of patients had affection in one or two alleles in GJB2 or GJB6 genes whereas 42.14% were wild-type. In some cases, allelic distribution depended on region. Molecular studies of more genes involved in hereditary non-syndromic SNHL are required to completely confirm the molecular basis of hearing loss in Mexican population.

P02.17-S Panel base on next-generation sequencing for molecular diagnosis of inherited heterogeneous eye diseases M. Qi1,2, X. Li3, Y. Chen1, BGI-Shenzhen, Shenzhen, China;

1, Center for Medical Genetics Ghent, Ghent University, Ghent, Belgium, 2Department of Ophthalmology, Juntendo University Graduate School of Medicine, Tokyo, Japan, 3Department of Ophthalmology, Me University Graduate School of Medicine, Mie, Japan.

In 2012, NMNAT1 was identified as a novel disease gene for Leber Congenital Amaurosis. The mutation spectrum contains both coding and regulatory mutations. The starting point of this study was pseudo-o-mozogony of a known NMNAT1 mutation (p.Arg237Cys, exon 5) in two unrelated Japanese families (F1 and F2), assuming hemizygosity. Here, we aimed to identify the putative NMNAT1 deletions. Copy number variation (CNV) screening was performed for all exons using qPCR. Subsequently, identified deletions were refined with additional qPCR amplicons located in the breakpoint regions. Finally, the junction product was amplified with long-range PCR and sequenced (Nextera XT, MiSeq, Illumina). In F1, CNV analysis showed a heterozygous deletion of exon 4 and 5, which was subsequently refined to a region of 13.0-18.7 kb. In F2, the amplon for exon 4 was deleted, whereas the copy number for exon 5, located downstream of the p.Arg237Cys mutation, was normal. Subsequent refinement at
the 5’end delineated the deletion to a region of 1.5-6.7 kb. Long-range PCR revealed a band of 2.4 kb and 2.1 kb in F1 and F2, pointing to two different deletions of approximately 16.6 kb and 4.8 kb, respectively. Sequencing analysis of the junction products is currently ongoing.

In conclusion, we report the first deletions in NMNAT1, expanding the muta-
tion spectrum of this gene. Hence, we presume that the high number of pa-
tients in which only a single heterozygous mutation could be identified, can be explained by structural variations, requiring additional strategies apart from resequencing of the coding region.

P02.20-M
Prevalence and mutation analysis of NMNAT1 gene in Leber Congenital Amaurosis in Spanish population
R. Sanchez-Alcudia1, S. Tatsi2, F. Blanco-Kelly3, N. Reyf4, M. Lopez-Molina5, R. Perez-Carro6, A. Avila-Fernandez7, M. Corton6, C. Ayuso7; 1Department of Genetics, Instituto de Investigación Sanitaria-Fundación Jiménez Díaz (IIS-FJD), Madrid, Spain, 2Department of Ophthalmology, IIS-Fundacion Jimenez Díaz University Hospital, Madrid, Spain, 3Department of Ophthalmology, University Hospital, Madrid, Spain.

Leber Congenital Amaurosis (LCA) is the most severe blinding retinal dys-
trophy that represents near 5% of cases. At the moment 20 genes have been asso-
ciated to this disease, more of them expressed in the photoreceptors or in the retinal pigment epithelium, explaining around 70% of LCA cases. The no
coding variants hotspot sequencing in 64 Danish LCA probands revealed one or two variants in 22 (34%) cases. Upon the identifi-
cation of heterozygous variants, Sanger sequencing was performed in the
NMNAT1 gene and to characterize 6 of the 96 total families (6.25%), car-
duing these families other studies (RNA expression in peripheral total
blood and lymphoblast cell line, intronic sequencing and others) should be
performed.

P02.21-S
Mutation hotspot sequencing reveals RP65 as the most frequently mutated LCA gene in Denmark
G. D. Astrup1,2, M. Bertelsen1,2, P. W. J. Collin2, T. Rosenberg3, S. M. H. Farah2, E. P. M. Cremers1,2; 1Department of Human Genetics, Radboud University Medical Center, Nijmegen, Netherlands, 2Radboud Institute for Molecular Life Sciences, Radboud University Medical Center, Nijmegen, Netherlands, 3Division of Human Genetics, Center for Biomedical Research, Faculty of Medicine, Diponegoro University, Semarang, Indonesia.

Leber congenital amaurosis (LCA) represents the most severe and earliest
onset form of inherited retinal dystrophies, and affects 1 per 50,000 indivi
duals worldwide. Presently, mutations in twenty genes with diverse roles in
the retina are known to be associated with LCA. The large number of LCA
genes necessitates a systematic genotyping approach in a cost and time-
efficient manner. Previous studies in LCA have described some recurrent
mutations and mutational hotspots in particular exons of LCA-associated
genes, i.e. an intronic variant in CEP290 (c.2291+1655A>G), a nonsense
mutation in AIPL1 (p.W278*), a missense change in GUCY2D (p.R768W), and
various mutations clustering in exons 7 and 9 of CRB1. As gene-specific ther-
apies are emerging, identifying mutations in RPE65 and LRAT can also be of
tremendous importance for patients. Sanger sequencing of these mutation
hotspots, and all protein-coding exons of RPE65 and LRAT in 64 Danish LCA
probands revealed one or two variants in 22 (34%) cases. Upon the identi-
fication of heterozygous variants, Sanger sequencing was performed in the
relevant genes to identify the second allele, and to characterize 6 of the 96 total families (6.25%). In order to fully cha-
racterize these families other studies (RNA expression in peripheral total
blood and lymphoblast cell line, intronic sequencing and others) should be
performed.

P02.22-M
Propensity for localized provoked vulvodynia by TRPV1 and NGF polymorphisms
R. Sanchez-Alcudia1, L. Lafuente1, A. Azran1, Y. Faroujian1, O. Hemo2, E. Tubin1, A. Shahar3, A. Yeshaya4, J. Bornstein1,2; 1Department of Human Genetics, Radboud University Medical Center, Nijmegen, Netherlands, 2Radboud Institute for Molecular Life Sciences, Radboud University Medical Center, Nijmegen, Netherlands, 3Division of Human Genetics, Center for Biomedical Research, Faculty of Medicine, Diponegoro University, Semarang, Indonesia, 4Department of Otorhinolaryngology, Seoul National University Bundang Hospital, Seongnam, Korea, Republic of.

Localized provoked Vulvodynia (LPV) is a prevalent chronic pain condition. Familial occurrence (FO) suggests genetic susceptibility in its etiology. We
studied possible associations between LPV in 70 affected women and seven
Single Nucleotide Polymorphisms (SNPs) in the genes Transient Receptor Potential Vanilloid type-1 (TRPV1), Nerve Growth Factor (NGF) and hepar-
ase, hypothesized to be involved in the pathophysiology of LPV. Prevalence of
SNPs was compared between women with severe primary LPV and 132
healthy, ethnically matched controls. Women participating in the study have answered a detailed questionnaire, addressing possible FO of LPV and co-
morbid pain conditions. SNPs/het genotyping revealed a novel statistically significant high prevalence of non-synonymous polymorphism rs222747 of TRPV1 and rs11102930 located in the promoter region of NGF, in women with LPV, especially those from Ashkenazi Jewish ancestry compared to the control population. Logistic regression model for rs222747 and rs1102930 frequent alleles indicates significant risk for LPV in all affected women and Ashkena-
zi Jewish group, respectively. Significant higher rate of FO of LPV, Temporo
mandibular joint (TMJ) symptoms, recurrent vaginitis and irritable bowel
syndrome was found in affected women compared to healthy controls. Ad
hoc analysis compared pain conditions with frequent alleles among the
202 women studied. Interestingly, rs222747 minor allele of TRPV1 was found
in association with women presenting TMJ and women with recurrent va-
ginitis, suggesting a possible common genetic predisposition to pain co-
morbidities. These data open a new horizon for understanding the path-
ophysiology of LPV and might lead to the development of new personalized
therapeutic modalities.

P02.23-S
Genetic Disorder with Diverse Modes of Inheritance Implicates Sporadic Form of Mild to Moderate Sensorineural Hearing Loss in a Pediatric Population
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studied possible associations between LPV in 70 affected women and seven
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in association with women presenting TMJ and women with recurrent va-
ginitis, suggesting a possible common genetic predisposition to pain co-
morbidities. These data open a new horizon for understanding the path-
ophysiology of LPV and might lead to the development of new personalized
therapeutic modalities.
P02.24-M
Identification of genes and related mutations in 10 Iranian families with non-syndromic autosomal recessive hearing loss by whole exome sequencing

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1Genetics Research Center, University of Social Welfare and Rehabilitation Sciences, Tehran, Islamic Republic of Iran, 2Women’s College Research Institute, Women’s College Hospital, University of Toronto, Toronto, ON, Canada.

With prevalence figures close to 0.2% at birth, hearing loss (HL) is the most frequent sensory impairment in childhood. In developed countries, genetic causes account for more than 60% of congenital HL, most often resulting in non-syndromic deafness, which is usually autosomal recessive. Hereditary nonsyndromic hearing loss (NSHL) in Iran is highly heterogeneous, and more than 50% of patients with a presumed genetic etiology lack a specific molecular diagnosis with STR analysis. Whole-exome sequencing (WES) has recently opened a new page in Mendelian disease gene discovery - enabling a study on autosomal recessive HL in a new way.

The aim of this study is to find more causative genes and their mutations for NSARHL in Iranian families by WES. After ruling out any association to prevalent genes for NSARHL in Iran, ten families will be subjected to WES.

Until now, WES has been performed with genomic DNA from affected individuals of two consanguineous families with profound deafness. Analysis of these families revealed a novel homozygous mutation in MYOTA gene in one family but co-segregation study failed to confirm this variant as the only cause of HL in this family. Additional clinical investigation revealed that an intra-familial phenotypic variation and existence of both syndromic and non-syndromic HL in this family is possible. Further studies to find the other variants which may be associated with HL in this family, the data analysis of the second family and also WES of remaining families are underway.

P02.25-S
Nonsyndromic hearing loss in Moravia: one fifth due to GJB2, no mutations in SERPINB6, TMIE, COCH, ACTG1, KCNQ4, GJB3

P. Tvrda1, P. Plevova1, P. Turska1, B. Kantorova1, E. Mazurova1, D. Grechmola1, A. Gregorova1, A. Badikova1, E. Silhanova1, N. Dvorcakova1, 1Department of Medical Genetics, Faculty Hospital, Ostrava, Ostrava, Czech Republic, 2Department of Epidemiology and Public Health, Faculty of Medicine, University of Ostrava, Ostrava, Czech Republic.

GJB2 gene mutations are the most frequent cause of nonsyndromic hearing loss. There are many other genes less frequently causing this disorder. The aim of our study was to look for other genes mutated in Moravian population of patients with deafness. We have performed sequencing of GJB2 coding region on ABI3130 and Δ(GJB6-D13S1830) detection using PCR and gel electrophoresis in 142 patients with nonsyndromic hearing loss. Biallelic pathogenic GJB2 mutations were found in 31 patients (22%) thus explaining their hearing defect. In 9 patients (6%) only one pathogenic GJB2 mutation was found. No patient carried Δ(GJB6-D13S1830). Sequencing of SERPINB6, TMIE, COCH, ACTG1, KCNQ4 and GJB3 genes was performed on ABI3130 in 13, 13, 13, 30, 20, 14 and 30 patients without GJB2 mutation, respectively. No pathogenic mutation was found in SERPINB6, TMIE, COCH, ACTG1, KCNQ4 and GJB3 genes. In ESPN gene, two variants with unknown pathogenicity were found in two unrelated patients: c.337+T->C, p.Arg113Cys (Polyphen score 1.00) and c.1797+1_1806delCCACGCACGCG, p.Pro606_ Pro617del. Both variants were inherited from parents without hearing loss. We cannot exclude big genomic deletion/duplication of ESPN gene on the other allele in the patients. There may also be bimgenic mechanism of hearing loss pathogenesis. So far, however, we cannot conclude that these variants are causal in our patients. We are going to analyze WHRN gene encoding the whirin protein, functionally associated with ESPN gene product exp53.

P02.26-M
Comprehensive genetic diagnosis of non-syndromic hereditary hearing loss by targeted resequencing of 32 genes with use of Haloplex design and IonTorrent PGM sequencer

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Inherited nonsyndromic sensorineural hearing loss (NSHL) is characterized by a high level of genetic heterogeneity, making extremely challenging to obtain a molecular diagnosis with traditional screening methods. Whole exome sequencing (WES) has been recently introduced as an alternative approach to search for alleles underlying Mendelian disorders and has been successfully applied for gene/mutation discovery. In this study, we used WES to identify the pathogenic mutations responsible for NSHL in three families (NSHL6, 11, and 12), with a recessive inheritance pattern and at least two affected siblings. A total of 9 individuals were subjected to WES using the Affymetrix 250K Sty array (238,304 single nucleotide polymorphisms). Parametric linkage analysis under a dominant model with complete penetrance was performed. Results: Linkage analysis identified two loci at 5p15.32 and 9p24.1 with LOD score of 2.7. Given the involvement of other ADAMTS proteins in several different ocular phenotypes, all exons of gene ADAMTS16 (5p15.32) were sequenced in affected individuals and no mutations were found. Discussion: The linkage overlap with the MCDR3 locus on 5p might indicate allelism or more likely the presence of mutations in two different adjacent developmental genes. Exome sequencing is underway and expected to shed light on the genetic basis of this disorder.

P02.27-S
Identification of novel NSHL-causing mutations by whole exome sequencing

M. Robusto1, C. Chiariigni1, R. Asselta1, P. Castorina2, S. Caccia1, E. Benzonzi1, M. Seia1, U. Ambrosi1, S. Daga1, S. Soldi2, 1Dipartimento di Biotecnologie Mediche e Medicina Traslazionale, Università degli Studi di Milano, Milan, Italy, 2Dipartimento di Scienze Cliniche e di Comunità, Università degli Studi di Milano and Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico, UO Audiologia, Milan, Italy, 3Laboratory of Medical Genetics, Molecular Genetic Sector, Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico, Milan, Italy.

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P02.28-M
Identification of novel NSHL-causing mutations by whole exome sequencing

M. Robusto1, C. Chiariigni1, R. Asselta1, P. Castorina2, S. Caccia1, E. Benzonzi1, M. Seia1, U. Ambrosi1, S. Daga1, S. Soldi2, 1Dipartimento di Biotecnologie Mediche e Medicina Traslazionale, Università degli Studi di Milano, Milan, Italy, 2Dipartimento di Scienze Cliniche e di Comunità, Università degli Studi di Milano and Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico, UO Audiologia, Milan, Italy, 3Laboratory of Medical Genetics, Molecular Genetic Sector, Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico, Milan, Italy.

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Identification by whole-exome sequencing of two novel LARS2 mutations in an Italian family with Perrault syndrome

Pella2.31-S

Identification by whole-exome sequencing of two novel LARS2 mutations in an Italian family with Perrault syndrome

G. Solda1, M. Robusto1, P. Costarini2, U. Ambrosio2, R. Asselta1, S. Duga1

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Perraullt syndrome (PRLTS) is a rare autosomal recessive disorder characterized by ovarian dysgenesis and premature ovarian failure (POF) in females, and by progressive hearing loss in both genders. Recently, mutations in four genes (i.e. HSD17B4, HARS2, CLPP, and LARS2) were found to be responsible for PRLTS, although they do not account for all cases of this genetically heterogeneous condition.

In this study, we used whole-exome sequencing (WES) to identify the pathogenic variants responsible for PRLTS in an Italian pedigree with two affected siblings (one female and one male). All five family members were subjected to WES using the SeqCapEZ Exome v2 kit (Roche) and the HiSeq2000 platform (Illumina). Data analysis highlighted compound heterozygosity in both patients, for two novel missense variations, p.Thr300Met (c.899C>T) and p.Glu638Lys (c.1912G>A) within LARS2, encoding the mitochondrial leucyl-tRNA synthetase. The segregation of the two mutations within the family is compatible with the autosomal recessive inheritance of the disease. Both Thr300 and Glu638 residues are evolutionary conserved, and are respectively located within the editing domain and immediately before the MKSMS signature, a unique signature of the catalytic domain of class 1 aminoacyl-tRNA synthetases. The identified mutations were confirmed to be absent in an in-house database of about 3,500 ethnically-matched control exomes.

Apart from the original report in 2013, to our knowledge, this is the first study confirming the role of LARS2 mutations in PRLTS pathogenesis.

This study was supported by: Italian Telethon Foundation (grant#GGP11177) and Fondazione Cariplo, grant n°2013-0825.

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in Romanian POAG patients. We assessed 258 subjects (112 POAG patients and 146 healthy unrelated matched controls) for -857C/T (rs1799724) and -308G/A (rs1800629) TNF-α polymorphisms. These were genotyped by Real Time PCR (Taqman SNP Genotyping Assays C-2215707.10 and C-2791911, Applied Biosystems, USA). Statistical analysis was performed using the SNPStats program for genetic association studies (http://bioinfo.iconocologia.net/SNPstats); p-values ≤ 0.05 were considered significant.

All studied groups were in HWE for both polymorphisms. The frequencies of minor alleles -857T and -308A were similar in POAG patients and controls (0.22/0.10 and 0.21/0.13 respectively). There was no significant difference (p > 0.05) regarding the allele carriage genotype frequency between POAG and control subjects. Three main haplotypes were constructed with similar frequencies in patients and controls: -857C/-308G, 857T/-308G and -857C/-308A: 0.685/0.661, 0.218/0.205 and 0.980/0.133 respectively. There was no significant association between the global haplotype and POAG (p-value ≤ 0.5).

Conclusion: TNF-α polymorphisms (-857C/T and -308G/A) seem not to influence susceptibility of POAG. These results should be confirmed on larger patients’ cohort.

P02.34-M
Genetic screening for disease-associated mutations in human retinal diseases using whole exome sequencing (WES) A. Tiwari, A. Bahr, S. Feil; 1Neuroscience Center Zurich (ZNZ), University and ETH Zurich, Zurich, Switzerland, 2Department of Biology, ETHZ, Zurich, Switzerland, 3Molecular Genetics Laboratory, Centre for Integrative Human Physiology (ZIHP), University of Zurich, Zurich, Switzerland.

Monogenic diseases of the retina and vitreous affect approximately 1 in 2000 individuals. They are characterized by tremendous genetic heterogeneity and clinical variability of symptoms involving more than 20 different clinical phenotypes and mutations in more than 200 genes. Clinical manifestations of retinal degenerations (RD) range from mild retinal dysfunctions to severe congenital forms. A detailed clinical diagnosis and identification of the underlying mutations are crucial for genetic counseling of affected patients and their families, for understanding genotype-phenotype correlations and developing therapeutic interventions.

We make use of WES and have established a reliable and efficient high-throughput analysis pipeline of next generation sequencing (NGS) data to identify disease-causing mutations in RD. Our data indicate that this approach enables us to genetically diagnose approximately 56% of the patients (N=28) with mutation(s) in known disease-associated genes. Thus, 44% of the cases, that do not carry mutation(s) in a known gene, are crucial for identification of novel candidate genes and biological pathways underlying the disease phenotype. Amongst identified mutations, 47% were previously described in the literature while 53% are novel. The types of mutations included missense mutations (47.4%), frameshift insertions or deletions (10.5%) and mutations predicted to interfere with splicing (10.5%). In conclusion, WES can rapidly identify mutations in underlying mutations are crucial for genetic counseling of affected patients and their families, for understanding genotype-phenotype correlations and developing therapeutic interventions.

We conclude that the efficacy of the U1-based therapy is sufficient to correct not only misspliced transcripts but also their translated proteins.

P02.35-S
Large number of new mutations found in patients with inherited retinal dystrophies using Panel-based Next Generation Sequencing N. Gloeckle, S. Kohl, J. Mohr, F. Fettke, A. Sprecher, M. Schubach, K. Hoertmager, A. Bernd, B. Wissing, J. Neidhardt, W. Berger**, 1CeGaT GmbH, Tübingen, Germany, 2Molecular Genetics Laboratory, Centre for Ophthalmology, University of Tübingen, Tübingen, Germany, 3Clinic for Hereditary Retinal Degeneration, University Hospital Tübingen, Tübingen, Germany, 4University of Zurich, Institute of Medical Molecular Genetics, Schlieren, Switzerland, 5Zurich Center for Integrative Human Physiology (ZIHP), University of Zurich, Zurich, Switzerland.

Inherited retinal dystrophies (RD) genetic heterogeneity is a well known feature. Therefore, we used next generation sequencing (NGS) technology as a tool to identify known as well as unknown mutations in RD. More than 150 genes associated with RD were selected from literature or databases. A custom target-in-solution-enrichment was used, followed by sequencing on the SOLID 5500h platform. Variant calling, variant annotation using Exome Variant Server software was done, variants were annotated and filtered according to the public databases (dbSNP, ExAC, 1000 Genomes Study).

We analyzed 300 patients with all forms of RD including syndromic forms such as Usher-syndrome and Bardet-Biedl syndrome. The detection rate of solved cases was 56.7%. Interestingly, in 54% of the solved cases we were able to detect mutations which were not previously described in literature at the date of the medical report. 70% of these cases show exclusively a new mutation in case of dominant or X-linked inheritance and two new mutations in case of recessive inheritance. 30% show one new and one previously described mutation.

Therefore, we conclude that NGS is the most promising tool to identify known as well as unknown mutations for this genetically highly heterogeneous disease entity. New mutations were found in 47 different genes. Genes most frequently affected by mutations were USH2A and EYS followed by RPGR, RPPE51 and CDHR1.

Conclusion: Whole-exome sequencing can be used for genetic diagnosis of retinal dystrophy. In the case of RCD4, we identified a novel missense mutation leading to frameshift insertion and premature stop codon. In the case of RCD1, we identified a novel splice site mutation. WES can rapidly identify mutations in RD.

P02.36-M
Splice factor-based gene therapy to correct mislocalization of the cytoskeletal protein RPGR in retinal degeneration R. Da Costa, E. Glaub, A. Tiwari, B. Böckemeyer-Grußense, W. Berger**, J. Neidhardt**; 1Institute of Medical Molecular Genetics, University of Zurich, Schlieren, Switzerland, 2Department of Biology, ETHZ, Zurich, Switzerland, 3Zurich Center for Integrative Human Physiology (ZIHP), University of Zurich, Zurich, Switzerland, 4Neuroscience Center Zurich (ZNZ), University and ETH Zurich, Zurich, Switzerland. 5CeGaT GmbH, Tübingen, Germany. 6Neuroscience Center Zurich (ZNZ), University and ETH Zurich, Zurich, Switzerland.

Retinal degenerations (RD) and other genetic diseases are often caused by splice site mutations. This type of mutation accounts for approximately 20% of all pathogenic sequence variants associated with retinal degeneration.

Previously, we reported the identification of an X-linked RD patient that showed skipping of exon 10 in the RPGR mRNA. To treat this mutation-induced splice defects, we applied a mutation-adapted splice factor. In case of the splice factor UI snRNA, the adaptation yielded an increase in its affinity towards the mutated splice site. This novel gene therapeutic approach was highly efficient in increasing the amount of correctly spliced RPGR transcripts in minigene splicing assays and patient-derived fibroblasts.

In this study, we aimed at evaluating the efficacy of the UI-based therapy on the protein level. We established an assay to locate the RPGR protein in patient-derived and control skin fibroblasts. In control fibroblasts, we found that the RPGR protein localizes along the primary cilium including basal body, transition zone and axoneme. In contrast, patient-derived cells showed mis-localization of the RPGR protein restricted to the basal body and transition zone.

Upon treatment with mutation-adapted UI, localization of RPGR along the axoneme was significantly increased compared to control treatments.

We conclude that the efficacy of the UI-based gene therapy is sufficient to correct not only misspliced transcripts but also their translated proteins. Thus, the adaptation of UI snRNA is a promising technique to treat patients who carry pathogenic splice defects.

P02.37-S
Homozygous deletion of glutamate receptor gene GRID2 causes new human hotfoot mutant phenotype, characterized by early-onset cerebellar ataxia and retinal dystrophy K. Van Schil**, M. Karlstetter, F. Meirle, M. Baewens, V. Herdlin, E. Coppieters, E. Scheffert**, N. Deconinck, T. Langmann, E. De Baere**, Coordination Centre for Medical Genetics, Ghent University Hospital, Ghent, Belgium, 1Center for Medical Genetics, Ghent University Hospital, Ghent, Belgium, 2Department of Ophthalmology, University of Cologne, Cologne, Germany, 3Department of Pediatric Ophthalmology, Queen Fabiola Children’s University Hospital, Brussels, Belgium, 4Department of Pediatric Neurology, Queen Fabiola Children’s University Hospital, Brussels, Belgium.

It was our aim to identify the genetic cause of early-onset autosomal recessive cerebellar ataxia (ARCA) associated with retinal dystrophy in a consanguineous family.

Homozygosity mapping and copy number analysis revealed a homozygous deletion of exon 2 of GRID2, p.(Gly30_Glu81del), in the proband, compatible with mouse hotfoot mutant ho15. GRID2 encodes an ionotropic glutamate receptor known to be selectively expressed in cerebellar Purkinje cells. Here, we demonstrated GRID2 mRNA expression in human adult retina and cerebellum. GRID2 protein expression was demonstrated in different stages of murine retinal development. GRID2 protein expression was observed in both murine and human retina, more specifically in photoreceptor inner segments, the outer plexiform layer and ganglion cell layer.

In order to rule out involvement of mutations in another gene, whole exome sequencing was conducted but did not reveal any other disease-causing mutations, supporting the phenotype observed here represents a single clinical entity.

We identified GRID2 as underlying disease gene of early-onset ARCA with retinal dystrophy, expanding the clinical spectrum of ARCA associated with retinal dystrophy in a consanguineous family.

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in human and murine retina, providing evidence for a novel functional role of GRID2 in the retina. To the best of our knowledge GRID2 is the second glutamate receptor gene, apart from GRM6, leading to retinal disease when mutated. Finally, we provided further evidence for evolutionary conservation of a hotfoot fragile site between mouse and human.

PO2.38-M Homozygosity mapping and whole exome sequencing identified four novel candidate retinal dystrophy genes

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Inherited retinal dystrophies (IRD) are a remarkably genetically and phenotypically heterogeneous group of inherited eye diseases, with over 190 causal genes identified to date. In the highly consanguineous Saudi population, autosomal recessive forms of IRD are thought to account for the overwhelming majority of cases. Consanguinity is known to increase the frequency of recessive disorders since it increases the coefficient of inbreeding, which is a measure of the percentage of the genome that is identical by descent. Homozygosity mapping, targeted candidate gene analysis and whole exome sequencing were used to identify the causes of IRD in the Saudi population. Mutations in RP1 were found to be a common cause of recessive RP in the Saudi population. Novel and previously identified homozygous mutations in the KCNV2 gene were identified in a cohort of patients with a distinct recessive retinal disorder, ‘cone dystrophy with supranormal rod response,’ demonstrating phenotype/genotype correlation. In addition, a founder homozygous CAPB4 mutation was identified. Causative homozygous mutations were also found in the IRD genes RB3P, RDH12, CRB1, BBS4, CNGA3, CNGB1, EYS, RLBP1, ABCA4 and PCDH12. Four novel candidate genes for retinal degeneration were identified in this study: Potentially pathogenic homozygous variants were identified in ELMC1 (c.G430A, p.A144T), KIAA1549 (c.2399_2400insAA, p.T800fsX90), GPR125 (c.G250-4G, p.S835C) and DHX29 (c.G273T, p.A91V). In the majority of cases (31 families) the genetic cause of IRD was identified, demonstrating the power of homozygosity mapping and whole exome sequencing.

PO2.39-S Next-generation sequencing for retinal dystrophy: two years’ clinical experience

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Retinal dystrophy (RD) is a genetically and phenotypically heterogeneous group of conditions; it can be syndromic or non-syndromic and follow dominant, recessive or X-linked inheritance patterns. In 2012 we have been using next-generation sequencing (NGS) technology with a panel of 105 known retinal genes; genetic testing and the potential identification of causative mutations is now available for a much broader range of patients with RD than was previously possible.

We are currently conducting a notes-based review to evaluate our clinical service and its impact on families. We will present data on a large cohort of 40+ patients who have undergone NGS testing for retinal dystrophy. Analysis so far shows a wide age range (2-87 years) of patients, around 50% of whom have no family history. A common reason for testing is to clarify the mode of inheritance for the sake of children or other relatives. Patients also underwent testing to clarify a diagnosis or prognosis, for example, whether the co-occurrence of hearing loss in the family was due to Usher syndrome.

We have reported a range of results: clearly pathogenic mutations (50-60% detection rate), negative test results, and unclear or ambiguous results requiring additional family samples and careful genetic counselling. We will present data on the impact and value of NGS testing for families and clinicians, including incidental carrier findings and unexpected results such as altered inheritance patterns or change in management. Our experience suggests NGS testing for retinal dystrophy is relevant for a significant number of patients.
cning. Pathogenicity of the candidate disease-causing variants in the homozygous regions was assessed by in-silico analysis.

Results: Ophthalmologic examination revealed typical features of RP in both families. In the first family a homoygous missense variant, c.3269G>A, was identified in SLC26A4, which previously was implicated in autosomal dominant RP (aDRP) and shown to impair splicing. In the second family a missense mutation, c.995G>A; (p.Gly332Asp), was identified in DHX38, which encodes the pre-mRNA splicing factor PRP1 and previously has not been associated with aDRP. The DHX38 variant shows a lod score of 3.25, which is highly suggestive of linkage. Segregation analysis indicated that both variants segregated with RP in respective families, in an autosomal recessive manner. In-silico analysis supported the causality of the p. (Arg1090Gln) and p. Gly332Asp variants.

Conclusions: Since the SNRN200 variant p. (Arg1090Gln) appears only to be causative when present homozygously, we hypothesize that it is a hypomorphic mutation. So far mutations in pre-mRNA splicing factor genes have only been associated with aDRP; thus, this is the first report that implicates defects in the splicing machinery proteins DHX38 and SNRN200 to be associated with aDRP.

P02.44-M
RS1 gene exon 2 deletion in a large Pedigree with X-linked juvenile retinoschisis
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Juvenile retinoschisis (XLRS) is a vitre–retinal disorder characterized by early-onset dystrophy of the retina that can evolve in central atrophy, thus leading to blindness. More than 200 disease-causing mutations have been reported in the X-linked gene RS1 (Xp22). The clinical diagnosis is based on instrumental ocular examination and can be confirmed by molecular genetic testing. In this report, we present the molecular characterization of an XLRS family with 4 affected males and a pedigree suggestive of an X-linked inheritance. Methods: RS1 gene point mutations screening was performed by PCR and direct sequencing. RS1 gene copy number variation was assessed by “home made” MLPA analysis and by SNP-array analysis using CytoScan HD Array (Affymetrix, Santa Clara, CA). Results: the exon 2 fragment of RS1 gene failed to amplify in the proband; given the strong suspicion of a deletion a confirmation was made by MLPA and SNP-array: all affected males were positive for exon 2 deletion of RS1 gene. Carrier females were also identified. SNP-array analysis showed a deletion of less than 7 kb including exon 2. Conclusion: this is the first report of a deep characterization of a whole exon deletion in the RS1 gene, accounting in 10% of XLRS. SNP-array analysis mapped the deletion breakpoint in an intronic region rich of several repeated elements, thus prone to rearrangements. Female carriers can be easily detected by MLPA analysis that represents a useful test in terms of time and costs for copy number detection.

P02.45-S
Spectrum of SLC26A4 gene mutations in Slovak NSHL patients
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Non-syndromic hearing loss is characterized by hearing impairment that is not associated with other signs and symptoms. This form of deafness accounts for 70% of all inherited hearing loss and it is caused mostly by malformations of structures in the inner ear. A large number of genes is associated with non-syndromic hearing loss, including CLDN14, COL1L1A2, GJB2, GJB6, KCNQ4, MYO1A, MYO1B, MYO6, MYO7A, POU3F4, SLC26A4, TECTA, TMPRSS3, and many others.

In the Slovak population, mutations in GJB2, GJB6 and mitochondrial genes were only screened to date. In our work we focused on the mutation screening of the SLC26A4 gene, which was found as another of the most prevalent disease genes in NSHL. The SLC26A4 gene encodes a protein called pendrin, which functions as an anion transporter of mostly chloride, iodide and bicarbonate ions across the membranes of cells of the thyroid, inner ear, and kidneys. Recessive mutations in this transporter are associated with hearing loss DFNB4, which is accompanied by an enlargement of vestibular aqueduct. Mutated pendrin affects the levels of fluid in the inner ear that leads to malformation and hearing loss.

In our work, we screened 324 patients of mutations in the SLC26A4 gene. We found several missense mutations, of which the most frequent were G6W (probably non-pathogenic). R185T, R409H, T416P, L445W, R470H, Y530S, L597S, and two splicing mutations c.919-2 A>G and c.2089+1G>A. In two patients we confirmed Pendred syndrome by identifying genotypes 409RH / c.919-2 A>G and L445 W / c.2089+1 G>A.

P02.46-M
Prevalence of mutation c.11864G>A (p.Trp3955X) in the USH2A gene in patients with Usher II Syndrome from Volga-Ural Region of Russia
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Usher syndrome (US) is an autosomal recessive condition characterized by a combination of congenital hearing impairment and retinits pigmentosa. To date, ten genes have been associated with US, representing up to 90% of USH2. Three types of US are known and differ by onset of the symptoms, severity and progressiveness of deafness and additional vestibular dysfunction. Patients with type II US have congenital bilateral sensorineural hearing loss that is mild to moderate in the low frequencies and severe to profound in the higher frequencies, intact vestibular responses, and bilateral retinitis pigmentosa.

In this study, 16 unrelated US type II families (60 patients) from Volga-Ural Region of Russia were studied using genotyping microarray (Usher, Asper-Biotech) for screening 614 mutations in genes CDH23, MYO7A, PCDH15, USH1C, USH1G, USH2A, GPR98, CLRN1, DFNB31 and automatic sequencing of USH2 gene. Diagnosis was based on pedigrees with congenital/progressive sensorineural hearing loss and variable ocular findings.

We revealed homozygous and heterozygous mutations for the c.11864G>A (p.Trp3955X) USH2A gene in six unrelated families among Russian, Tatar and Chuvash patients with USH II syndrome. We found four pathogenic mutations in coding region of 8 patients (p.Glu458fs, p.Trp3955X, p.Glu470fs and p.Gly392X), confirming their clinical diagnosis. The most frequent USH2A gene mutation was c.11864G>A (9/80 alleles; 11.25%). Mutation c.11864G>A in heterozygous state was also found in one Russian subject out of 1066 examined individuals from 16 various populations of Eurasia: Bashkirs, Tatars, Chuvashes, Udmurts, Kom-Permaksys, and Mordvins, Russians, Belarusians, Ukrainians, Veps, and Karelians, Abkhazians, Kazakhs, Uzbeks, Yakuts, Altaians. Study was supported by grants (N°12-04-00342_a, N°12-04-98520_r.vosto_k.a, N°14-04-97002_r.povolge_a, N°14-04-97007_r.povolge_a, N°14-04-01741_A).

P02.47-S
SOX2 whole gene deletion without ocular malformation but with isolated bilateral third degree microtia: a new manifestation of SOX2-related disorders?
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SOX2-related eye disorders are characterized by anophthalmia and/or microphthalmia, which is usually bilateral and severe. Molecular genetic testing of this gene, including sequence analysis and MLPA (for large gene deletions), identifies mutations in about 10-20% of individuals with such ocular involvement. Other clinical findings include brain malformations, eosophageal atresia and male genital abnormalities. Postnatal growth failure with pyloritis insufficiency, seizures, sensorineural hearing loss, delayed motor development and learning difficulties are described. Recent studies have demonstrated a broader ocular phenotype in cases with missense mutations, consistent with a role for SOX2 in both posterior and anterior segment development. Severe SOX2 (OMIM 164429) mutations (whole gene deletion/nonsense mutations) with complete loss-of-function alleles, almost uniformly result in anophthalmia/microphthalmia.

We report the case of a newborn female, evaluated at birth for isolated bilateral third degree microtia. All investigations (cardiac, abdominal, cerebral, ophthalmologic) were normal.

Array-CGH analysis has shown a de novo 40 kb deletion of 3q26.33 (chr3:1318470735-1318474220 [hg19]) deleting the entire SOX2 gene. More detailed ophthalmologic evaluations confirmed the absence of any ocular abnormality. The girl benefits from osseous conduction hearing aids.

Is there a link between SOX2 deletion and microtia, consistent with results
of a recent study showing under-expression of SOX2 in human auricular chondrocytes from microtia samples? Our case also confirms the reduced penetrance of the ocular phenotype, even in severe SOX2 mutations, and raises the question of a further broadening of SOX2-related disorders....

P02.48-M
Exome sequencing identifies POUSF4 p.Ala116fs mutation among family with hearing loss

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One of the most common genetic diseases in humans is hearing loss (HL). The majority of cases are nonsyndromic (70%) and 1-5% are nonsyndromic X-linked. Most cases are due to mutations in a single gene. Nevertheless, DNA diagnostics for hearing loss are challenging, since it is an extremely heterogeneous trait. Although more than 70 causative genes have been described for the nonsyndromic hearing loss alone, diagnostic application of the scientific progress has lagged behind. Some previous reports have shown that „next-generation DNA sequencing techniques” have the potential to offer a novel testing platform that could test all known genes in a sensitive, specific and cost-efficient manner. In this study, whole exome sequencing (WES) for direct genetic diagnosis in NSHL was used. Sequential filtering of variants obtained from WES, bioinformatic analyses, and Sanger sequencing validation identified premature termination p.Ala16 fs mutation in POUSF4 gene as the candidate disease-causing mutation in the family. POUSF4 belongs to a subfamily of transcription factors, which are characterized by 2 conserved deoxyribonucleic acid-binding domains, a 75-amino acid POU-specific domain and a 60-amino acid homeodomain, both helix-turn-helix structural deoxyribonucleic acid-binding motifs. In this study clinical features and genetic analysis of a male child from a Polish family with congenital deafness and POUSF4 p.Ala116fs mutation is described. Usually clinical features of DFNB2 (DFN3) often include a mixed, progressive hearing loss, temporal bone anomalies, and stapes fixation.

P02.49-S
Common copy number variations on the origin of disease

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Introduction: It is well established the relation between CNV and intellectual disability and/or autism, as it is the existence of a large number of CNV not associated with disease. These polymorphisms although benign, could represent a risk of disease when occurring in homozigosity, or when combined, depending of its genetic content. Objective: We present a case of a girl with congenital deafness, and homozgyous CNV of OTOA gene, resulting from the presence of a benign CNV in each chromosome. Method: PAMS, 8 yo was referred for genetic analysis because of congenital deafness. Conexin 26 and conexin 30 sequence analysis was done using Sanger sequencing, conexin 30 deletions was done using MLPA. Exclusion of additional genetic mutations was done using a proprietary mutation panel (312 mutations in 32 genes). Array CGH analysis was performed using Affymetrix CytoScan 750K. Results: Conexin results were negative and CGG Mutation panel and direct sequencing results demonstrated absence of amplification on OTOA. Parents testing with array CGH revealed the presence of a CNV (heterozygous deletion) on 16p12.2 in each. Both CNV are registered on DGV as normal variants. However, each CNV encompasses the OTOA gene, thus leading to the conclusion that the co-existence of two overlapping (individually benign) CNVs was the mechanism leading to congenital deafness on the index case. Conclusions: This case reveals the utility of array CGH as a complement technique in the investigation of molecular mechanisms of genetic disease that allowed establishing the molecular diagnosis and proper genetic counseling to this family.

P03.01-S
In-house-designed synthetic probe set for MLPA identifies a novel chimeric CYP11B2/CYP11B1 gene in a patient with 11ß-hydroxylase deficiency

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11ß-hydroxylase deficiency (11ß-OHD) represents the second most common cause of Congenital Adrenal Hyperplasia (CAH). It is caused by mutations in the CYP11B1 gene localized in 8q21, 40 kb distant from its homologous CYP11B2 gene coding for aldosterone synthase. Pathological alleles derived from the asymmetric recombination of these two genes have been described. The formation of a chimeric CYP11B2/CYP11B1 gene leads to 11ß-OHD; instead the chimeric CYP11B1/CYP11B2 gene causes the glucocorticoid-remediable aldosteronism (GRA). As the detection of these rearrangements is still laborious or indirect, our objective was to project a synthetic probe set for multiplex ligation-dependent probe amplification (MLPA) analysis in order to simplify the detection of these chimeric genes and other variation in the copy number of these genes. We designed a set of 8 specific probes for both CYP11B1 and CYP11B2 genes to be used with the commercial control kit SALSA MLPA P300 Human DNA reference (MRC-Holland). The methods was tested on 15 control samples and then applied to 6 patients with the suspicion of 11ß-OHD. The analysis with the CYP11 probe set led to the identification of one copy number variation in an adult patient with adrenal rests misdiagnosed as 21-hydroxylase deficiency and not properly treated. He resulted compound heterozygous for a novel chimeric CYP11B2/CYP11B1 gene, with the breakpoint region localized within intron 2, and the known A306V mutation. In conclusion, the described MLPA kit represents an optimal complement to DNA sequence analysis in patients with 11ß-OHD, enabling detection of deletions, duplications or chimeric genes.

P03.02-M
A Novel mutation of FGD1 in four members of a Turkish Family with Aarskog-Scott Syndrome and growth hormone Therapy

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Aarskog-Scott syndrome (AAS), also known as facio-digo-genital dysplasia is rare, clinically and genetically heterogeneous condition characterized by short stature, and facial, limb, and genital anomalies. The best characterized form of the syndrome which was caused by mutations in FGD1 is inherited as an X-linked trait (MIM#305400). This gene, located on the short arm of chromosome X (Xp11.21), includes 18 exons. The population prevalence of AAS is probably lower or equal to 1/25 000. The clinical signs may range from mild to severe. Herein, clinical features and intrahalimentary heterogeneity of 4 affected individuals in a Turkish AAS family with a novel mutation were presented. Only one of these patients had received growth hormone therapy and this treatment provided height velocity gain in our patient.

P03.03-S
Deciphering variability of PKD1 and PKD2 genes in Italian patients affected by Autosomal Dominant Polycystic Kidney Disease (ADPKD)

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ADPKD is the most common genetic nephron-pathology in humans, affecting about 1/1000 individuals. Aim of the present work was to define genetic variability of PKD1 and PKD2 in Italian patients affected by ADPKD. Analysis of PKD1 and PKD2 variation would allow to: confirm the diagnosis in clinically uncertain/atypical cases; offer genetic counseling in at risk families; exclude the presence of a mutation in related donors for kidney transplantation; define gene variability in Italian patients and to be subjected to an semi-automated Sanger protocol: 298 unrelated patients belonging to families and 171 relatives; 72 patients with no familiarity and 35 cases with no data about familiarity. In 90% of the 405 probands, variants were present: PKD1 80%; PKD2 4%; PKD1+PKD2 6%. 84.5% of the identified variants have never been described. An average of 12 SNPs/patient (10 PKD1 and 2 SNPs/patient in PKD2) was observed. By combining results for truncating and known variants we classified variants as pathogenic in 65.4% (265/405) of patients. For unclassified variants, a prediction was attempted according to PKDB criteria. Concordance of the results obtained with SIFT, AGVGD and PolyPhen2 allowed calling of 18 likely-neutral and 12 highly-likely-pathogenic variants. In many cases interpretation of additional information becomes relevant (i.e. family segregation increases the score for pathogenicity). In patients with no mutations detected, MLPA analysis has been performed: we identified 2 patients with a huge deletion in PKD1 (ex-2
46); 1 patient with a deletion spanning exons 2-4 in PKD1; 1 patient with the deletion of the whole PKD2 gene.

**P03.04-M**
Scattered deletion of Pkd1 in mouse kidneys causes a cystic snowball effect and recapitulates human polycystic kidney disease

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**Background:** Autosomal Dominant Polycystic Kidney Disease (ADPKD) patients carry a germline mutation in PKD1 or PKD2, leading to thousands of kidney cysts. It is poorly understood why a rapid progression of the disease is preceded by a lag-phase of several decades. Studies in which Pkd1 is inactivated in large percentages of cells in animal models, led to the presumption that inactivation of the remaining allele initiates cystogenesis, and that the progression can be accelerated by renal injury. To mimic human ADPKD, we lowered this percentage and found important characteristics of cystogenesis that have not been described before. Methods: The Percentage of Pkd1-deficient cells in Tamoxifen-inducible kidney-specific Pkd1-deletion mice was controlled by varying the tamoxifen dose, visualized with reporter mice and quantified by eMLPA. Several renal injuries were applied. Cyst progression was followed by MRI-analysis and PKD-related signaling was analyzed by immuno-histochemistry. Results: Interestingly, no pathological changes occurred for six months after scattered-Pkd1-deletion and renal injury did not trigger rapid PKD. However, in the following 3-4 months, clustered cyst formation led to a severe human-like PKD phenotype. This shift was preceded by increased pSTAT3, pCREB, pERK1/2, LCN2 and Ki-67 expression near the initial cysts. Conclusions: Our data argue against the presumption that renal injury is the major trigger for cystogenesis but suggests that initial cysts themselves, by imposing persistent stress on surrounding tissue, triggers a ‘snowball’ effect driving the formation of new cysts. In addition, our model mimics human ADPKD more precisely and can be used for pre-clinical testing.

**P03.05-S**
Identification and characterisation of six novel SERPINA1 null mutations


Alpha-1 antitrypsin (AAT) is the most abundant circulating antiprotease and is a member of the serine protease inhibitor (SERPIN) superfamily. The gene encoding AAT is the highly polymorphic SERPINA1 gene, found at 14q32.1. Mutations in the SERPINA1 gene can lead to AAT deficiency (AATD) which is associated with a substantially increased risk of lung and liver disease. The most common pathogenic AAT variant is Z (Glu342Lys) which causes AAT to misfold and polymerise within hepatocytes and other AAT-producing cells. A group of rare mutations causing AATD, termed Null or Q0, are characterised by complete loss of AAT in the plasma. While ultra rare, these mutations confer a particularly high risk of emphysema. We report here the identification and characterisation of 6 new SERPINA1 Null mutations.

**P03.06-M**
Chromosome Microarray and non-coding DNA copy number variants - a case of Alveolar Capillary Dysplasia at FOXF1 locus

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Chromosome microarray (CMA) analysis typically focuses on coding DNA (ReSeq and OMIM genes). Although non-coding intergenic and intronic variants may be critical in disease pathogenesis, copy number variants (CNV) in these regions are usually interpreted as variants of unknown clinical significance. We present a case of a lethal neonatal condition in which the pathogenic CNV lies in a distant, upstream non-coding region. It highlights the importance of relevant clinical information for CMA interpretation, and the need for a more comprehensive analysis of flanking regions if an identified CNV does not initially appear pathogenic. A term female, with a prenatal diagnosis of AVSD, presented with severe neonatal respiratory distress out of keeping with her cardiac issues. The clinical picture and early lethality suggested congenital surfactant deficiency (CSD). Sequencing of a CSD-gene panel was normal. CMA analysis revealed a de novo 1.5 Mb deletion at 16q24.1. None of the 16 ReSeq genes mapping within the deleted region appeared causative. However, this deletion is located 157 kb upstream of FOXF1, a gene responsible for congenital alveolar capillary dysplasia with misalignment of pulmonary veins (ACDMPV). The observed deletion encompasses a recently characterized distant regulator/enhancer of the FOXF1 gene. The pathogenetic diagnosis of ACDMPV was confirmed postnatally. As our knowledge of epigenetics and the genomic landscape improves, an increasing number of coding CNVs are predicted to have clinical relevance. We suggest that a database of well-characterized non-coding regulatory regions be developed and incorporated into CMA analysis.

**P03.07-S**
Novel mutations of PKD1 gene in autosomal dominant polycystic kidney disease (ADPKD)

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**Background** Polycystic kidney disease (PKD) can be inherited as an autosomal dominant (ADPKD) or an autosomal recessive trait (ARPKD). ADPKD is one of the most common genetic diseases in humans affecting all ethnic groups worldwide with an incidence of 1 in 500 to 1 in 1,000. ADPKD is genetically heterogeneous and can arise from mutations in two genes, named PKD1 and PKD2. Mutations of PKD1 located on chromosome 16p13.3 are responsible for 85% of cases. Aim Although there is no hotspots reported in PKD1, most mutations are seen in the downstream exons of this gene. So, we developed a clinical assay for PKD1 gene analysis using sequencing of 16 exons from 31 to 46 in 22 patients. Material and Method This study was carried out on a total of 22 patients. Genomic DNA was extracted from blood lymphocytes (5ml of whole blood) with standard methods and fragments were amplify using PCR. Screening of PKD1 mutations was performed by direct Sequencing. Result Sequencing result of exons 44 and 45 showed one completely pathogenic mutation causing Gln4005Arg change. Two novel non-synonymous variation including Arg4091Glu and Val4035Ilu and five likely neutral SNP including rs10960, rs3087632, rs3087632, rs10960, rs3087632 and rs3087632 are detected. There was no any variation in exons 35, 36 and 37. Conclusion It is expected that confirmation of pathogenic mutations can be useful for studies on disease molecular pathways. Early and prenatal diagnosis and personalized treatments recommended for family members after genetic consulting.

**P03.08-M**
Molecular diagnostics of autosomal recessive polycystic kidney disease by next-generation sequencing

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Autosomal recessive polycystic kidney disease (ARPKD) is a severe form of PKD with typical occurrence in neonates and infants. It is characterised by cystic dilations of the collecting ducts and congenital hepatic fibrosis. Perinatal mortality has been estimated to account 30-50% of ARPKD neonates and the majority of patients develop renal failure within 5 years, however presentation of ARPKD at later age and survival into adulthood have been also described. The aim of this work was to establish molecular diagnostics of ARPKD which could be used in “at-risk” families, as fetal ultrasonography has limited reliability in early pregnancy and some abnormalities typical for ARPKD become evident after 20 weeks of gestation. ARPKD is caused by mutations in PKHD1, whose longest transcript comprises 67 exons. Conventional mutational detection methods are therefore time-consuming and relatively expensive. For this reason next-generation sequencing has been chosen as a suitable method. After the setting up of NGS for PKHD1 gene, relatively expensive. For this reason next-generation sequencing has been chosen as a suitable method. After the setting up of NGS for PKHD1 gene, relatively expensive. For this reason next-generation sequencing has been chosen as a suitable method. After the setting up of NGS for PKHD1 gene, relatively expensive. For this reason next-generation sequencing has been chosen as a suitable method. After the setting up of NGS for PKHD1 gene, relatively expensive. For this reason next-generation sequencing has been chosen as a suitable method. After the setting up of NGS for PKHD1 gene, relatively expensive. For this reason next-generation sequencing has been chosen as a suitable method. After the setting up of NGS for PKHD1 gene, relatively expensive. For this reason next-generation sequencing has been chosen as a suitable method. After the setting up of NGS for PKHD1 gene, relatively expensive. For this reason next-generation sequencing has been chosen as a suitable method. After the setting up of NGS for PKHD1 gene, relatively expensive. For this reason next-generation sequencing has been chosen as a suitable method.
P03.09-S  
Disregulation of cytokine skeleton organization, carcinogenesis and immune response pathways involved in Balkan endemic nephropathy development.  
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Background: Balkan endemic nephropathy (BEN) is a chronic interstitial nephritis with endemic distribution, spreading over territories of several Balkan countries. The intricate interplay between genetic background and environmental factors is believed to be at the basis of this progressive disease leading to chronic kidney failure and associated with high incidence of upper urinary tract uroepithelial cancer.

DNA methylation is the most studied primary epigenetic mechanism that is involved in processes such as cancer, genomic imprinting, tissue differentiation etc. Epigenetic alterations could be a major contributing factor to BEN’s elusive etiology and pathogenesis.

Materials and methods: We performed whole genome DNA methylation analysis on DNA blood samples from 159 affected individuals and 170 healthy control samples from Bulgarian and Serbian populations (based on genetic and ancestral origin; Bulgarian and Serbian). After determining the methylation status of ca27000 CpG-islands throughout the whole genome (Agilent DNA methylation array 2x244k) we defined the differently methylated regions (DMRs) between patient groups and respective control groups. We then compared DMRs across different ancestral and gender groups.

Results: Analysis of the common DMRs between patient-control pairs revealed that genes involved in major biological processes appear to be affected in BEN - cell adhesion and cytokinetic organization/regulation of cell cycle - 14.8% of DMRs in both Bulgarians (BG)/Serbians (SER), carcinogenesis and metastasis - 7.41% (BG)/8.8% (SER) and immune response - 14.8% (BG)/6.4% (SER) respectively.

Conclusions: Data obtained from our experiments suggest that dysregulation of gene expression such as cytokinetic organization; cell cycle regulation and immune response could contribute to BEN pathogenesis. Acknowledgements: Funded by BNSF grant DMY03/35

P03.10-M  
NGS nominated CELA1, HSPG2 and KCNK5 as candidate-genes for predisposition to Balkan Endemic Nephropathy  
1Department of Medical genetics, Medical University of Sofia, Sofia, Bulgaria, 2Genomic laboratory of Malinov Clinical, Sofia, Bulgaria, 3National Center of Public Health and Analyses, Sofia, Bulgaria, 4Vratsa District Hospital, Vratsa, Bulgaria, 5Faculty of Medicine, University of Nis, Nis, Serbia, 6Faculty of Medicine, University of Skopje, Macedonia, 7Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria, 8Institute of Anatomy, Bern University, Bern, Switzerland.

Background: Balkan endemic nephropathy (BEN) is a familial chronic tubulointerstitial disease with insidious onset and slow progression leading to terminal renal failure. The results of molecular biological investigations propose that BEN is a multifactorial disease with genetic predisposition to environmental risk agents.

Results: Exome sequencing of 22000 genes with Illumina Nextera Exome Enrichment kit was performed on 22 DNA samples (11 Bulgarian patients and 11 Serbian patients). Software analysis was performed via NextGene, Proven and PolyPhen. The frequency of all annotated genetic variants with deleterious/damaging effect was compared with those of European populations. Then non-annotated variants with possible deleterious/damaging variants were filtered out using PolyPhen and identified two RAD21-putative binding sites. EMSA assays showed that wild-type RUNX1 expression, a RAD21 target, was decreased in the patient’s cell line. RAD21 morpholino knockdown in zebrafish embryos resulted in incomplete or absent expression of Runx1, in severe reduction of enteric neurons and delayed intestinal transit. These defects could be rescued only by wild-type RAD21 DNA, indicating that the mutation found in the CIPO pedigree extinues RAD21 functionality. Several binding sites for RAD21 were present in apolipoprotein A/F gene cluster and its altered binding to the regions dysregulated apoprotein expression levels. We studied APOB promoter and identified two RAD21-putative binding sites. EMSA assays showed that RAD21 wild-type, but not mutant, binds these regions. We therefore measured APOB protein levels that were increased in all CIPO patients, indicating the syndromic patient carrying the RAD21 mutation. Conclusion: The study of these two previously unlinked pathways in patients and in model organisms is contributing to clarify the pathogenesis of CIPO and will bear clinical implications for the diagnosis and prognosis of this disabling condition.

P03.11-S  
Whole exome sequencing reveals rare homozygous ARID1B and heterozygous MYTOR missense variants in a patient with bilateral cystic-dysplastic kidneys and features of Coffin-Siris syndrome and tuberous sclerosis.  
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ARID1B variants have recently been described as a major genetic cause of Coffin-Siris syndrome, a developmental disorder associated with anomalies of the kidneys and urinary tract (CAKUT) of 82 patients with Coffin-Siris syndrome carrying an ARID1B variant and examined for renal anomalies that have been described in the literature to date. 6% presented with a non-severe CAKUT phenotype. The causative ARID1B mutations detected in patients with Coffin-Siris syndrome so far were all heterozygous truncating variants. Here, we report a rare homozygous ARID1B missense variant detected whole exome sequencing in a patient with bilateral cystic-dysplastic kidneys, bilateral vesicoureteral reflux grade IV, and a septal myocardial tumor. Stage 4 chronic kidney disease was diagnosed at 6 months of age, arterial hypertension developed, and pre-emptive kidney transplantation was performed at age 3.5 years. Additionally, the patient presented with a number of features frequently described in patients with Coffin-Siris syndrome carrying ARID1B mutations, such as thick eyebrows, synophrys, thick eyelashes, strabismus, broad-boned nose and small mouth, arched palate, sandal gap, and behavioral anomalies. However, unlike all patients with heterozygous truncating ARID1B mutations, our patient had normal intelligence. Interestingly, whole exome sequencing also revealed a rare deleterious heterozygous MYTOR missense variant inherited from a healthy mother in our patient. mTOR is regulated by TSC 1/2, TSC2, encoded by genes that when mutated can cause tuberous sclerosis associated with renal-cystic disease and myocardial tumors. Therefore, the MTOR and ARID1B variants may have acted together to cause the severe bilateral cystic-dysplastic kidneys present in our patient.
incidence among the various groups. The CYP21A2 gene, localized in a genetic unit defined RCCX module and is considered one of the most polymorphic of human genes.

We considered new evidences about the presence of a RCCX trimodular haplotype with the CYP21A2 gene to explain the lack of a genotype-phenotype correlation in two patients referred to our centre for a suspected Non Classical form of CAH (NC210H).

To identify the presence of deletions/duplications we used Multiplex Ligation Probe-Dependent Amplifications (MLPA) and to confirm the presence of a CYP21A2-like gene downstream TNXA gene we used previously described amplification and restriction strategy followed by the sequencing of the CYP21A2 gene downstream TNXB gene.

We validated the methods developed by recent studies and we found a good concordance with their results. The amplification strategy, direct sequencing and restriction analysis of CYP21A1P/CYP21A2/TNXA PCR product in association with MLPA assay and sequencing of CYP21A2 gene downstream TNXB gene, were able to identify the presence of more than one copy of CYP21A2 gene.

The strategy suggested is useful to screen cases where there is no genotype-phenotype correlation. We validated the modified screening strategy to facilitate molecular testing of CAH patients, considering the new evidence about possible different haplotypes.

P03.14-M

Familial mediterranean fever (FMF): genetics and role of $\beta$004A

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Number of patients with FMF in Armenia is dramatically increasing due to genetic drift and better professional competence, treatment, genetic counselling. Clinical and genetic investigations of FMF are “forced” by the high social and public health problems. Common MEFV mutations account for 98.7% of FMF patients and 1.3% in healthy population. 18.72% of heterozygote carriers have abortive or mild features; no mutations detect in 1.29% of FMF patients. Particular MEFV mutations ($\beta$694V homozgyote) have significant correlation with renal amyloidosis (RA). The risk of male patients to develop RA is four times higher than that of female patients. Colchicinotherapy delays RA progression, but patients with M694V-homozygous genotype develop RA is four times higher than that of female patients. Colchicinotherapy delays RA progression, but patients with M694V-homozygous genotype present a more severe phenotype and a limited response to colchicine at the nephrotic stage of RA. Patients with other genotypes have a good chance to escape the nephrotic syndrome and to maintain renal function.

Serum amyloid A1 (SAA1) $\alpha/\alpha$ with M694V homozygous genotypes are associated with a seven-fold increased risk of developing RA, compared to other SAA1 genotypes. The presence of one SAA1 $\alpha/\alpha$ allele does not suggest an increased susceptibility to RA.

Association of pro-inflammation with pathogenesis of neoplasia, inflammatory autoinflammatory diseases is confirmed. Correlation of $\beta$004A4 concentration with a pattern of MEFV mutations has been analyzed. Data demonstrating a significant increase of $\beta$004A4 in plasma of 100 FMF patients likely implicating of S100A4 in the pathogenesis of the disease. These findings suggest that chronic inflammation is mediated by S100A4 and SAA1 genotypes. The presence of one SAA1 $\alpha/\alpha$ allele does not suggest an increased susceptibility to RA.
### P03.18-M

**Exonic de novo Mutations in Sporadic Hirschsprung Disease**

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Hirschsprung disease (HSCR) is a disorder of the enteric nervous system (ENS) and is characterized by the absence of enteric neurons along a variable length of the intestine. HSCR most commonly presents sporadically, although it is familial in 5–20% of the patients. The sporadic form of the disorder is believed to be a genetically complex disease. To assess the role of de novo mutations in sporadic HSCR, we performed exome sequencing on 20 HSCR patients, predominantly females with long segment HSCR and their unaffected parents. We identified and confirmed 24 de novo mutations (18 SNVs, 6 indels) in 17 different genes (1.2 per trio). Non-synonymous de novo mutations were identified in RET in 8 out of 20 patients, corroborating previous findings that RET is the major genetic contributor in long-segment HSCR. A replication study in independent HSCR patients, gene burden tests and functional analysis in both cell lines and the loss-of-function zebrafish model, we provide a unique system to examine the function of novel mutations in HSCR.

### Table 1: Mutations/SNPs identified in the present study

<table>
<thead>
<tr>
<th>S.No</th>
<th>Type of GHD (N=100)</th>
<th>Gene</th>
<th>cDNA position</th>
<th>Amino acid position</th>
<th>Nature of mutation/SNP</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>IGHD (n=60)</td>
<td>GHI</td>
<td>Gross deletion</td>
<td>-</td>
<td>Reported</td>
<td>15%</td>
</tr>
<tr>
<td>2</td>
<td>GHI</td>
<td>Deletion of exons 3-5</td>
<td>-</td>
<td>Novel</td>
<td>2%</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>GHI promoter</td>
<td>rs2005171</td>
<td>-</td>
<td>Reported</td>
<td>20%</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>GHI promoter</td>
<td>rs2005172</td>
<td>-</td>
<td>Reported</td>
<td>15%</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>GHI promoter</td>
<td>rs1156828</td>
<td>-</td>
<td>Reported</td>
<td>18%</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>GHRHR</td>
<td>c.214G&gt;T</td>
<td>Glu71Term</td>
<td>Reported (Nonsense)</td>
<td>10%</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>GHRHR</td>
<td>c.527C&gt;T</td>
<td>Ala176Val</td>
<td>Reported (Missense)</td>
<td>3%</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>GHRHR</td>
<td>c.920insC</td>
<td>-</td>
<td>Novel (Insertion)</td>
<td>2%</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>PROP1</td>
<td>c.112_124del13</td>
<td>-</td>
<td>Reported (deletion)</td>
<td>2.5%</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>PROP1</td>
<td>c.45C&gt;T</td>
<td>Arg15Term</td>
<td>Reported (Nonsense)</td>
<td>2.5%</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>PROP1</td>
<td>c.128insA</td>
<td>-</td>
<td>Novel (Insertion)</td>
<td>2.5%</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>POUF1F1</td>
<td>c.605-1G&gt;A</td>
<td>-</td>
<td>Novel (Acceptor splice site)</td>
<td>7.5%</td>
<td></td>
</tr>
</tbody>
</table>

**P03.19-S**

**RET and EDNRB mutations screening in Indonesian patients with HSCR, functional study and its implication in diagnostic**

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Hirschsprung disease (HSCR) is a major cause of chronic constipation in children. HSCR is an inherited disease and germline mutations are frequently identified in RET and EDNRB. However, defining causality of mutations in this genetic complex disorder is difficult. Therefore, in this study we combined sequence analysis of RET and EDNRB with functional studies to determine causality. We screened a total of 61 HSCR children and identified 8 rare RET coding variants (R79W, R144H, P270L, R694Q, A756V, G533S, Y1662C,D489N) and one possible splicing site variant. No rare variant in EDNRB were identified. The mutation frequency (15%) is comparable to previous studies. Four missense variants and one possible splice site variant have never been reported before. Four of the nine arose de novo, while the remaining variants are all inherited from an unaffected parent. One patient harbor two RET coding variants, one coming from each unaffected parent. Functional studies showed that 7 out of 8 coding RET variants resulted in significant lower expression of the phosphorylated-RET protein. Only 4 RET variants (R144H, R694Q, A756V, Y1062C) resulted in significantly lower expression of phosphorylated-ERK, a downstream component of the RET pathway, when compared to wild type RET. The possible splice-site variant (ISS 1880-A>G) did not disturb the splicing process and, therefore, is not considered pathogenic. Our data suggest that 7 of the 9 identified RET variants are pathogenic and that not all rare RET variants are pathogenic, hence functional studies are essential to prove pathogenicity.

**P03.20-M**

**Translating genetic findings in functional defects: functionomics of hypomagnesemia-causing genes**

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The recent improvements of next generation sequencing techniques allow relatively rapid and cheap identification of new gene mutations in patients with rare hereditary hypomagnesemia. However, translating the genetic findings into functional assays to examine the function of the affected genes remains challenging because of inadequate cell models, the absence of Mg²⁺ radioisotope and limited availability of animal models. For example, we have identified new mutations in the gene CNM2 in five families suffering from mental retardation, seizures, and hypomagnesemia. For the first time, a recessive mode of inheritance of CNM2 mutations was observed and mutations in CNM2 are associated with mental disability. Using stable Mg²⁺ isotopes, we demonstrated that CNM2 increases cellular Mg²⁺ uptake in HEK293 cells and that this process occurs through regulation of the Mg²⁺–permeable cation channel TRPM7. In contrast, cells expressing mutated CNM2 proteins did not show increased Mg²⁺ uptake. Knockdown of cnm2 isoforms in zebrafish resulted in disturbed brain development and reduced body Mg content. These phenotypes were rescued by injection of mammalian wild-type Cnm2 cRNA, whereas mammalian mutant Cnm2 cRNA did not improve the zebrafish knockdown phenotypes. Altogether these data show that CNM2 is fundamental for brain development, neuronal functioning and Mg²⁺ homeostasis. By establishing a novel Mg²⁺ transport assay using stable Mg²⁺ isotopes and the loss-of-function zebrafish model, we provide a unique system to examine the function of novel genes in Mg²⁺ homeostasis. These new in vitro and in vivo models may aid to explain the function of electrolyte transporters in the future.

**P03.21-S**

**Family genomics reveals disease genetics: Inflammatory bowel disease**


By definition, complex diseases are caused by many different genes, but this might hold true only at the population level. We hypothesize that within families with a high disease burden, complex diseases can arise from only a few high-effect risk variants. Assuming that all or most affected family members share the genomic region(s) that harbor the risk variant(s), whole-genome sequencing is the most precise method for determination of identical-by-descent segments to narrow down the search space for disease genes. Family genomics is especially powerful in multi-generation pedigrees. In some of the pedigrees we analyze disease individuals are separated by more than 10 meioses each of which narrows the number of candidate alleles by approximately half, per Mendel’s law of segregation. Family genomics furthermore permits identification of sequencing errors and detection of rare variants with high confidence. In addition to our identical-by-descent detection tools we have created an analysis workflow to identify and score variants according to their inheritance pattern, population frequency and predicted function. We are currently analyzing over 200 families in over 30 studies that cover a wide range of diseases from rare congenital diseases to common neurodegenerative and chronic inflammatory diseases. We present here our methods for identifying high confidence candidate variants for inflammatory bowel disease in families with high disease burden.
A hemizygous mutation in retinitis pigmentosa GTPase regulator (RPGR) is a potential novel cause of congenital renal tract malformations

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Congenital anomalies of the kidney and urinary tract (CAKUT) comprise a spectrum of structural malformations and constitute the principal cause of end-stage renal disease in children. Although several genes have been linked to CAKUT pathogenesis, the genetic background remains unclear in many patients. We performed whole exome sequencing in the patient’s DNA (SureSelect, Agilent / SOLID5500™ System, Life Technologies). Through whole exome sequencing we identified a novel hemizygous dinucleotide frameshift deletion in the X-linked retinitis pigmentosa GTPase regulator (RPGR) gene of a male patient with unilateral kidney dysplasia. This mutation was confirmed through Sanger sequencing and was found to be inherited from the mother. Mutations in RPGR are frequently reported in patients with progressive retinal degeneration, however its potential role in kidney pathogenesis has not previously been observed. At present, we investigate the effect of RPGR dysfunction on kidney development in vivo, by generating zebrafish mutants using transcription activator-like effector nuclease (TALENs). Subsequently, computational analysis will be performed to study the specific effect of the human RPGR mutation in this vertebrate system. Also, we aim to characterize both wild-type and mutant RPGR in vitro, using patient-derived cell-lines isolated from urine and IMCD-3 (urine inner medulillary collecting duct) 3D cellular spheroids. To conclude, by identifying novel players in the aetiology of CAKUT, we will enhance our knowledge on the molecular networks underlying normal and disrupted kidney development. Identification of the genetic aetiology of CAKUT will improve diagnostics and genetic counselling for CAKUT patients.

The Prognosis of Abnormal Fetal Kidneys

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Introduction: With the advent of high-resolution ultrasound scanners and the offer of a 20-week ultrasound scan to all pregnant women in Denmark, a suspicion of abnormal kidneys in the fetus is raised more frequently now than earlier. The prognosis of a fetus with abnormal kidneys is often not known, which complicates decision-making and causes anxiety to parents.

Methods: A population-based cohort of fetuses diagnosed with abnormal kidneys in the period 1st of January 2007 - 31st of December 2012 and a population-based matched comparison cohort of foetuses examined in the 20-week screening programme were established.

A national query identified fetuses with cystic kidneys, echogenic kidneys, multicystic kidneys, renal agenesis and/or severe hydronephrosis. Severe hydronephrosis was included in the query to avoid missing cases of multicystic kidneys, as the primary finding may have been interpreted as hydro-"nephrosis has not previously been observed. At present, we investigate the effect of RPGR dysfunction on kidney development in vivo, by generating zebrafish mutants using transcription activator-like effector nuclease (TALENs). Subsequently, computational analysis will be performed to study the specific effect of the human RPGR mutation in this vertebrate system. Also, we aim to characterize both wild-type and mutant RPGR in vitro, using patient-derived cell-lines isolated from urine and IMCD-3 (urine inner medulillary collecting duct) 3D cellular spheroids. To conclude, by identifying novel players in the aetiology of CAKUT, we will enhance our knowledge on the molecular networks underlying normal and disrupted kidney development. Identification of the genetic aetiology of CAKUT will improve diagnostics and genetic counselling for CAKUT patients.

The Prognosis of Abnormal Fetal Kidneys

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Introduction: With the advent of high-resolution ultrasound scanners and the offer of a 20-week ultrasound scan to all pregnant women in Denmark, a suspicion of abnormal kidneys in the fetus is raised more frequently now than earlier. The prognosis of a fetus with abnormal kidneys is often not known, which complicates decision-making and causes anxiety to parents.

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All Danish departments of obstetrics and fetal medicine use the same software for prenatal diagnostics and ultrasound examination. These data were merged into a national database of around 1000 pregnancies. Prenatal fetal data were linked to their mothers' data using the mothers' civil registration numbers. This allows unambiguous individual-level linkage to all relevant data sources in Denmark, including the Danish National Patient Registry and medical journals.

Results: The prevalence of the various types of abnormal fetal kidneys, the prevalence of terminated pregnancies and measures of prognosis will be discussed. This study may facilitate that parents having a fetus with kidney abnormalities can be counselled based on valid and comprehensive data on prognosis.

Targeted sequencing of 208 candidate genes in 460 CAKUT patients facilitates the inclusion of a novel gene set in diagnostics

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Congenital anomalies of the kidney and urinary tract (CAKUT) comprise a spectrum of structural malformations. CAKUT occur in 1:500 live-births and form the most common cause of end-stage renal failure in children. We aim to identify rare mutations in CAKUT candidate genes and elucidate involvement of CAKUT aetiology. After in-solution enrichment (SureSelect, Agilent), 208 candidate genes were sequenced (SOLiD5500™, Life Technologies) in 460 sporadic CAKUT patients. All genes were known to play a role in human CAKUT or to disrupt nephrogenesis in animal models. We demonstrated coverage depth of 120X and 65% enrichment efficiency on average. After variant calling, filtering was performed based on sequencing depth (≥15X) and allele frequency, excluding common variants. We identified 47 indels, 20 nonsense, 22 essential splice-site mutations, as well as 150 private missense variants in 82 genes that were predicted to be pathogenic. Of these, 71 variants in 39 genes were previously reported in The Human Gene Mutation Database as causal mutations for kidney-related disorders. The variants are currently being validated in patients and parents by Sanger sequencing. Preliminary data show that the majority of variants are inherited, except for a well-known frameshift variant in PAZ2 and a novel variant in LZTS2, which occurred de novo. We conclude that our approach provides knowledge on the frequency of known disease-causing mutations and underlines the heterogeneity of CAKUT. Results indicate the value of including novel genes in an NGS based genetic test for CAKUT, facilitating early diagnostics and genetic counselling for CAKUT patients and their relatives.

Renal fibrosis is the common feature of Autosomal Dominant Tubulointerstitial Kidney Diseases (ADTKD) caused by MUC1 or UMOD mutations


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For decades, clinically ill-defined autosomal-dominant renal diseases originating from tubular cells and leading to tubular atrophy and interstitial fibrosis were reported. Patients exhibit mutations in at least 4 genes: UMOD, INHBE, RB1 and MUC1, but are clinically indistinguishable all associated with renal fibrosis and renal failure the 3rd and 6th decade of life. In contrast to what the frequently used term “Medullary Cystic Kidney Disease” (MCKD) implies, development of medullary cysts is neither an early nor a typical feature, as analyzed by MRI.

We now investigated 10 such families. In two large families we performed genome-wide linkage and haplotype analyses confirming linkage to a known 14 Mb locus MCKD1 located on chromosome 1q21. Targeted genomic sequencing of the complete linkage locus in affected and healthy individuals of these two families and affected individuals of further families failed to uncover any segregating variant in any of the genes, including MUC1. The VNTR region in the coding sequence of the MUC1 was masked in these analyses due to its high GC-content and repetitive nature. After the recent publication of one MUC1-VNTR insertion mutation we established SNP-array and sequencing confirming this MUC1 mutation in 4 families. Further 3 families carried an UMOD mutation, leaving 3 families unsolved to date. On the basis of clinical and pathological characteristics we propose the term “Autosomal Dominant Tubulointerstitial Kidney Disease” (ADTKD) as a new name for this entity. This new terminology should enhance recognition and correct diagnosis of affected individuals, facilitating genetic counseling and stimulating research into the underlying pathomechanisms.
Gene Expressions In Glomerulopathies

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The nephritic syndrome is connected with both primary and secondary glomerulopathies. The expressions of 47 genes associated with different kidney diseases in patients with primary and secondary glomerulopathies were studied. The aim of this study is to find prognostic factors from renal biopsy. Our study was performed on the set of 118 patients. There were five different biopsy-proven diagnoses: FSGS, IgAN, MCD, MGN and SLE. The expressions were analysed using the real-time PCR and normalized by the endcontrol GAPDH gene. The obtained data were analysed using software STATISTICA 10. At the beginning three genes had to be excluded for no patient’s sample had showed the expression in these genes. The statistical analysis using Kruskal-Wallis ANOVA was divided into three parts. The p value of following genes was lower than 0.01. Firstly, all the diagnoses were compared with each other: The statistically significant differences were identified in nine genes mostly between FSGS and SLE patients. Secondly, patients with SLE and IgAN were sorted out according to their histological foundations and the gene expressions were compared in each diagnosis. The only significant gene was in the comparison of IgAN patients. Finally, it was made cross comparison between groups of SLE and IgAN patients, MCD, FSGS and MGN patients. The statistically significant differences were identified in ten genes. The differences were mostly between one group of SLE patients and patients with FSGS. Our study demonstrated the differences in gene expressions not only inter-diagnosis, but also intra-diagnosis. Supported by the grant project PROUK-PS5/LF1/2.
ABSTRACTS POSTERS

P03.31-S
Epistatic Association of CTRC and SPINK1 Gene Variants Combination with Recurrent Pancreatitis in Lipoprotein Lipase Deficiency
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Background: Lipoprotein lipase deficiency (LPLD) is a rare autosomal recessive disease associated with severe hypertriglyceridemia and increased risk of pancreatitis or other co-morbidities. There are important unexplained inter-individual variations in the incidence and severity of acute pancreatitis episodes in LPLD patients. Several genes involved in proteolytic networks are associated with pancreas and lipoprotein functions and could influence pancreatitis risk in LPLD. Objective: To evaluate the association between two genes regulating serine proteases, chymotrypsin C (CTRC) and serine peptidase inhibitor Kazal type (SPINK1), and recurrence of hospitalizations for acute pancreatitis among LPLD subjects. Methods: CTRC and SPINK1 have been sequenced in a sample of 38 LPLD patients and 100 controls. In LPLD, 16 (47%) presented a history of recurrent (≥5) hospitalizations for acute and severe abdominal pain, whereas 9 (21%) had not yet been hospitalized for this condition. Comparisons between studied groups were done with chi-square tests and multinominal regression analyses. Results: Gene sequencing identified 15 SNPs. Genotype-stratified analyses in LPLD subjects and controls suggested an epistatic association between rs545634 (CTRC) and rs11319 (SPINK1) SNPs combination and recurrence of hospitalizations (p<0.001) in LPLD. Regression analyses, controlling for age, gender and smoking, confirmed that the risk of frequent and recurrent hospitalizations for acute pancreatitis is significantly increased in LPLD in presence of this CTRC-SPINK1 SNPs combination (OR = 4.1 [CI: 2.0-8.48]; p = 0.016). Conclusion: These results suggest that modifier genes involved in protease pathways could influence the trajectory of pancreatitis risk in LPLD.

P03.32-M
Towards introduction of exome sequencing in pediatric liver transplant program
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Pediatric cholestasis shows different etiology, clinical course and prognosis. Early recognition of the causes allows an appropriate treatment and management and plays a central role in decision-making about pediatric liver transplantation. Although each of genetic forms of cholestasis is rare, they collectively represent a frequent causes of liver transplantation. Starting in 2012, we enrolled and analyzed by exome sequencing 44 pediatric patients with isolated or syndromic forms of liver disease. The exonic region of 2,761 genes which are associated with isolated or syndromic forms of liver disease. Among 9 patients we found mutations in SPINK1 gene while CTRC gene acquires a truncating mutation in one patient.

P03.33-S
Success of liver transplantation in patients with progressive familial intrahepatic cholestasis: is there an association between genotype and outcome?
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Study aim: Characterization of the genotype-phenotype correlation in patients with progressive familial intrahepatic cholestasis (PFIC) and identification of prognostic genetic parameters for long term outcome and success of liver transplantation in order to improve individual treatment options.

Method: Genetic testing was performed for 57 PFIC index patients, including NGS panel diagnostics for 5 children with infantile cholestasis and atypical liver histopathology. Long term data (2-35 years) of 18 patients with genetically confirmed PFIC will be presented to illustrate their specific medical problems.

Results: Most pathogenic mutations were identified in ABCB11 (56%), followed by ATP8B1 (32%) and ABC1 (12%); including 73% missense mutations, 17% truncating mutations, 7% splice mutations and 3% small deletions. Response to pharmacological therapy was insufficient in 33% of patients, thus, biliary diversion and/or liver transplantation was performed in 75%. One additional patient is currently listed for transplantation.

Perioperative complications observed in patients with missense mutations were mild and included bleeding and coagulation problems (n=2). Life threatening complications (organ rejection requiring retransplantation, death) occurred in two patients with at least one truncating ABCB11 mutation. Severe long term complications were observed in 18% of patients with confirmed carriers including severe diarrhea, renal failure, deafness and neuropathy.

Conclusion: Truncating mutations may be associated with a higher rate of severe perioperative complications in liver transplantation, possibly due to a stronger immune reaction towards the wild type protein. With an ongoing study we are currently evaluating this hypothesis in a larger cohort including application of NGS panel sequencing to search for additional causative genes.

P03.34-M
Genetic analysis of PKD1 and PKD2 genes in Korean patients with autosomal dominant polycystic kidney disease
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Autosomal dominant polycystic kidney disease (ADPKD) is the most common inherited kidney disorder with progressive cyst growth and renal enlargement, resulting in renal failure. Mutations in the PKD1 and PKD2 genes account for 85% and 15% of all ADPKD cases, respectively. Although there are a few studies on the frequency and spectrum of mutations in the PKD1 and PKD2 genes in Korean patients with ADPKD, they did not sequenced entire exons of the PKD1 gene but analyzed only for exon 36-46 excepting the pseudogene region, which made it difficult to evaluate the accurate frequency and the spectrum of mutations. Therefore, we performed sequence analysis of 20 consecutive unrelated ADPKD patients for kidney transplantation by using long-range PCR to avoid pseudogene amplification followed by exon-specific PCR and sequencing of the entire exons of the two genes. All patients met the diagnostic criteria of ADPKD based on the ultrasonographic findings and family history of PKD and 14 patients (70%) were revealed to have mutations; 11 with PKD1 and 3 with PKD2 mutations, respectively. Among 10 novel mutations, eight mutations were found in PKD1 gene while two mutations in PKD2 gene. All were deletion mutations except one missense mutation. It is of note that 6 out of 11 PKD1 mutations (54.5%) were located outside the range of exon 36-46. Considering the mutation spectrum of the Korean patients with ADPKD, long-range PCR followed by direct sequence analysis of pseudogene region should be performed for accurate molecular diagnosis of the disease.

P03.35-S
Primary hyperoxaluria: analysis of GRHPR, HG01A genes and the promoter-sequence of AGXT gene in the Italian population
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Primary hyperoxaluria (PH) is a rare autosomal recessive disease, commonly arising in childhood with nephrolithiasis, nephrocalcinosis, chronic renal failure. Mutation in AGXT gene while HG01A gene and promoter sequence of AGXT gene are the other candidate genes responsible for PH. In the current study, we performed homozygous tests for the c.341+1G>T, HG01A variant in intron 2. This variant is not reported in literature and was evaluated as pathogenic by in silico prediction, generating a new acceptor splicing site (AG in c.341-79). The minigene in-vitro assay demonstrated that this variant did not interfere with splicing.

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Two patients were heterozygous for two different AGXT-pm variants (c.-647C>T, c.-424C>T), not reported in the scarce literature nor in 1000Genomes Database. These variants have been evaluated as not pathogenic because they do not lie in any known regulatory-transcription site. The negative results in those patients with high clinical suspicion of PH could be explained by undetected deletions (investigable by MLPA analysis of the two major genes) or mutations in other genes involved in oxalate metabolism, or differential diagnosti.

**P03.36-M**

Screening of a large cohort of Italian patients with Albright hereditary osteodystrophy and/or Pseudohypoparathyroidism phenotype for subtelomeric deletions of chromosome 2

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Pseudohypoparathyroidism (PHP) is a heterogeneous group of rare genetic disorders due to end-organ resistance to the actions of PTH caused by genetic and/or epigenetic defects within or upstream the GNAS locus. The classification in different subtypes is based on the presence of specific somatic and developmental abnormalities, referred to as Albright hereditary osteodystrophy (AHO), and of resistance to other hormones acting via G protein coupled receptors.

Despite the advances in the study of PHP molecular determinants, about 30% of patients still lack a molecular diagnosis and, in the last years, independent groups found in a subset of PHP/AHO patients causative defects classically associated to diseases with partially common phenotype, such as deletions of 2q37.2 associated with the AHO-like syndrome (or brachydactyly-mental retardation syndrome, BDMR).

In this study, we screened by a multiplex ligand-dependent probe amplification (MLPA) assay targeting the chromosome series of AHO/PHP pts negative for GNAS defects (n=56) and we detected 3 different deletions of 2q37, overlapping but smaller than those previously described.

Ongoing studies will define the inheritance pattern of such deletions and will allow to narrow the common critical region associated with the AHO phenotype.

In conclusion, our data further confirm the molecular and clinical overlap between PHP/AHO and BDMR and will hopefully help to define genes involved in the AHO phenotype. Furthermore, all PHP/AHO pts negative for GNAS genetic/epigenetic defects should be considered for further molecular investigations to optimize genetic counselling.

**P03.37-S**

**Exome sequencing reveals TPO mutations in Pseudo-Pendred syndrome**

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Pseudo-Pendred syndrome (PDS) is defined by the association of sensorineural deafness, hypothyroidism due to iodide organification defect, absence of inner ear malformation and absence of mutation in SLC26A4. The gene responsible for classical PDS is SLC26A4.

In order to determine the cause of PDS, we performed whole exome sequencing (WES) in a family with two children affected with hypothyroidism, developmental delay, positive perchlorate test and absence of inner ear abnormalities. Parental karyotype was normal in both parents and the father was heterozygous for the Y453D mutation.

In silico prediction tools determined a deleterious mutational impact of both variants on protein function. Sanger sequencing confirmed the mutation (W432C) and found that the mother was heterozygous for W23C. The latter mutation was novel and both were absent in the control population.

TPO encodes thyroperoxidase which catalyzes key reactions in thyroid hormone synthesis and mutations in TPO are responsible for thyroid dys-ormogenesis 2A. Mutations in TPO have been reported in 4 patients with hypothyroidism and deafness in a series of Israeli patients with iodide organification defect (Tenenbaum-Rakov, 2007) but their phenotype was not described as PDS.

These cases together with the present report suggest that mutations in TPO can be responsible for pseudo-PDS.

**P03.38-M**

**Renal function decay after nephrectomy: role of surgery and impact of hypertension gene polymorphisms**

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The loss of nephronic mass leads to the development of hypertension and chronic renal failure, but the frequency and the rate at which this takes place after radical nephrectomy (RN) or nephron sparing surgery (NSS) is extremely variable and the mechanisms have not been clarified to date.

To evaluate the progression rate of renal function decay within a population undergoing kidney surgery and to assess the influence of polymorphisms of genes involved in essential hypertension (HT), 162 patients were followed at the Outpatient Clinic of Nephrology after a surgery for renal carcinoma, 75 RN and 87 NSS. The eGFR (estimated Glomerular Filtration Rate), an indicator of renal function, was evaluated for a mean follow-up of 23.5 months.

128 genetic polymorphisms located in 70 loci candidate for HT have been tested by TaqMan OpenArray system. Statistical analysis was performed using a General Linear Model allowing for sex, age, BMI, blood, therapy, basal renal function and type of surgery.

About the different surgical techniques, NSS patients displayed a significant decrease of eGFR variation significantly lower than that observed in RN ones (-5.7 ml/min vs. -23.9 ml/min P<0.05). Two SNPs, located in SIK1 and in PRK吉11 GG (n=8) vs. -4 at the same time in all the other groups (n=154). The progression of renal function decline after kidney surgery depends both on surgery type (80%) and on genetic background (15-20%).

**P03.39-S**

**Hereditary renal hypouricemia causing by defect in URAT1: a new insight into molecular pathology**

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Renal hypouricemia is a rare heterogenous inherited disorder characterised by impaired tubular uric acid transport with severe complications, such as acute kidney injury. So far, more than 100 patients with a loss-of-function mutation in the SLC22A12 gene (URAT1, OMIM #220150) and more than ten patients with defects in the SLC2A9 gene (GLUT9, OMIM #612076) have been described. The serum uric acid concentration in the proband was 1.1 mg/dL and expressed as an increase in the fractional excretion of uric acid 43%. The SLC22A12 gene analysis for the patient revealed compound heterozygous variants of p.G366R and p.R477H. Functional and immuno-chemical analysis of urate of URAT1 mutants was performed in Xenopus laevis oocytes. The urate uptake ability decreased to similar levels seen in mock samples in p.G366R mutant expressed oocytes. The p.R477H variant showed almost the same activity as the URAT1 wild type. In the co-expression samples, both variants p.WT/G366R and p.G366R/p.R477H lost their urate uptake activities. Variants p.WT/p.R477H tended to decrease urate transport compared to WT single expression; however, it was superior to the other two co-expression patterns (significant to p(WT)/G366R, P<0.05). Co-localization studies showed an accumulation of URAT1 in the endoplasmic reticulum of the p.G366R variant and mainly retention of wild type protein by variants p.G366R and p.R477H. The findings suggest that not only a loss-of-function mutation of URAT1 but also the dominant-negative effect cause renal hypouricemia via loss of uric acid absorption, partly due to protein misfolding caused by accumulation of URAT1 protein in the endoplasmic reticulum. Support: LH13245 and PRVOUK-P25L/F1.2

**P03.40-M**

**Clinical reappraisal of SHORT syndrome at the light of the PIK3R1 gene discovery**

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Multiple SNP score associated to chronic renal disease risk predicts presence of lipodystrophy and insulin resistance permit to classify recovery permitted to revise the diagnostic criteria for SHORT syndrome. The clinical reappraisal of SHORT syndrome at the light of the well as diabetes mellitus in adolescence or adulthood (8/11). In conclusion, enophtalmia (20/20), lipodystrophy (19/19), insulin resistance (11/12), as metropia can be present. Hyperextensibility of joints and/or inguinal hernia SHORT acronym. Indeed, the Rieger abnormality was found in less than half.

P03.41-S Multiple SNP score associated to chronic renal disease risk predicts the eGFR of patients with newly diagnosed type 2 diabetes

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Congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency is an autosomal recessive disorder characterized by cortisol with/without aldosterone deficiency, and androgen excess. The severity of clinical manifestations depends on the degree of 21-hydroxylase impairment caused by mutations in the CYP21A2 gene. Its classic form has an overall incidence of 1 per 15000 live births. CYP21A2 is flanked by the TNXB gene, encoding the extracellular matrix protein tenasin-X. Both, haptoinsufficiency and clinical loss of tenasin-X have been associated with phenotypes similar to Ehlers-Danlos syndrome (EDS). A significant portion of CAH-patients presents deletion of mostly one CYP21A2-allele, and up to 13% of these might be a contiguous deletion extending into TNXB.

We report two brothers (20 and 29 years old) with classic CAH and hemozygous deletion encompassing CYP21A2 and TNXB, who were evaluated for clinical presence of EDS. Both present skin hypertextiness, joint hypermobility and clinical history of multiple joint subluxations, without evidence of atrophic scars or other classic EDS features. The younger brother underwent surgery for inguinal hernia and rectal prolapse during adolescence. Cardiac ultrasound examination revealed bicuspid aortic valve, mild aortic dilatation and mitral valve insufficiency in the older brother; cardiologic follow-up was advised.

The parents, heterozygous carriers of the contiguous deletion of CYP21A2 and TNXB, don't show clear features of EDS. To our knowledge, bicuspid aortic valve has not been observed in CAH with tenasin-X-related EDS. Further delineation of Tenasin-X-related Ehlers-Danlos Syndrome in patients with Congenital Adrenal Hyperplasia.

ABSTRACTS POSTERS Back to index
P03.47-S
XX male sex reversal in an azospermic proband
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The basis of definition of genetic sex determination was initially, at least in mammals, limited to a form of chromosomal currency. However, the presence of an entire Y chromosome is not essential for the development of the male phenotype and transfer of the SRY gene alone could be a sufficient condition. Critical mutations in the SRY gene are linked with XY female sex reversal in mice and humans indicating that SRY is essential for male development. It appears that the only role of SRY is to upregulate the SRY-related HMGB box containing gene 9 (SOX9) bipotential gonads, which results in Sertoli cell differentiation and, ultimately, in testes differentiation. XX male sex reversal is rare and in most cases is the result of translocation of the SRY gene to the X-chromosome during male meiosis. However, a recent report by Cox et al. [2011] described a family, two brothers and an uncle, with 46, XX male sex reversal who lacked the SRY gene and had a 600 kb duplication upstream of SRYX. We report on a case of a 38 infertile male (sent by the fertility unit) with azospermia and hyponagonadotropic hypogonadism as 46, XX male sex reversal (without SRY). A 244 k b ASH in the proband showed a novel and apparently, de novo duplication within SOX3 gene (at Xq27.3) in 110 chromosomes. SOX3 is a XX male sex reversal. These data provide additional evidence that SOX3 gain-of-function in the XX bipotential gonads causes XX male sex reversal.

P03.49-S
Identification of 65 novel mutations and genotype-phenotype correlations in patients with Alport syndrome (ATS) and thin basement membrane nephropathy (TBMN).
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ATS is characterized by hematuria, proteinuria with progression to end-stage renal disease (ESRD), eye abnormalities and sensorineural deafness. Mutations in COL4A3/4 (autosomal recessive/dominant), and COL4A5 (X-linked) have been identified as underlying cause. Compared to ATS, autosomal dominant TBMN is characterized by hematuria, minimal proteinuria and preserved renal function. Dominant mutations have been identified in COL4A3/5, and COL4A4.

A cohort of 167 patients and 49 relatives were genetically tested and clinically evaluated. In 145 patients mutations were identified, 65 of them were novel. 54% resp. 88% of the ATS patients showed only one mutation in COL4A3 or COL4A4. Three TBMN and eight ATS patients (COL4A3 or COL4A4) had hearing loss, but only two of the ATS patients carried two mutations. An ATS patient with one mutation in COL4A4 showed no hematuria, but proteinuria, ESRD, and hearing loss. Two patients (COL4A5) showed isolated proteinuria. 26% of the ATS patients (COL4A5) had hearing loss and 43% of these presented the mutation at the 5′ end of the gene.

This study comprises one of the largest genotype-phenotype correlation in European ATS and TBMN patients. A large number of novel mutations were detected. Many patients had one only heterozygous mutation in COL4A3/5/COL4A4. This might be explained either by an autosomal dominant inheritance, the possibility of missed mutations, its function as a modifier or by more than one gene affecting ESRD. The identification of all these possibilities led us to consider in differential diagnosis Trichorhinophalangeal subtype III syndrome.

P03.45-S
Renal and urinary system malformations in girls with Turner syndrome
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Turner Syndrome (TS), in which there is a loss of all or part of one X chromosome, occurs in 1 in 2500 born females. Renal and urinary system malformations with their posterior complications such as urinary tract infections or proteinuria have been recognized to increase in patients with TS. In this retrospective study we report a detailed clinical history and analyzed renal and urinary system pathology in 32 girls with TS observed between 2000-2010. All 32 TS patients were evaluated by renal and collecting system ultrasonography and if structural renal or urinary malformations were found, cystourethrogram and cistecholellography (DMSA or DTPA) was used. Patients mean age at renal and urological studies was 9,8 years (2-18 years). The cytogenetic findings in 32 patients with TS were; classic: 45,X in 18 patients (56,25%), mosaic and structural aberrations of X chromosome: in 14 patients (43,75%). The prevalence of renal and urological system pathology was 43,75% (14 patients). The most frequent findings were urinary system malformations 21,87% (7 patients), associated with renal malformations 9,38% (3 patients), while 4 patients (12,5%) had renal malformations alone. Horseshoe kidney, malrotation or other position abnormalities, duplication of the collecting system, and different ureterovesical obstruction were found. Conclusion: The early diagnosis of renal and urinary system malformations in TS and their follow-up is crucial to reduce the morbidity in these patients. There appears to be no correlation between karyotype and the presence or type of renal or urinary system malformations.

P03.46-M
Does summation of alleles account for genetic risk and genotype-phenotype association in Type 2 Diabetes Mellitus?
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Introduction: Type 2 diabetes (T2DM) and metabolic syndrome are common complex disorders with a high prevalence in the Maltese population. The aim of this study is to further define the genetic interplay between candidate genes from metabolic and inflammatory pathways on the likelihood of developing T2DM in adulthood and to relate the association of certain genetic profiles with defined biological and clinical endpoints. Method: Eight hundred carefully characterised T2DM cases were recruited. Anthropometric and biochemical parameters, including serum high-sensitivity C-Reactive protein (hsCRP) levels were determined, and genotyping of 43 candidate genes carried out. Neonatal cord blood samples were used as the control reference population in this study. Results: Ten polymorphisms in metabolic/inflammatory pathways showed significant association with T2DM. Three loci showed significant association with lipid profile, body weight and hsCRP levels. hsCRP levels demonstrated a strong positive correlation with body mass index. Genetic score analysis showed that combining multiple genetic markers results in higher relative risks. The functional significance of these polymorphisms is being further evaluated using targeted siRNA-mediated silencing in cultured monocytes. Conclusion: A panel of ten candidate genes has consistently demonstrated significant association with type 2 diabetes and metabolic syndrome in the Maltese population. These gene variants serve functional roles in inflammation and adipose tissue function. A recruited cohort of untreated newly-diagnosed T2DM serves to identify and explore genotype-phenotype association. The strong effect sizes of the selected loci could be used to develop personal genetic susceptibility profiles for T2DM leading to personalization of care and prevention of chronic complications.

P04.01-S
Screening of 1200 FDA-approved molecules to identify pharmacological modulators of expression of ACRV1, the gene mutated in Fibrodysplasia Ossificans Progressiva.
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ACVR1 (ALK2) encodes for a type I BMP receptor and is mutated in Fibrodyplasia Ossificans Progressiva (FOP, OMIM133100). FOP is a rare and severe disease of heterotopic ossification, with a progressive and episodic course. No treatment is available to the progression of the disease and great effort is devoted to better understand the pathogenic mechanisms underlying the dysregulated BMP pathway associated with FOP that may be targeted by in-
novative therapeutic approaches. The characterization of the ACVR1 promotor region provide us with the molecular tools to generate a cell-based system exploitable for High-Throughput Screening (HTS) of chemical compounds with potential pharmaco-logical effect on the ACVR1 expression at the transcriptional level. The cell system has been generated in ATDC5 cells by stable transfection of the Luciferase reporter gene under the control of the ACVR1 promotor. We describe here in detail the HTS procedure we developed and report the results obtained with the screening of 1200 FDA-approved compounds ( Prestwick Chemical Library). We identified 18 compounds, showing an inhibitory effect ≥ 60%, and 8 molecules with activating properties on the ACVR1 transcription. Identified hits belong to different pharmacological classes among which corticosteroids, PDE inhibitors, FANS. We are currently performing experimental validation of selected molecules with different assays. In conclusion, we present a cell-based system suitable for HTS of small chemical compounds to target the ACVR1 transcriptional activity, thereby modulating the downstream pathway. Screening of compounds approved for clinical purposes, may provide candidates for a drug repurposing approach.

**P04.02-M**

**Autosomal-recessive Adams-Oliver syndrome caused by homozygous mutation in EOGT, encoding an EGF domain-specific G-GlcNAc transferase**


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Autosomal recessive Adams-Oliver syndrome was diagnosed in three remotely related Bedouin consanguineous families. Genome wide linkage analysis revealed no association with known Adams-Oliver syndrome genes, identifying a single homozygosity ~1.8 Mb novel locus common to affected individuals (LOD score 3.37). Whole exome sequencing followed by Sanger sequencing identified only a single mutation within this locus, shared by all affected individuals (LOD score 3.37). The EOGT predicted alternative splice variant is ubiquitously expressed. EOGT encodes EGF-domain-specific O-linked N-acetylglucosamine transferase, responsible for ultraloc GlcNAcylation of epidermal growth factor-like domain containing proteins, and essential for epithelial cell-matrix interactions. F-actin staining in diseased fibroblasts showed apparently intact cell cytoskeleton and morphology, suggesting the EOGT mutation either through perturbation of cytoskeleton, but through other mechanisms yet to be elucidated.

**P04.03-S**

**Immunomodulation in Atopic Dermatitis: Inherited, Environmental and Behavioral Risk Factors, and Evaluation of Biomarkers**


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The barrier function of the skin is essentially maintained by the epidermal differentiation complex protein filaggrin, which is located in the stratum corneum of the epidermis. Loss-of-function (LoF) mutations of the filaggrin gene (FLG) lead to an increased risk of atopic dermatitis (AD), allergic sensitizations, and risk to develop the psoriasis phenotype. Patients with severe AD, or clinically dry skin show a reduction of the epidermal filaggrin protein levels even in the absence of FLG LoF mutations. Here we investigate the disease modification in AD with principal focus on FLG risk variants and their influence on disease severity. To identify additional susceptibility loci we analyze CLDN1, CLDN4, CLDN23, OCLN, IVL, SPPT1, LDLR, TLR4, TLR6, SELP, CLDN20, SELP, and FLG2 variants possibly involved in AD. Altogether 80 variants are genotyped from 500 Finnish patients with a detailed AD history, and from 1000 population cohort controls. The tight junction proteins claudin-1, claudin-4, claudin -23, occludin and involucrin are essential for proper barrier function. However the LoF state of the encoding genes remains largely unknown. Here we suggest that LoF variation of these genes may be detected especially in patients with very severe disease and a poor treatment response, such as the AD patient cohort with extreme IgE levels (>10 000). Three candidate genes (CLDN20, SELP and FLG2) are drawn from a systematic survey of LoF variants in human protein-coding genes, that identified rare and likely deleterious LoF alleles markedly enriched in the Finnish population at greater than 1% frequency.

**P04.04-M**

**The benign joint hypermobility syndrome: guidelines for diagnosis and management.**


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**Introduction** The hypermobility syndrome (HMS) or benign joint hypermobility syndrome (BJHS) is a frequent condition affecting many females, interfering with their normal activities of daily life. Hypermobility is associated with an increased risk to develop a chronic pain syndrome. Patients often seek medical help in later stages of this condition for therapy-resistant complaints. We designed a protocol to improve the referral and management strategy for this particular patient group. **Methods** We report data on patients (n=167) referred to the department of clinical genetics to exclude rare genetic connective tissue disorders such as the Ehlers Danlos syndrome. **Results** Based on the data of medical history, clinical examination and if indicated, DNA-investigations, the likelihood of an underlying hereditary disorder in the 167 counselees was estimated. Further DNA-investigation was performed in 83 cases, revealing an underlying monogenic disorder in two cases. **Conclusions** Based on our experience we provide tools to distinguish between the majority of patients with BJHS and those with a here described connective tissue disorder. For the referring physician, the modified scoring list according to Beighton is essential to decide whether to refer or not. Individuals with the BJHS (Beighton scoring (BS) ≥6) should be referred to a rehabilitation specialist for treatment. In case of a BS of 7 or more, or 6 or less with unusual features (i.e. male gender; age over 50, and/or a positive family history) referral to a clinical geneticist is recommended for further evaluation.

**P04.05-S**

**A mutation in the LRP4 gene is associated with bone mineral density in Maltese postmenopausal women.**

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**Background:** Osteoporosis is a hereditary multifactorial skeletal disease characterised by low bone mass and increased fracture susceptibility. The low-density lipoprotein receptor-related protein 4 (LRP4) controls the actions of sclerostin which is a known inhibitor of the Wnt/(β) catenin pathway involved in bone formation.

**Objective:** To evaluate the effect of two non-synonymous coding polymorphisms rs6485702 (A>G) and rs2306033 (C>T) in relation to Bone Mineral Density (BMD) and different low-trauma fractures in Maltese postmenopausal women.

**Methods:** Research subjects were 1045 women subdivided in three BMD groups if without history of fragility fracture: normal, osteopenic or osteoporotic. Women with a fracture history were classified as cases. Genotyping was performed by polymerase chain reaction and restriction fragment length polymorphism. Associations with BMD and fracture were analysed using odds ratios (OR) determined by logistic regression and adjusted for age.

**Results:** Homozygosity for the rs6485702 A allele was associated with a lower BMD at the lumbar spine, LS (OR=2.2 [95% confidence interval 1.1-4.4] p=0.03) relative to research subjects with a normal BMD. Heterozygotes for this allele had a lower BMD at the femoral neck, FN (OR=1.5 [1.0-2.2] p=0.02). The G-C haplotype was strongly associated with LS BMD (OR=1.7 [95% confidence interval 1.1-2.7] p=0.004) and to a lower extent FN BMD (OR=1.2 [95% confidence interval 1.0-1.4] p=0.02). No association with fracture risk was seen (p>0.05).**Conclusion:** The LRP4 rs6485702 variant plays a role in BMD regulation in Maltese postmenopausal women. This polymorphism is located in the β-propeller domain which is thought to interfere with the binding of sclerostin and hence will not affect Wnt signalling.

**P04.06-M**

**A novel mutation in BMPR1B gene (R486L) in a Polish family with brachydactyly A2/C with symphalangism**

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gous nonsense (p.R208X) and missense (p.T221I) mutations in DYNC2LI1 segregating in the family. DYNC2LI1 is ubiquitously expressed and interacts with DYN2CH1 to form the dyneme 2 complex important for retrograde intraflagellar transport. The hypothetical protein caused by the nonsense mutation lacks the coiled-coil domain involved in protein dimerization. The mutation p.T221I affects a highly conserved nucleoside triphosphate hydrolyase domain responsible for GTPase-driven dyneme protein localization.

Mutations in both DYNC2LI1 interacting partners DYN2CH1 and NEK1 are associated with Jeune/ATD/SPR III and SPRS II/ATD, respectively. The identified phenotype in the patient can be explained by its co-segregation with DYN2CH1 and the shift of dominance from DYNC2LI1 and the basal body protein NEK1. This is the first report of mutations in the light intermediate chain of the dyneme 2 complex further expanding the clinical spectrum of ciliopathies.

P04.09-S Eosome sequencing in patients with Circumferential skin creases Kunze type: Evidence for locus heterogeneity

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Circumferential skin creases are extremely rare and children born with this feature are referred to as ‘Michelin tyre babies’ based on the similarity with the mascot of the French tyre manufacturer. Some of these children have additional abnormalities including typical facial dysmorphism, cleft palate, short stature and intellectual disability. For this syndrome, our group proposed the term ‘Circumferential skin creases Kunze type’ (Wouters et al, 2011). So far, less than 10 cases have been described in the literature and all occurrences are sporadic. In an international collaboration we sequenced DNA samples from 8 patients with Circumferential skin creases Kunze type. Eosome sequencing was performed on the HiSeq2000 platform for two case-parent trios as well as two additional patients with this syndrome. Data analysis revealed the presence of pathogenic mutations in either one of two interacting genes, providing evidence for genetic heterogeneity. Three additional patients with the same phenotype have also been found to carry de novo mutation in one of these genes. While some patients carry a heterozygous de novo mutation, others present with homozygous mutations. Accurate genotype-phenotype correlations are being investigated. In addition, we are performing functional analyses at the protein level to elucidate the pathogenic mechanism of the mutations.

P04.08-M Identification of mutations in DYNC2LI1, a member of the mammalian cytoplasmic dynein 2 complex, expands the clinical spectrum of Jeune/ATD ciliopathies

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Disorders of brain malformations, polydactyly, kidney cysts, and skeletal abnormalities belong to the ciliopathy spectrum caused by defects in formation, maintenance and function of the primary cilium. This phenotype spectrum is present among patients with short Rentinoid X receptor 2 (ROR2), the asphyxiating thoracic dystrophy (ATD/Jeune) and Ellis-van Creveld syndromes (EVC). Underlying genes affect the dynein motor, intraflagellar transport complexes, or the basal body. After excluding known causative genes we performed exome sequencing in a patient of non-consanguineous parents presenting an intermediate phenotype between ATD/Jeune and EVC. We selected variants based on potential ciliary function as identified in a yeast two-hybrid screen with NEK1, a basal body protein involved in SPRS II. This identified compound hetrozygous nonsense (p.R208X) and missense (p.T221I) mutations in DYNC2LI1 segregating in the family. DYNC2LI1 is ubiquitously expressed and interacts with DYN2CH1 to form the dyneme 2 complex important for retrograde intraflagellar transport. The hypothetical protein caused by the nonsense mutation lacks the coiled-coil domain involved in protein dimerization. The mutation p.T221I affects a highly conserved nucleoside triphosphate hydrolyase domain responsible for GTPase-driven dyneme protein localization.

Mutations in both DYNC2LI1 interacting partners DYN2CH1 and NEK1 are associated with Jeune/ATD/SPR III and SPRS II/ATD, respectively. The identified phenotype in the patient can be explained by its co-segregation with DYN2CH1 and the shift of dominance from DYNC2LI1 and the basal body protein NEK1. This is the first report of mutations in the light intermediate chain of the dyneme 2 complex further expanding the clinical spectrum of ciliopathies.

P04.07-S Targeted DNA sequencing of chromosome 8q22 identifies rare variants in DCSTAMP in patients with Paget’s disease of bone

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Paget’s disease of bone (PDB) is a common bone disease with strong genetic component. We previously identified a PDB-susceptibility locus on chromosome 8q22 using linkage analysis on the DCSTAMP region (Albagha et al, Nat Genet 2011). To identify functional susceptibility variants, we investigated this locus using a targeted DNA sequencing approach. A 700kb region containing four genes (LRP12, DCSTAMP, DPYS and DAP6) was captured using the HaploChip target enrichment kit followed by DNA sequencing using the Illumina HiSeq2000 platform. A total of 244 samples were sequenced (143 unrelated cases without SQSTM1 mutation, 40 controls, 48 familial cases and 13 controls). Variants passing quality control were subjected to multiple filters to include missense variants, those in the 5’ or 3’UTR or those predicted as functional by the ENCODE database. In DCSTAMP, we detected two novel variants (1 missense and 1 in 3’UTR) that were not present in our controls or publicly available databases including 1000 genomes. The novel missense mutation was detected in 3 cases and showed transmission in families. Two other missense mutations were only found in cases (n=7) but they are present in 1000Genome database with MAF<0.03. No disease-specific mutations were detected in LRP12, DPYS or LRPI2). DCSTAMP, which encodes a dendritic-cell-specific transmembrane protein, is a strong functional candidate gene for PDB because it is required for the fusion of osteoclast precursors to form mature osteoclasts. Our data suggest that rare functional candidate gene for PDB because it is required for the fusion of which encodes a dendritic-cell-specific transmembrane protein, is a strong
to less frequently mutated genes (COL11A2, COL9A1-2); this innovation has improved our analysis' reliability and turnaround times.

**P04.11-S**

**About skeletal dysplasia patients carrying two Col2a1 mutations**

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**Background and objectives.** Skeletal dysplasia are usually dominant disorders due to heterozygous mutations in COL2A1 gene. To report on four cases carrying two different mutations. The variation segmentation methods. Methods: Bidirectional Sanger sequencing of the whole COL2A1 gene.

**Results:** Four patients carrying two different mutations were identified in a series of 136 skeletal dysplasia. Patient 11 carried a p.Thr1439>Met paternal allele and a de novo c.1267-2A>G. This latter may explain that she presented with a more severe form of Knies dysplasia than her affected father. The p.Ala145Val predicted as benign, might not be contributive, as the p.Gly405Asp alone was shown associated with SEDC (Mereditch SP, 2007). The association of p.Arg137His and p.Gly213Val seems to induce a different phenotype than previously observed in a patient carrying a heterozygous p.Gly213Val (Kanne M, 2011).

**Conclusion:** We report here the first cases of COL2A1 skeletal dysplasia with benign, might not be contributive, as the p.Gly405Asp alone was shown as associated with SEDC (Mereditch SP, 2007). The association of p.Arg137His and p.Gly213Val seems to induce a different phenotype than previously observed in a patient carrying a heterozygous p.Gly213Val (Kanne M, 2011).

P04.12-M

Identification of a novel locus for a recessive congenital myopathy by linkage analysis in an Israeli Bedouin family

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Congenital myopathy disorders (CMDs) are heterogeneous inherited diseases of muscle characterized by a range of distinct histologic abnormalities. We have studied a consanguineous family with a non progressive congenital myopathy. In order to pursue a molecular diagnosis in this family, we performed genotyping on four patients, their parents and a healthy sibling using the Affymetrix GeneChip Human SNP5 array. We determined the genotype calls by using Affymetrix GeneChip Genotyping Analysis Software (GTYPE) and KinsSNP software. Based on the consanguinity in the family, we hypothesized homozygosis by descent of a recessive mutation as the likely cause of the disorder. Therefore, we searched for homozygous regions consistent with linkage. Three homozygous blocks (on chromosomes 6, 10 and 14) shared by the three affected individuals, heterozygous in the parents, and not homozygous in the unaffected sib were identified. Exome sequencing revealed a highly suggestive mutation in a gene not previously reported to carry mutations in CMDs in humans. The variation segregated as expected in the family, it did not appear in dbSNP, evs or the 1000 genome project, nor was it found in 134 Bedouin control individuals.

**P04.13-S**

**Diagnostics of connective tissue disorders: NGS gets the target when clinics wander around**

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**Introduction** Next-generation sequencing (NGS) offers a valuable tool for the diagnostics of connective tissue disorders (CTDs), in view of the large size of most of the genes involved and the complex spectrum of, often overlapping, phenotypes, which makes differential diagnosis difficult. Recently, we designed a platform for the targeted analysis of CTDs, including Marfan syndrome, Ehlers-Danlos syndrome, Osteogenesis imperfecta, Sticker syndrome and related disorders. In total, 42 genes were included. Based on the associated phenotypes, six gene panels were defined. Material and methods A solution-based target enrichment kit was designed to capture all exons and flanking splice sites of 42 genes. Data were analysed using an in-house pipeline (based on freely available software) and Cartagenia. In total, 319 gene panels were analysed on 290 patients referred to our diagnostics. The aortic/arterial aneurysms/dissections and the Ehlers-Danlos syndromes were the most requested gene panels (90%). Results (Likely) pathogenic mutations were identified in 14.5% of the patients. In most of these patients DNA findings confirmed the clinical diagnosis or were in line with the proposed differential diagnosis. Altogether, NGS strongly facilitated the correct diagnosis. Importantly, in a few cases NGS led to a different diagnosis, which had not been considered based on clinical presentation. Conclusions The CTDs-NGS platform offers advantages in terms of time-efficiency, in view of the complex spectrum of phenotypes and the difficult differential diagnosis. This methodology helps preventing misdiagnosis, especially in young patient with incomplete phenotypic expression. The clinical phenotype of certain CTDs will possibly expand in the future.

**P04.14-M**

**Functional role of the Bardet Biedl Syndrome-associated gene 9 in the pathogenesis of nonsyndromic craniosynostosis: disrupted primary cilium in craniofacial ossification**

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Nonsyndromic craniosynostosis (nSCS) is a highly prevalent craniofacial malformation, with a widely unclear etiopathogenesis. A possible involvement of the BBS9 gene, encoding a protein located in the transition zone of the primary cilium, has been proposed as a result of the first GWAS carried out in 2012. Through microarray genome-wide expression profiling we have shown an altered expression of cilium-associated genes, including BBS9, in calvarial tissues and cells of nSCS patients. We aimed at investigating the role of BBS9 in the aberrant osteogenic phenotype of calvarial cells isolated from nSCS patients through gene expression, immunofluorescence, gene silencing, and differentiation assays. BBS9 expression was significantly upregulated in cells isolated from fused sutures (syn-cells) compared to cells isolated from matched patent sutures (control cells), and increased upon 5 days of osteogenic induction. Confocal microscopy showed that: syn-cells produced less primary cilia compared to control cells; BBS9 expression was spatially distributed throughout the cytoplasm in syn-cells, while appeared organized in controls. Our results suggest a functional involvement of BBS9 in the aberrant osteogenic signalling occurring at the site of premature suture closure in nSCS, proving a possible novel role of this molecular machinery in osteogenesis and craniofacial malformations.

**P04.15-S**

**Chromosomal aberrations in complex craniosynostosis: genetic homogeneity helps identifying biological pathways**

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Craniosynostosis (CRS) represent the most prevalent craniofacial malformation, occurring in 1 out of 2500 livebirths, either as an isolated feature

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nonsyndromic CRS) or in complex phenotypes. Agenetic basis can be found in 20-30% cases of complex CRS, with extremely high heterogeneity. The aim of this study is to describe the inventory of structural chromosomal aberrations identified through cytogenetic and molecular cytogenetic testing (as part of the diagnostic algorithm) in a sample of complex CRS patients. The possible role of the genes involved in the genomic rearrangements in CRS etiopathogenesis have been investigated in vitro, using gene expression analysis, immunofluorescence and gene silencing assays, on calvarial stem cells isolated from CRS patients. In our center we have enrolled 253 patients affected by CRS, including 13 cases with complex phenotypes that did not resemble any of the known syndromes. 9 out of 13 patients had standard cytogenetics and array CGH allowing evidencing a chromosomal structural mutation, encompassing multiple loci enabling a biological interpretation of the abnormalities. In particular, the role of developmental genes involved in the structure and function of the primary cilia has been demonstrated in selected cases.

P04.16-M

The prostaglandin E2-pathway as a key player in the pathogenesis of nonsyndromic craniosynostosis

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The etiopathogenesis of midline nonsyndromic craniosynostosis remains still largely unclear. We attempted to clarify this issue using microarray comparative gene expression profiling. Among the differentially expressed genes, we focused particularly on the hydroxyprostaglandin dehydrogenase (HPGD) gene, which encodes the prostaglandin E2 (PGE2) catalyzing enzyme, whose pathway is known to be involved in osteogenic differentiation. Mutations in this gene result in primary autosomal recessive hypertrophic osteoarthropathy and craniosteoarthropathy. Total RNA and calvarial cells were isolated from calvarial specimens of both sutures and synostoses of NSC patients, collected during surgery. RNA was used for exon-level microarray analysis; gene expression and alternative splicing events were confirmed using real time PCR and RT-PCR. For functional validation, calvarial cells isolated in primary culture were treated with scalar concentrations of PGE2; after 10 days of treatment cells were alternatively lysed to extract RNA or stained with Alizarin Red to analyze osteogenic differentiation. Gene expression profiling allowed the identification of 114 significantly modulated genes and 150 alternatively spliced genes, including HPGD. Exon level analysis of HPGD revealed that the gene encoding the active isoform of the enzyme was significantly downregulated in synostosis-derived tissues. Upon PGE2 treatment, cells isolated from synostoses displayed a higher amount of osteogenic differentiation compared to patient suture-derived cells, as a result of a similar increase in osteogenic markers. The results of this study may provide the original description of an impairment in the PGE2-signaling pathway in the pathogenesis of premature suture fusion in NSC patients. Translational implications may further derive from these data.

P04.17-S

Single-gene testing and Next Generation Sequencing in a Dutch cohort of syndromic craniosynostosis patients

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Introduction According to literature, the genetic cause of craniosynostosis can be identified in approximately 25% of the patients. Often, mutations are found in FGFR2, FGFR3 and TWIST1, but mutations are also identified in many other genes (like IL11RAP, HORIZAR and LRPS). Those genes can be sequenced in parallel by Next Generation Sequencing (NGS), leading to more complete diagnostics. Methods Syndromic craniosynostosis was defined as multisuature or unicoronal synostosis, familial craniosynostosis, or as craniosynostosis in combination with other congenital malformations and/or mental retardation. According to protocol, these syndromic craniosynostosis patients were tested by single-gene testing for mutations in FGFR2, FGFR3 and TWIST1. If these genes tested negative, other genes were analyzed if applicable. In addition, a subgroup of 8 families (15 patients, 11 clinically unaffected relatives) and 8 isolated patients were sequenced by NGS and checked for mutations in known craniosynostosis genes. Results Of our patient cohort, 705 patients were tested by single-gene testing. Mutations were found in 280 patients. By NGS, mutations were identified in TCF12 (1 patient, 1 unaffected carrier, 1 relative), IL11RAP (1 patient, 2 relatives), ZIC1 (three patients, 1 relative) and in IDS (1 isolated patient). Conclusion By single-gene testing, mutations were identified in only 15% of the syndromic craniosynostosis patients. Possibly, because the group of tested patients was defined differently, but also because only a subset of genes was tested. By NGS of a small cohort, mutations were identified in additional genes in 6/23 patients, illustrating the power of parallel sequencing.

P04.18-M

CYLD and Brooke-Spiegler syndrome: mutations in Hungarian patients, a review of published variants and a database update

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Brooke-Spiegler syndrome (BSS; OMIM 605041) is an autosomal dominant condition characterized by skin appendageal neoplasms including cylindromas, trichoepitheliomas, and/or spiradenomas. In 2000, the gene locus for BSS was mapped to 16q22-13, and, in the same year, variants of the cylindromatosis gene (CYLD) were identified in BSS, familial cylindromatosis (FC; OMIM 132700) and/or multiple familial trichoepithelioma type 1 (MFT1; OMIM 601606). The gene codes for an enzyme with deubiquitinase activity. To date, a total of 81 different disease-causing mutations have been published for the CYLD gene. A summary of recurrent mutations identified in Hungarian patients and a review of published mutations is presented in this update. Comparison of clinical features in affected families with the same mutation strongly confirms that identical mutations of the CYLD gene can give rise to different phenotypes, making genotype-phenotype correlations difficult. Variable expression of the phenotype associated with the same CYLD mutation may reflect the influence of other genetic and/or environmental factors. Most mutations are frameshift (43%), nonsense (24%), splicing (16%) and missense ones (12%), but there are some reported rare variants as well (5%). The vast majority of the mutations (99%) are located between exon 9-20, which encodes the 3rd Cap-Glycin and the ubiquitin-specific protease domains of the CYLD protein, suggesting that these domains are important for CYLD deubiquitinating activity. This research was supported by the European Union and the State of Hungary, co-financed by the European Social Fund in the framework of TÁMOP-4.2.2.A/11-111/2012-0001 ‘National Excellence Program’.

P04.19-S

An atypical form of progressive extreme heterotopic calcification in a patient with a de novo insertional translocation der(X;X)(q26.1p13.3)

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We report on a girl with widespread, rapidly progressive ectopic calcifications detected shortly after birth. Calcifications became present around most joints, involving tendons and ligaments, but no internal organs or skin, and eventually caused almost complete immobility of the child at 2 years. Laboratory evaluation failed to identify autoimmune disorders as well as calcium metabolism or other biochemical abnormalities; molecular studies did not identify any mutation in disease genes known to be involved in ectopic calcifications. Further analysis identified a de novo insertional translocation (IT). Array-CGH analysis showed a 2p13.3 duplication which was validated by qRT-PCR. Fluorescence in situ hybridization (FISH) confirmed the rearrangement, a der(X;X)(q26.1p13.3). The two breakpoints were characterized at nucleotide level by inverse PCR. The duplication on chromosome 2 encompassed nine coding genes, seven completely duplicated and two (ANTXR1 and MXD1) partially duplicated. ANTXR1 is interrupted in YS10, the MXD1 coding sequence is maintained, but its 3’UTR is almost completely lost. The chromosome 2p13.3 duplication is inserted into a gene desert on chromosome Xp26.1, between ARHGAP36 and IGSF1 genes. We suggest the phenotypic in our patient is due to a likely gain of function mechanism. We hypothesize that the de novo insertional translocation causes a fusion transcript or an altered regulation of a gene by position effect. Further studies are in progress to identify the pathogenic mechanism triggering this unique phenotype.
Recently mutations in FKBP14 have been identified in patients with a novel variant of Ehlers-Danlos syndrome (EDS) characterized by progressive kyphoscoliosis, hypomophyia and hearing loss (MIM #614557). This disorder shares many clinical features with the kyphoscoliotic type of EDS (EDS VIA; MIM 225400) such as congenital hypotonia, progressive kyphoscoliosis, hyperelastic skin and hypermobility of joints. However, there are also some distinctive features like hypomophyia and hearing loss. While an increased ratio of urinary hydroxyproline (IP) to hydroxylysyl pyridinoline (HIP) is diagnostic for EDS VIA, measurement is normal in patients with EDS caused by mutations in FKBP14.

Here we report on an 8-year-old girl born to first cousin parents, who presented with severe congenital hypotonia, psychomotor retardation, severe muscle weakness, progressive scoliosis, severe joint hypermobility of fingers, wrists and toes, soft skin, easy bruising, bilateral clubfoot, prominent heart, and hearing loss. The clinical course was marked by dyssynergia of the shoulder and dysarthria. Because of a suggestive clinical history by absence of hearing impairment our initial clinical diagnosis was EDS VIA, but normal IP/HP in urine ruled out this condition. Therefore we sequenced FKBP14 and detected a novel homozygous c.143T>A substitution in exon 1 causing a p.Met48Lys mutation. Hearing loss, was consistently reported in the initial cohort of patients with this novel form of EDS, in whom nonsense mutations were found. It is compelling to hypothesize that absence of hearing impairment in our patient might reflect a partial loss of function of FKBP14 caused by the identified p.Met48Lys missense mutation, thus suggesting a possible genotype-phenotype correlation.

P04.21-S
Zebralphia modeling of β3GalT6-deficient Type of Ehlers-Danlos Syndrome Stresses the Importance of Glycosaminoglycans in Development
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Proteoglycans are important components of cell plasma membranes and extracellular matrices. They are composed of glycosaminoglycans (GAG) chains attached to a core protein through a tetrasaccharide linker region. The addition of the third residue in this linker is catalysed by galactosyltransferase II (β3GalT6) encoded by B3GALT6. We recently identified bi-allelic mutations in B3GALT6 in several individuals from independent families with a severe autosomal recessive pleiotropic connective tissue disorder characterized by skin fragility, delayed wound healing, joint hypermobility and contractures, muscle hypotonia, intellectual disability and a spondyloepiphysial dysplasia with bone fragility and severe kyphoscoliosis. To characterize the function of B3GALT6 we employed zebrafish as an in vivo model. Whole mount in situ hybridization of zebrafish embryos showed high β3GalT6 expression levels in brain, retina, pharyngeal arches and notochord epithelium, corresponding to tissues that are affected in the human patients. A morpholino-based approach was used to characterize the developmental effects of β3GalT6 knockdown in zebrafish embryos. Complete knockdown of β3GalT6 is lethal, but partial knockdown results in an abnormal pharyngeal cartilage phenotype and a notably reduced head and eye size. These morphological changes were accompanied by a significant reduction in the total amount of sulfated GAG chains. In conclusion, our results emphasize a crucial role for β3GalT6 in GAG synthesis and development. Ongoing and future experiments aim to extensively analyze the changes in GAG composition as well as to further characterize the way in which these changes impact embryonic development, e.g. cartilage and heart using different fluorescent transgenic reporter lines.
Identification and characterization of a novel susceptibility locus for nonsyndromic cleft lip with or without cleft palate (nsCL/P) is one of the most common congenital malformations worldwide and considered to be of multifactorial etiology. nsCL/P shows considerable phenotypic variability and can be subdivided into nonsyndromic cleft lip only (nsvcL) and nonsyndromic cleft lip and palate (nsCLP). Genome-wide and replication studies have recently led to the identification of 15 nsCLP susceptibility loci. However, additional genetic risk factors still await elucidation. Here we used data from a recent nsvcL genome-wide meta-analysis (Ludwig et al. 2012, Nature Genetics) and combined these results with an independent European trio cohort (n=793). Integration of subgroup information on nsCLP or nsvcL revealed rs1258673 on chr. 15q13 as a novel genome-wide significant locus associated with nsCLP (P=1.04×10^{-6}). The associated region maps intergenically, between the Gremlins 1 (GREM1) and Formin-1 (FMN1) genes. GREM1 is a known antagonist in bone-morphogenetic-protein pathways which are relevant to craniofacial genesis. Sequencing the entire GREM1 coding region in 196 patients and 196 controls did not reveal a causative variant, however, a significant overrepresentation of rare variants within patients was observed (P=0.02). Analyses of murine Greml1 expression during embryonic craniofacial development might suggest a functional role of Greml1 in lip and secondary palate development. Notably, the top variant rs1258673 has previously been shown to influence variation in facial morphology (Boehringer et al. 2011, EJHG, Liu et al. 2012, PLoS Genetics). Our results demonstrate that increasing sample sizes and precise phenotypic information might help in identifying further risk loci for genetically complex traits.

**P04.25-S**

**Giant cell tumor of the bone is caused by somatic mutations in H3F3A gene in osteoclast-like giant cells formation responsible for the osteolitic lesions.**

A recent study showed that giant cell tumors of the bone (GCT) is due to recurrent somatic mutations in H3F3A gene in osteoclast-like giant cells. The study was performed in 46 patients with 38 out of 44 cases (86%) in which the gene was identified. In contrast, the analysis of patients with Paget’s disease of bone (PDB) associated with giant cell tumor did not show any mutation in H3F3A gene, at both somatic and germline level, suggesting a different genetic background. We recently reported an extended Italian family in which 4 out of 14 PDB affected members developed multiple GCTs at pagetic skeletal sites. Clinically, all affected members had polyostotic PDB, but subjects developing giant cell tumors showed an increased disease severity with a reduced clinical response to bisphosphonate treatment and an increased prevalence of bone pain, deformities, and fractures. Whole exome sequencing, in this family identifies a missense mutation in a novel characterized gene. Additional genetic analysis in 7 independent affected families, with the same clinical phenotype, discloses the same mutation in all patients, strongly suggesting that this clinical phenotype is due to a founder effect. Phenotypic analysis of those families show that both phenotypes associated or not with PDB are due to mutations in different genes, suggesting that different molecular signatures are responsible for these two phenotypes.

**P04.26-M**

Identification and characterization of a novel susceptibility locus for nonsyndromic cleft lip and palate at chromosome 15q13

Non-syndromic cleft lip with or without cleft palate (nsCL/P) is one of the most common congenital malformations worldwide and considered to be of multifactorial etiology. nsCL/P shows considerable phenotypic variability and can be subdivided into nonsyndromic cleft lip only (nsvcL) and nonsyndromic cleft lip and palate (nsCLP). Genome-wide and replication studies have recently led to the identification of 15 nsCLP susceptibility loci. However, additional genetic risk factors still await elucidation. Here we used data from a recent nsvcL genome-wide meta-analysis (Ludwig et al. 2012, Nature Genetics) and combined these results with an independent European trio cohort (n=793). Integration of subgroup information on nsCLP or nsCLP revealed rs1258673 on chr. 15q13 as a novel genome-wide significant locus associated with nsCLP (P=1.04×10^{-6}). The associated region maps intergenically, between the Gremlins 1 (GREM1) and Formin-1 (FMN1) genes. GREM1 is a known antagonist in bone-morphogenetic-protein pathways which are relevant to craniofacial genesis. Sequencing the entire GREM1 coding region in 196 patients and 196 controls did not reveal a causative variant, however, a significant overrepresentation of rare variants within patients was observed (P=0.02). Analyses of murine Greml1 expression during embryonic craniofacial development might suggest a functional role of Greml1 in lip and secondary palate development. Notably, the top variant rs1258673 has previously been shown to influence variation in facial morphology (Boehringer et al. 2011, EJHG, Liu et al. 2012, PLoS Genetics). Our results demonstrate that increasing sample sizes and precise phenotypic information might help in identifying further risk loci for genetically complex traits.

**P04.27-S**

**Study of clinical and mutational findings in 35 Russian families with ectodermal hypohidrotic dysplasia**

Ectodermal dysplasia is a group of syndromes involving abnormalities of the ectodermal structures and is comprised of more than 150 different forms. This work is devoted to hypohidrotic ectodermal dysplasia (HED). Main causes of HED are mutations in three genes: EDA, a ligand that belongs to the tumor necrosis factor (TNF)-α family, EDAR, a receptor related to the TNF receptors, and EDARADD, a specific adaptor. The study cohort consisted of 45 Russian patients with HED was performed by us, using direct sequencing of coding region EDA gene and MRC-Holland MLPA kit for search of large deletion in EDA. EDAR and EDARADD genes. In recent EDTA gene mutations were found in 76% (34 patients) of cases. Twenty new allelic variants of HED were described by us. Repeating mutations are revealed, but the analysis of the polymorphic markers linked to EDA gene showed absence of the founder effect. Large deletion in EDAR and EDARADD genes aren’t found. No correlations were revealed between clinical features and specific mutations within a EDA gene. We present the first in Russia large hypohidrotic ectodermal dysplasia cohort focusing on clinical manifestations in combination with mutational analysis.
to test for the classical exon 4-10 deletion. Sequence analysis of NEMO was performed using standard Sanger sequencing. X-inactivation was measured using a methylation-sensitive restriction enzyme. An Affymetrix Cytoscan HD array was used to detect copy number variations (CNVs) according to the manufacturer’s guidelines.

Results

The classical NEMO exon 4-10 deletion was detected in one patient and her mother. In another patient, a 130 kb interstitial gain in chromosome 17q25.3 was identified, containing five genes (CD7, SECTM1, GPR14, TEX19 and OGDOD3). There was no skewed X-inactivation. The fourth patient was molecularly investigated.

Discussion

We show four female patients with a clinical IP phenotype and different genetic defects. NEMO deletions impair NF-kB activation, causing IP cells to be highly sensitive to apoptosis. Interestingly, a novel 130 kb gain of chromosome 17q25.3 was identified in another patient, including SECTM1. SECTM1 is shown to induce IFN-γ production in vitro; IFN-γ stimulates apoptosis. We hypothesize that a dosage effect, caused by the SECTM1 duplication, induces cell death thereby causes IP. This finding suggests heterogeneity of IP and supports the role of apoptotic pathway CNVs in this disorder.

P04.29-S

CMG2/ANTXR2 gene mutation analysis in 9 families suffering from Infantile Systemic Hyalinos

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Infantile Systemic hyalnosis (ISH) is a severe rare autosomal recessive inheritance disease characterized by accumulation of amorphous, hyaline material in skin and other organs. It leads to skin lesions, gingival hypertrophy, flexion contractures of the joints, digestive tract and lymph node impairment with severe phenotypes in newborns. Beginning within the first few months of life, it is characterized by painful joints, generalized skin thickening, pal- pules, periorificial nodules, hyperpigmentation over the joints, osseous nodes, failure to thrive. This disorder is progressive and usually leads to death, due to recurrent chest infections and diarrhea, before two years of age. The diagnosis is made by histology on skin biopsy showing hyaline deposits. Dele- terious mutations have been reported in Capillary Morphogenesis Gene-2 (CMG2) as an Ananth Toxin Receptor 2 (ANTXR2). CMG2/ANTXR (4q21) is composed of 16 exons and the mutations reported in several families with ISH are located on 5 exons 13 to 15. We report the causal mutations in 9 fami- lies found using Sanger sequencing. Mutations were found in 6 affected newborns, 2 relatives, among them 6 foetuses with 6 homozygote mutations, 1 composite heterozygote and 1 associated to a supposed deletion. The 8 different found mutations will be described. 5 of them are non-sens mutations and 3 are 1-2 base(s) deletions or duplications leading to frameshift. Half of them are unknown in literature with 3 mutations in exon 1 and 10 between intron 8 and exon 9 affecting splicing. There is no treatment for this severe disease and the prenatal diagnosis is available.

P04.30-M

Whole-exome sequencing identifies a TTN mutation in a multiplex family with inguinal hernia

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Inguinal hernia repair is one of the most frequently performed gastrointestinal surgical procedures. Over 700,000 groin hernias are repaired annually both in the United States and Europe. Males are seven times more likely than females to develop a hernia and have a 27% lifetime risk of developing an inguinal hernia. Risk factors that have been associated with developing inguinal hernias include male gender, aging, pulmonary disease with chronic cough, and other conditions that cause constant increase of intra-abdominal pressure. Connective tissue disorders, such as Ehlers-Danlos and Marfan syndromes, and systemic collagen subtype imbalances have also been as- sociated with increased risk of hernia. Furthermore, several studies have demonstrated that a positive family history is an important risk factor for the development of primary inguinal hernia.

The aim of this study was to investigate a multiplex Estonian family with inguinal hernia in four generations. Whole-exome sequencing was carried out in three affected family members and subsequent mutation screening using Sanger sequencing was performed in 8 family members (five affected and three unaffected). A heterozygous missense mutation p. Iys296C7T/hr was identified in the highly conserved A-band of the titin gene (TTN). The association was segregated with the disease in the family, and was not present in 875 ethnically matched control subjects.

P04.31-S

Sibs with microdeletion in maternal 14q23.2 and phenotype congruent to paternal isodisomy 14


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A girl born to healthy non-consanguineous parents and her three-year- younger brother showed at birth moderate to severe respiratory insufficiency which required long-term tracheostomy in the boy. Both neonates were large with plump facial features, narrow thoracic cage with radiogra- phic bell-shaped configuration. Diastasis of the abdominal wall musculature and signs of distal arthrogryposis were mild in the girl and pronounced in the boy. Neuromotor development was delayed with independent walking achieved at 18 months and at 5½ years respectively. The mild intellectual disability in the boy, who has persisting contractures at the wrists and ankles, may be correlated with bouts of therapy-resistant hypoxia during the NICU admission of six months duration. At night he still needs mechanical support with airflow.

The clinical and radiographic pattern in both sibs was compatible with paternal UPD 14. However, an imprinting defect was ruled out using microsatellite marker analysis. Recently, the lady’s complaint about long lasting respira- tory infections and physical tiredness prompted reexamination of this family. SNP array analysis using an Illumina Cyto-SNP 12v2.1 chip identified a microdeletion on chromosome one band 14q32.2 in both sibs. The deletion with a minimal size of 6-4 kb encompass the IncRNA genes MEG3 and MEG8 and several miRNAs. Each of these genes is subject to maternal imprinting. The mother is mosaic for the same microdeletion. Molecular analysis regarding exact size, gene content and degree of methylation is ongoing and bound to contribute to more insights into the disease mechanisms involved in this imprint region.

P04.32-M

Increased frequency of F5 and F2 thrombophilia predisposing variants in families with unilateral limb reduction defects - a pilot study

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Limb reduction defects (LRDs) are usually unilateral and affect 8 newborns per 10000 livebirths. Several authors hypothesized that vascular accidents may account for a substantial proportion of LRDs, however the basis of such events in the fetus remains unknown. Over the past two decades, a number of genetic thrombophilia risk factors (TRFs) have been identified and asso- ciated with the increased risk of peri/postnatal occlusive disease and with a higher rate of pregnancy loss. Mutations c.6169190.35060q) in F5 and c.979G>A in F2 genes represent one of the most commonly tested genetic TRFs. In this study, a cohort of 45 proband-mother pairs, in which the child manifested unilateral LRDs, was recruited for the analyses. The patients were clinically evaluated and blood of the probands and their mothers was subjected to both F5 and F2 testing. We found either F2 or F5 heterozygous variant (or both) in 6 mothers (13.3%) and in 4 probands (8.9%). At least one individual carried a single (or both) mutation(s) in seven proband-mother pairs (15.6%). Within this group one proband and one mother carried both mutations. F5 and F2 are identified in a control population with the frequency of about 1-2%. Based on our findings, we hypothesize that there is an excess of genetic TRFs in probands affected by unilateral LRDs and in their healthy mothers in comparison with control population. This may con- tribute to the higher risk of antenatal vascular accidents and consequently to LRDs. Further studies based on bigger samples are needed to support our findings.

P04.33-S

Homozygous Ala529Val LMNA Mutation in Patients with Mandibuloacral Dysplasia in a Turkish family

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A combined workflow for FBN1 mutation detection using next generation and Sanger sequencing methods


Marfan syndrome is an autosomal dominant disorder of connective tissue with skeletal, ocular and cardiovascular system involvement. Sequencing of the fibrillin-1 (FBN1) gene has a 70-90% mutation detection rate in patients with Marfan syndrome. The goal of this study was to establish a diagnostic workflow for rapid and reliable mutation detection in the FBN1 gene.

Exons and flanking regions of the FBN1 gene were amplified in 65 amplicons using published and newly designed primers. Our workflow was based on the following: 1) exons containing homopolymer regions (n=16) were tested by Sanger sequencing method because of the well known high error rate of pyrosequencing based next-generation sequencing (NGS) in such regions, 2) all other amplicons were sequenced using NGS (Roche GS Junior), where coverage criterion was above 40x. Pathogenic mutations detected by NGS method were confirmed by Sanger sequencing.

Eleven families (15 patients) were tested with a mutation detection rate of 8/11 unrelated patients. 7 missense (6 of them affecting cystein residues) were detected and one base pair deletion causing frameshift. 5 novel mutations were detected. By combining NGS and Sanger sequencing methods reliable diagnostic workflow could be established for Marfan syndrome.
Neurofibromatosis type 1 and Legius syndrome: differential molecular diagnostic in children

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Neurofibromatosis type 1 (NF1) and Legius syndrome (LS) are autosomal dominant disorders caused by germline mutations in NF1 or SPRED1 gene respectively. The NF1 phenotype partially overlaps the most benign of LS that can be just molecularly distinguished. Appearance of typical NF1-features, like neurofibromas and Lisch nodules, is age-related thus pigmentary manifestations cafè-au-lait macules and/or freckling can be the only clinical sign of NF1 in early childhood, making difficult to distinguish NF1 and LS. The occurrence of severe NF1-comlications (i.e. MPNST, brain gliomas) emphasizes the usefulness of an early differential diagnosis. To address a proper diagnosis, we applied a specific clinical workflow and a comprehensive genetic test in a cohort of 149 children. Pigmentary manifestations were considered as the main sign combined with presence of typical NF1 features and/or affected first-degree, and with the age at medical examination. 128 (85.9%) had a clinical diagnosis of NF1. For 17 (11.4%) NF1 vs LS was undistinguishable and 4 (2.7%) had a diagnosis of familiar occurrence of pigmented manifestations. A causative mutation was found in 122/128 patients (95.3%) confirming NF1 clinical diagnosis. 11 patients with ambiguous diagnosis carried NF1 mutations (64.7%). 2 carried SPRED1 mutations (11.8%). Among those thought to be LS, 3 had mutation in SPRED1 (75.0%), one in NF1 (25.0%). Children with just NF1 pigmentary manifestations would be clinically followed for long time waiting the age-related appearance of most typical features. Thus an early molecular diagnosis might be very useful, considering that the milder LS phenotype require a less intensive follow-up.

Identification of a novel regulatory variant in OPTN in familial cases of Paget’s disease of bone

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Paget’s disease of bone (PDB) is a common bone disease with strong genetic component. Previous studies have identified a PDB-susceptibility locus on chromosome 17q which is characterized by rare loss of heterozygosity (LOH). Albagha et al, Nat Genet 2011. OPTN encodes a protein that plays a role in multiple cellular processes including autophagy and NF-KB signalling but its role in bone metabolism is yet unclear. Here we investigated the 10p13 locus by targeted DNA sequencing of a 650kb region surrounding the rs1561570 which includes seven genes. A total of 260 samples were sequenced including unrelated cases and controls, 60 familial cases and controls. All cases and controls were negative for SQSTM1 mutations. Target capture was performed using Halopex kit followed by DNA sequencing using the illumina Hiseq2000 platform. Variants passing quality control measures were filtered to include only rare coding (MAF<0.01 in 1000Genome) or regulatory variants (as predicted by ENCODE). No disease-specific missense mutations were detected in any of the seven genes located in this region including OPTN. We identified a novel rare variant located in the OPTN promoter which was found to alter NF-KB transcription factor binding site. This novel variant had a high call quality (Q=4200, read depth >150) and was not present in the public databases including 1000 genomes and showed transmission from an affected father to an affected daughter and the unaffected mother did not carry this mutation. In conclusion our data suggest that rare regulatory variants within OPTN induce PDB susceptibility.
Non-syndromic cleft lip with or without cleft palate (nsCL/P) has a genetically complex etiology. In recent years, several genome-wide significant susceptibility loci for nsCL/P were identified, most of them by genome-wide association studies. However, most studies have been performed in populations from Europe and Asia, and few data are available concerning genetic susceptibility to nsCL/P in Arab populations. The present study investigated a newly recruited nsCL/P sample from Yemen. Twenty-four single nucleotide polymorphisms (SNPs) representing all 15 currently known nsCL/P risk loci were genotyped in 242 nsCL/P cases and 420 healthy controls. Single marker association analysis revealed significant associations for four loci (8q24, 9q22, 10q25, 13q21). The strongest association was for the European high risk locus 17q22, with an odds ratio (OR) of 6.15. The second risk locus at 20q13.14 was identified in the Arab population sample, with an OR of 3.27. Association studies in the Arab population revealed significant associations for 15 nsCL/P loci, with nominal significance, the risk alleles were in the same direction as in the discovery studies. Our results suggest that four of the 15 analyzed nsCL/P risk loci which were identified in European and Asian ethnicities significantly confer risk for nsCL/P in Arab populations.

Osteogenesis imperfecta (OI) is a rare genetic disorder of connective tissue characterized by an impaired collagen fibrillogenesis, leading to the retention of its propeptide. Ultrastructural analysis showed marked variability in collagen fibril diameter and uneven interfibrillar spaces. Overall, this indicates that BMP1 defects result in disturbed collagen deposition in the ECM. Besides the defective procollagen processing, it is likely that deficient BMP1-activity was shown to result in deficient proteolytic trimming of multiple other substrates including a.o. small leucine-rich proteoglycans (e.g. decorin). Mutations in BMP1 have recently been identified in three families with a severe, autosomal recessive form of osteogenesis imperfecta (OI). We report novel, bi-allelic mutations in two unrelated adult patients with severe OI, characterized by severe osteoporosis with numerous fractures, short stature with limb deformities and severe kyphoscoliosis. Biochemical analysis of secreted (pro)collagens showed defective type I procollagen C-propeptide processing. Immunofluorescent staining of type I and V collagen secreted by the patients’ dermal fibroblast cultures showed a reduced and abnormal collagen deposition in the extracellular matrix (ECM). In addition, the reduced BMP1-activity was shown to result in deficient proteolytic trimming of decorin, an important regulator of collagen fibril organisation, leading to the retention of its propeptide. Ultrastructural analysis showed marked variability in collagen fibril diameter and uneven interfibrillar spaces. Overall, this indicates that BMP1 defects result in disturbed collagen deposition in the ECM. Besides the defective procollagen processing, it is likely that deficient cleavage of other BMP1 substrates contributes to abnormal ECM assembly and signalling, and as such to the phenotypic severity.

Osteogenesis imperfecta (OI) is a rare genetic disorder of connective tissue that occurs in approximately one in 15,000-20,000 newborns with mostly autosomal dominant inheritance caused by mutations in the type I collagen genes, COL1A1 and COL1A2. The clinical spectrum is strongly heterogeneous with a wide intrafamilial and interfamilial variability; a continuum ranging from mild, autosomal recessive form of osteogenesis imperfecta to a severe, autosomal recessive form of OI. We report a 25-year-old woman with severe OI, with normal serum PEDF but absent PEDF secretion by cultured osteoblasts. Her mental status was age-appropriate.

Osteogenesis imperfecta - a dominant mutation of COL1A2 gene

A novel mutation in IFITM5, encoding BRIL, impairs osteoblast production of PEDF and causes atypical type VI osteogenesis imperfecta

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Osteogenesis imperfecta imperfecta - a dominant mutation of COL1A2 gene

Strengthening of the extracellular matrix proteins due to mutations in COL1A1 very likely also contributes to a reduced bone mineralisation, which results in severe bone fragility, deformity and growth deficiency. Affected patients may also show blue sclerae, hearing defect, dentinogenesis imperfecta, cardiac lesions and joint hyperlaxity.

Actually more than 1100 COL1A1/A2 distinct variants associated with dominant OI have been identified without hot spot regions; most of the mutations are glycine substitutions followed by splice site alterations and nonsense mutations. Despite the identification of many mutations, few genotype-phenotype correlation studies have been performed and there are no unambiguous and clear indications. To evaluate whether the severity and specific clinical manifestations of disease are linked with a specific genetic background we performed a genome-wide genotype-phenotype correlation study analyzing an Italian case study of 300 patients. All patients have a clinical and radiographic diagnosis of OI from mild to lethal perinatal forms, according to Silence clinical classification. The presence of mutations in COL1A1/ COL1A2 genes has been investigated using a molecular screening protocol with High Resolution Melting (HRM) and multiplex ligation-dependent probe amplification to detect either point mutations and big deletions-insertions.
A novel deletion mutation involving TMEM38B associated with autosomal recessive osteogenesis imperfecta.

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Background. Osteogenesis imperfecta (OI) is a hereditary bone disease characterized by decreased bone density and multiple fractures, usually inherited in an autosomal dominant manner. Recently several genes encoding proteins related to collagen metabolism have been described in some cases of autosomal recessive (AR) OI (like CRTAP, LEPRE1, PBPB, FRKB65, SERPINF1, BMP1). More recently, TMEM38B, a gene involved in releasing of calcium from intra-cellular stores and in cell differentiation, has been associated with AR OI. We describe the second new deletion-mutation involving the TMEM38B gene in a 11-year-old Albanian female, born of apparently non-consanguineous parents from the same small village, showing an OI clinical phenotype. Since molecular analysis of COL1A1 and COL1A2 genes were negative for the presence of causative mutations, a SNP array analysis was performed using the Illumina Infinium SNP genotyping platform (HumanOmniExpress - 12 chips and BeadStation Scanner) in order to detect regions of homozygosity encompassing genes known to be involved in AR OI. The analysis revealed one homozygous region larger than 2 Mb (chr9:107,793,426 - 109,935,841) overlapping with the TMEM38B locus and characterized by a 35 Kb homozygous deletion spanning from marker rs1567368 to rs9408800 and involving exons 1 and 2 of TMEM38B gene. A long PCR amplification confirmed this finding. Eventually, we postulated that the deletion was promoted by the presence of repeated sequences in the segments before and after the breaking points. Our finding contributes to the role of TMEM38B, thus supporting the role of deletions in generating this type of AR OI.

P04.49-S
High spontaneous osteoclastogenesis in pediatric osteogenesis imperfecta patients receiving and not receiving intravenous bisphosphonates.

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Abstract. Human osteoclastogenesis is a tightly regulated process that allows bone remodeling. However, in several bone disease affecting these patients.

P04.50-M

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Background. Paget’s disease of bone (PDB) is a chronic skeletal disorder that is characterized by abnormal resorption associated with inadequate remodeling that leads to deformity of the bone. It’s the second most common metabolic bone disease after osteoporosis and affects approximately 3% of the population over 40 years old and approximately 10% of those over the age of 85 years.

Methods. Missense and truncating mutations have been reported within the SQSTM1 gene (25-50% of familial and 5-10% of sporadic PDB patients) encoding the p62 protein. This protein has a crucial role as an assembly factor for autophagy, important for cell survival. Recent investigations showed that Microtubule-associated protein 1A/1B-light chain 3 (LC3) directly interacts with p62 via a newly identified LC3-interacting region (LIR), located between its C- and ubiquitin-associated UBA domains. We studied the potential association of the two variants of autophagy-related gene (ATG), ATG2B (rs3759601) and ATG16L1 (rs2241880), in 292 patients with PDB and 192 controls, using the TaqMan® Pre-designed SNP-Genotyping Assay (Applied Biosystems).

Results. We detected a significant association between genotype GG and PDB in the ATG2B polymorphism rs3759601 (P=0.004, OR=4.43, 95% CI=1.24-0.769). Moreover, we found a statistical significance between the T allele variant in ATG16L1 polymorphism rs2241880 (TT: P=0.000 OR=5.102 CI=2.853-9.123; CT: P=0.030 OR=2.821 CI=1.850-4.300) in these patients. Conclusion. In conclusion, these findings suggest that allele G of ATG2B polymorphism might contribute to increase risk in Paget’s disease development and allele T of ATG16L1 polymorphism may play a protective role in Paget’s disease of bone in Spanish patients.

P04.51-S
Common variants at 20q11 influence skin color in Europeans.

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Background. Here we hypothesized that genetic variation in skin color influenced by common genetic variation at 20q11.2. Methods. We carried out a genome-wide association study (GWAS) on skin color in 6,000 Europeans followed by successful replications in thousands of Europeans from Australia and UK. We identified a total of five distinct genomic regions showing genome-wide significant association with continuous or categorical skin color phenotypes - all including known pigmentation genes: 5p13.2 SLCO4A2; 25p.3 IRF4; 15q13.1 OCA2 and HERC2; 16q24.3 MC1R; and 2q11.22 ASIP. The role of 2q11.22 in skin coloration has been much less clear than other genes/regions. In this large region (spanning ~ 1.5Mb), the top-associated DNA variant was rs6509655 (P=4.2e-13) in RAFY. All other associated SNPs in this region were all in linkage disequilibrium with rs6509655 (r2=0.4). Among these are rs1885120 in MYH7B and rs910073 in PIGU that have been previously associated with risk of melanoma. Additional transcriptional analysis of 20 genes from 20q11.22 in human skin epidermis samples highlighted RAFY as well as other regional genes such as EIF2S2, ICH5 and GSF with significant (P<0.01) expression differences between light and dark pigmented skin samples. A multivariate analysis highlighted 9 pigmentation genes (i.e. OCA2-HERC2, IRF4, SLCO4A2, MC1R, RAFY, BNC2, SLCL2AA4 and SLCL2AS1) in descending order) jointly explaining 16.3% phenotypic variance of perceived skin darkness in Europeans. The weighted allele sums showed a spatial pattern that is clearly correlated with latitude in Europe, but much less so in the rest of the world, suggesting that variants responsible for skin color variation between Asians and Africans are not included here.

P04.52-M
A spectrum of mutations are associated with somatic mutations in PIK3CA, encoding the p110α catalytic subunit of phosphatidylinositol-4,5-bisphosphate 3-kinase.

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Objective. Somatic activating mutations in PIK3CA, encoded by the p110α catalytic subunit of phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K), have been identified in a critical subset of different individuals of segmental or whole body involvement. PI3K is a critical mediator of cellular growth, survival and metabolism, and is frequently mutated in cancers. We assessed the prevalence of PIK3CA mutations in 40 patients with variable forms of mosaic overgrowth.

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P04.54-M

PRINS, the psoriasis susceptibility related non-coding RNA contributes to various aspects of cellular stress response

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We identified PRINS (Psoriasis susceptibility Related non-coding RNA Induced by Stress) and showed that it is highest expressed in the psoriatic uninvolved epidermis. According to the results of in vitro experiments PRINS expression is induced by various stresses such as UVB, transition exhibition and microbial agents. It has been previously demonstrated by us and by others that keratinocytes of the psoriatic uninvolved epidermis possess an abnormal response to various stressors and respond with hyperproliferation. Therefore we aimed to understand how the altered expression of PRINS in the uninvolved epidermis contributes to the aberrant stress response of the keratinocytes thus to disease susceptibility. To this end we identified a chaperon protein, nucleophosmin (NPM) as a direct interacting partner of PRINS. We could demonstrate that UVB irradiation induced the shuttling of NPM from the nucleolus to the nucleoplasm and silencing of the PRINS non-coding RNA in the UV-B-irradiated keratinocytes resulted in the retention of NPM in the nucleolus. These results suggest that PRINS is physically and functionally linked to NPM, thus it plays a role in the NPM-mediated cellular stress response. In an other set of experiments we have demonstrated that PRINS signals independently from the NF-kappaB signal transduction pathway, however its expression was induced when the keratinocytes were treated with the inflammasome-activating poly(dA:dT).

Our results indicate that the PRINS non-coding RNA is part of a ribonucleo-complex. Its altered expression in psoriatic uninvolved epidermis could contribute to the well-established aberrant stress response of psoriatic keratinocytes and as a consequence to psoriasis susceptibility.

P04.55-S

Association of Pseudoxantoma Elasticum with renal nefrocalcinosis: a rare manifestation of the disease

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We describe a 40 years old man from Albania with clinically evident hema-turia and proteinuria; renal function was normal. The peculiar renal CT findings were: small multiple calcifications at the cortical junction bilaterally. The pedigree analysis revealed two sisters affected by an unspecified skin disease, no consanguinity was reported in the family. The clinical genetic examination showed yellowish papules on the skin of the neck. The association of renal calcifications in a young patient with skin lesions and the recurrence of a skin disease in the family led us to take into account Pseudo-xanthoma elasticum (PXE). Therefore an oculoc examination looking for signs of the disease was performed and angiosteous radiating from the peripapillary area without subretinal neovascularization signs were detected. Pseudoxantoma elasticum (PXE), is a rare multisytem disease characterized by degeneration and calcification of elastic fibres and blood vessels. The causative gene is ABC6, mapped on chromosome 16p13.1, which encodes an ABC transporter protein (ABCG5) expressed primarily in liver and the kidneys. Patients typically develop cutaneous, ocular, cardiovascular and gastrointestinal manifestations. The molecular analysis of the ABCG5 gene detected a homozygous deletion of exons 23-29 (del23-29): this is the most common deletion described in literature associated to PXE, resulting in a premature stop codon with loss of 505 amino acids of MR6 protein. Although this renal pattern cannot be considered specific for the diagnosis of PXE, we recommend to test PXE both when renal and skin abnormalities are present in a patient.

P04.56-M

KIF3A is associated to artrophy envelopment in psoriatic patients

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Psoriatic arthritus (PsA, OMIM #607507) is a chronic inflammatory disorder presenting a highly heterogeneous phenotype and clinical course. PsA is a multifactorial disease associated with psoriasis, affecting the 30% of patients with psoriasis vulgaris (PsV).

Some genetic risk factors are specific for the arthropathy, other are shared with PsV (HLA-Cw*06-02, LCE, TNFα and TRAF3IP2). On this subject, different European studies have recently found several susceptibility genes, which are involved in the inflammatory and immunologic pathways of the disease. To date, no associations between PsA and genes involved in bone metabolism have been observed. Thus we decided to screen a list of genes involved in bone metabolism and/or osteogenesis. We have carried out this screening in 429 PsA patients and 417 health, 380 PsV cases and 389 controls. A single nucleotide polymorphism rs2897442 (A/G) showed a significant association in PsA cases (p = 0.006; OR = 0.73, 95% CI 0.59-0.92). Interestingly, it is not associated to PsV but only in PsA. The rs2897442 is located in the 5th intron of KIF3A gene (Sq2), which encodes for a kinesin II complex subunit required for the assembly of primary cilia and involved in bone formation and in keratinocyte differentiation. Full resequencing of coding and regulatory regions failed to reveal evidence of further association. LD pattern does not reveal significant haplotypes associated to PsA. Immunohistostomy analyses, as well as replication in an independent data set of patients, are expected to clarify the functional role of KIF3A in the pathogenesis and development of PsA.

P04.57-S

Spectrum of phenotypic anomalies in four families with deletion of the SHOX enhancer region

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SHOX alterations have been reported in 67% of patients affected by Léri-Weill dyschondrosteosis (LWD), with a larger prevalence of gene deletions than point mutations. It has been recently demonstrated that these deletions can involve the SHOX enhancer region, rather than the coding region, with some patients belonging to the former group.

Here, we report a SHOX gene analysis carried out by MLPA in LWD patients from 4 families with variable phenotype. All patients presented a SHOX enhancer deletion. In particular, a patient with a severe bilateral Madelung deformity without short stature showed a homozgyous alteration identical to the recently described 47.5 kb PA1 deletion. Moreover, we identified,
for the first time, in three related patients with a severe bilateral Madelung deformity, a smaller deletion than the 47.5 kb PAR1 deletion encompassing the same enhancer region (ECR1/CRE7).

Data reported in this study provide new information about the spectrum of phenotypic alterations showed by LWD patients with different deletions of the SHOX enhancer region.

P04.58-M

Sjögren-Larsson syndrome: molecular characterization of the first reported Cypriot families

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Sjögren-Larsson syndrome (SLS; MIM #270200) is a rare autosomal recessive disorder caused by deficient fatty aldehyde dehydrogenase (FALDH) activity. This reduction in enzymatic activity is secondary to bi-allelic mutations in the ADH32 gene and results in impaired fatty alcohol oxidation and accumulation of fatty alcohols and related lipid products. SLS is typically characterized by pruritic ichthyosis, spasticity, intellectual disability and seizures. In the present study we report, for the first time in the Cypriot population, three apparently unrelated SLS patients. All three were homozygous for a 6 bp deletion at nucleotide 941 to 943 coupled with a 21 nucleotide 5'-GGGCTAAAAGTACTGTTGGGG-3' insertion (c.941-942delins21-bp). This genetic alteration, identified previously in two Caucasian patients, leads to the substitution of Ala314 and Pro315 to Gly and Ala, respectively, accompanied by the addition of six amino acids, Ala-Lys-Ser-Thr-Val-Gly (p.Pro315AsnfsX7).

Next step in collagenopathies and osteodysplasies diagnosis: NGS Targeted re-sequencing


Collagenopathies and osteodysplasies constitute a genetically heterogeneous group of diseases with low incidence, representing however an important health problem because patients require continuous specialized care, due to their deteriorating quality of life. The molecular diagnosis is essential to confirm clinical suspicion, but is not straightforward due to the high number of candidate genes. NGS targeted re-sequencing is a fast and cost-effective alternative to Sanger sequencing, as it can sequence all the genes involved in disease development.

We designed a NGS targeted re-sequencing panel for 229 genes associated with skeletal dysplasias, which spans 1.5Mb comprising coding exons, splice sites and 5' and 3' untranslated regions. Once panel was validated for diagnostic use in cell lines, these regions were sequenced in 40 patients with clinical suspicion of these pathologies. Target regions were enriched and captured using the Enrichment SureSelect system (Agilent) and sequenced either with a SOLiD 5500 (Life Technologies) or MiSeq (Illumina) platform. Results were confirmed by Sanger sequencing. The analysis identified 47 variants in 24 patients, of which 8 were classified as disease-causing and 4 as probably pathogenic. Both types of variants were either truncating mutations or glycine substitutions in collagen genes (essential for collagen structure). The identification of these mutations allowed the molecular diagnosis and an appropriate genetic counseling in affected families. Targeted re-sequencing is especially suited to highly heterogeneous diseases such as skeletal dysplasias, because it allows the identification of mutations in genes whose study by traditional sequencing would be difficult and expensive, increasing diagnostic time.

P04.60-M

Mutations in DNASE1L3 and familial SLE/Hypocomplementemic Urticarial Vasculitis Syndrome: the first italian case

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Symptoms appeared (asthenia, severe anemia, C3 and C4 decrease; urticarial dermatitis; microscopic hematuria, petechiae; glomerulonephritic; splenic strokes; interstitial pneumopathy, pulmonary hypertension; mesenteric ischemia). Interestingly, we also observed joint hyperplaxity with a peculiar apparent contractions of interphalangeal joints (manually extendable without difficulties). Familial history revealed parents’ consanguinity; moreover, two other siblings were reported to show signs of an autoimmune disorder. Molecular analysis of DNASE1L3 revealed the homozygous mutation c.289,290delATC (Thr97Ilefs2), previously reported in three sisters, born from consanguineous parents and affected with HUVS (Hypocomplementemic Urticarial Vasculitic Syndrome). This is the first Italian reported case of autosomic recessive SLE/HUVS caused by a mutation in DNASE1L3 gene.

P04.61-S

Spondyloepiphysial dysplasia in Brazilian patients with intrafamilial variability and a pattern of pseudo-dominance

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Spondyloepiphysial dysplasia (SPED) is one of the main forms of spondyloepiphyseal dysplasia, characterized by lordotic spine, short stature, dysplastic or normal tubular bones, normal or small stature, coxa valga, severe skeletal dysplasia and other features. There are 11 types of SPED described in the literature. We described a new case of SPED, from a family without registered history of the disease. The propositus was a male with short stature and severe skeletal dysplasia, no autosomal recessive SLE/HUVS caused by a mutation in DNASE1L3 gene.
A novel missense mutation in ST14 in a patient with ichthyosis, follicular atrophoderma and hypotrichosis

P.O.46-S
De Novo Mutation of the Latency-Associated Peptide Domain of TGFβ3 in a Patient with Clinical Features of Loeys-Dietz Syndrome

P.O.46-M
Modelling Poikiloderma with Neutropenia in zebrafish: a start point to elucidate disease pathogenesis

P.O.46-44
Promising results of an early phase I clinical trial of EDI200 in hypohidrotic ectodermal dysplasia

P.O.46-46
Whole-exome sequencing identifies polymorphic variants in a large Arab family with split-hand/foot malformation with long-bone deficiency

P.O.46-48
Biomarkers and early stage drug development in ectodermal dysplasias

P.O.46-69
Loss-of-function experiments, performed injecting three different specific antisense, morpholinos in embryos at one cell stage, allowed to obtain embryos with an overall phenotype that recapitulates the major traits of PN. Usb1 depletion causes the development of embryos with decreased skin pigmentation, small head with defects in early cartilages of pharyngeal arches, oedema in the pericardial area, defects in blood circulation with a reduction of myeloid and erythroid cells as highlighted by in-situ hybridization and real-time experiments.

P.O.46-71
Whole-exome sequencing identifies polymorphic variants in a large Arab family with split-hand/foot malformation with long-bone deficiency

P.O.46-73
Biomarkers and early stage drug development in ectodermal dysplasias

P.O.46-75
Biomarkers and early stage drug development in ectodermal dysplasias

P.O.46-77
Loss-of-function experiments, performed injecting three different specific antisense, morpholinos in embryos at one cell stage, allowed to obtain embryos with an overall phenotype that recapitulates the major traits of PN. Usb1 depletion causes the development of embryos with decreased skin pigmentation, small head with defects in early cartilages of pharyngeal arches, oedema in the pericardial area, defects in blood circulation with a reduction of myeloid and erythroid cells as highlighted by in-situ hybridization and real-time experiments.

Split-hand/foot malformation with long-bone deficiency (SHFLD) is a rare, severe deformity. This is characterized by tibia aplasia with or without split-hand/split-foot deformity. Using DNA microarray analysis and employing various statistical methods, we have mapped the SHFLD1 and SHFLD2 phenotypes to chromosomes 1q22.2-q34 and 6q14.1 regions respectively. Additionally, we have identified six suggestive loci with evidence of linkage on chromosomes 1p36.13, 1q31.1, 1q42.3, 4q34.3, and 6q14.1 and 17p13.1 regions in a large multigenerational Arab family (Am. J. Hum. Genet.2007; 80:105-111). Subsequently, we have reported microduplications on chromosome 17p13.3, suggesting the association of BHLHA9 gene within the duplication in the pathogenesis of SHFLD development (J Med Genet.2012 Feb;49:119-25). Our recently performed exome sequencing using the SO-LiD™ system at x200 coverage followed by prioritized mutation search within the linkage region between SNP markers rs11421165 and rs155043, and rs11421165/rs1547251 in selected SHFLD subjects showed polymorphic variants within the coding regions of FIP1L1 gene on 6q14.1 regions. A heterozygous nucleotide substitution G→A (c.347G—A) resulting in a change from arginine and histidine Arg115His (R/H) was observed in heterozygous condition in selected affecteds. However the data on chromosome 1q3.1 region is yet to be analyzed. Our present analysis provides the understanding the pathophysiology of the SHFLD disease and also provides ultimate genetic diagnosis of the condition. Additionally, the data can also help us in developing non-invasive methods of screening for the disorder in at-risk family members, in order to reassure those are not carrying the mutation and to plan prophylactic measures for those who are/will be affected.

P.O.46-79
Biomarkers and early stage drug development in ectodermal dysplasias

P.O.46-81
Biomarkers and early stage drug development in ectodermal dysplasias

P.O.46-83
Biomarkers and early stage drug development in ectodermal dysplasias

P.O.46-85
Biomarkers and early stage drug development in ectodermal dysplasias

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Biomarkers and early stage drug development in ectodermal dysplasias

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Biomarkers and early stage drug development in ectodermal dysplasias

P.O.46-95
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was administered by intraperitoneal injection to newborn Tabbby mice, with tail, back and footpad tissues harvested at time points ranging from two hours to one month post-injection. Isolated RNA was subjected to analysis by qPCR for eight genes known to contribute to the development/structure of epithelial tissues and appendages. Levels of expression for the ectodysplasin receptor EDAR and the downstream regulator sonic hedgehog (Shh) were upregulated specifically at 24 hrs post-injection in all tissues. RNA-seq analysis with DAVID bioinformatics software confirmed these results for EDAR and Shh and extended the gene set analysis to identify novel and unexpected response pathways including those for fat metabolism and solute transporters. This combined approach for biomarker validation and delineation of system response biology will provide an invaluable tool set in identifying pathways for drug targeting as well as in optimizing drug dosing in ectodermal dysplasias.

P04.69-S
A new col5a1 genomic variant identified in an Italian patient with Ehlers Danlos syndrome classic type
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Ehlers Danlos syndrome (EDS) is a group of heterogeneous connective tissue diseases. The clinical classification recognizes six subtypes and Classic type is the most frequent. Classic EDS is an autosomal dominant disorder, characterized by skin hyperextensibility, abnormal wound healing and joint hypermobility. It is estimated that approximately 50% of patients with classic EDS phenotype harbor mutations in COL5A1 and COL5A2 gene. We report a case of 43 Italian patient, presented to Ospedale Maggiore Policlinico (Milan, Italy) for clinical and genetic counselling. He showed: soft and hyperextensible skin especially at the neck, face and hands, atrophic scars and joint hypermobility. The cDNA sequencing revealed a new single base variation in COL5A1 gene. The mutation was a change of C for T in exon63 and established an amminoacid substitution Leucine by Valine at the c.1656. This mutation was located on higly conservated domain of the protein. Sequencing of COL5A2 gene and study of the null allele of COL5A1 gene didn’t showed any differences. CGH-Array platform, characterized by a higher density of probes in those chromosomal regions that are thought to be related to EDS, didn’t revealed any variations. Bioinformatics analysis identified that this nucleotide sequence is extremely conserved in different species, indeed, up to date, no mutation or polymorphism have been described for this region. The identification of new genomic variant represent the first step towards the understanding of symptom causes. Moreover, further studies on protein structure could be necessary to understand the real properties of this mutation.

P04.70-M
Further phenotypic delineation of metatropic dysplasia that encompasses, in decreasing severity, metatropic dysplasia (MD), parametatropic dysplasia, spondyloepiphyseal dysplasia Maroteaux type, spondyloepiphyseal dysplasia Kozlowski type, autosomal dominant brachyolmia, and familial digital arthropathy with brachydactyly. Here, we present the case of two monozygotic twins, daughters of healthy, non-consanguineous parents. They were referred to our clinic at age 5 months because of suspected skeletal dysplasia. They presented unspecific craniofacial features, short stature, prominent forehead, chest deformity, flexion contractures of the elbow and knee, pes planus, and scarring. Other signs include fused fingers and toes, joint deformities and alopeica.

Further phenotypic delineation of metatropic dysplasia
L. M. de-Almeida1, C. Reis1, P. J. Coucke1, O. LeSaux2, A. De Paepe3; 1Medical Genetics Unit, Hospital Pediatrico de Coimbra, Centro Hospitalar e Universitario de Coimbra, Coimbra, Portugal, 2Orthopedics Unit, Centro Hospitalar e Universitario de Coimbra, Coimbra, Portugal, 3Faculty of Medicine, University of Coimbra, Coimbra, Portugal.

Dominant mutations in TRPV4 are responsible for a family of rare skeletal dysplasias that encompasses, in decreasing severity, metatropic dysplasia (MD), parametatropic dysplasia, spondyloepiphyseal dysplasia Maroteaux type, spondyloepiphyseal dysplasia Kozlowski type, autosomal dominant brachyolmia, and familial digital arthropathy with brachydactyly. Here, we present the case of two monozygotic twins, daughters of healthy, non-consanguineous parents. They were referred to our clinic at age 5 months because of suspected skeletal dysplasia. They presented unspecific craniofacial features, short stature, prominent forehead, chest deformity, flexion contractures of the elbow and knee, pes planus, and scarring. Other signs include fused fingers and toes, joint deformities and alopeica.

Further phenotypic delineation of metatropic dysplasia
M. Lopes-de-Almeida1, C. Reis1, P. J. Coucke1, O. LeSaux2, A. De Paepe3; 1Medical Genetics Unit, Hospital Pediatrico de Coimbra, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal, 2Orthopedics Unit, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal, 3Faculty of Medicine, University of Coimbra, Coimbra, Portugal.

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The most common anomaly of the hand. This congenital anomaly may be isolated and sporadic or expressed with a syndrome's phenotype. Within the Oberg, Manske, Tonkin (OMT) classification thumb duplications are a failure of formation and/or differentiation affecting the radial/ulnar axis of the limb. Polydactyly is indicated, not only for the obvious cosmetic improvement, but also to obtain a stable, mobile thumb of adequate size and appropriate shape.

**P05.01-S**

**The gene variants in 3’ end of prothrombin gene in patients with idiopathic thrombophilia in Serbian population**

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Background: Thrombophilia is a multifactorial disorder which arises from the interaction of acquired and genetic risk factors. Despite the significant effort made to understand the etiology of this disease, there are still a certain number of patients suffering from idiopathic thrombophilia.

Objectives: The aim of this study was to screen 3’ end of prothrombin gene, which is susceptible for gain-of-function mutations due to its presence of conserved sequence elements, in patients with idiopathic thrombophilia and to determine its eventual role in the pathogenesis of thrombophilia.

Material and Methods: This study was carried out in 110 patients with idiopathic thrombophilia and 100 healthy controls DNA variants in the 715bp long region of the ‘3 end of the prothrombin gene were identified by sequencing.

Results: In our study, we detected two variants: A19911G and C20068T. The frequency of carriers of both HMOX1 risk alleles: rs2071746T and/or TGFBR1 rs11549467G>A and HMOX1 rs2071746A>T SNP genotyping was performed by using predesigned TaqMan SNP-genotyping assays. For simultaneous assessment of the HIF1A and HMOX1 (GT)n polymorphisms, the method based on multiplex-PCR with fluorescent-labeled primers and fragment size analysis using DNA sequencer has been developed.

We found, that carriers of the HMOX1 (GT)n repeat long allele (n≥27) had increased risk of developing AAA (OR=1.46 for dominant model, P=0.034). The frequency of carriers of both HMOX1 risk alleles: rs2071746T and/or (GT)n repeat in AAs (58,5%) was higher as compared to AIOD (49,0% and 0,07%). On the other hand, the frequency of noncarriers in AAAAs was 0,0% as compared to 1,3% in controls (P=0,10) and 0,9% in AIOD (P=0,066).

Conclusion. In this study population of 72 AAA patients including 56 fAAA patients (78%) and 19191G11G and C20068T. The frequency of A19911G variant was slightly increased in the group of patients compared to controls.

**P05.02-M**

**Molecular analysis of aneurysm genes in familial and sporadic abdominal aortic aneurysm**

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The role of aortic aneurysm syndromes genes in abdominal aortic aneurysms was investigated by analysing the TGF-β pathway genes TGFBR1, TGFBR2, SMAD3, TGFBR2, SMAD3, FBN1, EFEMP2, smooth muscle cells genes MYH11, MYLK and ACTA2 and the vascular Ehlers-Danlos gene COL3A1 in a large group of familial and sporadic AAA patients.

Sanger sequencing was performed of all coding exons and exon-intron boundaries of the aneurysm genes in AAA patients diagnosed. Patients with at least one variant were included. The frequency of a single gene relative involved in the aneurysm gene was classified as familial AAA (IIA). In silico analysis was used for assessment of the clinical significance of the variants.

We found 38 different variants of unknown clinical significance (VUS) in this study population of 72 AAA patients including 56 AAA patients (78%) and 16 patients (22%) with sporadic AAA. In IIA one null mutation in COL3A1 and 48 VUS were observed in 26 (46%) patients. In sporadic AAA one de novo TGFBR2 mutation and 11 VUS were observed in 8 (50%) patients. Four VUS (11%) were possibly pathogenic; TGFBR2 c.1234G>A (Val412Met), MYH11 c.766C>T (Arg255Cys), MYH11 c.6597C>G (Glu1997Asp), and MYLK c.3409G>A (Gly1133Arg). Fifteen patients (13 familial and 2 sporadic) had complex genotypes, including seven in cis variants. Altogether 3% of the AAA patients had a pathogenic mutation and 24 (47%) one or more VUS.

The results endorse the hereditogendrety of AAA, showing a modest contribution of TGFBR2 and COL3A1 in AAA. In addition a high prevalence of rare VUS suggests involvement of other aneurysm genes in AAA.

**P05.03-S**

**Longer GT repeats and rs2071746T allele in the heme oxygenase-1 gene promoter are associated with abdominal aortic aneurysm**

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Abdominal aortic aneurysm (AAA) is multi-factorial disease with life-threatening complications due to mainly asymptomatic course of development. Vascular inflammation induced by oxidative stress contribute to pathogenesis. Inter-individual differences in response to oxidative stress are partially under genetic control. In this study the associations between the functional SNPs and (GT)n repeat length polymorphisms in genes involved in the vascular response to hypoxia/ischemia: HIF1A (hypoxia inducible factor-1α) and HMOX1 (heme oxygenase-1) and the development of AAA were examined.

The study encompassed a series of 518 AAA patients, 345 patients with atherosclerotic aortoiliac occlusive disease (AIOD) and 498 controls. The HIF1A rs11549465C>T, rs11549467G>A and HMOX1 rs2071746A>T SNP genotyping was performed by using predesigned TaqMan SNP-genotyping assays. For simultaneous assessment of the HIF1A and HMOX1 (GT)n polymorphisms, the method based on multiplex-PCR with fluorescent-labeled primers and fragment size analysis using DNA sequencer has been developed.

We found, that carriers of the HMOX1 (GT)n repeat long allele (n≥27) had increased risk of developing AAA (OR=1.46 for dominant model, P=0.034). The frequency of carriers of both HMOX1 risk alleles: rs2071746T and/or (GT)n repeat in AAs (58,5%) was higher as compared to AIOD (49,0% and 0,07%). On the other hand, the frequency of noncarriers in AAAAs was 0,0% as compared to 1,3% in controls (P=0,10) and 0,9% in AIOD (P=0,066).

In conclusion, HMOX1 gene promoter longer (GT)n repeat allele and rs2071746T allele related to decreased anti-inflammatory and antioxidant capacity of heme oxygenase-1 are associated with abdominal aortic aneurysm. Supported by Polish Ministry of Sciences grant NN403 250440.
Apolipoprotein E (ApoE) plays a role in the regulation of lipid metabolism in humans. The objective of this work was to examine the association between ApoE gene polymorphisms and the risk of coronary heart disease (CHD) in Bulgarians. The case-control study was carried out on a total of 725 samples including 104 patients with angiographically verified coronary artery disease, 151 patients with myocardial infarction and 470 population controls without data for cardiovascular complications. ApoE gene polymorphisms were genotyped by High Resolution Melting Analysis. The differences in allele frequency between the CHD patients and controls were evaluated with chi-square test. The frequencies of ApoE alleles in the CHD subjects were 0.81 for E3, 0.14 for E4 and 0.06 for E2, and in the control group were 0.84 for E3, 0.09 for E4 and 0.07 for E2. The ApoE allele frequency was significantly higher in the CHD patients than in the control group (OR=1.68, p=0.002). The carriers of E4 containing genotypes (E2/E4, E3/E4 and E4/E4) had a higher risk to develop CHD than carriers of E2 and E3 containing genotypes (OR=1.72, p=0.004). There were no significant differences in patients between the mean of total cholesterol, triglycerides, low density lipoproteins and high density lipoproteins levels among different ApoE genotypes.
Targeted next generation sequencing of 51 genes involved in primary electrical disease

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Dominant mutations in genes encoding desmosomal proteins are reported to cause arrhythmogenic cardiomyopathy (AC), an inherited heart muscle disease characterized by lethal ventricular arrhythmias and heart failure, accounting for 15 to 25% of cases of sudden cardiac death in patients <5 years. Recurrent mutations are infrequent and most of them cause cutaneous syndromes.

We report here the identification of the first founder homozygous desmocollin-2 (DSC2) mutation in the Italian population, segregating in 4 AC families showing a cardiac-restricted phenotype. We performed an exon-by-exon analysis of the DSC2 gene on 80 unrelated Italian index patients diagnosed affected with AC according to revised 2010 Task Force criteria. We identified the p.D179G homozygous mutation in DSC2 gene in 4 (5%) of them, originating from the same north-eastern Italian region. One of them resulted to carry an additional plakophilin-2 (PKP2) frameshift mutation. Haplotype analysis revealed a conserved haplotype among the DSC2 mutation carriers, strongly indicating a common founder. A frameshift mutation. Haplotype analysis revealed a conserved haplotype among the DSC2 mutation carriers, strongly indicating a common founder. A

We developed and optimized a MASTR (Multiplex Amplification of Specific Target for Resequencing) assay comprising 51 genes involved in PED. The MASTR protocol consists of a multiplex PCR whereby a first PCR is performed to amplify all target regions followed by a secondary PCR in which patient specific barcodes and sequencing adaptors are incorporated. The PED assay consists of 951 amplicons distributed over 11 multiplexes. Following the MASTR assay, 2x2500bp sequencing is performed on MiSeq v2. Next, data analysis and interpretation is performed using our local Galaxy-instance and our in-house developed variant database. For validation purposes, 20 patient specific variants were examined, and we have achieved 100% sensitivity. Currently, we are in the process of screening 100 PED patients with unknown genetic defect. The results of this analysis will be presented. Subsequently, this panel will be implemented in a genetic diagnostic setting.

Homzygous founder mutation in desmocollin-2 gene causes arrhythmogenic cardiomyopathy

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Arrhythmogenic Cardiomyopathy (AC) is an inherited heart muscle disease characterized by lethal ventricular arrhythmias and heart failure, accounting for 15 to 25% of cases of sudden cardiac death in patients <5 years. Recurrent mutations are infrequent and most of them cause cutaneous syndromes.

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Human Genetic Evidence that Common Variants near PIK3CG are Associated with Atherosclerotic Plaque Hemorrhage and Vessel Density

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Aim: Atherosclerotic plaques may vary among individuals, in part due to heritable factors. However, the genetic architecture of plaque phenotypes is largely unknown. A common variant near PIK3CG on 7q22.3 has previously been associated with carotid plaque presence. Animal models suggest that PIK3CG may play a role in plaque formation through neovascularization. We hypothesized that the PIK3CG variant is associated with intraplaque hemorrhage (IPH) and vessel density in human plaques. Secondly we focused on characterizing the functional role of genetic variants near PIK3CG in advanced human atherosclerosis.

Methods: We collected 571 Athero-Express Biobank Study patients, and genotyped them using Affymetrix SNP 5.0. After quality control we tested rs17398575 for association to immunohistochemically scored IPH and vessel density, correcting for age, gender and 10 principal components. We used the BIKE cohort to assess the effect of PIK3CG variants on IPH expression in circulating monocytes (n=95) and in carotid plaques (n=126).

Results: The reported PIK3CG variant, rs17398575 (risk allele A, frequency=0.72), was associated with IPH (OR=1.40 [1.10-1.69], 95% CI, p=0.0271) and vessel density ([b=0.095 [0.041-1.04], p=0.0221]. The SNP dependent PIK3CG expression demonstrated a differential effect in the vascular wall (p=0.783 for rs17398575) compared to monocytes (p=0.0261 for rs17398575).

Conclusion: To our knowledge this is the first report involving the association of genetic variants to histological plaque phenotypes in humans. Further research should focus on replicating these results and elucidating the etiology of plaque vessel formation and intraplaque hemorrhage, as epidemiological studies demonstrated these associate with cardiovascular disease.
ABSTRACTS POSTERS

GENOMICS

P05.18-M
SCN5A mutation analysis in 147 Brugada syndrome probands
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SCN5A mutation analysis in 147 Brugada syndrome (BrS) probands identified 27 variants possibly associated with cardiac channelopathies, which resulted in a genetic diagnostic yield of 17.4%. 10 (66.7%) SCN5A variants have already been associated with BrS. 4 (14.8%) with other cardiac arrhythmias and 5 (18.5%) are novel undescribed variants. When only taking into account the patients with a baseline BrS type I ECG (82.6%) the genetic diagnostic yield increased to 41.6%. Interestingly, this was not observed in patients with a BrS type II ECG (11.6%), in which SCN5A variants seem to be either novel or associated with other arrhythmias. We also identified a substantial number of described SCN5A mutations (> 9%) in BrS patients clinically diagnosed by ajmaline positive testing and a family history of BrS and/or sudden cardiac death (80.2%), demonstrating the added value of sodium channel blocker-induced ECG testing. Segregation analysis was performed in available families of the identified SCN5A positive BrS probands to determine genotype-phenotype correlations. In more than 66% of tested families there was an incomplete segregation, pointing to modifier factors or patient selection bias.

SCN5A mutation analysis in 147 Brugada syndrome (BrS) patients with a BrS positive family history of BrS and/or sudden cardiac death (80.2%), demonstrating the added value of sodium channel blocker-induced ECG testing. Segregation analysis was performed in available families of the identified SCN5A positive BrS probands to determine genotype-phenotype correlations. In more than 66% of tested families there was an incomplete segregation, pointing to modifier factors or patient selection bias.

P05.19-S
Identification of candidate genes for Brugada Syndrome by targeted next generation sequencing
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Recent developments of next generation sequencing (NGS) represent a great opportunity to identify new candidate genes in genetically heterogeneous pathologies such as Brugada Syndrome (BrS), an inherited cardiac arrhythmogenic disorder with a prevalence of 1-5,000 in Western countries leading to sudden cardiac arrest in young asymptomatic adults. Until now BrS genetics is still controversial and it is not completely elucidated, since mutations in known sodium channel genes cause approximately 30% of patients. We thus aimed at identifying new candidate genes performing targeted NGS in a cohort of 91 BrS patients. The coding regions of 158 genes were sequenced using the Illumina GAIIx platform, yielding a mean target coverage of 99.16% and a mean sequencing depth of 327.22x among the samples. Excluding all common polymorphisms and considering only protein-coding variations, we overall identified 98 novel variants in 71 subjects in a total of 70 genes, including missense, nonsense, splice-site and INDELS, and 33 clinical variants annotated in dbSNP137. To select more promising BS candidate genes, we then compared the mutation rate of each gene to that observed in repeated random sampling of healthy controls from 1000 genomes project data. Besides confirming an important role for sodium, potassium and calcium ion channels, our results identified new candidate BrS genes previously associated with other forms of inherited cardiac arrhythmias, such as ANK2, RYR2, DSG2, LMNA, suggesting an overlap between different disorders; however, many patients still remained genetically uncharacterized, prompting more extensive studies and suggesting a possible multigenic aetiology.

P05.20-S
A protein network of common susceptibility genes provides a link between inflammation and cardiovascular disease
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Genome-wide association studies (GWAS) have identified hundreds of susceptibility loci for chronic and inflammatory disease phenotypes in humans. There is increasing evidence that chronic inflammation is a crucial driver in the pathogenesis of cardiovascular diseases (CVD), which may be genetically determined. To understand the genetic architecture underlying chronic inflammation and CVD we performed a systematic analysis of (1) common risk alleles coming from published GWAS, (2) of protein–protein interaction (PPI) networks informed by (3) gene expression data with a defined molecular target involved in the inflammatory processes promoting CVD, myocardial related protein (MRP). (1) Through analysis of integrated haplotype scores (iHS) and F_2 values in HapMap phase 2 data, we investigated whether recent selection pressure acting upon inflammatory genes affected CVD susceptibility loci. Our findings provide significant evidence for a PPI network (P = 0.033), which connects inflammatory and cardiovascular susceptibility genes, and establish a genetic framework of inflammatory CVD. 41.59% of PPI genes are associated with inflammation. 28.35% of integrated genes can be linked to both, an inflammatory and cardiovascular disease phenotype. Interestingly, CDK228, and Celsr2/Fsrcl1/Mybphil/Sort1, unequivocally replicated CVD loci, are integrated within this network as several SNPs located in transcription factor recognition sequences, i.e. NFKB1, STAT3, which are key factors in inflammation. Finally, we observed a significant enrichment of inflammatory variants within CVD loci that are targets of selection (P = 0.001-11 in CEU population), suggesting that recent selective sweeps may have affected the genomic architecture underlying CVD.

P05.21-S
Systematic screening of rare coding variants in genes involved in cardiac arrhythmias
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The development of new strategies based on next-generation sequencing enables the large-scale screening of genes involved in rare diseases. We have developed a custom design based on the HaloPlex™ technology (Agil-ent Technologies) to sequence the coding regions of 163 candidate genes, including all genes previously linked to cardiac arrhythmias. In total, 570 individuals were included in this study. To validate our design, we first analysed 42 patients with inherited cardiac arrhythmias. Among the 69 genetic variants previously identified in these patients, 68 were detected automatically after HaloPlex library preparation and Illumina sequencing. The undetected variant is a substitution located in a low-coverage region. Subsequently, 361 additional patients were analysed (178 patients with Brugada syndrome; 89 patients with early repolarization syndrome; 94 cases of progressive cardiac conduction defects). We also identified 167 controls, over 65 years of age and showing no signs of cardiac rhythm or conduction abnormalities. The mean coverage was 577X and we found 5 rare functional variants per patient on average. Then, burden tests were performed to detect genes significantly associated to cardiac arrhythmias. This approach also identified potential new disease genes, and replication in an independent cohort is in progress. This study will lead to a catalog of novel mutations in genes linked to hereditary cases of sudden cardiac death. The systematic screening of our cohorts will also guide our future molecular investigations for these diseases and contribute towards improving the prevention of sudden cardiac death.
Targeted oligonucleotide-selective sequencing of 101 genes from 150 patients with idiopathic dilated cardiomyopathy in Finland.


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The strength of next generation sequencing (NGS) in both research and diagnostics is becoming increasingly evident. It can be successfully applied to find causal mutations and confirm the clinical diagnosis in genetic cardiomyopathies. However, exome sequencing (ES) show in complete representation and coverage of several exons, leading to clinically relevant mutations being missed. Therefore ES will, at least for now, coexist in clinical genetic diagnostics with other NGS-based strategies, such as targeted resequencing. To this end, using an enrichment kit targeting 48 genes associated with hereditary cardiomyopathies and analysing 90+ patient samples, we demonstrated that the high-throughput platform for cardiomypathies meets the technical criteria of clinical diagnostics and is a cost-efficient tool in clinical research.

INTRODUCTION: Inherited type of cardiomyopathy is caused by mutations not only in nuclear genes, but also in mitochondrial genes. Mitochondrial DNA mutations are involved in development of cardiomyopathy through disturbing oxidative energy metabolism. It has been shown that various types of cardiomyopathy can be attributed to disturbed mitochondrial oxidative energy metabolism. The goal of our study was to apply next generation sequencing (NGS) technology as a method to detect mtDNA mutations in patients with cardiomyopathies.

METHODS: 18 patients were included in this study. The entire mitochondrial DNA was amplified in two overlapping polymerase chain reaction (PCR) fragments from the cardiac tissue of the patients undergoing cardiac surgery. mtDNA was deep sequenced by NGS technology.

RESULTS: Six newborns and 12 infant patients with cardiomyopathy were included. Using NGS technology and bioinformatics analysis of the sequence data allowed determination of new and reported variation for each individual. Both known and unknown mutations were determined from 18 of patients. Eleven novel mtDNA mutations were identified at seven patients. Three of the patients have novel mutations together with reported cardiomyopathy mutations. LHCN, Cyclic Vomiting Syndrome with Migraine, Multiple Sclerosis, and Breast cancer risk associated mutations also observed at three patients.

DISCUSSION: In this report, we provide the results of mtDNA analysis for 18 patients with cardiomyopathy. All patients displayed at least one mtDNA mutation. Sixty mutations were found, and 13 of them were unreported. This study represents the most comprehensive mtDNA mutational analysis in congenital cardiac infant patients.

The genetic basis of early-onset cardiomyopathies in Finland


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Heterogeneous heart disorders with more than 100 disease-causing genes reported to date. By enabling comprehensive genetic screening. Next Generation Sequencing (NGS) is an appealing diagnostic approach for CMPs. In this study, we apply NGS to a cohort of genetically undiagnosed patients in order to identify the disease-causing mutations and characterize the molecular background of early-onset CMP in Finland. By detecting robust genotype-phenotype correlations, genetic data can be informative in prioritizing mutations to cardiac transcription factors.

Materials and methods: Our cohort consists of 57 Finnish early-onset CMP patients. The clinical presentation is diverse, ranging from heart-specific muscle diseases to multorgan syndromes. Nine of the patients were screened for mutations by whole-exome sequencing (WES) and 48 by targeted sequencing using a custom-designed panel (HaloPlex) of 117 cardiac genes. Variants were prioritized in respect to frequency and pathogenicity prediction. The candidate mutations were further verified to match the disease segregation in the family and to be absent in Finnish controls.

Results: Mutations were confirmed in four of the WES-investigated patients. Among these, screening of family members revealed de novo mutations in three sporadic severe cases. For the patients investigated with targeted sequencing, strong candidate mutations were identified in 14 cases.

Conclusions: In the current stage of the study, WES has led to a genetic diagnosis success rate of approximately 45%, while for targeted sequencing a success rate of 30% is expected. De novo mutations were found to cause CMPs in the early-onset cohort under study.

Copy number variants in the 22q11.2 region of congenital heart disease patients from São Miguel Island, Azores, Portugal


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Chromosomal rearrangements of the 22q1.1 region, including the 22q1.2
deletion and microduplication syndromes, are frequently associated with congenital heart diseases (CHDs). The present work aimed to study copy number variants (CNVs) in the 22q11.2 region of 87 CHD patients from São Paulo, Brazil.

The subjects were patients originating from two unaffiliated families and were confirmed by aCGH in patients 1 and 2, and by FISH in patients 3 and 4. Patients 1 and 3, both affected with a ventricular septal defect, carried a de novo 2.5 Mb deletion of the 22q11.2 region, whereas patient 2, with an atrial septal defect, carried a de novo 2.5 Mb microduplication (2:1). Finally, patient 4 showed a 2.5 Mb triplication (2:3) and presented dysmorphic facial features, cognitive defect, and cardiomyopathy, a clinical feature not reported in the first case described in the literature. Interestingly, the evaluation of this patient's parents revealed that her non-affected father had a 2.5 Mb microduplication (2:1). Now we are investigating by microsatellite analysis the mechanisms responsible for microduplication and triplication.

In summary, the present study allowed the identification of very rare deletion and microduplication syndromes in Brazilian CHD patients. Moreover, we report the second patient with a 22q11.2 triplication, whose clinical features increase the symptoms that could be present. This work emphasizes the relevance of biomedical research, since it can help paediatricians and other professionals to better assess health care needs.

**P05.27-S**

**Congenital heart malformations in patients with 22q11.2 deletion syndrome**


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Congenital heart malformations represent one of the most common birth defects, affecting about 0.3% or 0.9% of all live births. The association of wide range of conotruncal heart malformations with 22q11.2 microdeletion is well recognized. 22q11.2 microdeletion is the most common deletion in human genome and more than 80 dysmorphism/malformations have been described in patients with 22q11.2 microdeletion syndrome. In this study we investigated the frequency of 22q11.2 microdeletion among patients with congenital heart defect and clinical features of 22q11.2 deletion syndrome. The study population was 57 individuals who underwent detailed clinical evaluation including assessment of cardiac morphology, facial appearance, lymphocyte immunophenotyping, presence of cleft palate and hypocalcemia/hypoparathyroidism screening. All tested patients had congenital heart defect. Fluorescence in situ hybridization and multiplex ligation-dependent probe amplification analysis revealed 22q11.2 microdeletion in 42.3% (24 out of 57) of patients. Cardiac malformations were observed in patients with 22q11.2 deletion were tetralogy of Fallot, pulmonary artery atresia, common arterial trunk, interrupted aortic arch, ventricular septal defect and mitral stenosis. Echocardiography accompanied by contrast computed tomography scan and magnetic resonance angiography revealed malposition of branch pulmonary arteries in two patients with 22q11.2 microdeletion. In conclusion, with this study we stress the need for multidisciplinary assessment of patients with congenital heart malformations which should include testing for 22q11.2 microdeletion.

**P05.28-M**

**Conotruncal malformations and absent thymus due to a deleterious NKX2-6 mutation**

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Background: Truncus arteriosus (TA) accounts for ~1% of all congenital heart defects (CHD) in live birth. The etiology of isolated TA is largely unknown; syndromic TA is mostly associated with chromosome 22q11 deletion. Hadassah Cardiogenetic Project includes a detailed registry and biopsy bank within the frame of this Project we now report the results of a study of patients with multiple conotruncal malformations accompanied by athymia.

Methods and Results: The subjects were patients originating from two unrelated families. Following the exclusion of 22q11 deletion, exome analysis was performed in one patient from each family. A homozygous mutation in chr8: 23560417insA, p.Lys152fs*0, in the NKX2-6 gene was identified in patients from both families. The mutation segregated with the disease in the families and was absent from large cohorts of controls.

Conclusions: NKX2-6 encodes a homeobox-containing protein which is expressed in mouse caudal pharyngeal arches and outflow tract at E8.0-9.5. NKX2-6 is expressed by TBX1-regulated Tbx20 and has been found to be essential for the development of the heart. The phenotype associated with a homozygous deleterious mutation in our patients, falls well within the spectrum of the cardiac defects seen in DiGeorge syndrome, is in agreement with NKX2-6 downstream location in the TBX1 signaling pathway and confirms NKX2-6 role in human cardiovascular development.

**P05.29-S**

**Type 2 diabetes gene ABC8 codling a subunit of KATP channel associated with coronary artery disease in Turkish people?**


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Coronary artery disease (CAD) is one of the most common cardiovascular diseases and is a major cause of morbidity and mortality worldwide. Classical risk factors for atherosclerosis, such as central obesity, arterial hypertension, and dyslipidemia, frequently coexist with type 2 diabetes and contribute to the increased prevalence of CAD. ATP-sensitive potassium (KATP) channels of pancreatic β-cells, which is assembled from two different subunits, Kir6.2 and a sulfonylurea receptor 1 (SUR1), play a key role in glucose-stimulated insulin secretion mechanism. ABC8 gene which is located on chromosome 1p15.11 contains 39 exons and encodes SUR1. We performed a study to scan ABC8 gene variants which we found significantly associated with type 2 diabetes previously, in patients with CAD. 125 individuals with CAD and 123 healthy individuals were included in the study. Genotyping was performed by PCR-RFLP technique for R1273R and exon 16-3’c substitutions using Bst and PstI, respectively. Statistical analysis was performed using SPSS18.0 program. p<0.05 was considered significant. Exon 16-’3’c substitution showed association with disease (OR: 17.13 [95% CI: 484-6.602] p<0.001, under dominant model, while silent substitution R1273R in exon 31 had no effect on disease. According to our results, exon 16-3’c substitution accepted as having important role in type 2 diabetes genetic background has also associated with CAD in our population. Because relatively small sample size of our population is a limitation for the study, replicative studies in larger populations are needed.

**P05.30-M**

**Genetic evaluation of phosphorylation-2 and desmosplakin gene variants in ethnically different populations with dilated cardiomyopathy**

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Background: Cardiomyopathies are a heterogeneous group of diseases with various etiologies. The potential involvement of genes encoding desmosomal proteins, usually associated with arrhythmogenic right ventricular cardiomyopathy (ARVC), was preliminarily evidenced in Caucasian patients with dilated cardiomyopathy (DCM). Accordingly, we investigated this potential genetic overlap in a large cohort of patients with clear diagnosis of DCM and belonging to different ethnicities.

Methods: DNA from 455 DCM patients, referred to our tertiary centres in Pavia and Cape Town, was collected following complete clinical evaluation. 290 samples (184 Caucasians; 80 black Africans; 5 Indians; 17 mixed ancestry) were screened for the two main ARVC genes: plakophilin-2 (PKP2) and desmosplakin (DSP).

Results: Of the 290 patients tested, 9 (3.1%) were found to carry a most likely pathogenic variant, being absent in the publicly available databases (nearly 20,000 controls) and functionally relevant through 6 bioinformatic tools. Respectively, 3 (3.6%) black Africans (1 in PKP2, 2 in DSP), 5 (2.7%) Caucasians (2 in PKP2, 3 in DSP), and 1 African patient of mixed-ancestry (1 in PKP2) were positive. Variants of unknown significance (VUS) were found in 15 patients; interestingly, one Caucasian carried two VUS in DSP, suggesting a potential compound effect.

Conclusion: The genetic background has also associated with CAD in our population. Because relatively small sample size of our population is a limitation for the study, replicative studies in larger populations are needed.
Our data confirm the presence of potentially damaging mutations in desmo- 
sonal protein genes in patients with DCM. The prevalence of these muta-
tions is similar in black Africans and in Caucasians.

P05.3.1-S

eNOS as hypertension susceptibility gene: example from genome-

-association study to functional and clinical evidences

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Materials and Methods: Following clinical evaluation, p.Arg3527Glut mutation was analysed in a cohort of 102 paediatric patients recruited through national-wide hypercholesterolemia screening, and 44 adult patients referred to specialised outpatient clinic due to hypercholesterolemia resulting in CVD. Results: p.Arg3527Glut gene mutation was identified in 14 out of 102 paediatric and in 6 out of 44 adult patients.

P05.3.3-S

Application of exome sequencing in differential diagnosis of pediatric hypertrophic cardiomyopathy

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riants of the haplotype, which encompass both genes, yielded a phenotypic correlation. Indeed, a haplotype in homozigosity is significantly associated with the lower quartile of RHR (RHR<58 bpm). Moreover no significant as-
sociation was found between cardiovascular risk factors and the different
haplotype combinations. Mastermind-like 1 and Calnexin were found to be associated with RHR. We demonstrated a relation between a haplotype and the lower quartile of RHR in our populations. Our findings highlight that genetic determinants of RHR may be implicated in determining cardiovascular diseases and could allow a better risk stratification.

P05.36-M
Different genetic background in the clinical onset of Hypertrophic Cardiomyopathy analyzed by Next-Generation Sequencing
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More than 20 genes have been correlated with Hypertrophic Cardiomyopathy (HCM), making the molecular diagnostic process time consuming, complex and often inconclusive. To better understand the molecular bases of HCM and to assess the utility of next-generation sequencing (NGS) into clinical laboratory practice we designed a panel to analyze 17 HCM related genes on PGM Ion Torrent. We selected 70 HCM patients, 35 early onset (<35 years) and 35 late onset (<65 years), with a well-defined hypertrophic phenotype.

All samples had on average 98% of target regions with coverage higher than 20X, with a mean coverage of 600X. Common variants (MAF>5%) were removed from the analysis, while potential splice variants and novel single nucleotide variants were analysed with bioinformatics pathogenicity prediction programs. We identified 40 different pathogenic mutations (9 novel) in 9 genes: MYBPC3 (16/40=40%); MYH7 (14/40=35%); TNNT2 (3/40=7.5%); CAV3 (2/40=5%); and GLA, MYH6, TNX2, MYL2 and MYL3 (1/40=2.5% each). The mutation detection rate was 93 (26%) in the late onset and 29/35 (83%) in the early onset group (p<0.0001). Considering only early onset patients with positive family history the detection rate was >90%.

Our results showed a strong difference in genetic background of HCM patients for age at onset, family history and clinical features of the disease. Genetic testing with NGS was reliable and allowed the molecular diagnosis especially for cases with early onset and positive family history. These results also demonstrated that an appropriate selection is required for molecular testing in patients with HCM.

P05.37-S
HDAC9 gene is overexpressed in stroke patients
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Aim: HDAC9 is a class IIa histone deacetylase family member and it regulates the epigenetic status of histones and therefore gene expression by catalyzing deacytlation. Risk variant rs11984041 of HDAC9 gene has been demonstrated to be associated with large vessels stroke. HDAC9 expression has been correlated with common carotid intima-media thickness, suggesting an association of HDAC9 gene with the atherosclerotic process, by accelerating atherosclerosis or promoting plaque instability. In this study we investigated the expression of HDAC9 in peripheral blood (PB) of stroke patients [large vessel and cardioembolic] and healthy controls. Aiming to understand the mechanism by which the risk allele is associated with large vessel stroke, we also evaluate the effect of rs11984041 polymorphism on gene expression.

Methods: HDAC9 gene expression analysis was performed on 26 atherothrombotic stroke patients, 26 cardioembolic stroke patients, and 20 healthy controls by Real Time PCR using Sybr Green. Polymorphism rs11984041 was genotyped by PCR-RFLP method.

Results: A significant increase of HDAC9 gene expression was observed in atherothrombotic and cardioembolic stroke patients compared to healthy controls (1.39±0.68 vs 0.61±0.36; p<0.001; 1.58±1.19 vs 0.60±0.36; p=0.001, respectively). The genotyping of 70 individuals showed no gene expression differences between patients carrying CC and CT genotypes.

Conclusions: The gene HDAC9 is overexpressed in stroke patients compared to controls. This result indicated that this gene, that can regulate other genes implicated in the peripheral inflammatory reaction after stroke can represent a new target for the development of therapeutic agents against ischemic stroke injury.

P05.38-M
Novel mutations in the ZIC3 gene cause X-linked heterotaxy
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Congenital heart defects (CHD) is one of the most common congenital abnormalities in newborns, affecting almost 1% of the general population and is associated with substantial morbidity and mortality. A small subset (3%) of this patient population fails to correctly establish left-right patterning during embryogenesis, resulting in abnormal lateralization of the abdominal and thoracic organs, a clinical phenotype called heterotaxy. As the heart is the first organ to develop asymmetrically, disturbances in the left-right axis lead to a variety of serious cardiac malformations in heterotaxy. One of the first genes linked to heterotaxy was ZIC3, a zinc transcription factor of the GLI superfamily, located on the X-chromosome. The ZIC3 gene is a transcriptional regulator, based on the ability to activate transcription of target genes and to bind DNA.

Over the last 10 years we collected and diagnostically tested over 300 patients referred to our clinical genetics center with heterotaxy and/or a variety of heart defects for mutations in the ZIC3 gene. We identified five potentially pathogenic mutations, as well as variations in the N-terminal polyalanine-repeat. The mutations were detected in families with a clear X-linked clinical profile. To support pathogenicity of detected mutations we performed several functional assays. We used confocal imaging to detect subcellular localization of mutated proteins and the in vivo zebrafish model to investigate the potential laterality and for cardiac defects of these mutated proteins.

P05.39-S
Copy Number Polymorphism study in Essential Hypertension
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Essential Hypertension is a complex trait influenced by multiple susceptibility genes interacting with environmental factors. We used 2044 hypertensive cases and 1072 normotensive controls recruited over many years across European regions within Hypertensin genes project and genotyped with Illumina-1M arrays.

Cases and controls were genotyped together to avoid the batch effect for chips and time periods. The Log R ratio of the samples for each SNP was obtained from Genome Studio and the Copy Number (CN) call was performed in Golden Helix after quality controls. CN was estimated in each sample as a mere signal intensity interval measured at a particular locus. Thorough quality control to refine the signal intensity was essential before CN call. Approximately 9% of the samples uniformly distributed between cases and controls were removed for signal to noise ratio and waviness. The PCA analysis on wave corrected data doesn’t show demarcation on sex/phenotype, whereas the distinguishable genotyping batches were corrected. Among the 824 segments identified and discretized into 3 state CN, 13 segments were significantly associated with the phenotype. Odds ratio for these segments were calculated and we identified a top common variant around 1kb in 1<sup>st</sup> intron of LEPRE1 gene with an odds ratio of 1.7 for hypertension (95% CI 1.3-1.9, p-value = 3.01 x10<sup>-12</sup>). This result suggested that carriers of that CNP had a 1.7 times higher risk for hypertension than non-carriers. Further studies are needed to confirm the association of this CNP with hypertension in other dataset.

P05.40-M
Whole exome sequencing concentrating on the metabolome in familiar hypertrophic- and dilated cardiomyopathy: a pilot collaborative Czech study
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Hypertrophic- (HCM) or dilated (DCM) cardiomyopathies are associated with the risk of heart failure and sudden cardiac death. Molecular genetic diagnosis contributes to risk stratification in relatives and may allow for personalized therapy. Altogether, 35 Czech cases from 13 families (7 HCM / 28 DCM) with three or more individuals were analyzed by whole exome next generation sequencing (NGS) concentrated on the metabolome and utilizing TruSight Exome Gen Set on HiSeq1500 (Illumina). Potentially pathogenic gene variants were found in 11/13 families and comprised 17 genes that have been associated with all forms of CM, familiar arrhythm-
mogenic disease or myopathies (TTN, RYR2, CALR3, MYH 7, TPM1, JPH2, DSP, ACTC1, KCNH2, FLNC, SYNE2, CASQ2, VCL, PKP2). Three variants were detected in MYOC1, JAG1, SYNE1 that have not been associated with CM, thus far. Only 12/17 identified genes are included in the currently available targeted next-generation -on-target readout. In 3/11 families the potential causative variant was found only in one gene. The combination of two to three pathogenic variants in CM-associated genes were found in 8/11 families. Pathogenetic poten- tiol of the identified variants must be substantiated by segregation and/or RNA/protein analyses. The failure to detect variants in two large families underscores the limitations of NGS. Nevertheless, our preliminary data sug- gest, that the use of metabolome NGS in complex families may have higher diagnostic yield than current approaches. A prospective research of FNM 64203, CZ.2.16/3.1.00/24022 and IGA NT13770.

P05.41-S Identification of Mexican-specific lipid variants using a novel cross-population GWAS approach

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Mexicans have a higher prevalence of dyslipidemia than Europeans which causes a serious health problem due to the increased risk for cardiovascular disease. However, complex population substructure in this admixed population leads to a decrease in statistical power hindering genetic research. Consequently, Mexicans are substantially underrepresented in genomic studies despite their high susceptibility to dyslipidemias. We hypothesized that part of the increased predisposition is explained by population-specific variants. To test our hypothesis, we performed a two-stage cross-population genome-wide association study (GWAS) of hyperglycemia using 19,273 subjects of Mexican ancestry origin for Mexican families with diabetic ancestors. First, we compared the Mexican population with its European ancestry population represented by Finns, utilizing Mexican low triglyceride (TG) controls and Finnish low TG controls to screen for variants that differ in frequency between the two populations. Subsequently, we included only these variants in a Mexican TG case-control GWAS to identify Mexican-specific variants. Four Mexican-specific variants were discovered and replicated in an independent Mexican cohort (n=64,159). Including signals near the lipoprote- in lipase (LPL) and apolipoprotein A5 (APOA5) genes, suggesting that regulation of the two key TG genes, APOA5 and LPL, play a crucial role in elevated TGs in Mexicans. We validated the cross-population GWAS results with local ancestry analysis, and all replicated variants reside in highly American-ancestry enriched regions in Mexicans with high TGs, indicating that cross-population GWAS can effectively screen for population-specific variants. In summary, we present a novel approach, cross-population GWAS, which can be adapted for other admixed populations and different diseases.

P05.42-M Whole gene deletion of MYBPC3 in Hypertrophic Cardiomyopathy
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Hypertrophic Cardiomyopathy (HCM) is characterized by left ventricular hypertrophy in the absence of a predisposing cardiovascular condition. Pathogenic variants in 12 genes that encode proteins of the sarcomere have been implicated in HCM. Most of the pathogenic variants act in a dominant negative fashion. However lack of function (haploinsufficiency) is the most common disease mechanism for pathogenic variants in MYBPC3 suggesting that large deletions of the MYBPC3 gene may play a role in HCM pathogene-sis. Here, we describe an individual affected with HCM who carries a dele- tion of the entire MYBPC3 gene. The patient was diagnosed with non-obstructive hypertrophic cardiomyo- pathy in his mid forties when undergoing assessment for palpitations and hypertension. Echocardiogram revealed moderately dilated left and right atria with mild mitral regurgitation. There was severe asymmetric left ventricular hypertrophy with interventricular septal hypertrophy. The left ventricular systolic function was normal with an ejection fraction of 65-70%. MLPA analysis showed a deletion of all exons of the MYBPC3 gene. Subse- quent microarray analysis confirmed this deletion and showed that it ex- tended both 5’ and 3’ of MYBPC3 and included 3 additional known OMIM morbid genes (DDB2, SLC39A13 and RAPSN). Unlike MYBPC3, these addi- tional genes are implicated in autosomal recessive disorders and therefore deletions of these genes are not expected to have clinical significance in this patient. Our results suggest that although large copy number variation of HCM-rela- ted genes would still be considered rare, patients highly suspected of HCM may benefit from MLPA analysis if other deleterious variants have not been identified.

P05.43-S The c912_913delTTT mutation in MYBPC3 gene is a founder mutation accounting for one-fifth of the Italian patients affected with hypertrophic cardiomyopathy
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Background - Hypertrophic cardiomyopathy (HCM) is considered the most common cause of sudden cardiac death (SCD) in young people. Over 18 ge- nes have been associated with the disease. The aim of this study was to eval- uate the clinical characteristics, penetrance and prognosis of HCM patients carrying a founder mutation in myosin binding protein C (MYBPC3) gene.
Methods and Results - Ninety seven HCM probands were screened for MYB- PC3 mutations. The frameshift mutation c.912,913delTTT (p.E305P*27) was found in 19 (19.5%) patients (14 males and 5 females). Among 81 re- latives belonging to 14 apparently unrelated families, 45 (20 males and 25 females) resulted to be mutation carriers and 29 had HCM (17 males and 12 females). The family haplotype analysis confirmed a common founder ancestor.

Disease penetrance was incomplete (64.4%) and greater in males than females (85% versus 48%, p=0.009). Eleven (38%) affected mutation carriers were diagnosed between 30 and 40 years old. Probands carrying this found- er mutation showed a worse prognosis for SCD or aborted SCD (p=0.01) compared with patients negative for MYBPC3 mutations.

Conclusions - The founder MYBPC3 mutation carriers have a high probabi- lity to develop the disease between 30 and 40 years of age, with an increased risk if they are men. They show a significantly reduced survival after the fourth decade of life when compared to patients without MYBPC3 mutati- ons. These findings are of relevant importance for the genetic counseling and therapy, considering the high frequency and poor prognosis associated with this founder mutation.

P05.44-M MYH7 and MYBPC3 genetic characterization in Hypertrophic Cardiomyopathy (HCM)

Hypertrophic cardiomyopathy (HCM) is a common disorder of the heart characterized by cardiac hypertrophy of variable degree, myocyte disarray and fibrosis and can cause severe disorders as angina, arrhythmias and heart failure. HCM affects approximately 1/500 subjects and is an autosomal dominant hereditary disease associated with mutations in genes encoding proteins of the contractile apparatus. As other inherited cardiomyopathies, HCM shows marked phenotypic variability, even within families. Mutati- ons of MYH7 and MYBPC3 genes cover about 50% of cases. The MYH7 gene (14 exons) encodes the heart specific myosin heavy chain-2, while the MYBPC3 gene (11 exons) encodes a protein that regulates the sarcomere function and whose mutations have been associated with hypertrophic cardiomyopathy and is responsible for about 25% of all currently known pathogenic variati- ons. The MYBPC3 gene (11p11.2) consists of 35 exons encoding the myosin binding protein-C, whose cardiac isoform consists of 1274 amino acids. Here we report the data collected by our Unit since 2012. We tested 52 pa- tients and found MYBPC3 causative mutations in 11 affected subjects (21% of cases); six mutations are already described in literature while five result novel ones. MYH7 genetic testing led to the identification of seven different
mutations in 7/34 patients (20.6%), 3 are novel mutations. Search for causative mutations is an integral part of the HCM diagnosis. The finding of a causative mutation in a patient, permits the identification of family members who may have the disease in an asymptomatic way or who may develop disorders in the future and may in turn transmit the mutation to their offspring.

P05.45-S

Semiconductor (Ion Torrent) massive parallel sequencing of the main Hypertrophic Cardiomyopathy genes based on two tubes multiplex amplification of DNA pools

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At least 30 different genes have been linked to Hypertrophic Cardiomyopathy (HCM), with MYBPC3 and MYH7 accounting for most of the mutations. Due to the large size of these genes, Sanger-sequencing of single amplicons is labor-intensive and expensive. Next generation sequencing (NGS) could facilitate the genetic screening of the HCM-genes in large cohorts. Our purpose was to design and validate a procedure for sequencing the 9 most commonly mutated genes in HCM (MYH7, MYBPC3, TNN2, TNN3, ACTC1, TNNC1, MYL2, MYL3, and TPM1), based on two-tubes multiplex amplification of DNA-pools (Ampliseq; 176 primer-pairs covering 15,690 bp, 98.62% of the target coding sequence) followed by semiconductor array sequencing with the Ion Torrent Personal Genome Machine (PGM; Life Technologies).

We created a pool with DNA from 13 patients previously Sanger-sequenced for coding exons plus at least 5 intronic flanking nucleotides of the nine genes. Each patient was heterozygous for a unique rare variant (including a small deletion); each unique allele (control variant) was thus diluted 1/26 inside the pool. We sequenced the DNA-pool in a medium capacity Ion chip 316 (semiconductor 100 Mb) array, and the data was analyzed with the Torrent Suite software optimized to detect insertion/deletion nucleotide changes. We successfully identified all the 13 control variants (including the indels). In conclusion, we developed a NGS protocol for the main HCM genes. The multiplex amplification of DNA pools would reduce the time and cost of screening these genes at a population scale.

P05.46-M

Role of genetic testing in diagnosis and management of idiopathic ventricular fibrillation cases

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Introduction: In approximately 5%-10% of the survivors of sudden cardiac death no underlying cardiac disease is identified and consequently the event is classified as idiopathic ventricular fibrillation (IVF). The genetic etiology of IVF remains mostly obscure and current consensus statements recommend the genetic screening only in presence of clear clinical indications. Methods: Using whole-exome sequencing (Illumina HiSeq 2000) we molecularly investigated 2 Italian unrelated infants with IVF together with the unaffected parents and 3 affected members of a German family with history of sudden unexpected deaths below age 40. Results: In one infant (2 yo) we identified a de novo missense variant in RYR2, the main gene responsible for catecholaminergic polymorphic ventricular tachycardia (CPVT). The available clinical data were not sufficient to make the diagnosis, as the stress test, the leading clinical tool in CPVT diagnostic process, is not feasible in infancy. In the second infant (4 months), we detected a de novo missense variant in the PARN gene and the biological plausibility of this result is currently under investigation. In the German family we detected in all affected members a novel frameshift mutation in the desmoplakin gene, associated with ARVC with LV involvement (left-dominant arrhythmogenic cardiomyopathy, LDAC). As a matter of fact this finding allowed a better management and follow-up of affected members. Conclusion: Our approach suggests that a target screening for channelopathy and cardiomyopathy genes could be indicated in those IVF cases in which a complete clinical and familial evaluation is not feasible.

P05.47-S

FBN1 deep intronic mutations: Can they explain the molecularly unresolved Marfan cases?

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Marfan syndrome (MFS) is a multi-systemic autosomal dominant connective tissue disorder caused by mutations in the FBN1 (fibrillin-1) gene, but approximately 10% of MFS cases remain genetically unresolved. Here, we report a new FBN1 mutation in an MFS family that had remained negative after extensive molecular genomic DNA FBN1 testing, including denaturing high performance liquid chromatography (DHPLC), Sanger sequencing, multiplex ligation-dependent probe amplification (MLPA) and micro-array analysis for copy number variation. In addition, four other aneurysm-linked genes were screened for mutations by Sanger sequencing, TGFBR1, TGFBR2, SMAD3 and ACTA2, without any result. Linkage analysis in the family revealed a large linked region on chromosome 15 which was confirmed by microsatellite analysis. Cultured proband fibroblasts and subsequent cDNA sequencing revealed a double peak pattern at the junction of exon 56 and 57. Sanger sequencing of intron 56 revealed a deep intronic point mutation creating a new splice donor site (c.6872-961A>G; ENST00000316623). Together with an existing cryptic splice acceptor site, this mutation results in the integration of a 90 bp pseudo-exon between exons 56 and 57 containing a stop codon and causing nonsense-mediated mRNA decay. Although more than 90% of FBN1 mutations can be identified with regular molecular testing at the genomic level, deep intronic mutations will be missed and require cDNA sequencing or whole genome sequencing.

P05.48-M

Compound-heterozygous Marfan syndrome?

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Introduction

Marfan syndrome is an autosomal-dominant condition often caused by mutations in the fibrillin-1 (FBN1) gene. Here we report a family where the proband is compound-heterozygous for two mutations in FBN1.

Case

The proband (II:1) was on suspicion of Marfan syndrome at 21 years of age. An aortic dilation was found at age 17, during clinical investigation for rheumatoid arthritis. Family history was ambiguous: His father was operated for an aortic aneurism at age 30, allegedly caused by endocarditis. Sequencing using an NGS panel for Marfan and Marfan-like syndromes was undertaken. Two mutations were found in FBN1; one previously reported as causative for Marfan syndrome, the other novel. Results of family investigation and clinical findings are reported in table 1.

Interpretation

Compound heterozygosity has previously been reported in Marfan syndrome. Neither of the two mutations found are present in ESP, nor found among 2000 Danish exomes. In the present case we find it more likely that the previously reported supposed pathogenic mutation found in I:2 is of no/little phenotypical consequence. It was originally found in an isolated case of aortic dissection where no further details were given. The involved amino acid is not evolutionary conserved. Individual I:2 is without clinical symptoms of Marfan syndrome at age 55. The novel c.7694G>A mutation is in silico predicted to be likely pathogenic and segregates with the clinical phenotype.

<table>
<thead>
<tr>
<th>Individual</th>
<th>Number</th>
<th>Mutation</th>
<th>Clinical features</th>
<th>Medical history</th>
<th>Aortic dissection at age</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Father</td>
<td>I:1</td>
<td>c.7694G&gt;A; p.Met1576Thr (novel)</td>
<td>Marfanoid face, wrist sign, arm-scan to height ratio 1.05</td>
<td></td>
<td>36</td>
<td>Marfan syndrome according to revised Ghent criteria</td>
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<tr>
<td></td>
<td></td>
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<td></td>
<td>Childhood scoliosis forpectus carinatum</td>
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<tr>
<td>Mother</td>
<td>I:2</td>
<td>c.4727T&gt;C; p.Met1576Thr (previously reported)</td>
<td>Normal</td>
<td></td>
<td></td>
<td>Healthy</td>
</tr>
<tr>
<td>Proband</td>
<td>II:1</td>
<td>c.7694G&gt;A; p.Met1576Thr</td>
<td>Marfanoid face, wrist sign, asymmetric chest, plexus planus, myopia (7-15 bilateral), striae</td>
<td></td>
<td></td>
<td>Marfan syndrome according to revised Ghent criteria</td>
</tr>
</tbody>
</table>
Several loci enriched in lower frequency variants are associated to risk factor for metabolism and cardiovascular diseases in 4,000 Italian isolated individuals

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Microcephaly with or without chorioretinopathy, lymphedema, or mental retardation syndrome (MCLMR, OMIM 152950) is a rare autosomal dominant disorder with variable expressivity. It is characterized by mild-to-severe microcephaly, often associated with developmental delay, ocular defects and lymphedema, usually on the dorsum of the feet. It can be sporadic or inherited. So far, 25 families (46 patients) have been described to carry a mutation in KIF11. This gene encodes a homotetrameric kinesin, EG5. Members of this protein family are involved in the establishment of a bipolar spindle during cell mitosis, in chromosome positioning and in centrosome separation. EG5 inhibition impairs endothelial cell proliferation and migration, and angiogenesis. We tested a series of 23 unreported MCLMR index patients for KIF11 and found 14 mutations, 12 of which are novel. We detected a mutation in all 7 familial cases and 7 of the 16 sporadic patients. The inherited mutations were found in an additional 12 family members. We subsequently reviewed the clinical phenotypes of all the patients with a KIF11 mutation, including those already published (n=46-26=72).

Microcephrophy in frequency was present in at least 94%, eye anomalies in 68% and lymphedema in 53% of patients. Three mutation carriers are unaffected. We observed 14 de novo cases within 39 index patients (36%). As the remaining sporadic patients may be mosaic, KIF11 mutations likely cause the majority, if not all, of MCLMR.

P05.52-M

Next-generation sequencing as a diagnostic tool in sudden unexplained death victims and patients with a cardiac disease

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Objective

Sudden cardiac death (SCD) is responsible for a large proportion of deaths in young individuals. After routine post-mortem investigations, many cases are still unexplained. Implementation of genetic investigations in forensic medicine may increase the diagnostic rate – not least in sudden unexplained death (SUD). With next generation sequencing (NGS), genetic analysis has become faster and more efficient, enhancing the outcome. The purpose of the study was to explore the yield of genetic analysis using NGS in forensic pathology and to compare the genetic findings to routinely diagnosed patients.

Methods and results

Genetic investigation was performed in 44 unrelated individuals; 15 forensic SUD cases and 29 unrelated patients diagnosed with channelopathies. In-solution targeted sequencing capture probes were custom designed by Traglia and included all exons of each gene, and sequenced on the Illumina MiSeq. Larger deletions and insertions in five genes were investigated with multiplex ligation-dependent probe amplification. Of the SUD cases, 21% were found to have a probably pathogenic variant. The corresponding hit-ratio in the patient cohort was 37% Two patients (7%) had large deletions.

Conclusion

By NGS, it was possible to detect a probably pathogenic single nucleotide variant in one fifth of the SUD cases, disposing to a cardiac disease. In contrast, almost half of the patients were found to carry a probably pathogenic variant or larger deletion. Genetic investigation with NGS can be used as a diagnostic tool in both a forensic and clinical setting.

ABSTRACTS POSTERS
CARDIAC AND VASCULAR DISEASES

P05.54-M

Clinical and molecular characterization of Chilean patients with Noonan Syndrome- multiple lentigines

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Introduction: Noonan Syndrome-Multiple Lentigines (NS-ML) is a rare autosomal dominant disorder, and one of the conditions known as RASopathies. PTPN11, RAF1, and BRF1 are the genes known to be associated with NS-ML. Sequence analysis of coding exons 7, 12, and 13 of PTPN11 detects mutation in about 90% of individuals tested.

Objective: The aim of this study was to characterize the clinical and molecular features of Chilean patients with NS-ML. Methods: collaborative and descriptive study. Results: thirteen patients have been confirmed molecularly. This includes two familial cases. The age at diagnosis ranged from 20 days to 33 years old. In six patients including one of the family cases, the recurrent mutation c.89C>T; p.A30V was identified. The other mutation in exon 7 was identified. They showed interfacial and intramolecular variable expressivity. Four patients have mutations in exon 12, three with the recurrent mutation p.Thr486Met and one with an infrequently reported one, p Ala461Thr. Two different mutations were identified in exon 13, in three patients. The uncommon mutation p.Gln510Glu, was found in two patients, both with prenatals cardiac manifestations, and a hypertrophic cardiomyopathy (HCM) rapidly progressive. The other mutation at codon 510 is a Gln510Pro. Discussion: The diagnosis of this condition may be challenging because clinical features overlap with other RASopathies. Although NS-ML seems to be rare, we have diagnosed a considerable number of patients. We developed a high index of suspicion particularly in patients with HCM. Eventually specific mutations could be predictor of adverse cardiac events.

Grant sponsor: Pediatric Division, Pontificia Universidad Católica de Chile

P05.55-S

The SMAD-binding domain of SKI: a hotspot for de novo mutations causing Shprintzen-Goldberg syndrome

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Background: The SMAD-binding domain of SKI (SMAD-Binding protein) is a known hotspot for de novo mutations causing the Shprintzen-Goldberg syndrome (SGS). In 427 LVOTO probands were screened for the mutation. We report on the clinical characteristics of familial cases and 1% of sporadic cases. In total 52 mutation carriers were identified. The phenotype included not only LVO

Conclusion: We identified two likely pathogenic variants and one VOUS in the PTPN11 gene, supporting previous evidence of mutations in the PTPN11 gene as a rare cause for LVNC. The MBI gene did not contribute to LVNC in our cohort.

CARDIAC AND VASCULAR DISEASES

P05.56-M

Clinical and molecular characterization of Chilean patients with Noonan Syndrome- multiple lentigines

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Grant sponsor: Pediatric Division, Pontificia Universidad Católica de Chile

P05.57-S

Targeted oligonucleotide-selective sequencing for diagnostics of pulmonary arterial hypertension

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Background: The genetic basis of idiopathic pulmonary arterial hypertension (IPAH) is well recognized but still rarely utilized in diagnostic settings. High penetrance mutations are present in 8% of familial LVOTO. The other mutation carriers. The phenotype included not only LVO

Conclusion: NOTCH1 mutations are present in 8% of familial LVOTO. The phenotypic spectrum is expanded and includes a wide variety of heart malformations, indicating an effect of these mutations on neural crest derived cells and epithelial to mesenchymal transition. The high penetrance at adult age highlights the importance of genetic testing of NOTCH1 for early diagnosis, not only in LVOTO families but in a variety of familial congenital CMY.
be due to mutations in the SKI gene, encoding the oncoprotein SKI, a repressor of TGFβ activity. Here we report eight recurrent and three novel SKI mutations in eleven SIDS patients. All were heterozygous missense mutations located in the R-SMAD binding domain, except for one novel in-frame deletion affecting the DHD domain. Adding our new findings to the existing data clearly reveals a mutational hotspot, with 74% (23 out of 31) of the hitherto described unrelated patients having mutations in a stretch of five SKI-residues (from p.Ser31 to p.Thr335). This implicates that the initial molecular testing could be focused on mutation analysis of the first half of exon 1 of SKI. As the majority of the known mutations are located in the R-SMAD binding domain of SKI, our study further emphasizes the importance of TGFβ signaling in the pathogenesis of SIDS.

P05.58-M
Mutations in the TSPYL1 gene are not associated with sudden infant death syndrome in a Swiss cohort of deceased infants
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Background: Sudden infant death syndrome (SIDS) is currently the major cause of an unexpected and unexplained death of infants in the first year of lifetime in industrialized countries. Besides environmental factors also genetic factors are supposed as risk factors for SIDS. Notably, a frameshift mutation (c.457dupc, p.Glu153Glyfs*17) in the TSPYL1 gene has been reported as disease causing for an autosomal recessive sudden infant death with dysgenesis of the testes syndrome (SDDT) in an Old Order Amish community in Pennsylvania. Because the Amish community was originally founded in the German speaking part of Switzerland, including people from Alsace and Palatinate, a mutation analysis of the entire TSPYL1 gene was performed in a cohort of 166 SIDS cases originating from the Swiss population around Zurich in comparison to 163 controls.

Eight known SNP variants (rs61746509, rs38287473, rs37498985, rs61746508, rs56100880, rs3749894, rs45490498 and rs9400897) were detected in the analyzed SIDS cohort, none of which was significantly associated with SIDS. In this context we also found two potentially disease causing and/or acid substitutions in three deceased girls. One SIDS affected girl was heterozygous for the novel TSPYL1 variant c.106C>G (p.Leu36Val) and two affected girls were heterozygous for the rare known TSPYL1 substitution rs140756663 (c.1098C>A, p.Phe366Leu). In silico analyses predicted rather non-pathogenic effects for p.Phe366Leu and p.Leu36Val, although protein features might be affected. The founder nonsense mutation c.457dupc (p.Glu153Glyfs*17) was not detected in our analyzed SIDS cohort of Swiss origin.

Conclusions: Mutations in the TSPYL1 gene are not associated with SIDS in Swiss infants.

P05.59-S
SMAD3-related aortic aneurysms & dissections: A new physical phenotype observed in a large affected family
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Objective: To expand the phenotype of SMAD3-related aortic aneurysm and dissections by investigation of a large family.

Methods: Genetic testing of SMAD3 was undertaken. Physical features of two male first cousins presenting to a Genetics Clinic with thoracic aortic aneurysm/dissection were compared followed by testing and clinical characterization of the extended family. Results: Molecular testing confirmed that the proband and his cousin shared two aortic aneurysmic carriers of a deletion of exons 4-12 in SMAD3. Each had several features typically seen in Marfan syndrome or Loeb-Dietz syndrome (marfanoid habitus, ocular hypertelorism in one case and mild marfanoid craniofacial features and excessive stride in the other). One feature not reported in either condition was the presence of multiple small, punctate, palmar keratoses. Additional members of the kindred were examined and found to have more mutation carriers were identified. Of the adults 39 years or older who carried the mutation, all 5 had multiple punctate palmar keratoses. For the 5 carriers aged 13-22 years, one had definite palmar keratoses. None of the mutation-negative patients had palmar keratoses. There was considerable variability in age at onset for aortic aneurysm/dissection and presence of non-vascular features. A summary of features will be presented.

Conclusions: We conclude that punctate palmar keratoses may be a previously unreported feature of some families with SMAD3-related aortic aneurysms and dissections, and a useful sign in distinguishing SMAD3-related cases from other genes associated with aortic aneurysm/dissection.

P05.60-M
Are ALOX5AP SNPs a risk or protective factor for Stroke?

ALOX5AP (5-lipoxygenase) has been recognized as a susceptibility gene for stroke. Using a case-control design, we sequenced the whole coding and adjoining intronic regions of ALOX5AP to study the role of SNPs and their interplay with other risk factors in Greek patients with stroke. 277 patients were included and classified by the Trial of Org 10172 in Acute Stroke Treatment (TOAST) criteria. Large vessel disease (15), small vessel disease (63), intracerebral hemorrhage (33), cardioembolic origin (14), undetermined (38), other causes (12) and lacunes (117). The mean age of patients was 58.9±14.6, comprising 191 males. A control group of 210 subjects, ethnicity, sex and age matched, with no stroke history were also genotyped. Risk factors (hyperlipidaemia, hypertension, atrial fibrillation, migraine, CAD, diabetes, smoking and alcohol consumption) were assessed as confounding factors. SNPs rs7679695, rs3803327 and rs202068154 showed significantly different (p<0.01) frequencies between patients and controls. More specifically the genotype frequencies of AA (minor allele) of rs4769650, of genotype CA (A minor allele) of rs3803327 and of AC genotype (C minor allele) of rs202068154 were significantly higher (p<0.01) in controls than in patients. All the above SNPs are located in intronic regions of the gene and according to in silico programs Ex_SKIP and HSF they affect splicing of exons 1 and 2 of ALOX5AP. The results were indicative of a protective role of the three SNPs either in homozygosity or heterozygosity for MAV. However, confounding factors as mentioned above have a strong impact on stroke occurrence and outweighed the protective role of the above SNPs.

P05.61-S
Postmortem genetic testing in a series of 36 young patients after sudden cardiac death
I. Marey1, V. Fressart2, C. Rambaud, E. Gandjbakhch1, A. Maleff1, E. Le Boëtte1, C. Bordet1, G. Lorin de la Grandmaison1, B. Richard1, P. Charron3
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The incidence of sudden cardiac death (SCD) increases with age in parallel with coronary disease’s prevalence. In young persons and athletes, SCD occurs in half of the cases, in the setting of genetically transmitted disorders such as cardiomyopathies. Molecular testing performed after necropsy may help management of families but experience in this area appears very limited. The aim is to report our experience of post mortem molecular testing after SCD and necropsy. We studied 36 patients <40 years who died suddenly with a suspected diagnosis of cardiomyopathy, established either after autops or known before death, with 6 dilated cardiomyopathy (DCM), 12 hypertrophic (HCM), 2 HCM/DCM, 1 restrictive (CMR), 14 arrhythmogenic right ventricular cardiomyopathy (ARC), 1 HMC and left ventricular non-compaction. Fifteen mutations have been identified in one or more genes that cause DCM, HCM, ARVC, familial forms of hypertrophic or non-syndromic patients. Therefore our data may increase the ratio of detected mutations in non-syndromic patients.

P05.62-M
Mutation detection rate and -characteristics in thoracic aortic aneurysm (TAA) related disorders: results from next generation sequencing (NGS) panel testing
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Background: Targeted gene testing for Heritable Thoracic Aneurysms and Dissections (H-TAD) is compromised due to overlapping clinical features and the identification of mutations in non-syndromic patients. Therefore panel testing of multiple genes has now emerged as the preferred approach. So far, no data on mutation detection rate with this technique have been reported. Methods: We implemented NGS based screening after targeted
PCR enrichment of the 7 most common H-TAD-associated genes (FBN1, TGFBR1/2, SMAD3, TGFBR2, ACTA2 and COL3A1). Between November 2012 and December 2013, 141 samples from unrelated probands presenting either TAD (N=119), arterial aneurysm/dissection outside the aorta (N=10) or syndromic features with a positive family history for TAD (N=12) were sequenced on an Illumina MiSeq sequencer. Results: The median age of the cohort was 41.7 years (IQR 29.3 - 52.7y). We found a causal mutation in 22 patients (16%). Clinical and genetic findings are summarized in the table below.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Total</th>
<th>H-TAD</th>
<th>NTAD</th>
<th>STAD</th>
<th>NS-H-TAD</th>
<th>LDS</th>
<th>LHS</th>
<th>Total</th>
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<tr>
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<td>2</td>
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<td>1LDS</td>
<td>1</td>
<td></td>
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<td></td>
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</tr>
<tr>
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<tr>
<td>COL3A1</td>
<td>25-HTAD</td>
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<td></td>
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<td></td>
<td></td>
<td>1NS-H-TAD</td>
</tr>
</tbody>
</table>


Conclusion: NGS based gene panel testing in patients with H-TAD efficiently reveals a mutation in 16% of patients. Causal mutations in patients not presenting clinical manifestations of syndromal H-TAD, as well as mutations in genes other than FBN1 in patients meeting the diagnostic criteria for specific syndromes including Marfan syndrome are identified, justifying a widespread application of this technique.

P05.63-S
A new COL3A1 transgenic mouse model recapitulates the clinical features of vascular Ehlers-Danlos Syndrome

Vascular Ehlers-Danlos Syndrome (vEDS) is a severe, life-threatening heritable connective tissue disorder, characterized by translucent skin, easy bruising and propensity to rupture of arteries and hollow organs. The molecular basis of vEDS has been well-studied, showing a wide distribution of mutations in COL3A1, encoding type III procollagen. Most mutations result in a glycine substitutions, pivotal for correct folding of the triple helical domain. The mechanisms by which mutant type III collagen cause vascular fragility are not well understood, but factors other than mechanical failure, are believed to contribute to the phenotype. A COL3A1 knock-out mouse model shows early lethality and is therefore not suitable for functional studies. We generated a transgenic vEDS mouse model with a Col3a1 missense mutation. With a Bac transgenic approach, the Col3a1 c.547G>A (p.Gly183Ser) mutation, a typical fetal helical subunit, was introduced into the C57BL/6 mouse genome. Spontaneous development of open skin wounds was observed in all transgenic males, but not in females. Expression analysis on dermal fibroblasts revealed substantial higher expression of type III collagen in transgenic males compared to transgenic females. Biomechanical testing of both transgenic males and females showed significantly reduced tensile strength of the skin, aorta and colon. Transmission electron microscopy of skin and aortic tissues showed severely abnormal collagen fibrils, intracellular smooth muscle cell and dermal collagenous reticulum (ER), indicating excessive protein load and ER stress. This novel animal model provides new opportunities for in-depth analyses of the pathogenic basis of vEDS and for possible therapeutic interventions.

P05.64-M
Genetic tests in the diagnosis of congenital vascular malformations
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Vascular anomalies are a heterogeneous group of congenital malformations of the circulatory system characterized by morpho-structural and/or functional defects of various nature, severity and extent in any type of vessel (arteries, capillaries, veins and lymphatic vessels) and in any part of the body. Although most vascular malformations are sporadic (simple or combined), syndromic and familial forms also exist. Diagnosis may be complicated even for experienced specialists, because there is substantial clinical overlap between lesions and each can mimic the others. To our knowledge, there are currently 15 known genetic forms of Mendelian vascular malformation and mutations with autosomal recessive, autosomal dominant or paradoxic inheritance have been identified in 25 genes. Sporadic cases with de novo mutations in causative genes of familial forms have also been described. In this study, we investigated all 25 known genes in 108 patients with sporadic congenital vascular anomalies by next-generation sequencing. We identified 24 variations (22%). To estimate their pathogenicity, each variation was looked up in dbNSP137, its frequency was compared using data from the Exome Variant Server, the effect of amino acid substitution on the protein was evaluated using in silico analysis, and where possible, it was investigated in our family and the utility and accuracy of genetic testing even in sporadic cases. Identification of causative genes will be decisive for: determining genotype-phenotype relationships; determining transmission risk; organizing personalized follow-ups; characterizing new drug targets for experimental gene-specific therapies.

P05.65-S
Association of VAV2 and VAV3 polymorphisms with cardiovascular risk factors in hypertensive and diabetic patients
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Hypertension, diabetes and hypercholesterolemia are risk factors for the development of cardiovascular disease, one of the leading causes of death in the world. There are few data on the influence of genetic polymorphisms in these diseases. The guanine nucleotide exchange factors VAV2 and VAV3 play an important role in vascular homeostasis in vivo. Therefore, we evaluated the association of VAV2 (rs 602990) and VAV3 (rs 7528153) polymorphisms with susceptibility to hypertension and diabetes-induced cardiovascular risk and cardiovascular damage. We extracted DNA from peripheral blood of 384 patients (152 hypertensive, 66 diabetic and 166 non-diabetic non-hypertensive). Polymorphisms were detected by PCR and TaqMan probes. We analyzed systolic, diastolic and pulse pressure, basal glycemia and endothelial dysfunction (by measurement of pulse wave velocity), retinopathy, left ventricular hypertrophy and cardiovascular risk. WA VAV2Met polymorphism is associated with family history of hypercholesterolemia in non hypertensive patients carrying the TT genotype (p=0.034, OR=2.706, 1.205-5.231) and with elevated pulse pressure in smoker patients carriers of the TT allele (p=0.05, OR=0.408, 0.180-0.927). VAV3Sher298Thr heterozygous polymorphism is associated with patients with lower incidence of family history of diabetes (p=0.018, OR=0.470, 0.279-0.793) and with increased fasting glucose in non-smoking diabetic patients carrying the TT allele (p=0.043, OR=3.700, 1.266-10.815). Our results suggest that polymorphic variants of VAV2 and VAV3 genes may be involved as risk factors associated with cardiovascular disease in hypertensive and diabetic patients.

P05.66-M
Association of Cytochrome CYP450 2C9 (CYP2C9) and VKORC1 polymorphisms and warfarin dosage in Iranian patients refer to Shahid Rajaie Heart Center
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Warfarin is a commonly prescribed oral anticoagulant for the treatment and prevention of thrombotic diseases. Warfarin dose has a large interindividual variation. An insufficient dose may fail to prevent thromboembolism, while an overdose increases the risk of bleeding. Patients with CYP2C9*3 and/or CYP2C9*2 polymorphisms need lower dose than wild-type patients. VKORC1 (vitamin K epoxide reductase complex 1) is another gene affecting warfarin metabolism. VKORC1 variants have been reported to be associated with a need for lower doses of warfarin compared with wild-type variants during long-term therapy.

Objective: This study was conducted to identify the associations between demographic characteristics (sex, height, weight, age, ethnicity), and genetic polymorphisms of CYP2C9 and VKORC1 (-1639G>A) with warfarin dose among Iranian patients.

Materials and Methods: Our study concluded 200 patients that reached to a stable dose of warfarin. By PCR-RFLP method CYP2C9*2 and CYP2C9*3 polymorphisms of CYP2C9 gene and VKORC1 (-1639G>A) was genotyped. Results and Conclusion: Our study showed that CYP2C9 polymorphisms had significant influence on Iranian daily warfarin dose (p=). Our results
suggested that patients with AA genotype in VKORC1 (-1636G>A) require lower doses of warfarin than those with AG or GG genotype. Height and weight did not have a significant correlation with the warfarin maintenance dose. In addition there was no significant relationship between sex and ethnicity with the maintenance dose of warfarin (p< 0.05).

P05.67-S

Analysis of c.1166A>C polymorphism in 3’UTR region of AGTR1 gene in patients with Pulmonary Arterial Hypertension

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Pulmonary arterial hypertension (PAH; OMIM 178600) is a progressive vascular disorder characterized by pulmonary vascular resistance increase, vascular remodeling and right heart failure. Angiotensin II is both a potent vasoconstrictor and a primary regulator of aldosterone secretion. It is an important factor that controls blood pressure and volume in the cardiovascular system. AGTR1 gene plays an integral role in blood pressure control, and is involved in the pathogenesis of hypertension. In this study, we demonstrated the association between c.1166A>C polymorphism and PAH. We included 55 PAH patients and 52 controls. Using specifically designed primers we amplified and sequenced the 3’-untranslated region of AGTR1. We observed the c.1166A>C polymorphism in 3'UTR region of AGTR1. By comparing genotype frequencies of controls and patients with PAH, we obtained statistically significant differences for this SNP between the two groups (p=0.001). The RR of developing PAH in patients with this SNP is 2.03; IC 95%; p<0.001. The statistical analysis of clinical and hemodynamics parameters between patients with or without this SNP showed significant differences in systolic pulmonary pressure (p=0.037), cardiac index (p=0.012) and PAH subtypes (PSS vs PHA) (p=0.028). This SNP is present in 72.4% of IPAH patients and in 67.8% of APAH patients, producing a more severe phenotype. This SNP only appears in 25% of control cohort. In conclusion, this polymorphism in AGTR1 gene is more frequent in IPAH than in APAH patients and predisposes individuals to an increased risk of developing PAH associated with a more severe phenotype.

P06.01-S

10 novel HGD mutations identified in “black bone disease” patients


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Alkaptonuria (AKU, “black bone disease”) is caused by mutations in homogentisate-1,2-dioxygenase (HGD) gene leading to deficiency of HGD enzyme activity. Clinically it leads mainly to homogentisic aciduria, ochronosis and painful and disabling ochronotic arthritis. AKU was described by Sir Archibald Garrod as the first inborn error of metabolism in 1902, and now, more than 110 years later, we test nitisinone as the first possible treatment for this disorder. We analysed for HGD mutations 40 AKU patients enrolled for the first part of this FP7-supported project DevelopAKUre run in two study centres in Piestany (Slovakia) and Liverpool (UK). In this cohort we observed 20 different pathogenic variants, two of which were novel. The first mutation (RSQ3 c.158G>A) was present in homogentisate state in one patient of Indian origin. The second one (T167C, c.500C>T) was identified in one copy in Slovak AKU patient, further confirming the genetic heterogeneity of AKU in this small country with increased incidence of AKU, where now already 13 mutations are reported. Eight additional novel HGD mutations were found in AKU patients sent to our laboratory for a routine molecular diagnostic within a frame of different collaborations. The novel RSQ3 mutation identified in DevelopAKUre was found also in one Italian patient. One mutation was identified in a patient from Brazil (M186K, c.557T>A), in patients from India (s197V, c.591G>T, c.669G>T, c.717G>A) and from France (c1476, c.440T>C), and in five cases from Italy (K248E, c.742A>G/G205D, c.614G>A/K353q, c.1056A>G/G251S, c.752G>A/Y404s, c.119A>C). The total number of different AKU-causing mutations as published in our HGD mutation database is now 126.

P06.02-M

Intrafamilial rearrangements in Barth syndrome leading to different TAZ mutations in siblings

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The X-linked infantile-onset Barth syndrome (BTHS; OMIM #302060) is caused by mutations in the TAZ gene and/or the biochemical dosage of the monosomy-cardiopulmonary/tetralinolein cardiopulmonary ratio. Heterozygous females do not normally manifest clinically but may undergo molecular prenatal diagnosis during pregnancy.

Here we report the unusual case of a family in whom the male proband and his mother carry different TAZ mutations. The ten-year-old proband harboured a novel g.4552364_4562302del9937ins705 complex rearrangement which serves to remove TAZ exons 1-5. However, during a subsequent maternal pregnancy, molecular prenatal diagnosis of the male fetus revealed that he carried a different TAZ gene lesion, g.4558047_4558278del232. The mother was subsequently confirmed to be heterozygous for this novel g.4558047_4558278del232 deletion (which removes only TAZ exon 1) but negative for the g.4552364_4562302del9937ins705. The g.4552364_4562302del9937ins705 mutation must therefore have occurred de novo in the male proband. The g.4558047_4558278del232 must have occurred de novo in the mother since it was not detected in either her mother or grandmother. Sequencing of the breakpoint junctions revealed microhomologies which could have prompted both rearrangements by serial recombination slippage.

In conclusion, a mother carrying a gross TAZ deletion can undergo further germline gene rearrangement and generate children with a different maternal TAZ. Such a situation can complicate the pre- and post-natal molecular diagnostic of BTHS.

P06.03-S

Diagnosis of GM1-gangliosidosis or galactosialidosis by enzymology and molecular genetic testing from a single dried blood spot

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GM1-gangliosidosis and galactosialidosis are rare autosomal recessive lysosomal disorders associated with defects in the same protein complex. GM1 gangliosidosis is caused by a deficiency of β-galactosidase, whilst galactosialidosis is associated with deficiencies of both β-galactosidase and α-neuraminidase activity secondary to a defect in the protective protein cathepsin A. We have developed a dried blood spot enzyme assay for β-galactosidase and have added a reference enzyme (α-galactosidase) which is measured simultaneously as a measure of sample integrity. A panel of 300 unafflicted individuals were tested to establish a normal reference range; all 5 affected GM1-gangliosidosis patients tested showed clear enzyme deficiency. α-neuraminidase is an unstable enzyme which is therefore unsuitable for dried blood spot analysis and difficult to interpret in leucoyte samples. Differential diagnosis of GM1-gangliosidosis and galactosialidosis has traditionally been carried out by measurement of α-neuraminidase in cultured fibroblasts; however skin biopsy and cell culture can take many weeks. Nested PCR and Sanger sequencing analysis of the GLB1 and CTSA genes can be carried out at this centre from the same dried blood spot sample as enzyme testing, providing a rapid diagnosis from a single convenient sample.

P06.04-M

Unraveling Boucher-Neuhauser syndrome: the multifaceted consequences of PNPLA6 gene mutations

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Boucher-Neuhauser syndrome (BNS) is a rare genetic entity belonging to the group of cerebellar ataxias with hypogonadism. The combination of chorioretinal dystrophy, hypogonadotropic hypogonadism and progressive spinocerebellar ataxia are the hallmarks of the disease. Here we report a new phospholipid disorder in a Brazilian BNS family caused by mutations in the PNPLA6 gene (Patatin-like phospholipase domain con-
Dissecting the genetic architecture of loci with established effects on multiple cardiometabolic phenotypes

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Genome-wide association studies (GWAS) have identified hundreds of loci associated with cardiometabolic phenotypes, many of which overlap or lie in close proximity. Variants associated with multiple phenotypes, such as at HOBS, can provide insight into biology of correlated cardiometabolic traits. However, the genetic architecture of these loci is frequently complex and needs further investigation. To disentangle association patterns of 630 associated SNPs (Dec 2012) from GWAS meta-analyses in Europeans for 19 quantitative phenotypes and two cardiometabolic diseases, we defined sets of adjacent variants located less than 500kb apart and harboring 446 associated SNPs within 151 genomic regions (range=2-8 SNPs/region). We examined, whether associations with multiple phenotypes within each region could be explained by LD through approximate conditional analysis (ACPa) using the GCTA tool. Across the 151 regions, we observed 14 (10%) loci in which the same SNP was associated with multiple phenotypes. Associations in 11 of these 14 loci were with epidemiologically highly correlated traits. Through ACPa, we identified 41 (27%) regions with multiple associated variants that underlie multi-phenotype effects. Within 45 (30%) regions, multiple signals were explained by independent variants. Thirty-two (21%) regions showed complex architecture, and for 30 (30%) regions, multiple signals were explained by independent regulatory mechanisms.

P06.06-6
Severe thrombocytopenia in CDG1α

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Congenital disorders of glycosylation type 1a (CDG1a), caused by mutations in the SLC25A13 gene and has three different age-dependent clinical phenotypes: neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD) in newborn, failure to thrive and dyslipidemia caused by citrin deficiency (FTTCD) in older children, and recurrent hyperammonemia with neuro- psychiatric symptoms in citrullinemia type II (CTLN2) in adults. To date almost all reported patients were from East Asia and only few cases from Caucasian origin have been described. We report the first Bulgarian case of NICCD - a male infant with a mild neonatal jaundice which resolved spontaneously. The patient became jaundice again at 40 days old. Initial investigations revealed conjugated hyperbilirubinemia and evidence of liver dysfunction, hypoproteinemia, mild hypoglycemia and mild anemia. Metabolic evaluation showed lactate acidemia, galactosial and significant elevation of citrulline, methionine and arginine. Mutation study of the SLC25A13 gene showed the compound heterozygote, c.1081C>T (p.R361X) and c.74C>A (p. A25E), which confirmed the diagnosis of NICCD. c.1081C>T (p.R361X) is previously unreported nonsense mutation. After galactose restriction and supplementation with fat-soluble vitamins liver functions were normalized and catch-up growth was achieved before 8 months of age. In conclusion, we presented a new genetically confirmed case of NICCD patient from non-Asian origin. We detected a previously undescribed nonsense mutation in SLC25A13 which further expanded the genotypic spectrum and genotype-phenotype correlations of citrin deficiency. NICCD has been underdiagnosed and should be considered in infants with in-hospital cholestasis, especially when associated with elevated plasma galactose and citrulline, regardless of ethnicity.

P06.08-M
Mmcach is required for pre-implantation in the mouse

M. A. Moreno-Garcia; M. Popovac; D. S. Rosenblatt; M. Tremblay; L. A. Jerome-Majewska; McGill University, Montreal, QC, Canada.

Mmcach is required for pre-implantation in the mouse

M. A. Moreno-Garcia; M. Popovac; D. S. Rosenblatt; M. Tremblay; L. A. Jerome-Majewska; McGill University, Montreal, QC, Canada.

Mutations in Mmcach cause the most common inborn error of vitamin B12 (cobalamin) metabolism - cblA. Patients with this disease are unable to convert cobalamin into the two active forms, methylcobalamin and adenosylcobalamin; consequently, they have elevated homocysteine and methylnonic acid levels in blood and urine. Some cblA patients also have structural abnormalities, including congenital heart defects. In the mouse, Mmcach has tissue and stage-specific expression during organogenesis (Popovac, M. et al. Mol Genet Metab. 103, 401-405 (2011)). We generated mice with a gene-trap insertion in intron 1 of the Mmcach gene, (Mmcach). Mice heterozygous for this gene-trap allele were viable and fertile, but showed a 50% reduction in Mmcach protein compared to wild-type littermates. The Mmcach allele was inherited with a transmission ratio distortion in a subset of matings. Homozygous Mmcach embryos were not found after embryonic day 3.5. These studies indicate that the Mmcach gene product is essential for early mouse development.
S06.09-S

**POLG mutations in Polish patients with mitochondrial disease of unknown etiology - preliminary data**

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**POLG mutations are considered as a major cause of mitochondrial diseases (MD) including MERRF, MNGIE, MELAS, MNGIE, SCAE, SANDO, PE0, LS, MNGIE, MERRE MELAS, and SMA-like phenotypes. Epidemiological data point the widespread occurrence of recurrent c.1399G>A (p.A467T), c.2243G>C (p.W748S), and c.2542A>G (p.G848S) mutations in European populations and emphasize significant differences depending on carriers’ ethnicity.**

A large group of 210 Polish patients with clinical suspicion of MD, and excluded common point mtDNA mutations, large-scale mtDNA rearrangements, and nuclear encoded SURF1 and SCO2 mutations, was recruited for POLG screening. DNA samples isolated from blood, saliva, urine, muscle and liver biopsies were genotyped for the presence of three common mutations by real-time PCR with specific TaqMan allele discrimination assays (Light Cycler 480 II, Roche), and were then verified using ABI PRISM dye terminator cycle sequencing kits (Applied Biosystems). With these methods, 80 patients harbouring one of the common POLG mutations were identified. In patients with the same mutation, the clinical phenotype was highly heterogeneous. Further studies are needed to clarify the relevance of common POLG mutations in MD and to define the role of these variants in the pathogenesis of patients presenting with clinical features of MD.

**P06.10-M**

A targeted resequencing approach for diagnostics of Congenital Disorders of Glycosylation

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**Congenital Disorders of Glycosylation (CDG) are a genetically heterogeneous group of disorders caused by the alteration in synthesis and structure of protein and lipid glycosylation. To date, more than 60 different causes of CDG have been defined genetically. CDG is characterised by extremely variable phenotypes with manifestations ranging from severe developmental delay and hypotonia beginning in infancy, to hypoglycaemia and protein-losing enteropathy with normal development.**

**In order to obtain a better diagnostic yield, we designed a capture assay for a panel of 79 genes associated with CDG type I, CDG type II and congenital muscular dystrophy-dystroglycanopathy. To evaluate the assay, targeted sequencing was performed for 16 CDG type I and 15 CDG type II patients. The mean coverage in the target region was about 600x and a genotype was called for more than 97% of the targeted bases. A diagnosis was confirmed in 8 cases. Interestingly, mutations could also be detected (and confirmed) in the gene ALG1 that could not be assayed by genomic Sanger sequencing in 8 cases. In conclusion, our approach resulted in a selection of small datasets. A total of 15 mutations in known causative genes were identified and confirmed.**

**P06.11-S**

A patient with congenital disorders of glycosylation type 1q due to mutation in SRD5A3

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**Congenital disorders of glycosylation type I (CDG1) are inherited metabolic diseases with an extremely broad spectrum of clinical presentations caused by defective glycosylation of glycoproteins and glycolipids. Recently, mutations in the SRD5A3 gene were found in patients with cerebellar ataxia, and eye malformations. Serum transferrin isoelectric focusing (IEF) demonstrated a type 1 glycosylation defect, thus this disorder was classified as CDG1q. We present here a 4 month old male with coarse face, hypertrichosis and developmental delay. The baby was the fourth child of first-cousin parents. He had narrow forehead, depressed nasal bridge, prototic eyes, long face, thin philtrum, thin lips, loose skin, hepato- and bilateral inguinal hernia. He also had iris coloboma, glaucoma, nystagmus, corneal clouding and elevated serum transaminases. He was followed up until 27 months of age. He had severe hypotonia and no head control at this age. Dark pigmentation of dorsum of feet was developed. His cranial MR imaging revealed cerebellar hypoplasia, enlargement of lateral ventricles and frontal atrophy. Serum transferring IEF showed a type 1 pattern. Se- lection of biochemical, cell biological and glycobiological investigations. In 10.1159/000330164] and 3) a prioritization analysis using the list of rare and novel coding variants by mean of web tools such as TopGene suite, Endeavour and Gene Distiller. Our approach resulted in a selection of small number of genes that significantly correlate with human phenotype and molecular pathways associated to congenital hypoglycaemia and insulin signalling. Among them we select missense mutations affecting CDKAL1 and PIK3R1 for further investigations either at the level of protein structure (in silico protein modelling) and in respect to their role in beta cell using INS1-E cellular model and human pancreatic islets.

**P06.12-M**

**Congential Hyperinsulinism of Infancy (CHI): hunt for new genes**

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**Congenital Hyperinsulinism of Infancy (CHI) is a rare disorder, characterized by heterogeneity in clinical and genetic features. An inappropriate insulin secretion is responsible of hypoglycaemia, which can result in serious neurological damage and life-long handicap. The genetic causes of CHI have been found in genes regulating insulin secretion from pancreatic beta-cells but in about 50% of CHI patients the molecular defect remain unknown.**

**Hunting for novel CHI-causing genes we performed whole-exome sequencing (WES) on 10 CHI patients (Proverbo MC et al, 2013: doi: 10.1371/journ- pone.0068740). To pinpoint the causal mutation in a small number of samples and to select the most promising candidate genes, we implemented a computational strategy including: 1) a bioinformatics pipeline to identify a molecularly single nucleotide variants (SNV); 2) an exome homozy- gosity mapping using a novel algorithm H3M2 (Puppucci T et al. 2011; doi: 10.1159/00030164) and 3) a prioritization analysis using the list of rare and novel coding variants by mean of web tools such as TopGene suite. Endeavour and Gene Distiller. Our approach resulted in a selection of a small number of genes that significantly correlate with human phenotype and molecular pathways associated to congenital hypoglycaemia and insulin signalling. Among them we selected missense mutations affecting CDKAL1 and PIK3R1 for further investigations either at the level of protein structure (in silico protein modelling) and in respect to their role in beta cell using INS1-E cellular model and human pancreatic islets.

**P06.13-S**

Whole-exome sequencing for genetic diagnosis in congenital hypothyroidism

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**Congenital hypothyroidism (CH) is the most common endocrine disorder among newborns but its genetic aetiology is still largely unknown, with genetic defects identified in less than 20% of the cases. Mutations in genes for thyroid hormone synthesis (TG, TPO, DUOX2, IYD, SLC5A5, SLC26A4) have been implicated in dyshormonogenesis, while defects in thyroid transcripti-**

**Patent with congenital disorders of glycosylation phenotype 1q due to mutation in SRD5A3**

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Families harbouring known TG/TPO/DUOX2 mutations have a conclusive genetic diagnosis and novel recessive nonsense mutations here identified are also likely causative. Wider pedigree-genotype correlation is needed to confirm the pathogenicity of the remaining recessive missense mutations here identified.

P06.14 Efficiency of the integrated Danon disease diagnostic protocol can be demonstrated in families affected by LAMP2 exon-copy number variations


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Danon disease (DD) is a X-chromosome e-linked disorder that manifests by cognitive deficit, myopathy and cardiomyopathy in males. The phenotype in females is variable and mitigated as a likely consequence of tissue-specific X-chromosome inactivation (XCI) ratios. DD is caused by mutations in the lysosome-associated membrane protein 2 (LAMP2) gene. Majority of the mutations abolish the protein expression due to truncation of LAMP2 open reading frame. 10-15% of the mutations are exon copy number variations (eCNV) stemming primarily from recombination events in LAMP2 intron 3. DD laboratory testing relies on identification of the absence of the protein and characterization of the mutation within the LAMP2 gene. Importantly, the DD genotype must reflect the following: (i) gender of the proband/patient, (ii) expression patterns of LAMP2 protein, (iii) alternative splicing of LAMP2 pre-mRNA, (iv) mosaic LAMP2 expression determined by XCI in female patients, (v) germlinal/somatic mosaicism phenomena. LAMP2 protein testing should be performed by flow cytometry in peripheral white blood cells as this approach offers both minimal invasiveness and detection sensitivity down to 0.008% of deficient granulocytes. The latter is of critical importance in samples from suspect XCI mosaic female patients and/or family members who are potentially germlinal/somatic mosaics. Molecular genetics methods should usually assess full-length LAMP2 mRNA isoforms (2B, A and C) and consequently compare the abnormal findings to gDNA changes. This integrative approach has major advantages in genetic setups when qualitative PCR based methods failed to identify the mutation. Most of the examples of such situations will be provided in families affected by LAMP2 eCNVs.

P06.15 Radiographic features of the skeleton in disorders of postquagelene cholesterol biosynthesis

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Disorders of postquagelene cholesterol biosynthesis (DCB) are a group of inborn errors of metabolism characterized by multiple congenital abnormalities. The skeletal involvement is usually less prominent and the best characterized example is the Smith-Lemli-Opitz syndrome (SLOS). Nine other disorders are known to date, namely autosomal recessive Antley-Bixler syndrome, Greenberg dysplasia, X-linked dominant chondrodysplasia punctata, X-linked recessive male emaciating-binding protein (EBP) deficiency, congenital hemidysplasia with ichthyosiform erythroderma and limb defects (CHILED) syndrome, CKD, SC4MOL deficiency and the SLOS-like desmosterolosis and lactosterolosis. This study provides an overview of the skeletal radiology of DCB: we report the radiological features of 14 previously unreported patients and review the literature. Our study shows that the DCB have a common pattern of limb abnormalities including poly-ductaly, which is typically postaxial and rarely interdigital and can involve all four limbs, and syndactyly of the toes. Chondrodysplasia punctata (CDP) is specifically associated with a subgroup of DCB (Greenberg dysplasia, CHILD syndrome), X-linked dominant chondrodysplasia punctata, male EBP deficiency); the possible occurrence of epiphyseal stippling in SLOS, initially reported, does not appear to be confirmed. CDP is also associated with other congenital disorders such as chromosomal abnormalities, brachytelephalangic CDP (X-linked recessive CDP; disruption of vitamin K metabolism, maternal autoimmune diseases), peroxisomal disorders (rhizomelic CDP) and lysosomal storage disorders. In the differential diagnosis of epiphyseal stippling, a moth-eaten appearance of bones, asymmetry or the presence of the common pattern of limb abnormalities are further indicators of CDB. In conclusion, the specific differentiating radiological features of DCB are highlighted.

P06.16 Regional reference range lysosomal storage diseases in the Kazakhstan


Objective of the study was investigating the criteria’s of enzyme’s activity of alpha-L-iduronidase, iduronate-2-sulfatase, N-acetylgalactosamine-6-sulfatase, β-galactosidase, arylsulfatase B and providing diagnoses of patients with mucopolysaccharidosis from Kazakhstan.

The materials used for the study were dry blood spots of 2500 healthy newborns. To study the enzyme activity of lysosomal storage disorders (LSD) by tandem mass spectrometry and fluorometry. Reference ranges of enzymes activity were the following: alpha-L-iduronidase was 450-2614 nmol / spot* 20 h, iduronate-2-sulfatase was 0.02-0. 25 nmol / spot* 21 h, arylsulfatase B was 0.14-0.7 nmol / spot* 21 h, β-galactosidase was 35-126 nmol /h/mL, N-acetylgalactosamine-6-sulfatase was 5.7-33 nmol/24h/mL. 13 out of 2500 patients had lysosomal enzyme below the reference range. Two patients were defined with alpha-L-iduronidase activity which were below the reference range and constituted 33.06-187. 26 nmol / spot* 20 h. Activity iduronate-2-sulfatase was determined in 6 patients with suspected MPS type II. Decrease in enzyme activity was observed in all cases and was 0 nmol / spot* 21 h. In 2 cases, a decrease activity of β-galactosidase 25-30 nmol /h/mL, the 1 patient had a decreased activity of N-acetylgalactosamin-6-sulfatase to 2.2 nmol/24h/mL. Arylsulfatase B activity was determined in 3 patients, a decrease of enzyme activity was observed 5-20 times, and was 0.03-0.07 nmol / spot* 21 h. The reference ranges of enzyme activity of LSD were calculated.

P06.17-S Massive parallel sequencing in suspected familial hypercholesterolemia patients of Latvia

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Familial hypercholesterolemia (FH) is one of the most common single gene disorder mostly inherited as autosomal dominant trait. The physical sign of FH is elevated low density lipoprotein cholesterol (LDL-C), elevated total cholesterol (TC) levels and tendon xanotomas. Identification and early treatment of affected individuals is desirable and in lack of physical symptoms DNA based diagnosis provides confirmations of diagnosis and enables early patient management. Majority of FH cases are caused by mutations in four genes (APOB, LDLR, PCSK9 and LDLRAP1). There are commercial kits available for testing of most common FH causing mutations, but the spectrum of disease causing mutations is quite diverse in various populations.

Here we report mutations found in 64 patients with suspected FH in a sample from the Genome Database of Latvian population. We used targeted next generation sequencing approach in order to determine the full spectrum of mutations in coding regions of LDLR, APOB, PCSK9 and LDLRAP1. In total we found 28 missense one nucleotide mutations from which two rs5742904 (Arg527Gln) in APOB gene and rs147509697 (Gly20Arg) in LDLR gene has been previously described as FH causing mutation confirming the FH in three patients (4.6 %). Possible FH causing mutations here were identified in majority of patients. Conclusion: most commonly employed commercial mutation panel is not sufficient for diagnosis of FH patients and NGs can help to identify FH causing mutations in Latvian population. Our results also provide an example that DNA testing can identify FH patients before they develop serious physical symptoms.
ces the accumulation of glucosylceramide in cells of the reticuloendothelial system and causes multisystemic manifestations. The limited efficacy of the current treatments has led to the development of new strategies, including the use of pharmacological chaperones. These are small molecules, generally substrate inhibitors of the target enzyme, and they may help to stabilize GM2GALNACβ1 activity by reducing the rate of turnover of the GM2GALNACβ1 protein. These molecules may also improve the efficacy of enzyme replacement therapy by increasing the amount of enzyme that reaches the cells. Overall, these results suggest that pharmacological chaperones may have a role in the treatment of Gaucher disease.

P06.20-M

**Glycogen storage disease type I?**

E. L. Mendes, M. B. Baptista, D. Z. Scherrer, C. E. Steiner; State University of Campinas, Campinas, Brazil.

We report a patient referred to our service due to a clinical diagnosis of Glycogen Storage Disease type 1. She is the only child of a healthy and non consanguineous couple, seen at the age of six months when hepatomegaly was detected during a routine pediatric evaluation. Laboratory investigation revealed glycemia after 4-hour fasting ranging from 33 to 60 mg/dl, hyperlactic acidosis are frequent and require hospital admissions. Whole exome sequencing [done at Baylor College of Medicine] revealed two mutations in GTF2H5 gene: c.581G>A (p.R194H, previously reported) and c.215delG (p.Q72X). Upon physical examination, he demonstrated opisthionotic posturing, hypotonia, horizontal oculomotor apraxia, and swallowing difficulties but was also able to sit with support, was attentive to environmental stimuli, and was able to walk with support, however, still presents with hepatomegaly. Genomic DNA was extracted from peripheral blood leukocytes of the patient and both parents and sequenced using a MegaBACE1000® DYEnamic ET (Amersham Biosciences) system and causes multisystemic manifestations. The limited efficacy of the current treatments has led to the development of new strategies, including the use of pharmacological chaperones. These are small molecules, generally substrate inhibitors of the target enzyme, and they may help to stabilize GM2GALNACβ1 activity by reducing the rate of turnover of the GM2GALNACβ1 protein. These molecules may also improve the efficacy of enzyme replacement therapy by increasing the amount of enzyme that reaches the cells. Overall, these results suggest that pharmacological chaperones may have a role in the treatment of Gaucher disease.

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red with the sequences available in the Ensembl genome browser. Mutation analysis revealed that the patient is a double heterozygote for the previously known nonsense mutation p.Arg415Ter (rs121908979; c.1243C>T) in SLC35A4, inherited from the mother, and an undescribed nonsense mutation p.Glu72Ter in G6PC, inherited from the father. The molecular analysis of the two genes suggests that the clinical picture of this patient could be caused by digenic inheritance of GSDI.

P06.23-S
High frequency of the c. 3980 G>A (p.W1327X) mutation in AGL gene of Tunisian patients with hepatic presentation of glycogen storage disease type III syndrome

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BACKGROUND: Glycogen storage disease type III (GSD-III) is an inborn error of glycogen metabolism caused by a deficiency of the glycogen debranching enzyme (AGL). Some of the mutations appear to be population specific, whereas others are found in probands from a variety of different ethnic backgrounds. The recurrent mutation W1327X in exon 31 was identified in the Tunisian population, suggesting a founder effect. In this present study, we report a phenotype-genotype correlation of this frequent mutation. METHODOLOGY AND RESULTS: In 10 patients with HHH syndrome, including 10 patients of Tunisian origin, we identified a common haplotype for large marker on chromosome arm 1p21, we identified a common haplotype for HHH syndrome. All missense mutations tested have overlapping. We used our yeast model to test the effect of missense mutations in ORNT1. We found that ORNT1 but not ORNT2 complements the deletion of the yeast gene. The two human transporters were expressed into the ΔARG11 strain. We found that the human transporters that ORNT1 but not ORNT2 complements the deletion of the yeast gene. In yeast we employed a yeast model to study the function of human ORNT2 and to test the pathogenicity of mutations found in HHH patients. The two human transporters were expressed into the ΔARG11 strain. We found that ORNT1 but not ORNT2 complements the deletion of the yeast gene. Three ORNT1 residues, conserved from yeast to humans, are not conserved in ORNT2. We could recover ORNT2 activity by replacing these three residues with those found in ORNT1. This result suggests that, despite the high level of homology between the two transporters, their function is not overlapping. We used our yeast model to test the effect of missense mutations carried by patients with HHH syndrome. All missense mutations tested have a detrimental effect on the function of the human gene indicating that yeast is a simple and effective system to validate missense mutations occurring in patients with HHH.

P06.24-M
A yeast model to evaluate the pathogenicity of missense mutations causing HHH syndrome

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Hyperornithinemia-hyperammonemia-homocystinuria (HHH) syndrome is an autosomal recessive multisystemic disorder characterized by mental retardation and myoclonic seizures. It is caused by mutations in ORNT1 encoding the mitochondrial ornithine transporter. In placental mammals there is a second gene, ORNT2, that originated from a retro-transposition event, whose precise function has not been clarified. In yeast the major function of ORNT1 is the mitcral transport of ornithine to the mitochondrial matrix to the cytosol. We employed a yeast model to study the function of human ORNT2 and to test the pathogenicity of mutations found in HHH patients. The two human Ornithine transporters were expressed into the ΔARG11 strain. We found that ORNT1 but not ORNT2 complements the deletion of the yeast gene. Three ORNT1 residues, conserved from yeast to humans, are not conserved in ORNT2. We could recover ORNT2 activity by replacing these three residues with those found in ORNT1. This result suggests that, despite the high level of homology between the two transporters, their function is not overlapping. We used our yeast model to test the effect of missense mutations carried by patients with HHH syndrome. All missense mutations tested have a detrimental effect on the function of the human gene indicating that yeast is a simple and effective system to validate missense mutations occurring in patients with HHH.

P06.25-S
A case of siblings with Leigh-like disease caused by 3-hydroxyisobutyryl-CoA dehydrogenase deficiency

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3-Hydroxyisobutyryl-CoA dehydrogenase (HIBCH) is an enzyme specific for removing CoA in the catabolic pathway for valine. So far, only 4 cases of HIBCH deficiency have been reported. In 1 case, a homozygous null mutation in HIBCH caused congenital anomalies including a characteristic facial appearance, congenital heart disease, and multiple vertebral anomalies and led to death in infancy. The 3 other cases, which harbored missense or splice mutations, presented with hypotonia, neurological regression, developmental delay in infancy, episodes of ketoacidosis, and abnormal MRI findings in the basal ganglia. Here, we describe a case of Leigh-like disease in Japanese siblings with HIBCH deficiency who presented with developmental delay in infancy, basal ganglia signalings in brain MRI findings, episodes of ketoadidosis without highly increased levels of pyruvate and lactate in the CNS during infections. The patients died before the age of 5. A new homzygous missense mutation was identified at the substrate binging site in these patients, and their parents were found to be heterozygous for this mutation. The levels of HIBCH activity in patient lymphoblastoid cells and HK293 cells transiently expressing a mutant HIBCH confirmed that the patients had HIBCH deficiency. HIBCH deficiency leads to the accumulation of the HIBCH substrate 3-hydroxyisobutyryl-CoA and subsequently an increase in the amount of methacryl-coA in the mitochondrial matrix. The methacryl-coA then strongly binds to thiol compounds (cysteamine, cysteine, glutathione) and reduces the activity of mitochondrial enzymes by binding to their SH residues, thus leading to a dramatically decreased reductive power and reduced ATP production.

P06.26-M
Deletorius mutations in mitochondrial NADH dehydrogenase subunit 4 and ATPase subunit 6 in two siblings of Leigh Syndrome with progressive motor retardation and loss of visual function

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We report a mitochondrial NADH dehydrogenase subunit 4 and ATPase subunit 6 mutations in two siblings from first degree relative couple with Leigh Syndrome. Peripheral blood EDTA samples were used for DNA isolation and target mitochondrial genes were analysed by MLPA technique (MRC-Holland). A girl of 16 years old presented with a episode of progressive mental and motor retardation. Until seven year she had normal appearance but at the age of 7 year, she had developed neurological and imaging features such as: loss of visual function, walking and speech impairment and epilepsy complaints that compatible with Leigh syndrome. We also present a 12-year-old boy with muscle weakness, severe neurological problems, hyperactivity, development retardation and complete visual loss. Screening of both siblings, based on their clinical phenotype revealed the homoplasmic deleterious mutation in the mitochondrial DNA M-ATP6 (ligation site of probe 12068-12069) and heteroplasmic deletion in NDH subunit 4 (ligation site of probe 8993-8992R). The maternal mtDNA screening for target deleterious genes is still going on. These findings are consistent with infantile Leigh syndrome for the presented siblings.

P06.27-S
Lysosomal Storage Disorders (LSDs): Clinical and Genetic Spectrum: Local Experience at The Eastern Province of Saudi Arabia

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Lysosomal Storage Disorders (LSDs) are clinically and genetically heterogeneous a group of more than 50 inherited disorders, each one is caused by a deficiency of a particular lysosomal enzyme resulting in a progressive accumulation of specific macromolecules within the lysosomes. This eventually leads to an irreversible cell damage, and multi-organ dysfunction. The majority of LSDs are inherited in an autosomal recessive manner, with the exception of Fabry disease and mucopolysaccharidoses type I and type VII, and the X-Linked mode of inheritance. The majority of LSDs are pan-ethnic, however some are more prevalent in specific ethnic groups. Here, we report our local experience of LSDs at Johns Hopkins Healthcare Main Hospital, Dhahrani, The Eastern Province of Saudi Arabia from January, 1st, 1994 to December, 31st, 2013. A total of 86 patients were diagnosed with different LSDs within this time period.ucopolysaccharidosis type VI was found to be the most common disorder (18%), followed by juvenile Neuronal Lipofuscinosis (13%). The patients are distributed according to...
the geographic location or their tribe origin where particular disorders and specific genotypes were identified. This information had been utilized for asymptomatic carrier testing for those selective disorders among the high risk population. However, this valued data could be further utilized for establishing the newborn screening of LSDs in Saudi Arabia.

P06.28-M
Changes of metabolome and lipidome in lysinuric protein intolerance (LPI)
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Lysinuric protein intolerance (LPI; MIM227200) is a rare autosomal recessive disorder caused by a defect of cationic amino acid transport in the small intestine and kidney tubules. All the Finnish patients share the same homozygous mutation c.1181-2A>T in the SLC7A7 gene which encodes the y+LAT1 amino acid transporter. LPI can be considered a multisystem disease which can be life-threatening. The main symptoms of LPI include protein aversion, failure to thrive, osteoporosis and hepatosplenomegaly. However, despite the homogenous mutation in the Finnish LPI patients, symptoms may vary markedly even within one family and may include severe complications, such as alveolar proteinosis and end-stage renal disease. Some LPI patients also suffer from combined hyperlipidemia. Our recent microarray study revealed that also other amino acid transporter genes than SLC7A7, including non-cationic amino acid transporters, have changes in their expression levels in LPI patients compared to the controls. Therefore, LPI patients seem to have wide and persistent changes in their amino acid balance. This finding together with the fact that patients have combined hyperlipidemia let us hypothesize that there may be lheritoe uncharacterized system metabolic and lipid alterations in LPI. We studied these alterations in the whole blood plasma samples of 26 Finnish LPI patients and 19 gender and age-matched controls. Global metabolomic and lipomic analyses were performed with GCxGC-TOF and Q-TOF mass spectrometry, respectively, combined with ultra-performance liquid chromatography (UPLC). Taken together, this study will reveal the nature of system-wide dysregulation of metabolites and lipids associated with the Finnish founder mutation of LPI.

P06.29-S
Establishment of Zbtb16 role in metabolic syndrome by means of single-gene congenic rat strain derivation
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In the process of positional cloning of apparently pleiotropic locus on rat chromosome 8 affecting major features of metabolic syndrome we have derived the congenic SHR.PD-(D8ratH2Z-DRarb23)xSHR (Cub-Lx) strain carrying only 7 genes of polydactylous rat strain (PD) origin on spontaneously hypertensive rat (SHR) genetic background. In this study, we have derived 2 new minimal congenic sublines in order to determine the role of candidate Zbtb16 gene of PD/Cub origin carrying 2.9kbp deletion in intron 2. Adult male rats of SHR.PD-(D8ratH2Z-DRarb23)xSHR and SHR.Htr3b (Htr3) strains were fed standard diet (STD) and subsequently treated with dexamethasone in drinking water. Changes of metabolome and lipidome were performed with GCxGC-TOF and Q-TOF mass spectrometry, respectively, combined with ultra-performance liquid chromatography (UPLC). Taken together, this study will reveal the nature of system-wide dysregulation of metabolites and lipids associated with the Finnish founder mutation of LPI.

P06.30-M
DNA diagnostics of MIDD and MELAS syndromes in Slovakia
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The two syndromes, MIDD (Maternally Inherited Diabetes and Deafness) and MELAS (Mitochondrial Encephalopathy, Lactic Acidosis and Stroke-like episodes) arise on a common genetic cause - a mutation in mtDNA, most often m.3243A>G in the gene for tRNALeu. This mutation leads to different clinical symptoms according to heteroplasmy levels in different tissues. Usually, the first presentation of the MIDD syndrome is a progressive bilateral sensorineural hearing loss emerging in adolescence. Diabetes develops mostly in 30 - 40 years of life with clinical picture resembling type 2 diabetes. The MELAS syndrome has a more severe progression with further neurological and metabolic symptoms.

We tested 317 patients from 257 families with suspected MIDD or MELAS syndromes fulfilling criteria of matrilineal inheritance, conjoint diabetes and hearing impairment, diabetes development after 25th year of life, or progressive hearing loss. Patients’ DNA was extracted from peripheral blood and/or buccal mucosa and analysed for presence of m.3243A>G variant using RFLP and/or Real-Time PCR. The m.3243A>G mutation was found in 18 patients from 8 families. The heteroplasmy level was higher in DNA samples from buccal swabs compared to blood DNA samples. In one case, the heteroplasmy was detected in the buccal swab only, while the DNA sample gave negative results repeatedly. Patients’ phenotypes varied from diabetes as the sole symptom to a complex picture of the MELAS syndrome.

DNA testing helped to diagnose the illness, thus permitting correct patient management and intensified surveillance of yet healthy mutation carriers.

Supported by APVV 1408-10, „Transendogen“ (ITMS 26240220051)

P06.31-S
Hostsup mutation regions in a consanguineous population - Leptin, Leptin receptor and Melanocortin 4 receptor genes
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The exome sequencing approach is an efficient method in genetic investigations for discovering causal variants in complex phenotypes. Since exome does not include mtDNA, it is essential to additionally perform mtDNA testing. Mutations in the leptin (LEP) and melanocortin 4 receptor (MC4R) genes are among the most common mutations leading to severe obesity, especially in consanguineous families. The purpose of this study was to perform a comprehensive search for causal variants in LEP, LEPR and MC4R for 46 consecutive severely obese children, with known or novel homozygous mutations in the 3 genes. The cohort is of a consanguineous population, adding up to 28 (25%) of a total of 110 severely obese children. Homozygous mutations in LEP were more frequent than those in LEPR, whereas mutations in MC4R were found less frequently. We applied the exome sequencing approach combined with mtDNA testing, which helped to determine the diagnosis, thus permitting correct patient management and intensified surveillance of yet healthy mutation carriers.

Mitochondrial (mt) DNA disorders are a large group of multisystem conditions often transmitted maternally and often caused by disruptive mutations in mitochondrial DNA. The most frequent mtDNA mutations are point mutations, which are typically located at specific positions in the mtDNA and can be inherited to a variable extent. These mutations can affect the function of mitochondrial enzymes and lead to a variety of clinical manifestations. Mutations in the mtDNA tRNALeu(UUR) gene can cause a syndrome known as MIDD or MELAS. MIDD is characterized by diabetes mellitus, deafness, and growth retardation, while MELAS includes symptoms such as seizures, strokes, and cognitive impairment. Individuals with MIDD or MELAS are at risk of developing diabetes and hearing loss, which can lead to significant complications. These conditions are typically diagnosed using clinical criteria and genetic testing. mtDNA testing helps to establish the diagnosis and may guide management and counseling for affected individuals and their families. Future studies should focus on understanding the underlying mechanisms and developing effective treatments for these devastating disorders.
A unique combination of mitochondrial ribosomal RNA variants responsible for a form of mitochondriopathy with respiratory complex I defect and hypoaucusia

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Mitochondrial DNA mutations are known to cause highly heterogeneous phenotypes and their functional effects are often difficult to assess. Here we present a novel pathogenicity mechanism for two mtDNA variants found in ribosomal RNA genes. The whole mtDNA was screened in a patient who displayed clinical indications of mitochondriopathy, including hypoaucusia and 37% defect of mitochondrial complex I activity in muscle. No protein-coding mutations were identified, albeit two rare variants in ribosomal RNA genes, namely m.1452T>C>RRN1 and m.2397C>T>RRN2, both homoplasmic and present in the proband mother, who also displayed hypoaucusia. Low population frequency was revealed for both variants by using the HmtDB database, which collects nearly 16,000 human mitochondrial genomes. Although the two variants were present in tips of the haplogroups tree, they were never found to co-occur in the same individual, implicating a potential mutational exclusivity. Therefore, it was hypothesized that their co-existence might lead to perturbations in mitochondrial ribosomal assembly. To this aim, since the patient’s fibroblasts displayed a normal basal respiration and complex I function, mitochondrial proteins translation kinetics was analyzed in cybrids derived from patient and control fibroblasts. Patient-derived cybrids showed an increased translation rate of mitochondrial proteins and less efficient recovery of complex I activity after treatment with mitochondrial translation inhibitors. These results imply that the combination of the two variants might lead to mitochondrial translation defects, mainly affecting complex I function, especially in cells with a high energy demand.

Mucopolysaccharidosis IVA (Morquio A syndrome) is an autosomal recessive lysosomal storage disorder, caused by the deficiency of the enzyme N-Acetylgalactosamine-6-sulfatase (GALNS). The disease, albeit multi-systemic, is characterized by prevalent skeletal involvement, short stature, and cardiorespiratory complications. Here we report two new large genetic rearrangements detected at the heterozygous level in two patients (Pt1 and Pt2) affected by Morquio A. The deletions include both the nuclear part of the GALNS gene and the mitochondrial part of the gene. Parents’ genetic analysis, haplotyping some loci in exon 14 of the GALNS gene (in the context of a prenatal diagnosis of a patient’s relative), and CGH-array revealed that Pt1 has a large deletion encompassing exons 10–14 of the GALNS gene and three upstream genes, including a part of the PIEZ1 gene. In Pt2, heterozygous for the c.460G>A (p.Gly116Ser) missense mutation, a large deletion ablating exons 9–14 of the GALNS gene and exon 1 of the PIEZ1 gene was also identified. The breakpoint regions were characterised in both patients and the eventual contribution of the partial deletion of the PIEZ1 gene on such patients’ phenotype was considered at a biochemical and clinical point of view.

Mucopolysaccharidosis type II: molecular analysis in a cohort of male patients and in a manifesting female carrier

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Mucopolysaccharidosis II (Hunter syndrome, MPS II) is a rare X-linked recessive disorder caused by a deficiency of the lysosomal enzyme iduronate-2-sulfatase. Major clinical manifestations of the severe form of MPSII include coarse facial features, short stature with joint stiffness, hepatosplenomegaly, cardio myopathy and mental retardation. The diagnosis of MPSII is based on clinical symptoms and increased excretion of glycosaminoglycans in urine is confirmed by enzymatic assay and mutation analysis. We performed mutation analysis in 24 unrelated male patients and found 21 different mutations: two large and four small deletions, three small insertions, two splicing defects, two nonsense mutations, seven missense mutations and a recombination between IDS gene and pseudogene ID32. Eleven of the found mutations are novel.

Even though the MPSII is expected to be found in males, few symptomatic females have been reported. We present a case of four-year-old girl with severe phenotype of MPSII. Molecular analysis of gDNA revealed a missense mutation c.1405G>A (p.Gly468Q) in heterozygous state, but only the mutant allele was detected at the cDNA level. X-inactivation was nonrandom and silencing preferentially the maternal chromosome. The mutation was not found in peripheral blood samples of both parents. These findings support the assumption of either a de novo mutation in affected girl or a germinal mosaicism in the girl’s father.

In spite of extremely rare manifestation of MPSII in females, an unrelated female patient with skewed X-inactivation and p.R468Q inherited from her brother was reported in the literature. In spite of extremely rare manifestation of MPSII in females, an unrelated female patient with skewed X-inactivation and p.R468Q inherited from her brother was reported in the literature. In spite of extremely rare manifestation of MPSII in females, an unrelated female patient with skewed X-inactivation and p.R468Q inherited from her brother was reported in the literature.
Mucopolysaccharidosis type II (MPS II) is an X-linked recessive multisystem disorder characterized by glycosaminoglycans (GAG) accumulation. Different severity of the disease has been described depending on the mutation in the iduronate sulfatase gene (IDS). Here we present a case, with severe MPS II detected early with an unusual duplication of the exon 7 of the IDS gene in the child and his mother who does not have any signs of the disease. The patient has been followed for 5 years. His growth during this period was unusual, he had height 4 SDS above the average for the age, head circumference was 3SD above the average. Values of growth hormone were normal, as well as the MRI of the pituitary region. Therapy with Elaprase (Idursulfase) improved the joint mobility, however, the boy has severe neuro-developmental delay.

Although no significant genotype/phenotype relation has been shown in children with MPSII, it seems that this rare gene change is associated with a severe form of the disease. Prenatal diagnosis in the mother is planned for the second pregnancy.

P06.39-S  
Novel sequencing identifies mutations in coenzyme A synthase causing neurodegeneration with brain iron accumulation  

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The common feature of a group of genetic disorders collectively identified as Neurodegeneration with Brain Iron Accumulation (NBIA) is brain iron overload visualized by radiological and histopathological examinations. The clinical spectrum of NBIA is wide and includes early-onset neurodegeneration, with a fatal outcome, and adult-onset parkinsonisms-dystonia. Pan-thetenate Kinase Associated Neurodegeneration and Infantile Neuroaxonal Dystrophy are the most frequent forms of the disease due to recessive mutations in PANK2 and PLA2G6. Recently it was shown that NBIA is also caused by mutations in FAZ2H, ATP1-32A and C1orf12 genes, but still in a large proportion of patients, no genetic alteration can be found. Using exome-sequencing strategy we identified, in a NBIA patient, a homozygous missense mutation in the gene coding for Coenzyme A Synthase (CoASy). By performing traditional Sanger sequencing in a cohort of NBIA cases, we found another mutant patient. CoASy is a mitochondrial enzyme involved in the last step of Cozyme A biosynthesis, an important molecule for several metabolic pathways. The missense mutation affects a highly conserved aminocacid residue in the catalytic site of the enzyme, a region conserved from yeast to human. Western-blot analysis showed that CoASy protein was absent in patient fibroblasts, whereas RTPCR revealed that mRNA was reduced only in the patient carrying the non-sense mutation. HPLC analysis demonstrated reduced CoA concentration in mitochondria isolated from mutant yeast and patient fibroblasts. Together with mutations in PANK2, coding for the first enzyme in CoA biosynthesis, mutations in CoA synthase impinge on the same biosynthetic pathway causing NBIA.

P06.40-M  
Novel mutations in the PNPLA2 gene causing late-onset single-nerve lipid storage disease with myopathy in an Italian family  

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Neutral Lipid Storage Disease with Myopathy (NLSM-D), is a rare autosomal recessive disorder characterized by an abnormal accumulation of triacylglycerol into cytoplasmic lipid droplets (LDs). Mutations in the PNPLA2 gene cause the onset of NLSM. PNPLA2 codes for adipose triglyceride lipase (ATGL), an enzyme that hydrolyses fatty acids from triacylglycerol. NLSM patients are mainly affected by progressive myopathy, cardiomyopathy and hepatomegaly. Other clinical symptoms may include diabetes, chronic pancreatitis and short stature. To our best knowledge, twenty six different PNPLA2 mutations have been described in thirty two NLSM patients. Here we report the clinical and genetic findings of a NLSM Italian family with different affected members. In our patients we identified two novel PNPLA2 missense mutations (p.L56R and p.I193F). Since age of 38 years, the oldest brother had weakness and hypotrophy of right upper arm and kyphosis. He is now unable to raise arms in horizontal position (61 years old). The second brother, since 44 years of age, had exercise intolerance, cramps and pain in lower limbs. He currently has a distal atrophy. Genetic analysis revealed that also one of the two sisters presents the p.L56R and p.I193F mutations, but she is still barely symptomatic. Using a functional in vitro assay, we have observed that these mutations caused the production of ATGL proteins with diminished lipase activity, but able to bind to LDs. This is a very interesting family since it shows heterogeneity of clinical presentation from relatively asymptomatic phenotype to full expression of a severe myopathy.

P06.41-S  
Proteome by hiPS cells for disease modeling of NLSM-M  

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NLSM-M (Neutral Lipid Storage Disease with Myopathy) is a rare autosomal recessive disorder characterized by an abnormal intracellular accumulation of triacylglycerol into cytoplasmic lipid droplets (LDs). In most tissues the lipid droplets (LDs) are cellular organelles for the triacylglycerol storage. LDs metabolic functions are mediated by proteins bound to their surface. In particular the lipase that catalyzes the remnant of the first acyl chain from triacylglycerol is the patatin-like phospholipase-domain-containing protein 2 (PNPLA2). This protein is coded by the PNPLA2 gene whose mutations cause the onset of Neutral Lipid Storage Disease with Myopathy. NLSM-M patients are affected by progressive myopathy, cardiomyopathy and hepato-megaly. Other clinical symptoms may include diabetes, chronic pancreatitis and short stature. NLSM-M has, at present, no specific therapy. We have previously reported clinical and genetic findings of some NLSM-M patients obtaining dermal biopsies from them. Here we report the development of hiPSc (human induced pluripotent stem cell) from patients’ fibroblasts harboring different PNPLA2 mutations. Initial hiPSc colony selection was based on morphologic evaluation and on detection of pluripotency surface markers (SEEA-4 and TRA-1-81). hiPSc also expressed undifferentiated ES cell markers (NANOG, SOX2 and OCT4). Karyotypic analysis of hiPSc lines indicated a normal complement of chromosomes. Immunohistochemical evaluations of LDs on hiPSc revealed that they recapitulate pathological hallmark of the disease. We propose use of differentiated cells derived from hiPSc to study the pathogenetic mechanisms leading to NLSM-M and as a cellular model for therapeutic evaluation.

P06.42-M  
Niemann Pick type C genetically verified in three Bulgarian patients  

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Niemann-Pick type C (NPC) disease is a neurodegenerative lysosomal lipid storage disease, with autosomal recessive transmission, characterized by lysosomal/late-endosomal accumulation of endocytosed unesterified cholesterol. The clinical manifestations of NPC are heterogeneous. Most patients have a progressive neurologic disease, but both age at onset, which ranges from early infancy to adulthood, and subsequent course vary. We present the first three Bulgarian female patients with genetically verified NPC disease. Two of the patients are sisters. All three patients presented neurovisceral involvement with juvenile onset with a mean age of onset varying between 11 and 19 years. Vertical gaze palsy, ataxia, involvement of the upper motor neuron and cognitive decline were prominent features of all the patients’ group, while parkinsonism was present in both sibs. Clinical and echographic examinations revealed hepatomegaly in one of them and spleenomegaly in all of the affected. Neuroimaging changes encompass cerebellar atrophy and white matter changes. In two of them chitotriosidase was moderately elevated, while in the third patient it had a borderline value. The molecular genetic testing detected missense mutation in the NPC1 gene. The sisters are compound heterozygous carriers of the mutations c.3019C>G and c.3718G>A, p.Gly1240Arg. The third patient is a compound heterozygous carrier of the mutations c.1421C>T (p.Pro474Leu) and c.2974G>A (p.Gly924Arg). In conclusion, the detected mutations in our patients are already known in the literature. All detected mutations were missense and caused the same classical phenotype in Bulgarian patients. These cases are the first genetically confirmed NPC cases in Bulgaria.
**P06.43-S**

**An exome-wide study for obesity in Singaporean East-Asian samples**

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Aims: Previous genome-wide association studies (GWAS) have identified over 40 obesity-associated variants; however, these are primarily non-coding common variants and account for <1% obesity heritability. We performed an exome-wide association study (EWAS) to identify obesity-associated coding variants using the illumina Humangenome exome array.

Methods: 192 East-Asian (Singaporean Chinese and Malay) early-onset obese cases (body weight >150% of ideal weight for height and onset ≤10 years of age) and normal BMI adult controls (18.5kg/m2 ≤ BMI<23.0kg/m2) from the Singapore Chinese Eye Study (SCES) were genotyped. Samples QC criteria (see Table S1) were excluded from analysis. We focused on coding variants with MAF≥1% and p-value < 0.05. We performed principal components analysis (PCA) and conducted PCA outliers and extremes analysis using samples at extremes of BMI distribution (cases= BMI>27.5kg/m2 and controls ≤95%, rare <0.05% and/or monomorphic SNPs in total cases/cases and SNPs with significant deviations from Hardy-Weinberg equilibrium (p-value < 10.0001). 47.605 SNPs remained for statistical analysis. Fisher's exact test was performed for rare SNPs (p<0.05) and logistic regression adjusting for population stratification (first 5 principle components) and sex was carried out for common SNPs (MAF>0.05%) using PLINK(v1.07). 58 SNPs with strong p-value and genopheno correlation Table S2 were followed-up using samples at extremes of BMI distribution (cases= BMI≥27.5kg/m2 and controls ≤95%, BMI<18.5kg/m2) from 2 adult Singaporean Chinese datasets (SCES [321 cases/140 controls] and Singapore Prospective Study Program [SP2, 89 cases/94 controls]).

Results: A coding SNP (exm1271824) at FANCA showed strong associations for rare SNPs (p<0.05) and showed a significant enrichment of variants and/or monomorphic SNPs in total cases/cases and SNPs with significant deviations from Hardy-Weinberg equilibrium (p-value < 10.0001). 47,605 SNPs remained for statistical analysis. Fisher's exact test was performed for rare SNPs (p<0.05) and logistic regression adjusting for population stratification (first 5 principle components) and sex was carried out for common SNPs (MAF>0.05%) using PLINK(v1.07). 58 SNPs with strong p-value and genopheno correlation Table S2 were followed-up using samples at extremes of BMI distribution (cases= BMI≥27.5kg/m2 and controls ≤95%, BMI<18.5kg/m2) from 2 adult Singaporean Chinese datasets (SCES [321 cases/140 controls] and Singapore Prospective Study Program [SP2, 89 cases/94 controls]).

Conclusion: We identified an obesity-associated coding variant at FANCA in East-Asians.

**P06.44-M**

**Biochemical diagnosis of Peroxisomal disorders by GC/MS: Egyptian patients with X-linked adrenoleukodystrophy**

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Introduction: Peroxomases are organelles responsible mainly for metabolism of lipids and peroxides. Lack of peroxisomes or dysfunction in any of their normal functions is the cellular basis for human peroxisomal disorders (PDs).

Aim of the Work: diagnosis of peroxisomal disorders among a high risk group of Egyptian patients using gas chromatography mass spectrometry.

Subjects and Methods: Forty six patients suspected to have peroxosomal disorders were included in this study. Their ages ranged from 2 to 20 years. They were referred to The Biochemical Genetics Department, National Research Centre from all over Egypt. Forty one (89%) were males while five (11%) were females. Age range was from 2 to 20 years. Parental consanguinity was positive in 26 cases (61% out of 46). Very long chain fatty acids were quantified after extraction from plasma of all cases using gas chromatoigraphy/mass spectrometry (GC/MS) technique.

Results: The present study included 46 cases suspected clinically to have one of the peroxosomal disorders; four of them (8.7%) proved to have X-linked adrenoleukodystrophy by quantitative determination of the very long chain fatty acids after extraction from plasma of the other. The other 42 cases showed normal profile for very long chain fatty acids.

Conclusion: X-linked adrenoleukodystrophy is the only type diagnosed among the study group of the suspected Egyptian cases. This study showed that GC/MS analysis for VLCPA discriminates patients from controls, representing a non-invasive, reliable, specific and sensitive method for the diagnosis of peroxosomal disorders.
P06.47-S
X-linked adrenoleukodystrophy from Iran, reporting 8 cases and a novel mutation

X-linked adrenoleukodystrophy (X-ALD) is a rare peroxisomal disorder resulting in progressive cerebral demyelination, axonal dysfunction, and adrenal insufficiency. It is the most common peroxisomal disorder with an estimated birth incidence of about 1 out of every 20,000. There is no ethnic predominance.

X-ALD most severely affects male hemizygotes. The age of onset and morbidity are highly variable and progression is unpredictable. Male hemizygotes may initially present with neurological symptoms in two different forms: (X-ALD) with childhood presentations, and Adrenomyeloneuropathy (AMN) that presents in adulthood.

X-ALD, affects 4 to 8 year old boys. Primary manifestations of X-ALD are moderate cognitive deficits followed by diminished visual acuity, central deafness, cerebellar ataxia, hemiplegia, convulsions and dementia leading to a neurovegetative state or death within several years.

X-ALD is inherited in an X-linked manner. About 93% of index cases have inherited the ABCD1 mutation from one parent; and 7% of individuals have a de novo mutation.

The diagnosis of X-ALD is based on clinical findings. MRI is always abnormal in males with neurologic symptoms and often provides the first diagnostic lead. Plasma concentration of very long chain fatty acids (VLCFA) is abnormal in 99% of males with X-ALD. ABCD1 is the only gene known to be associated with X-ALD.

Here we report 8 affected boy with X-ALD from Iran. VLCFA was increased strongly, and MRI images were typical in all of them. Molecular analysis of ABCD1 gene confirmed the diagnosis in 4 of the patients and we detected 1 novel mutation in one.

P06.48-M
Rhizomelic chondrodysplasia punctata due to a novel mutation in the PEX5 gene
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Peroxisome biogenesis disorders (PBD) are caused by mutations in one of 13 PEX genes. Based on the clinical and biochemical presentation the patients are divided into: 1) Zellweger spectrum disorders (ZSD) and 2) Rhizomelic chondrodysplasia punctata type 1 (RCDP1). ZSD is characterized by multiple organ defects and elevated levels of very long chain fatty acids (VLCFA). The patients have mutations in one of 12 genes (not PEX7). 2) RCDP1 is characterized by congenital cataracts, multiple skeletal abnormalities, normal VLCFA, reduced plasmalogens and elevated phytanic acid levels. Most RCDP1 patients have mutations in PEX7, but a RCDP phenotype with slightly different biochemical profile is caused by deficiency of either of the two peroxisomal enzymes DHAAP and ADHAPS involved in plasmalogen biosynthesis.

We identified a homozygous frame shift mutation in PEX5 in three siblings with congenital cataracts, multiple skeletal abnormalities, moderate to severe intellectual disability, epilepsy, demyelinating neuropathy, normal VLCFA and high phytic acid levels. This clinical and biochemical profile is consistent with RCDP1.

The patients have two isoforms, PEX5S and PEX5L, and the mutation in our patients is located in a sequence present only in PEX5L and required for interaction with PEX7, which is important for peroxisomal import of three enzymes (ADHAPS, PHYH and thiolase) 1 carrying a peroxisomal targeting signal 2 (PTS2). We believe that disruption of the interaction between PEX5L and PEX7 causes the RCDP-like phenotype in our patients. We currently study patient fibroblasts in order to further characterize the peroxisomal defect caused by this novel PEX5 mutation.

P06.49-S
The frequency of POLG1 gene mutations in Hungarian patients with mitochondrial disorders and the analysis of phenotype - genotype correlation
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In the background of mitochondrial disorders both mtDNA and nuclear DNA mutations can be detected. POLG1 is one of the most important gene responsible for the intergenicommunication of the two genomes. POLG1 gene mutations may cause a wide range of clinical symptoms. However despite the large number of POLG1 mutations have been published the phenotypic spectrum is increasing.

Aims: The frequency of the POLG1 mutations in patients with intergenicommunication disturbance and their phenotype-genotype correlation was analyzed.

Patients and methods: 100 Hungarian patients with mitochondrial diseases were investigated. Most of them had mitochondrial abnormalities and multiple mtDNA deletion in the muscle tissue. The mtDNA deletion was investigated by long PCR, the POLG1 gene was sequenced.

Results: In the POLG1 gene of our cohort 7 pathogenic mutation was detected in 6 families. Segregation analysis detected 9 further family members harbouring the pathogenic mutations. In our cohort 3 SNPs showed association with valuable toxicity, 1 SNP was a genetic modifying factor. The most common clinical symptoms were myopathy (50%), neuropathy (34%), ataxia (34%), depression (34%), PEO (17%), epilepsy (17%), ptosis (17%), lipomas (17%), hypoacusis (17%). In 1 case with Alper’s syndrome valproate toxicity resulted in fatal outcome.

Conclusion POLG1 mutation is a common genetic cause of the mitochondrial disorders. It is recommended to investigate at first in patient with a mitochondrial clinical disease having mendelien inherited PEO, ataxia, neuropathy, epilepsy and psychiatric disorders. In cases with epilepsy the predisposing SNPs to valproate toxicity in the POLG1 gene shall be screened to avoid the serious side effects.

P06.50-M
Very high prevalence of infantile Pompe disease in the Bushinegue population of French Guyana as a result of founder effect and endogamy
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Pompe disease (Glycogen storage disease type II or acid maltase deficiency) is an autosomal recessive metabolic disorder caused by an accumulation of glycogen in the lysosome due to deficiency of the lysosomal acid alpha-glucoisidase enzyme. The infantile form of Pompe disease is a rare lysosomal storage disorder usually symptomatic before age 1, with universally fatal outcome before age 4 in absence of enzyme replacement therapy. Incidence of neonatal Pompe disease is usually 1/15000 births in most country, except Taiwan, where its incidence is evaluated around 1/15000. We have identified 17 newborns with infantile Pompe disease born in 10 years in the same maternity to parents from the Bushinegue tribes living at the frontier between French Guyana, an overseas French department, and Suriname. This population descends from African-onset slaves who escaped to the Amazonian forest and settled along the lower Maroni River in the nineteenth century, where they grew as a geographical and cultural isolate population till recently. Genetic investigation revealed that all patients were homozygous or compound heterozygous for 2 mutations: p.Arg854* that has already been reported in the african-american population, and p.Gly648Ser, previously considered to be a mild variant. Despite probable incomplete detection, the raw incidence of infantile Pompe disease in the area is at least 1/2000 births corresponding to a heterogeneous frequency of 1/22. Early detection allowed us to put under enzyme replacement therapy the last 3 cases, where all CRIM positives. Implementation of systematic newborn screening will be implemented in Guyana by mid 2014.

P06.51-S
New insights into PPARγ regulation mechanisms: the unexplored impact of alternative splicing on its function
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The transcription factor PPARγ is involved in a variety of biological processes, including adipogenesis, glucose homeostasis, and immune response. Alternative splicing of PPARγ transcripts results in the production of multiple PPARγ isoforms with different tissue distributions and functions. The biological significance of PPARγ isoforms and the mechanisms underlying their formation have been largely unknown. Recent studies have shown that alternative splicing of PPARγ transcripts is affected by various stimuli, including hormones, growth factors, and environmental factors. The impact of alternative splicing on PPARγ function is still poorly understood.

In the present study, we investigated the effects of alternative splicing on the function of PPARγ. We used RT-PCR to measure the expression of PPARγ isoforms in various tissues and cell lines. We also used reporter assays to study the transcriptional activity of PPARγ isoforms and their ability to bind to DNA. Our results showed that alternative splicing of PPARγ transcripts affects the transcriptional activity of PPARγ and its ability to bind to DNA. These findings suggest that alternative splicing of PPARγ transcripts may play a role in the regulation of PPARγ function.

In conclusion, our study provides new insights into the regulation of PPARγ function and highlights the importance of alternative splicing on its function. Further studies are required to fully understand the mechanisms underlying alternative splicing of PPARγ transcripts and their impact on PPARγ function.
The nuclear receptor PPARG is a key regulator of cell proliferation and differentiation, including adipogenesis. Defects in PPARG signaling are implicated in metabolic syndrome, cardiovascular diseases and cancer. The wide number of ligands, coregulators and target genes, combined to different protein isoforms and alternative transcripts, determines the complexity of PPARG biological role.

Notably, our group identified a new PPARG isoform (γORF4) with a dominant negative effect toward PPARG, suggesting underestimated aspects of its regulation. We also recently described the differential contribution of PPARG canonical transcripts and ORF4 variants to adipocyte differentiation of human mesenchymal stem cells. The analysis demonstrated that PPARG transcripts can be regulated in a time-specific manner, through differential usage of distinct promoters, also indicating that dominant negative isoforms are actively transcribed and regulated throughout the adipogenesis. Moreover, during this study we identified another PPARG transcript, named ΔS5, with features similar to ORF4. Through transfection experiments, the usage of a PPARY agonist and luciferase assay, we demonstrated its dominant negative activity and an altered ability to regulate cellular proliferation. In order to understand, on a genome-wide scale, the effects of ΔS5 expressed on PPARY target genes’ expression, we also performed a transcriptome analysis by RNA-Sequencing in HEK293 cells over-expressing ΔS5, in presence of a PPARY agonist. Our results indicate that different dominant negative isoforms encoded by PPARY gene may have a relevant, and yet unexplored, role in the biological function of PPARY, with a potential involvement in physiologic and pathologic conditions, of which PPARY is a key player.

**P06.54-M**

**Association of rs780094 with metabolic syndrome and related traits were confirmed in Tehran lipid and glucose study**

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Background: The minor T-allele of rs780094 in intronic region of glucokinase gene (GCKR) is reported to be associated with a number of metabolic traits including higher triglyceride levels and lipid metabolite factors in GWA studies mostly European ancestry. Here we report replicating these findings in Iranian population using samples from the Tehran lipid and glucose study (TLGS), a large population-based cohort study. Methods: Among TLGS participants (n=1777) cases with metabolic syndrome according to ATP III criteria, were selected and compared to 3999 control. In GCKR gene, rs780094 genotyped using the Centaurus (Nanogen) platform in DeCODE genetics. Association of T-allele with metabolic syndrome and lipid related variables (e.g. triglyceride, cholesterol, HDL and LDL) was tested using plink. Results: Replicating previous findings TLGS participants showed that T-allele of rs780094 was associated with higher triglyceride levels (p<0.05), higher cholesterol levels (p<0.005) and metabolic syndrome prevalence (p<0.0025). Conclusion: Our findings showed the association between the presence of T allele in rs780094 and metabolic syndrome and related traits among Iranian population and confirmed previous result in other ethnicity.

**P06.53-S**

**Sengers syndrome: case report and review of literature**

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Sengers syndrome is a rare autosomal-recessive condition (prevalence <1/1 000 000) associating congenital cataract, hypertrophic cardiomyopathy, skeletal myopathy and exercise-related lactic acidosis. We report on a non-consanguineous, full-term, eutrophic child with bilateral congenital cataract. Metabolic investigations were normal. Cataract surgery was early performed. At three weeks and three months of age, during an infectious episode, the infant developed respiratory distress, and decreased cardiac efficiency. Krabh revealed cardiomyopathy, and severe lactic acidosis was found in blood sample. No genetic analysis could be performed in the patient. Thus we decided to analyze acylglycerol kinase (AGK) gene in both parents in this context. We identified heterozygous mutations in AGK gene in both parents allowing retrospective diagnosis of Sengers syndrome in the child and clear genetic counseling for the family. The management of congenital cataract should be very early and exhaustive performed, to exclude Sengers syndrome, considering the risk of cardiac failure during cataract surgery or during any intercurrent infection.

**P06.55-M**

**A homozygous mutation in the transcription factor THAP11 in a patient with methylnalonic aciduria and a severe neurological phenotypes**

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Inborn errors of vitamin B12 (cobalamin) metabolism result in homocystinuria and methylnalonic aciduria, either alone or in combination. The most common error, cblC caused by mutations in MMACHC gene has catastrophic consequences. We recently described a biochemically similar inborn error, cblK caused by mutations in the gene for the X-linked transcriptional regulator HCF1, which activates the transcription of MMACHC. The cblK patients were originally diagnosed as cblC; however they have a severe neurological and mild biochemical phenotype than most cblC patients [Yu et al. AJHG, 2013, 93:5]. One patient, characterized as cblC by complementation analysis, had no mutations in either MMACHC or HCF1 by Sanger sequencing. Clinically, he had methylnalonic aciduria, encephalopathy, profound mental retardation, seizures and tetraplegia and died at age 10. His biochemical manifestations were mild, with higher cellular function of both cobalamin-dependent enzymes than normally seen from cblC patients and no homocystine elevation. Sequencing of patient genomic DNA identified a homozygous mutation, c.240C>G (p.F80L) in THAP11, the gene encoding THAP11 a homologous transcription factor that functions with HCF1. The mutation affects a residue located in a conserved zinc finger–containing domain, two residues away from P78, responsible for interacting with the zinc ion, and is predicted to be likely damaging by Polyphen-2. F80 is conserved to zebras, monkeys, and human. The F80L change is predicted to affect the zinc coordination and decrease zinc finger interaction, possibly leading to inactivation of THAP11, leading to severe neurological phenotype. This patient demonstrates the importance of comprehensive genetic analysis in patients with inborn errors of metabolism.

**P06.56-M**

**Rogers syndrome (thiamine responsive megaloblastic anemia syndrome phenotype): the success of multidisciplinary approach**

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Thiamine responsive megaloblastic anemia syndrome is a rare autosomal recessive metabolic disorder. Only ~80 cases have been described mainly in consanguineous families. A gene SLC19A2 coding high affinity thiamine transporter mediating vitamin B1 uptake through cell membrane has been identified. Classic triad of features is characteristic to the disease - megaloblastic anemia, deafness and non-type 1 diabetes.

We report a 3y old boy born in nonconsanguineous family. From the early days the boy was easy irritable and suffered with common infecto-respiratory problems. The psychomotor abilities developed according to age. The slow-down of speech development was noticed from the 7th month of life. Insulin dependent non-type 1 diabetes was diagnosed at the age of 1y. At the age of 1.5y the profound bilateral hearing loss was diagnosed and cochlear implantation performed with good auditory and speech outcomes. During 3rd year of life severe megaloblastic anemia without folic acid or vitamin B12 deficiency and bilateral maculopathy has developed. The coding sequence of GI2 gene was analyzed and genotype c.[13.326delAGGTCATCGAGG
G]=s [p.[Lys105GlyfsX5]]=s was identified. MDNA 155AA mutation was not revealed. The patient had slightly elevated branched chain amino acids (Leu, Ile, Val) in plasma. The clinical diagnosis of FTMDRA syndrome was suspected and daily supplementation with thiamine 100mg started. The course of the patient markedly improved several days after the initiation of treatment – the psychomotor development and also psychophysical status of the child clearly improved. The results of SLC19A2 gene molecular testing will be achieved in the near future.

**P06.59-S**

Tyrosinemia type II (Richner-Hanhart syndrome): a new mutation in the TAT gene

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In the present study we report the clinical features and the molecular genetic investigation of the tyrosine aminotransferase (TAT) gene in a young girl from Croatia with Richner-Hanhart syndrome, mainly suffering from photophobia, hyperkeratoses of the palms and soles and slight neurological abnormalities. Sequencing analysis of the TAT gene revealed a novel homozygous missense mutation c.1250G>A (p.R417Q) in exon 12, and herewith confirmed the clinical diagnosis. Showing the first symptoms in babyhood, at the age of 8 years it was diagnosed for the first time clinically diagnosed that the patient suffers from tyrosinemia type II and a therapy with tyrosine and phenylalanine reduced diet has been started successfully. All symptoms disappeared within 2-4 weeks. Since that time, we have been following the girl until today for more than ten years. She is in a good condition, and attends the normal high school program.

**P06.59-M**

Neurological impairment is frequent among heterozygote women for X-linked Adrenoleukodystrophy - A clinical, neurophysiological and biochemical study

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Background: Neuropathic impairments in female heterozygotes for X-linked Adrenoleukodystrophy (X-ALD) are poorly understood. Our aims were to describe the neurological and neurophysiological manifestations of a cohort of X-ALD heterozygotes, and to correlate them with age, disease duration, mutations, X-inactivation and serum concentrations of a marker of neuronal damage. 45 heterozygotes of our institution were assessed with previous VLCFA and molecular diagnosis, were invited to be evaluated through myelopathy scales JOA and SSPROM, nerve conduction studies and somato-sensory evoked responses. X-inactivation pattern was tested by HUMARA methylation assay. Serum NSE was measured by electrochemiluminescence.

Results: Thirty-three heterozygotes were recruited: 29 (87%) were symptomatic and 4 asymptomatic. The coding sequence of the TAT gene revealed a novel homozygous mutation c.1250G>A (p.R417Q) in exon 12, and herewith confirmed the clinical diagnosis. Showing the first symptoms in babyhood, at the age of 8 years it was diagnosed for the first time clinically diagnosed that the patient suffers from tyrosinemia type II and a therapy with tyrosine and phenylalanine reduced diet has been started successfully. All symptoms disappeared within 2-4 weeks. Since that time, we have been following the girl until today for more than ten years. She is in a good condition, and attends the normal high school program.

Discussion: Neurologic manifestations, clearly related to age, were more common than expected. JOA and SSPROM scales discriminated asymptomatic from symptomatic heterozygotes. Both might be useful tools to follow disease progression, in future studies.
abnormalities, including hydrocephaly, ventriculomegaly, and white matter lesions, as well as other symptoms, such as facial and thoracic deformities, joint stiffness, exudative otitis media, hepatosplenomegaly and intellectual disability. After at least 9 months of treatment, urinary glycosaminoglycans levels had decreased, and liver and spleen size were reduced. Idrusulfase treatment was well tolerated (adverse reactions occurred in none of the patients). However, cognitive development had shown clear tendency to decline over time, resulting in severe mental retardation by the end of second year of treatment. Our experience of enzyme replacement therapy of Hunter syndrome provides an evidence of the beneficial somatic effects and also suggests the importance of early diagnosis of the disease which might lead to more favorable impact on cognitive development of patients.

**P07.01-S**

ACTN1 : identification of novel mutations in a cohort of Italian IMTP patients

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Inherited macrothrombocytopenia (IMTP) is a highly heterogeneous group of inherited disorders characterized by a low platelet count and abnormally platelet size. Even though IMTP-causing mutations have been reported in several genes, only 5-10% of patients have to date a molecular diagnosis. In March 2013 Kanishima and colleagues identified alpha-actinin1 in (ACTN1) as a new gene responsible for IMTP which accounted for 5,5% of cases in Japanese population. To evaluate the frequency of ACTN1 mutations in the Italian population, we performed a screening in 160 probands in which all the known forms of IMTP were previously excluded. Ten, including 8 novo, different missense have been identified in 11 patients. All except one (p.D666V) segregated with the macrothrombocytopenia within the families. In vivo transfection experiments in Hela cells were performed to demonstrate the pathogenicity of the variants identified. Except for p.D666V, preliminary data indicate that the missense mutations are associated with disorganization of cytoskeleton, as determined different co-localization of mutant and wild type alpha-actinin with actin. Further studies will be needed to determine how these mutations may lead to the onset of disease.

**P07.02-M**

Simultaneous silencing of Mcl-1 and survivin expression by small interfering RNA and enhancement of chemosensitivity in human acute myeloid leukemia cells

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Anti-apoptotic genes such as Mcl-1 or survivin may be responsible for resistance to apoptosis induced by chemotherapeutic drugs. The aim of this study was to investigate whether the knockdown of Mcl-1 or survivin expression by small interfering RNA (siRNA) would sensitize HL-60 acute myeloid leukemia cells to etoposide. Knockdown of Mcl-1 or survivin expression was confirmed by quantitative real-time PCR and Western Blotting. The effects of Mcl-1 or survivin down-regulation on the chemosensitivity of the cells was assessed by the MTT assay. Cell viability and apoptosis were also determined using the trypan blue exclusion assay and the annexin V/PI double staining method, respectively. Transfection of siRNAs markedly decreased the expression levels of both Mcl-1 and survivin genes in a time-dependent manner. Down-regulation of Mcl-1 or survivin significantly inhibited the proliferation and enhanced the chemosensitivity of the cells. Furthermore, pretreatment with siRNAs clearly enhanced the etoposide-mediated apoptosis of the leukemia cells. Surprisingly, siRNA co-transfection had a more antiapoptotic effect relative to the single siRNA transfection. Our study demonstrates that a triple approach involving siRNA-mediated silencing of the Mcl-1 and survivin along with chemotherapeutic drugs could potentially be used to lower the effective doses of the chemotherapeutic drugs and reduce drug-related toxicities.

**P07.03-S**

Role of the mutations identified in the 5’UTR of ANKRD26 responsible for an inherited form of thrombocytopenia

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The joint application of clinical and genetic investigations has greatly expanded the knowledge of inherited thrombocytopenias, leading to characterization of new forms, such as ANKRD26 related disease (ANKRD26-RD). ANKRD26-RD is characterized by thrombocytopenia and bleeding tendency, as well as an increased risk of leukemia. Since the identification of ANKRD26 as the gene responsible for the disease, 12 different mutations, all localized in a short stretch of 22 nucleotides in the 5’UTR, have been identified. To further investigate their effect, we tested the activity of the basal promoter together with the wt or the mutant 5’UTR in a reporter assay. In a megakaryocytic cell line, mutations generated a statistically significant increase of the luciferase activity, suggesting that the 5’UTR plays a role in inhibiting the expression of ANKRD26 in megakaryocytes. Consistent with this hypothesis, ANKRD26 is expressed in human CD34+ and BFU-E but it is hardly detectable in megakaryocytes. Bioinformatic analysis revealed putative binding sites for transcription factors in the region hit by mutations. In order to identify these factors, we performed electrophoretic mobility shift assay (EMSA). Among different complexes of the same size in both wt and mutant samples, we observed an additional band generated by two mutant probes. Experiments of super-shift EMSA are in progress to characterize the pathogenetic factor(s) that could interfere with the physiological control of the ANKRD26 expression.

**P07.04-M**

HBB gene mutation spectrum of beta-thalassemia patients from Turkey

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Beta-thalassemia is defined by the absence or decrease of beta globin via mutations of the HBB gene and is one of the most common hereditary disorders existing in Turkey. With the mean carrier frequency of β-thalassemia being 2.1% in the general population, and rates as high as 10% concentrated in certain regions of the country, hemoglobin electrophoresis of the individuals at premarital stage and molecular diagnosis of the carrier individuals for genetic counseling cannot be overstated. Targeted diagnosis of the HBB gene mutations can be readily obtained using commercially available reverse dot blotting kits. A sequence analysis of the complete HBB gene covering UTR and near-gene regions provides a 99% mutation detection rate. We report here a summary finding of HBB gene analysis for 163 Turkish patients, along with their family members totaling 248 individuals, referred to beta-thalassemia indications covering the period of 2010-2014. 39 were found to have homozygous, 31 possessed compound heterozygous and 63 possessed heterozygous mutations. Overall, a total of 205 alleles were found to have mutations. The first 15 frequent mutations covered 88% of the entirety of all mutations. The summary range is as follows: c.93-21-G>A (IVS1+106G>A) 30.7%; c.135G>C (pser45fs) 7.8%; c.92+1-G>A (IVS-1+2G>A) 7.5%; c.260C>T (p.Ala87Val) 5.9%; c.260T>C (p.Ala87Thr) 4.9%; c.92-67C>T (IVS-1-46) 4.9%. We discuss that the commercial targeted kits detect up to 80% of the HBB mutations for our patients. Sequence analysis of the HBB gene from 5’ promoter (-250bp) to 3’ promoter region (+250bp) contributes 15% to the mutation detection rate.

**P07.05-S**

Identification of two novel mutations in NLRP3 gene in Italian patients with Cryopyrin-associated periodic syndrome (CAPS)

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Cryopyrin-associated periodic syndrome (CAPS) is an autoinflammatory syndrome caused by mutations in NLRP3 gene. Three clinical types exist: familial cold autoinflammatory syndrome (FACS), Muckle-Wells Syndrome (MWS) and chronic infantile neurological, cutaneous and articular (CINCA) syndrome. More than 150 different diseases-causing mutations have been reported in NLRP3 with more than 80% of them localized in exon 3. We report here two cases of CAPS in Italian patients harbouring two de novo mutations. The first case is a 20 years old boy with CINCA. He has severe phenotype with clinical features mimicking those of juvenile rheumatoid arthritis, including recurrent episodes of skin rash, fever, arthralgia, and cen-
tral nervous system involvement. In this patient was identified a c.913G>A mutation in the exon 3 of the NRLP3 gene. The analysis of the parents did not show the same mutation. Mutations at the codon 305 of the NRLP3 gene have been described but not the Asp>Asn substitution. The second case is a 35 years old woman with FCAS. She showed recurrent episodes of skin rash, fever, arthralgia and conjunctivitis after generalized exposure to cold. In this patient the c.2113C>A (p.Q705K) mutation in exon 3 of the NRLP3 gene has been identified. Also in this case the analysis of the parents did not show the same mutation. In conclusion, we report two de novo mutations in NRFLP3 gene in CAP5 Italian patients and we suggest the molecular screening of NRLP3 gene in patients with a strong suspicion of autoinflammatory syndrome.

P07.06-M
IPEX-like syndrome: beyond FOXP3 analysis

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IPEX-like patients have clinical features resembling IPEX syndrome (Immunodeficiency, polyendocrinopathy, enteropathy, X-linked) without mutations in the FOXP3 gene (Xp11.23), that encodes a transcriptional regulator critical for the development and function of CD4+CD25+ regulatory T cells. These cells are indispensable for the maintenance of immune self-tolerance and homeostasis by suppressing aberrant or excessive immune responses.

IL2Ra and STAT5b are two genes whose deficiency has been associated with IPEX-like syndrome and both encode proteins involved in FOXP3 pathway. IL2Ra, IL2Ra codes for the alpha subunit (CD25) of the receptor complex for IL2, and the interaction between IL2-IL2Ra is the first step that trigger FOXP3 transcription.

In this study we describe the identification of three different point mutations affecting IL2Ra gene of three newly published by our group and the other two were never reported in literature. All variations are missense mutations leading to a single aminocaid substitution that modify the protein structure. Two are located in exon 4 and alter the “sushi” domain that seems to be fundamental for IL2-IL2Ra interaction, the third mutation determines the substitution of a cysteine that may be involved in the formation of a disulfide bond, thus causing the alteration of the tertiary structure of the receptor.

Cyt fluorimetric analysis revealed total absence of CD25 expression in all three patients, strengthening the hypothesis that these mutations cause the disruption of the receptor structure thus leading to its degradation. Further studies are ongoing to better characterize the consequence of these mutations from the molecular and cellular point of view.

P07.07-S
Immunophenotypic profile of erythroid extracellular vesicles obtained from peripheral blood of patients with Diamond-Blackfan anemia

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Diamond-Blackfan anemia (DBA) is a rare inherited anemia. Heterozygous mutations in one of 11 ribosomal protein genes cause defective ribosome biogenesis. Erythroid progenitors (BFU-E and CFU-E) in bone marrow (BM) show a proapoptotic phenotype. Suspicion of DBA is raised after exclusion of other causes of BM failure syndromes and the diagnosis of BM failure syndrome is confirmed by mutations analysis. To improve DBA diagnosis, we tested a new approach based on the study of extracellular vesicles (EVs). EVs have been isolated from plasma of patients with DBA and appropriate controls by differential centrifugations and analyzed by flow cytometry. To study erythroid EVs we evaluated three erythroid markers: CD34, CD71 and CD235a. EVs immune phenotypic profiles of 8 patients with DBA, 20 healthy controls and 10 patients with other hematological diseases have been characterized. We were able to identify different clusters: CD71+/CD34+, CD34+/CD71 low and CD235a+. In all cases the absolute number of EVs/µl has been evaluated.

Only the CD34+/CD71 low population is significantly different between patients with DBA and controls (p<0.05). This population that is always present in healthy controls, is absent in patients with DBA. The absence of CD34+/CD71 low population in DBA patients plasma is in agreement with the low level of erythroid progenitors in the patients’ BM. The area under the ROC curve that compares patients with DBA and healthy controls is 0.92. Further analyses are needed to ascertain whether this assay may be used in the clinics.

P07.08-M
Analysis of rRNA maturation in patients with Diamond-Blackfan anemia

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Diamond-Blackfan anemia (DBA MIM # 105650) is an inherited erythro- id aplasia caused by mutations in 11 genes encoding ribosomal proteins of the small (RPS) or the large (RPL) subunit. The 28S, 5.8S and 18S rRNAs are transcribed as a single precursor that is processed into mature RNAs, which form the ribosomal subunits with the rPs. Mutations in a RPS or a RPL alter the processing of 18S or 28S and 5.8S rRNAs, respectively. The analysis of pre-rRNA by Northern blot in cell models or lymphoblastoid cells from patients shows different patterns of precursors depending on which RP is mutated.

Mutation analysis of 11 genes is needed to confirm the diagnosis. We have prepared both rRNA analysis of activated lymphocytes could improve the DBA diagnostic approach. We extracted total RNA from activated lymphocytes of patients with DBA (4 mutated in RPS19, 1 in RPL17, 2 in RPL11, 4 in RPL5 and 5 with unknown mutations). Northern blot analysis was performed using appropriate probes. All the patients with a mutation in a RPL showed the accumulation of a 325 precursor that is visible also by ethidium bromide staining. Northern blot analysis allowed to discriminate patients with mutations in RPS19 from those with mutations in RPS17. We observed the rRNA maturation defect also in a patient with a mutation in RPL5 after stable remission from the disease.

This is the first demonstration of rRNA abnormalities in patient lymphocytes. rRNA processing analysis is a useful approach to direct the subsequent mutational screening.

P07.09-S
A case of factor X (FX) deficiency caused by novel mutations Q56K, Q104X

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Background
Inherited deficiency of coagulation factor X (FX) is a rare bleeding disorder with prevalence of 1 per 500,000 in general population. Mostly, the condition is associated with missense mutation, which is a valuable clue.

Case and results
A six month-old infant was hospitalized due to fever, lethargy and seizure with no significant medical history. Symptoms were attributable to brain abscess subsequently diagnosed. For operability evaluation, prothrombin time (PT) and activated partial thromboplastin time tests were performed, and the results were prolonged to 121.6 seconds and 109.1 seconds, respectively. Coagulation factor activity were also measured. Factor II, V, VII, IX, XI and XII activities were 80%, 99% and 1%, respectively. Factor VII, VIII, IX, XI and XII activities were all in normal ranges. FX activity of the parents was not tested due to refusal of family study. FX of the patient was sequenced on coding regions and the results were prolonged to 121.6 seconds and 109.1 seconds, respectively. FX activities were all in normal ranges. Factor VII, VIII, IX, XI and XII activities were all in normal ranges. FX activity of the parents was not tested due to refusal of family study. FX of the patient was sequenced on coding regions and the results were prolonged to 121.6 seconds and 109.1 seconds, respectively. FX activities were all in normal ranges. Factor VII, VIII, IX, XI and XII activities were all in normal ranges.

Conclusion
The amino acid substitution of glutamine to lysine at EGF-like-domain 1 is probably related to functional inactivation of coagulation FX. Confirmation by expression study will be required.
Study of IL-1β and IL-1RA gene polymorphisms in familial Mediterranean fever (FMF)

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Objectives. Familial Mediterranean fever (FMF) is a recessively transmitted autoinflammatory disease, caused by mutations in the MEFV gene. The pro-inflammatory cytokine IL-1β has been implicated in the pathogenesis of FMF and the balance between IL-1β and its receptor antagonist IL-1RA plays an important role in the development of the disease. Since polymorphisms in the IL-1 gene cluster have been suggested to have an effect on IL-1β and IL-1RA production, our aim was to determine a possible association of specific polymorphisms in IL-1β and IL-1RA genes with susceptibility to and/or severity of FMF.

Subjects and Methods. Forty-two genetically confirmed FMF patients and 42 controls were genotyped for IL-1β(-511C/T), IL-1β(-31C/T) and IL-1β(+395T/C) polymorphisms by PCR-digestion. The IL-1RA VNTR was identified by fragment-size analysis. IL-1β and IL-1RA levels were evaluated by Luminex in supernatants of PBMC cultures of 30 FMF patients with and without 24h stimulation of monocytes by LPS.

Results. The CC genotype and C allele at positions -31 and +3954 of IL-1β gene were observed more frequently in FMF patients than in controls. No significant difference was observed in the genotypic and allelic frequencies of the IL-1β(-31) polymorphism and IL-1RA VNTR. FMF patients carriers of II-1β(-31) CC genotype were associated with a 2 fold increase in LPS-induced IL-1β secretion compared to patients carrying other genotypes.

Conclusion. These results indicate that IL-1 β gene polymorphisms at positions -31 and +3954 may be associated with susceptibility to FMF. IL-1β(-31) may also contribute to the severity of the disease, probably by modulating IL-1β secretion.

Host genotype-pathogen interactions in the PBMC cells of healthy individuals identify critical immune regulators

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Immune response to infection or vaccination is significantly influenced by host genetic components and autoimmune disease associated genetic variants may have stronger influence on such a response. One way to investigate the role of genetics in differential susceptibility to infection is to correlate the gene expression levels response to stimulation with autoimmune disease associated SNPs. We performed a pilot experiment by stimulating peripheral blood mononuclear cells (PBMC) of 46 volunteers with four different pathogenes for 4 and 24 hours. Gene expression profile was determined with Illumina arrays and genotype of 356 autoimmune disease-associated SNPs were genotyped using Illuminchip. Linear model with stimulation status, genotype and stimulation-genotype interaction term was fitted for each probe and SNP. Due to the small sample size, we were not able to identify globally significant pathogen-genotype interactions after multiple testing correction, so possibly relevant interactions were prioritized based on uncorrected p-values, allelic trends and effect size differences between unstimulated and stimulated conditions. 133 SNPs showed interaction effect for at least one probe. The most significant interaction was between rs6087990 and a gene encoding macrophage receptor (uncorrected P=3.72×10⁻⁵). We were also able to identify SNP-gene pairs showing similar stimulation-genotype interaction in case of several different stimulations.

P07.12-M The influence of a short-term gluten free diet on the human microbiome and the genotype of gray platelet syndrome

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A gluten free diet (GFD) is the most common diet worldwide. It is not only an effective treatment for celiac disease, but also commonly followed by individuals with gut complaints. How a GFD affects the human microbiome is largely unknown. We studied changes in the gut microbiome in healthy individuals following a short-term GFD. 23 healthy volunteers followed a GFD for 4 weeks. Stool samples were collected before the start of the diet, then at weekly intervals during the GFD and again at weekly intervals for 4 weeks on normal diet after a wash-out period. The samples were sequenced using 454 sequencing of the 16s rDNA (hyper variable region 3 to 4). We used closed reference picking to cluster reads into OTUs using the 2013 GreenGenes reference. Function imputation of the GFD intervention, with the intra-subject variation being larger than the effect from a short-term diet change. However, we did observe that family Veillonellaceae (class Clostridia) was significantly less present in the GFD samples (p=6.58e⁻⁵, q=0.0132). Based on richness of the samples we were able to define two groups which show a difference in response to a GFD period. The lower richness group showed more bacterial variation in composition during the diet change. Furthermore, sixteen gene pathways showed significant association to the change in diet. In conclusion, in this we observed changes in microbiome and gene composition associated to a GFD.
conservation might also be the result of a specific biological effect, as HLA-G*01:04 has been associated with reduced sHLA-G production and that HLA-E could also be incriminated. We showed that 2 haplotypes in Tswana Pygmies, HLA-G*01:04–E01:03:01 and G*01:04–E01:01 exhibited LD values.

The aim of our study is to explore the region between HLA-A and -E. We genotyped HLA-A, -G, -H and -E alleles in 71 French samples and explored their association.

We found that 21 haplotypes represent 75% of all the haplotypes. HLA-H deletion was exclusively associated with A*23–HLA-G01:04–U4R-3–HLA*E01:01 and HLA-A*24–HLA-G01:04–U4R-3–HLA*E01:03:01 haplotypes. A new HLA*H02:04(02) allele was described and associated with A*11–HLA-G01:01–U4R-7–HLA*E01:01. This study suggests that the HLA region between HLA-A and -E loci displays a haplotype conservation that might be the results of biological function. This haplotype conservation has to be further studied to understand their clinical implication.

P07.16-M HLA-DRB1 genotyping in romanian patients with early rheumatoid arthritis
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As being the most debated polygenic disease, rheumatoid arthritis elicits great interest in the study of association with genetic factors in various ethnic and racial groups. Some of the HLA-DRB1 alleles are encoding shared epitope amino acids that are not conferring the same risk in various populations.

Our study focuses on the evaluation of the distribution of HLA-DRB1 alleles in Romanian patients with early rheumatoid arthritis, along with controls by using PCR–SSP method. HLA-DRB1 allele genotyping showed statistically significant differences given by a higher allele frequency for *04, *01 and *14. Also, in our study was observed a lower frequency for *03,*11,*13 and *15 alleles in patients group compared with controls. Using a high resolution kit for HLA-DRB1 *04 group we found a high frequency for *0404 and *0408 alleles, in contrast with *0401 and *0402 which were significantly lower in patients than in controls. *0403, *0405 were not associated with early rheumatoid arthritis in our group diagnosed according with new classification criteria ACR/EULAR 2010.

Results of our study are demonstrating the need of a continuous work of allele tracing and associating with rheumatoid arthritis, especially in cases early diagnosed in order to create sufficient premises for instituting a correct and possibly long term remissive treatment. Keywords: early rheumatoid arthritis; HLA-DRB1; allele distribution

P07.17-S Genetic association of childhood psoriasis to the IL22 promoter is linked to higher promoter activity and increased IL-22 production in T cells
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Psoriasis is a common, immune-mediated and genetically complex skin disease. Recent large-scale studies have reported a number of psoriasis susceptibility genes. Most of these genes were identified in large cohorts not defining the clinical phenotypes and exploration of genes in distinct subtypes of psoriasis is missing. We recently reported that stratification according to age-at-onset was helpful in dissecting the genetic profile of early onset psoriasis. Here we investigated the genetic association to IL22 in three psoriasis populations: disease onset between 0-9, 10-20 and 21-40 years. IL22 encodes for a cytokine with an established role both in psoriasis skin pathology and in host defense, thus exemplifying delicate balance between autoimmune and control of infection. Herein we report strong association to regulatory elements in the IL22 promoter confined to onset of psoriasis before puberty. The associated IL22 variants contain putative binding sites for the transcription factor aryl hydrocarbon receptor, which is a potent inducer of IL22 expression in T cells. We next compared the transcriptional activity between a high-risk and a low-risk gene variant in a luciferase assay which consistently resulted in significantly higher activity from the high-risk construct. Furthermore, in children carrying a high risk variant, T cells from peripheral blood produced significantly more IL-22 after ex vivo stimulation compared to children with a low-risk genotype. These data indicate that genotypes in the IL22 promoter enhancing IL-22 production is preferentially enriched in psoriasis with onset before puberty and may predispose to development of psoriasis at an early age.

P07.18-M Whole exome sequencing as a tool for detection of the genetic basis of inherited thrombocytopenias

Thrombocytopenia is a common aspect of platelet function disorders (PFDs) which account for a significant proportion of bleeding diatheses. Identification of the genetic basis of congenital thrombocytopenias is often a difficult task due to the variability in clinical presentation and the relative redundance of known platelet receptors and signalling pathways. DNA-based analysis has therefore previously played a confirmatory role dependent on platelet function testing to validate candidate mutations. Using a novel approach undertaken through the Center of Expertise in platelet disorders (GAPP) study we have extended the DNA-based analysis, especially in cases where a qualitative defect is observed, to unravel the underlying genetic defects in patients diagnosed with inherited thrombocytopenia. The combination of platelet function testing, including flow cytometry, with whole exome sequencing provides a complete and complementary platform for efficient and effective investigative procedures. Initial integration of a database of PFDs and platelet-related genes into our analysis improves detection and allows for the identification of variants in novel genes for further functional studies. To date our unique workflow has confirmed 14 mutations in 21 index cases, including two in novel genes with previously unreported mechanisms within platelet formation or function. Whole exome sequencing is therefore an effective tool to study the molecular genetics of inherited thrombocytopenias with excellent applicability for patient investigation when coupled with platelet function testing.

P07.19-S Mutation spectra of the ITGB2 gene in Iranian families with Leukocyte Adhesion Deficiency type 1
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Leukocyte adhesion deficiency (LAD) type 1 is a rare, autosomal recessive disorder characterized by the triad of symptoms including recurrent bacterial infections, impaired wound healing, primarily localized to skin and mucosal surfaces. Beta-2 integrin subunit (ITGB2) gene located on 2q22.3 responsible for neutrophil dysfunction and impaired leukocyte cell adhesion. A total of 19 consanguineous families with LAD1 were investigated. Blood samples were collected after informed and written consent was obtained. Isolated DNA derived from subjects was amplified using intronic primers. The entire sequence of the ITGB2 gene, including regulatory regions, coding regions and exon-intron boundaries were analyzed for any mutations. A total of 10 mutations scattered throughout the ITGB2 gene were ascertained in the 15 subjects. Six different types of mutations were identified including IVS6+4C>A, c.3082T>A (Asp1218Ter), c.715G>A (Ala239Thr), IVS7+1G>A, c.1907DelE (Lys636fsX22) and four novel mutations consist of IVS5+11G>T, c.567delP (Asn193GlnfsX27), c.706G>A (Gly236Arg), IVS7+1G>T were identified. Moreover, two compound heterozygote and five homozygote mutations were detected in exon 6, suggested this region of ITGB2 gene might be a hot spot. This is the first comprehensive report of ITGB2 gene analysis in Iranian families with LAD1. Our results indicated that mutations distributed across the ITGB2 gene. Every population should develop a mutation database for their own rare genetic disorders. However, we suggest that to make a private database, appropriate screening strategy could be started with common alleles initially.

P07.20-M Identification of monoallelic forms of Bernard-Soulier syndrome
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Bernard-Soulier syndrome (BSS) is a rare inherited macrothrombocytope-
nia caused by mutations in GP1BA, GP1BB and GP9, the genes encoding for three subunits of the GP1b-IX complex, which is the platelet receptor for von Willebrand factor (vWF). In addition to the biallelic form, a less severe autosomal dominant form (monoallelic) of BSS is due to mutations identified in GP1BA or GP1BB Except for A.172Val (Noris et al, 2012), which is relatively frequent at least in the Italian population, other mutations have been reported only in single families. In order to evaluate the frequency of the monoallelic form in our thrombocytopenic cohort, we selected 120 probands with large platelets and no mutations in candidate genes. In 11 cases we found heterozygous mutations in GP1BA (n=4), GP1BB (n=6) and GP9 (n=1). In addition to 4 variants previously identified in biallelic BSS patients, the others are all novel mutations. They are classified as missense (n=8), nonsense (n=1) or frame-shift (n=2) mutations. Segregation analysis within the families showed that only the affected individuals carry the mutations. Functional studies are in progress to determine the effect of the mutations on GP1b-IX complex formation and its capacity to interact with vWF. Considering that half of the patients with inherited thrombocytopenia remain without a molecular diagnosis, we could estimate that the frequency of the monoallelic form of BSS could account for 5% of the cases. This suggest that monoallelic BSS should be always taken into consideration in the differential diagnosis of inherited thrombocytopenia.

P07.21-S

MHC and the 1000 genomes: genotyping from exome data
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The publicly available 1000 genomes (1KG) data is still a valuable source of scientific discovery after years of its release. We have demonstrated previously that HLA typing of MHC-I genes is possible from 1KG exome data for HapMap samples. In this study we are aiming to determine types for MHC-II genes and for any 1KG exome samples passing certain quality control (QC) conditions. We are presenting the generation and interpretation of these QC values. Besides the HLA genes we are showing typing results for other polymorphic MHC genes like TAP1, TAP2, MICA and MICB. The HLA genotype assignments were compared to classical HLA typing obtained by sequencing techniques. Typing a diverse set of genes of MHC sheds light on lineage deficiencies and represent the effect of the genetic background on their expression. Furthermore, differences in results from different exome capturing kits are also to be presented.

P07.22-M

SNP variants in MHC are associated with sarcoidosis susceptibility and subgroups - a joint case-control association study in four European populations
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Sarcoidosis is a multiorgan inflammatory disorder of unknown etiology. The most probable pathophysiology of sarcoidosis, the dysregulation of the immune response strongly suggests benefits from a better understanding of the role of the immune mediating genes (e.g. MHC genes) in sarcoidosis susceptibility. We present results from a Finnish case-control discovery sample as well as three independent replication studies from the Swedish, Dutch and Czech populations. We studied four genes in the MHC Class III region (LTα, TNF, AGER, BTN2L2) and HLA-DRA in relation to IL1-DRB1 alleles to detect variants predisposing to sarcoidosis and to identify genetic differences between patient subgroups. Patients with sarcoidosis (n=805) were further subdivided based on the disease activity and the presence of Löfgren syndrome. In a meta-analysis, seven SNPs were associated with non-Löfgren sarcoidosis (NL; the strongest association with rs3177928 in HLA-DRA, P=1.79e-07, OR=1.9) and eight with Löfgren syndrome (LS; the strongest association with rs3129843 in HLA-DRA region, P=3.4e-12, OR=3.4) when compared with healthy controls (n=970). The high LD between SNPs and an HLA-DRB1 challenged the result interpretation. In addition to these SNPs, population-specific associations for sarcoidosis were observed. In conclusion, there is clear evidence that polymorphisms in the BTN2L2 and HLA-DRA have a role in sarcoidosis susceptibility. Most importantly, our study revealed sarcoidosis-related variants that were shared across ethnicities as well as ethnicity-specific markers. Future functional studies are required to reveal the causal variants of these associations and the immunogenetic basis related to sarcoidosis.

P07.23-S

Impact of immunogenetic polymorphisms in predisposition to lymphoid malignancies in NBS patients

The Nijmegen breakage syndrome (NBS) is a recessive genetic disorder, resulting in high predisposition to developing a malignancy. The 5bp deletion (c.657-661del) in the NBN gene is founder mutation for Slavic populations. In total, 80 NBS cases were studied, and it was observed that lymphoid malignancies has been developed at age 5-12 years. It remains unclear why the carriers of same mutation are implemented in a different morbidity. The purpose of this study was looking for immunogenetic criteria for tumor developing in NBS patients. Interleukin-10 (IL-10) and interferon-gamma (IFN-γ) play a key role in controlling the immune response and SNPs IL-10-1082A/G and IFN-γ-874T/T respectively can significantly affect their expression. Patients with severe outcome (multiple chronic recurrent inflammatory, developing of tumor or death) and moderate course of disease were comparatively investigated. The distribution of IL-10 1082AA, 1082GG and 1082AG genotypes in cases of severe and mild outcome was 42.9% and 33.3%, 35.7% and 33.3% respectively. The distribution of IFN-γ 874AA, 874AT and 874TT genotypes in groups of patients with severe and mild outcome was 20.0% and 29.4%, 40.0% and 58.8%, 40.0% and 11.86% respectively. The results allow to suggest, that severe outcome in NBS may depends on carrying of IL-10 1082AA genotype, associated with decreased anti-inflammatory activity, and IFN-γ 874TT genotype, known by increased pro-inflammatory properties. In conclusion, disruption in balance of pro- and anti-inflammatory cytokines may strongly affect NBS course.

P07.24-M

MEFV gene mutations in pediatric patients with PFAPA syndrome in Slovenia
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Introduction: PFAPA syndrome is the most common autoinflammatory fever disorder in childhood, characterized by recurrent fever, aphthous stomatitis, pharyngitis and adenitis. Mutations in the MEFV gene are known to cause syndrome with PFAPA overlapping symptoms (Familial Mediterranean Fever), which is common in eastern Mediterranean population, but rarely reported in patients from Slovenia. Objective: The aim of this study was to assess the frequency of MEFV gene mutations in pediatric patients with PFAPA syndrome from Slovenia. Methods: We collected clinical and laboratory data and results of genetic testing of MEFV gene of PFAPA patients under the age of 18, who were followed from the beginning of 2006 to the end of 2013. All 10 exons and intron/exon boundaries of gene were directly sequenced. Results: In total, 91 PFAPA patients were tested for MEFV gene mutations. All of them were under the age of 18, mean age at diagnosis was 7 years. The ratio of women to men was 1:1. 15 patients (16%) were found to have at least one mutation. 11 patients (12%) were heterozygote and 4 patients (4%) were compound heterozygote, 3 with R408Q/P369S and 1 with K695R/I591T mutation. The overall number of mutation found was 12. The most frequent was K695R (26%), followed by R408Q (16%), P369S (16%), E148Q (10%), I591T (10%), M694V (5%), S703F (5%), A289V (5%) and A745S (5%). Conclusion: In order to evaluate effect of these mutations on PFAPA phenotype, we are planning to determine the carrier rate in healthy Slovenian population and evaluate genotype-phenotype correlation in MEFV gene mutation positive patients.
A new gene involved in an autosomal dominant form of common variable immunodeficiency (CVID)

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Common variable immunodeficiency (CVID, MIM#607594) is the most common primary antibody deficiency in adults. It includes a heterogeneous group of disorders characterized by defects in the terminal stage of B lymphocyte differentiation, whose underlying genetic defects remain unknown in the majority of cases.

We studied a five-generation Italian family with an autosomal dominant form of CVID through whole exome sequencing, giving priority to the variants associated with a candidate genomic interval on chromosome 3q27-3q29 previously identified by genome-wide linkage analysis. Since no causative mutation was identified by whole exome sequencing, we performed a whole genome high resolution SNP array analysis, which allowed to detect a ~880 kb tandem duplication in 3q27.3, located inside the candidate linkage region and involving 8 genes: ST6GAL1, BCL6, RTP2, RT1, MASP1, RTPI, RT4, SST, RTF2 and BCL6.

The expression pattern analysis in control peripheral blood lymphocytes (PBL) of all genes included in the duplicated region revealed that only ST6GAL1, BCL6, RTP4 and BCL6 are expressed in mature circulating lymphocytes, allowing us to exclude the remaining four genes from further investigations. Preliminary qRT-PCR analyses conducted on affected vs unaffected subjects showed a significant upregulation of RTP4 in affected members (t-test, p < 0.0001). This finding suggests RTP4 overexpression as a possible pathogenic mechanism underlying CVID in this family. RTP4 is a Golgi chaperone and we hypothesize that it might be involved in the regulation of the Unfolding Protein Response (UPR), a key process of plasma cell differentiation.

Primary immunodeficiency diseases (PIDD) encompass a wide and heterogeneous group of disorders caused by mutations in genes with immune function. The molecular mechanisms behind many forms are not yet known.

The significant clinical and immunological heterogeneity of PIDD often delay the diagnosis making treatment challenging. Few thousand individuals are expected to suffer from PIDD in Finland, where the unique genetic background has proven to be very useful in identifying genes for monogenic disorders. An exome sequencing analysis of a Finnish PIDD family characterized by recurrent aneurysms, has identified compound heterozygous mutations in the CECR1 gene not previously implicated in PIDD. The affected individuals carried a common synonymous variant, highly conserved and predicted deleterious in silico, in the allele and a rare missense variant, highly conserved and predicted deleterious in silico, in the gene not previously implicated in PIDD. The affected individuals carried a common synonymous variant, highly conserved and predicted deleterious in silico, in the allele and a rare missense variant, highly conserved and predicted deleterious in silico, in the unique genetic background has proven to be very useful in identifying genes for monogenic disorders. An exome sequencing analysis of a Finnish PIDD family characterized by recurrent aneurysms, has identified compound heterozygous mutations in the CECR1 gene not previously implicated in PIDD.
stranded RNA ligand poly-IC. Cytokine production was measured in patients and compared to controls, and a specific defect in poly I:C-induced interferon-gamma was detected. Substitution therapy with interferon-gamma was successfully administered to all patients, leading to impressive amelioration.

Additionally, we performed exome sequencing in all 3 patients, aiming to understand the genetic basics of the underlying immune deficiency. While this genetic analysis did not identify one common cause of the primary immune deficiency, rare variants in candidate genes SYK, IFI1H and EIF3E were identified that may have caused the aberrant interferon signaling. In summary, these preliminary results indicate that patients with recurrent viral HSV infections may have genetic heterogeneous forms of primary immune deficiency; however they show a common functional defect in double stranded RNA-induced IFN-gamma, and common replacement therapy with interferon-gamma was shown to be an effective treatment.

P07.30-M
Allele specific expression of HLA haplotypes associated to autoimmune diseases A. Zheleva, J. Pu, D. Zhernakova, L. Franke, C. Wijmenga; University medical centre Groningen, Groningen, Netherlands.

HLAs are strongly associated with many autoimmune diseases, including rheumatoid arthritis (RA), celiac disease (CD) and type 1 diabetes (T1D). For many diseases the association is established, but the underlying mechanisms are unknown. For example, heterozygosity for DR3-DQ2/DR4-DQ8 is a stronger risk factors to T1D compared to homozygosity for both DR3-DQ2 and DR4-DQ8. The goal of our study was to study the downstream effect of HLA haplotypes by looking for allele specific gene expression (ASE) and haplotype expression quantitative trait loci (eQTLs) in HLA alleles associated to autoimmune diseases. We selected individuals heterozygous for DR3-DQ2/DR4-DQ8 and homozygous for both alleles (T1D and CD risk haplotypes), and individuals heterozygous for DR4-DQ8/DR4-DQ8 and homozygous for these alleles (RA risk haplotypes). We run RNAseq to quantify gene expression and performed eQTL and ASE analysis in these individuals. In total, 90 individuals were selected for these analysis. We observed different expression of multiple genes in HLA locus in DR3-DQ2/DR4-DQ8 heterozygous individuals, in compare with both homozygous group. In particular, HLA-DQA2, HLA-DQB1 and HLA-DQB9 (P-value Wilcox test <0.001 for all three genes). ASE analysis of individuals heterozygous for DR3-DQ2/DR4-DQ8 indicated ASE effect for multiple variants located in TAP2 gene, which plays a role in antigen presentation. In DR4-DQ8/DR4-DQ4 analysis we identified multiple eQTLs in the HLA locus, as well as allele specific effect on SNPs located in DOA genes.

P07.31-S

Rheumatoid arthritis (RA) is well-known as an autoimmune disease and is a chronic inflammatory disorder characterized by the destruction of multiple joints. Many genome wide association studies were performed and multiple RA-susceptibility loci and autoimmune-susceptibility loci have been identified. These studies suggested that multiple genes and their functions were associated with disease causing and development. Previously, peptidylarginine deiminase type 4 (PADI4) was identified as a susceptibility gene for RA in a Japanese population by case-control association study [Ref 1]. PADI4 is a member of the PADI gene family and converts arginine residue (peptidylarginine) to citrulline residue (peptidylcitrulline). PADI4 is highly expressed in bone marrow, macrophages, neutrophils and monocytes. PADI4 genes are important genes in RA, because only PADI4 (translated protein from PADI4 gene) can provide antigens of ACPA, which are specific antibodies of RA. To evaluate these, however, they show a common functional defect of RA, we generated Padi4-/- DBA1 mice. We used Padi4-/- mice to show that Padi4 is significantly affected to progress of collagen induced arthritis (CIA), well generated Padi4 -/- CIA mice are also significantly lower than those from WT CIA mice. As the results, we suggested that Padi4 enhanced collagen-initiated inflammatory responses.

P07.32-M
Identification of susceptibility loci associated with primary Sjögren’s syndrome by genome-wide association study L. Song1, H. Chen1, C. Chen1, J. Wu1; 1Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan, 2Rheumatology, Immunology and Allergy Division, Tri-Service General Hospital, National Defense Medical Center, Taipei, Taiwan.

Primary Sjögren’s syndrome (PSS) is one of the most common autoimmune connective tissue diseases which primarily affect women, with a female-to-male ratio of 9:1. To identify the susceptibility genes predisposing individuals to PSS, we conducted genome-wide association analyses comparing 242 PSS female patients with 1444 female controls, recruited from the Han Chinese population residing in Taiwan. In discovery GWAS, SNPs in the MHC region of chromosome 6 and the GTF2I gene of chromosome 7 were found to be associated with PSS. In particular, SNP rs11702632 on chromosome 7 showed the highest association (P value = 6.71 x 10-13). The two loci were previously reported to be associated with PSS. Replication with additional samples is now under process. Our study confirmed the associations between the two loci and PSS.

P07.33-S
Functional analysis of genetic risk factors for canine SLE-related disease complex and identification of genetic risk factors for human SLE F. H. G. Farías1, M. Wilber2, S. V. Koypuru2, D. Leonard3, H. Bremer3, J. Dahlqvist4, A. Hellstrand5, G. R. Perrelli5, U. Gustafson2, M. Eloranta5, H. Hansson-Hamlin6, G. Andersson7, L. Bäckström1, K. Lindblad-Toh2,3,4; 1Uppsala University, Science for Life, Department of Medical Biochemistry and Microbiology, Uppsala, Sweden, 2Swedish University of Agricultural Sciences, Department of Animal Breeding and Genetics, Uppsala, Sweden, 3Uppsala University, Section of Rheumatology, Uppsala, Sweden, 4Swedish University of Agricultural Sciences, Department of Clinical Sciences, Uppsala, Sweden, 5Broad Institute, Cambridge, MA, United States.

Dogs represent an excellent model to study complex genetic disorders including many autoimmune diseases that they share with humans. A systemic lupus erythematosus (SLE)-related disease complex, which shows similarities to human SLE, has high prevalence in Nova Scotia duck-tolling retriever (NSDTR) dogs. Genome-wide association analyses have identified five candidate loci in NSDTR containing several genes. Detailed analysis of the candidate loci identified variants that alter the expression of several of these genes. Multiple genes involved in T-cell activation show expression differences, but regulatory mutations also alter expression of genes such as BANK1, involved in human SLE. As a proof of concept, the genes and pathways identified in dogs and also genes relevant for human SLE were sequenced in 140 Swedish human SLE patients. Patients were divided in 9 pools according to disease manifestation and a group of healthy Swedish controls was also sequenced. Targeted Nimblegen + Illumina sequencing of 219 genes and their regulatory elements resulted in approximately 250x coverage per individual. We detected 4276 novel SNPs (not present in 1000genomes or dbSNP137) of 1258 SNPs were found only in cases. Seventeen genes showed 5 novel case-only variants. Of these, three genes were previously associated to human SLE and 14 were novel candidate genes. From the top genes, ten variants that show strong regulatory potential have been selected and being evaluated for their importance for gene expression and correlation with clinical phenotypes. Several variants appear to be potential regulators of specific SLE sub-phenotypes.

P07.34-S
Deep analysis of TCR repertoires of twins by next generation sequencing M. V. Pogorelykh1, J. V. Zvoyagin2, M. E. Ivanova2, D. A. Bolotin2, D. M. Chudakov2, Y. B. Lebedev2; 1Shemyakin–Ovchinnikov Institute of bioorganic Chemistry RAS, Moscow, Russian Federation.

Immune system interacts with great diversity of pathogens. Receptors involved in antigen recognition - BCRs (B-cell receptors) and TCRs (T-cell receptors) - are not encoded in genome due to its limited capacity, but generated by V(D)J recombination. For a long time, no instrument to estimate TCR diversity in individual organism has been available. Next generation sequencing methods (NGS) have created the possibility of deep TCR profiling in vitro, we aimed to estimate the role of genetic factors in TCR repertoire formation by sequencing TCR repertoires of monozygotic and dizygotic twins. 16 cDNA TCR libraries were generated and sequenced on Illumina platform. Surprisingly, the overlap of amino acid sequences of CDR3 region in TCR clonotypes was not greater in MZ twin pairs and depended on sample size.
only. However, the number of identical clonotypes was higher for monozygotic twins in the abundant clonotypes subset, representing mainly antigen-experienced T cells. At the same time V-segment usage is more similar in twin pairs for clonotypes before and after thymic selection. We also showed that the ratio of clonotypes with identical CD3r and V-segments (coding for CDR1 and CDR2) to clonotypes with identical CD3r is higher in twin pairs in the abundant clonotypes subset. All these features were not observed in a dizygotic twin pair, which probably reflects the impact of genetic factors on TCR repertoire formation.

**P07.36-M**

A novel StripAssay identifies genetic variants modifying beta-thalassemia disease severity

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Background: The clinical phenotype of patients with beta-hemoglobinopathies is extremely heterogeneous, ranging from nearly asymptomatic forms of thalassemia intermedia to severe transfusion-dependent thalassemia major. The wide phenotypical variability is associated with the type of beta-globin mutation, the co-inheritance of alpha-thalassemia and the ability for persistent production of fetal hemoglobin (Hbf) in adult life. For the latter, three different quantitative trait loci, accounting for 20-50% of Hbf variation, have been identified by now. Single nucleotide polymorphisms (SNPs) in the gamma-globin gene promoter (HBG2), in the BCL1A1 gene and the HSBS1-MYB intergenic region lead to increased residual Hbf levels in adults. In our study, we developed a novel Strip-based reverse-hybridisation assay was developed for the simultaneous detection of SNPs in the HBG2 (g.-158 C>T), BCL1A1 (rs1447407, rs101889857), HSBS1-MYB (rs28384513, rs9399137) genes. Results: The new StripAssay enables the concomitant identification of genetic variants known to influence beta-thalassemia disease severity. Based on the presence of positively modifying alleles, and combined with alpha- and beta-globin genotyping, it allows the prediction of patients likely to display less severe phenotypes. Favorable properties, such as the rapid DNA extraction protocol, ready-to-use reagents and teststrips, as well as the potential for automation of the hybridisation/detection and interpretation steps, make the StripAssay convenient and easy to perform within less than six hours. Conclusions: Testing for genetic modifiers influencing disease severity will lead to more specific and effective treatment, and support clinical decisions regarding the beginning of transfusion therapy in beta-thalassemia patients. Furthermore, the knowledge about prognostic markers has implications for genetic counselling and prenatal diagnosis.

**P07.37-S**

Family with inherited thrombocytopenia and homozygous pathogenic variant in FYB gene

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Inherited thrombocytopenias (IT) are a heterogeneous group of rare diseases characterized by a reduced number of blood platelets. The frequency of IT is probably underestimated because of diagnostic difficulties and because not all the existing forms have yet been identified, and some patients remain without a definitive diagnosis. We report a family with IT with small size platelets seen in several members of a highly consanguineous Kurdish family from Northern Iraq. Genotyping of all affected, their unaffected siblings and parents, followed by exome sequencing revealed a strong candidate pathogenic variant in a homozygous state: a frameshift mutation was detected in the FYB gene. The protein encoded by this gene is a cytosolic adaptor molecule expressed by T cells, natural killer (NK) cells, myeloid cells and platelets and is known to be involved in platelet activation and controls the expression of interleukin-2. This is the first report to hypothesize that pathogenic variants in FYB gene could cause thrombocytopenia in humans. We propose that FYB is the causative gene for this phenotype.

**P07.38-M**

Distribution of A736V variant of TMPRSS6 gene in beta-globin mutation carriers

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The genome-wide association studies on the genes related with iron metabolism has been showed that some of the genetic variants were associated with serum iron level and erythrocyte parameters. One of these common variants was A736V in TMPRSS6 gene which regulates serum iron level. Inactivation of TMPRSS6 gene causes iron-deficiency anaemia and impairment of iron absorption. It has been found that A736V variant of TMPRSS6 gene is associated with lower levels of serum iron and erythrocyte MCV with high hemoglobin. We have performed a study of 68 patients of hereditary microcytic anemia. It was also shown that inheritance of A736V variant of TMPRSS6 has a the nephrectic effect on Beta-thalassemiaemic. However the frequency of this variant among Beta-thalassemia patients is unknown. Related with these data, we aimed to investigate frequency of A736V variant of TMPRSS6 gene among patients carrying beta-globin gene mutations. 93 patients investigated for beta-globin gene mutation with DNA sequencing method were enrolled in this study. A736V (rs550791) variant of TMPRSS6 gene was detected by real-time quantitative PCR method. Erythrocyte parameters and hemoglobin levels were measured and their distributions according to gene variants were analyzed. Frequency of TMPRSS6 gene A736V variant was 0.47 among all patients. There was no association between A736V variant of TMPRSS6 gene and beta-globin gene mutations, hemoglobin levels or erythrocyte parameters. However, frequency of A736V variant was higher among the patients having low levels of hemoglobin or erythrocyte MCV with a wild type beta-globin gene. Further studies are needed for better understanding the relationship between A736V variant and Beta-thalassemia prognosis.
and marked deficiency of blood and lung iNKT cells in patients with newly diagnosed sarcoidosis. During 4 years of disease follow-up, there was a significant increase in expression of SLAM-SAP signaling factors, mainly SLAMF6, SLAMF7, and PFKY, and blood iNKT cells. This increase clearly correlated with improvement in patients’ clinical symptoms as after 4 years the disease had gone into remission in the great majority of patients.

Our longitudinal study showed that an increase in expression of SLAM-SAP signaling factors and iNKT cells characterizes the clinical remission of pulmonary sarcoidosis.

P07.43-S
A late diagnosis of NOMID-syndrome / CINCA-syndrome - lessons to learn
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The case of a 10 ½ year old boy from Germany is presented with a new diagnosis of NOMID syndrome, also known as CINCA-syndrome, the rarest and most severe of the Cryopyrin-associated periodic syndromes (CAPS), which are genetic syndromes of autoinflammation. The diagnosis was delayed despite many clinical clues including the typical non-itching maculopapular rash which was present from the neonatal period, raised inflammatory markers on many occasions without detectable infectious pathogens, progressive macrocephaly with hydrocephalus, evolving short stature, progressive optic atrophy, progressive deafness, progressive arthropathy and other non-specific haematological and immunological abnormalities. In addition, the patient reported malfunctions of activities and finger clubbing. Periodic fever does not always occur in this condition and in the case of this patient was completely absent. This patient was treated for several years with growth hormone in the absence of a diagnosis without any improvement of his short stature. Early diagnosis of these patients is vital because treatment with interleukin -1 receptor antagonists is now well established and very effective. Untreated, the patients may develop a destructive arthropathy, blindness, profound deafness and renal failure secondary to amyloidosis. The macrocephaly, hydrocephalus and short stature result from the chronic aseptic meningitis. The disease is caused by mutations (mostly new dominant) in the NLRP3 gene, which may be mosaic and thus are sometimes only detectable by next generation sequencing.

P08.01-S
Chromosome 15q11.2 imbalances associated with neuropsychiatric and developmental disorders - array-CGH findings in a cohort of 1000 patients
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Array-Comparative Genomic Hybridization has led to the knowledge that some copy number variants (CNVs) correspond to susceptibility loci for developmental disorders. CNVs at chromosomal region 15q11.2 involving 4 known genes, TUBGCP5, CYFIP1, NIPA2 and NIPA1, are of challenging interpretation due to their presence both in normal populations and in individuals with diverse developmental disorders. In a cohort of 1000 patients analyzed for 15q11.2 imbalances by array-CGH we identified 12 patients with 15q11.2 imbalances, 9 deletions and 3 duplications, 7 females and 5 males. Four of the 12 patients had additional genomic imbalances. The patients presented with global developmental delay, dysmorphisms, ID, epilepsy, microcephaly, amongst others. To date, we were only able to determine inheritance in 4 patients, 2 deletions maternal in origin, 1 paternal, and a de novo duplication. The proximal breakpoint was common in 11 of the 12 patients, while the distal breakpoint was variable, but similar in some patients. The 4 previously mentioned genes were involved in the genomic imbalances of all the patients, except in the patient with the distinct proximal breakpoint, where TUBGCP5 gene was in normal copy number. Functional data have revealed that TUBGCP5, CYFIP1 and NIPA1 genes are expressed in developing mammalian brain and are involved in processes such as mitotubule nucleation, interaction with other proteins and nervous system development and regulation, respectively. To date, there is still no straightforward interpretation when a 15q11.2 genomic imbalance is detected, but in this cohort further evidence was given that this region is associated with neuropsychiatric disorders.
Small RNAs (miRNA, siRNA, and piRNA) regulate gene expression through RNA interference (RNAi), a process that has emerged as a fundamental principle of normal cellular function. The Argonaute (AGO) proteins are critical mediators of the RNAi pathway and constitute a highly conserved family of genes found in almost all eukaryotes. Four AGO genes are present in humans, three of which (AGO1, 3, and 4) reside in a cluster on chromosome 1p35p34. The possible effects of germline AGO mutations or dosage alterations in humans is not known, however, animal models deficient for AGO proteins display development brain defects including a reduction in total number of neurons and glia. Moreover, different studies have established that miRNA and siRNA are prevalently or exclusively expressed in the brain, where they play an essential role in the development and function of the central nervous system (CNS). We describe five patients with hypotonia, microcephaly, intellectual disability, and facial dysmorphism, in whom array-Comparative Genomic Hybridization revealed overlapping de novo microdeletions of the chromosomal region 1p34.3. The minimal critical region is a segment of approximately 694 Kb that encompasses the AGO1, AGO3, and AGO4 genes. We propose that the neurocognitive deficits present in these patients are due to deletion of the 1p34.3 region and resulting haploinsufficiency of several AGO genes. It seems plausible that the 1p34.3 deletion syndrome may eventually be recognized as a neurodevelopmental disorder associated with RNAi deregulation, however, further investigation is necessary to prove this.

**P08.05-S**

**Two brothers with 2q23 microdeletion syndrome inherited from their mosaic father**

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Patient 1, the older brother, is 13 years old and has moderate intellectual disability. He communicates by single words, pointing and some signing. He walked at 4 years. He uses diapers, suffers from constipation and manifests aggressive outbursts and stereotypic movements. His sleeping pattern is disturbed by periods of awakening. He has teeth enamel erosions. His twin sister, the younger brother, who has been evaluated at 2 years, the younger child is 6.5 years and has moderate intellectual disability. He speaks a few words and infrequently combines two words. He has a heart murmur, enamel tooth erosion and periodic sleep problems. He is restless and active. ArrayCGH of the patients and father showed a deletion of chr2q23.1(1148705701-148926786 bp), including the MBDS, considered critical in the 2q23.1 microdeletion syndrome. By PISH the deletion in the father was found to be a mosaicism found in 73% of lymphocytes. Assessment of the mosaicism level in additional tissues is ongoing. The father received speech therapy in childhood and has dyslexia. He completed secondary school with low-average marks, and did not finish high school. He has been working as a manual worker in the same factory for 18 years. In patient 2 and his father aGHD detected also a chr15q26.1(9022468-90255068 bp) deletion, including PLIN1. PLIN1 mutations cause dominant familial partial lipoatrophy type-4, but the effect of PLIN1 haploinsufficiency is unknown. Patient 2 and his father, but not Patient 1, are obese. To our knowledge this is the first familial case of the 2q23 microdeletion syndrome.

**P08.06-M**

**Microdeletion of 8q21 region: clinical and molecular analysis based on a new case**

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Submicroscopic deletion of 8q21.11 is a rare case of intellectual disability, developmental delay and craniofacial dysmorphology. Hypotonia, impaired balance, poor feeding, frequent crying, swallowing difficulties, flat facial behavior as well as mild fingers and toes anomalies are frequently observed. To date, 13 cases, including 5 from the same family, have been clinically and molecularly characterized. Here we report on the case of a 14-year-old girl with moderate mental retardation, numerous dysmorphic features (a round face with full cheeks, high forehead, ptosis, long and downslanting palpebral fissures, short philtrum, Cupid’s bow of the upper lip, down-turned corners of the mouth, micrognathia, high palate, low-set and prominent ears, and short neck), short stature and overweight. At birth, microtia of the right ear with external auditory duct stenosis and atrial septal defect were diagnosed. Muscle tone was unremarkable. Other features comprise small hands with camptodactyly of fifth and second fingers of the opposite hands, unilateral transverse crease, and valgus, flat feet. Auto-aggressive behaviour, autism and sleep problems were also noted. Whole-genome microarray analysis revealed a 5.19 Mb deletion 8q22.2-8q22.31. The sisters of the patient encompassing 19 genes. The phenotypic and genetic findings of our patient will be compared with those of previously reported patients. We indicate several candidate genes, providing new data supporting further genotype-phenotype studies. Our results suggest that haploinsufficient genes within the deleted region, e.g. ZFHX4/STMN2, FA-BMP/FAIRP and HEY1, could underlie the intellectual impairment, excess weight and cardiovascular disorders, observed in 8q21 microdeletion. This study was supported by MNiSW Grant No. 019/13/P1/2013/72.

**P08.07-S**

**Clinical and molecular delineation of the emerging 8q22.3 microdeletion syndrome**

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**Background:** Five patients with deletions that involving chromosome 8q22.2-8q22.3 has been recently reported. Four patients shared a similar craniofacial phenotype with microcephaly, blepharophimosis, uni or bilateral cleft palate and congenital heart anomaly. They have moderate to severe intellectual disability (ID) and impairment or absent speech. They presented the shorter deletion of 1.92 Mb (hg19:102.01-103.93).

**Methods and Results:** Here, we described on 18-year-old female patient with microcephaly, unilateral ptosis, moderate ID and speech impairment. CytoScan HD array analysis identified a 2.95 Mb 8q22.2-8q22.3 deletion. The comparison of the present patient with the above cases contributed to narrow down the critical region in 8q22.3 of ~1.47 Mb (hg19:101.95-103.42). Furthermore, we analyze the gene functional roles and probability of haploinsufficiency (HI) of this genomic region; resulting the ubiquitin protein ligase E3 component N-ras-5 (UBR5) gene as the best candidate for the phenotype. UBR5 belongs to ubiquitin/proteasome system (UPS). Neurodevelopmental genetic disorders involving alterations in UBR5 have been described such as Angelman syndrome (UBR3), Johnson-Bliizard syndrome (UBR1) and X-linked intellectual disability type Nascimento (UB2A). Recently, mutations in ubiquitin protein ligase E3B (UB3EB) in a blepharophimosis-ptosis-intellectual disability syndrome, which fits Kaufman oclocerebrofacial syndrome have been recognized.

**Conclusions:** We suggested that this novel 8q22.3 microdeletion syndrome had a variable expressivity and proposed that the HI of UBR5 could lead to this peculiar craniofacial phenotype and ID of this neurogenomic disorder.

**P08.08-M**

**An atypical inherited ATR-16 syndrome unrelated to SOX8 haploinsufficiency**

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We report the case of a girl affected by mild mental retardation, macrocephaly and microcytic anemia. Clinical examination did not reveal significant dysmorphic features, except for frontal bossing and mild hypertelorism. Also her mother presents with mild intellectual disability. Array-CGH analysis detected a 49.31 Kb telomeric deletion on chromosome 16p13.3, which was found to be maternally inherited. Further examination of mother’s medical history revealed that she was also affected by microcytic anemia, which had been treated with oral iron therapy without benefit. The deletion involves the two alpha-globin genes (HBA1 and HBA2), explaining the haematological defect. Deletions of 1.5 and 2 Mb involving the telomeric short arm of chromosome 16 cause the contiguous gene syndrome ATR-16 (MIM #141750) characterized by alpha-thalassemia, mental retardation and variable dysmorphic features (downskinning palpebral fissures, mild hypertelorism, broad nasal bridge, small ears and a short neck with webbing). Haploinsufficiency of SOX8 (MIM #605923) is thought to be responsible for intellectual disability. The deleted region in our patient does not include SOX8, whereas it comprises 31 genes whose function is still partially unknown, except for HBA1 and HBA2.

To our knowledge this is the smallest 16p13.3 telomeric deletion characterized by mental retardation and microcytic anemia, narrowing the critical region and pointing at other candidate genes for intellectual disabilities. Moreover this is the first case of inherited ATR-16 syndrome.
Intelligence is a complex trait that may be influenced by a combination of genes and environment. Understanding the genetic basis of intelligence is crucial for the development of interventions and personalized treatments. Recent advances in genetic research have revealed that certain genes play a role in intelligence, with a particular focus on the brain-gut axis, which highlights the interplay between the central nervous system and the gut microbiome. These findings underscore the importance of a holistic approach to understanding and improving intelligence, incorporating both genetic and environmental factors.
Mutation in CAPN10 Causing Intellectual Disability in Two Independent Iranian Families with Overlapping Phenotypes

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Intellectual disability (ID) is a broad diagnosis encompassing a wide variety of phenotypes and severities. There are many reasons why the genetics of intellectual disability have been difficult to unravel, but the most important are extensive genetic and phenotypic heterogeneity in autosomal-recessive (AR) inheritance of ID. Therefore, our joined researches on ID started in 2003 with abroad centers to elucidate the molecular causes of ARID in Iran. As a part of this collaboration, whole genome homozygosity mapping and exome sequencing was performed to identify novel genes and mutations in two Iranian families affected with ID.

In our previous study, published in Nature 2011, we reported on a mutation in CAPN10 gene in a consanguineous Iranian family with syndromic ID. In the current study, two novel nonsense mutations were discovered in two unrelated Iranian families with ID in CAPN10 gene, which encodes calpain10 protein eight isoforms and involves in brain function, NIDDM and other cellular functions. In this study, in addition to the above mentioned data, we observed overlapping clinical findings including microcephaly, ID and distinct brain MRI features in two independent Iranian families. Here, our aim was to introduce CAPN10 as a promising candidate gene in syndromic ID.

De novo single exon deletion of AUTS2 in a patient with profound intellectual disability

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The autism susceptibility candidate 2 (AUTS2) gene has a critical role in early brain development with its strong expression in fetal and adult brain. Association of AUTS2 with intellectual disability (ID), autism spectrum disorders, and other neurodevelopmental disorders has recently gained more attention. Genetic rearrangements and copy number variations (CNVs) involving AUTS2 have been implicated in a range of neurodevelopmental disorders with or without congenital malformations and dysmorphic features. Here we report a 127 kb de novo encompassing exon 5 of AUTS2 at 7q11.22 which result in frame deletion of 10 amino acids. The deletion was detected by SNP-array analysis applying InfiniumHD whole-genome genotyping assay with the HumanCytoSNP-12 BeadChip (Illumina Inc.). Obtained data were analyzed with Illumina Genomestudio and QuantiSNP software.

The single exon deletion was detected in a 10 year-old female patient with severe speech disorder, intention tremor, fine motor activity deficit, behavior disturbance, residual lesion in the CNS, and intracranial hypertension.

This is one of the smallest de novo intragenic deletions of AUTS2 described in patients with neurodevelopmental disorders. Along with the review of previously reported cases, we detected 20 cases with small pathogenic CNVs and 4 cases with different de novo balanced translocations of 7q11.2 interrupting the AUTS2 gene with severe profound to borderline ID without autistic features or delayed psychomotor development with mild to moderate autism, this report provides additional insight into the clinical spectrum of AUTS2 disruptions.
In conclusion, these findings indicate that NR2F1 plays an important role in the neurodevelopment of the visual system and that its disruption can lead to optic atrophy with intellectual disability.

P08.19-S
Chromosomal microarray analysis of patients with intellectual disability, autism or multiple congenital anomalies presenting for genetic services


Copy number variations (CNVs) are the most common identifiable causes of intellectual disability/developmental delay (ID/DD), autism spectrum disorders (ASD), or multiple congenital anomalies (MCA). Chromosomal microarray analysis (CMA), with a 10-20% diagnostic yield, can identify CNVs as a microarray. We report our experience with the use of the Affymetrix SNP Arrays in 1600 Italian patients during the past 6 years (2008-2013). We identified CNVs with a high score of pathogenicity in 415 (27%) patients. Among them 143 (34.4%) showed a CNV overlapping with a known syndrome. The most common CNVs were the ones in 2q24.1q24.2, in 2q24.1q24.2, in 22q11.22 and in 11p13. Some CNVs were useful to describe new syndromes such as a 1.7 Mb deletion in 3q13.2q13.31. Also, we have identified a large group of small CNVs (< 1.0 Mb) encompassing, either in whole or in part, functionally related genes to the same chromosome(s) such as CASK, CNTN4, SNT2, HIP1, DLD2, NRXN1, MCPH1 and CHL1 genes. Among these small CNVs, we have reported a FOPX1 gene microdeletion in a boy with autistic and speech delay, and a de novo interstitial deletion of 0.122 Mb at 2q24.2 region harboring only TRB1 gene in a boy with moderate to severe intellectual disability. Variants of uncertain significance (VOUS) because unreported, containing genes of uncertain clinical significance or non-genic but potentially regulating nearby gene expression, were identified in 128 individuals (8%).

P08.20-M
The Cohen syndrome-associated protein COH1 functions as Golgi matrix protein required for Golgi integrity

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Mutations in the COH1 (VPS13B) gene cause autosomal recessive Cohen syndrome, which is mainly characterized by mental retardation, postnatal microcephaly, pigmentary retinopathy, and intermittent neutropenia. However, the clinical characteristics, tissue localization, or functional role of the encoded protein COH1 (3997 aa) have so far not been addressed. Our cell biological analysis showed strong co-localization of COH1 with the cis-Golgi marker protein GM130, which was preserved even upon chemical disruption of the Golgi architecture. Further biochemical analysis showed that COH1 is a peripheral membrane protein similar to its remote homologs. Vps13p in yeast. Vps13p has been found to regulate anterograde and retrograde vesicular transport of transmembrane proteins between the prevacuolar compartment and the trans-Golgi network. Consequently, we found that loss of COH1 upon RNAi impairs the ability of the Golgi ribbon to (re)assemble and thus induces fragmentation into mini-stacks. Moreover, we found that COH1 regulates the formation of Golgi-derived membrane tubules in a dominant negative function in isolated cells. Finally, further protein-protein interaction studies identified COH1 as a potentially effector protein of the Golgi-associated small GTPase Rab6, emphasizing a role of COH1 for Golgi-related vesicle transport. Thus, our accumulated evidence suggests COH1 as a molecular regulator of antero- and retrograde Golgi membrane trafficking. As Rab6-associated Golgi transport critically regulates neuronal development and neuron function the Cohen syndrome pathology is likely caused by a failure of intracellular vesicle transport.

P08.21-S
De novo heterozygous mutations in beta-catenin 1 (CTNNB1) appear to be a frequent cause of intellectual disability (ID)

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De novo heterozygous mutations in beta-catenin 1 (CTNNB1) appear to be a frequent cause of intellectual disability (ID). Our finding of five individuals in our cohort of 250 (2.0%) suggests that CTNNB1 loss-of-function mutations might be a more frequent cause of ID than estimated from the data of deloit and colleagues. Our data further emphasize the importance of Wnt signalling in human brain development and/or function.

P08.22-M
Deletions limited to CTNN2D cause mild intellectual disability

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Using chromosomal microarray testing we detected a 113 kb de novo out of frame deletion encompassing exons 4-7 of CTNN2D in a patient with borderline ID. This gene was mapped to the cri-du-chat syndrome critical region in chromosome 5p15.2 and encodes a regulator of neuronal migration. CTNN2D was considered responsible for the severe intellectual disability in cri-du-chat syndrome patients with terminal deletions. Extended deletion mapping however indicated that interstitial deletions restricted to the CTNN2D locus produce a milder level of intellectual disability. The girl was born at term with no complication and normal measurements. Apart from 2 episodes of acute subglotic laryngitis there were no remarkable health problems. Developmental milestones were within normal limits. The patient was referred to developmental testing because of behavioural issues and mild intellectual disability. She showed a dissociated cognitive profile with better language than nonverbal functions (full scale IQ 77) and suffered from short attention span, poor executive functioning and impaired working memory. Three other patients with deletions limited to CTNN2D were found in the DECIPHER database. One patient had a 413 kb deletion with mild intellectual disability, autism and hypotonia. The other patient showed a 479 kb deletion with learning difficulties, behavioral problems and autism spectrum disorder. In the third patient a 54 kb deletion was detected. He showed intellectual disability and neurological problems which may be caused by an additional unidentified disorder. We assume that CTNN2D haploinsufficiency is a novel cause of mild neurodevelopmental features.

P08.23-S
A familial case of 15q26.3 microduplication

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The “15q overgrowth syndrome” has been associated with the very distal 15q duplication. At least 26 cases with trisomy of 15q25-26ter have been published, of which approximately 70% have presented with overgrowth and about 65% have been the result of a parental balanced translocation. Overgrowth has been associated with the dosage effect of IGFIR (insulin- like growth factor 1 receptor) gene located on 15q26. However, about 50% of
patients with larger duplications of distal 15q (15q13.1-21qter, including the IGFIR gene) on the contrary presented with growth retardation, although a few authors have reported overgrowth as well. We report 12 and 8 years old brothers with identical 0.77 Mb microduplications in 15q26.3 region (chr15: 101,051,538-101,802,565, HG19). The elder sibling presents with postnatal overgrowth - at the age 12 his height is 175 cm (+4 SD), but his birth length was 52 cm (0 SD). The younger sibling has normal growth (+1 SD). In addition, they both present with expressive speech disorder, some facial and hand microanomalies, and poor fine motor skills. Their father has overgrowth (+2.5 SD), prominent facial features and presented with dysarthria in childhood. Therefore, the duplication is with high probability of a paternal origin (analysis in work). This is the smallest “pure” 15q26 duplication reported so far. Interestingly, the IGFIR gene is not duplicated in our patients. Furthermore, they demonstrate variable clinical phenotype. Therefore, we give further evidence that a more complex pathogenesis for the development of somatic overgrowth should exist in case of distal 15q duplication.

P08.24-M
6p21.33 microdeletion associated with EHMT2 haploinsufficiency and intellectual disability
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Microdeletion of 6p21 is a rare condition that has been described in patients with multiple congenital malformation and intellectual disability. We report on a patient with a 0.4 Mb microdeletion in 6p21.33 and intellectual disability. The genomic loss contained 30 RefSeq genes including EHMT2 which is a primary enzyme for mono- and dimethylation of lys 9 of histone H3 (H3K9me1/2), and plays critical roles in various biological processes. The 6-year-old boy was referred for evaluation of intellectual disability and macrocephaly. He was born at 37 weeks of gestation to a 32-year-old G1 P1 mother and 34-year-old father after uneventful pregnancy. The birth weight was 2418 g, length 45 cm, and head circumference 33 cm. His psychomotor development was delayed. He achieved head control at 4 months, sitting at 10 months, and walking alone at 2 years. He spoke his first word at 2.5 years. He never had seizures and his hearing was normal. His height was 106 cm (-1.7 SD) length 84 cm (-0.3 SD) and head circumference 53.5 cm (+1.4 SD) respectively. Brain MR at age of 4 years showed incomplete rotation of the bilateral hippocampus. EHMT2 is a human homologue of mouse G9a that exists predominantly as a G9a-GLP heteromeric complex. The human homologue of the GLP is EHMT1, which is a causative gene responsible for Kleefstra syndrome with characteristic facial dysmorphism and severe intellectual disability. This case provides insight into the etiological mechanisms of histone modification and human development.

P08.25-S
EPHA1 as a new candidate gene for autosomal recessive non-syndromic intellectual disability
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We report a Ukrainian family consisted of healthy and non-consanguineous parents and two affected children with moderate intellectual disability (ID) and similar psychoneurological symptoms. Biochemical and CGH-array investigations revealed no genetic abnormalities in children. Whole exome sequencing performed in family members identified two non-synonymous variants c.1475G>A and c.1891G>A in the EPHA1 gene. Both affected siblings were compound heterozygotes while father and mother were heterozygous carriers for the c.1891A and c.1475A variants respectively. C.1475G>A and c.1891G>A frequencies analyses in 300 healthy Ukrainian controls revealed that the c.1475T allele frequency was 1.2% while the c.1891A was not found. Epha1 belongs to EPH receptors family implicated in axon guidance control but it was not previously associated with ID. To understand a possible effect of these substitutions the mutated EPHA1 proteins’ tertiary structures were predicted. As a result it turned out that substitutions are located in important functional domains of Epha1. The substitution of positive charged Arg492 to uncharged Gin492 (c.1475G>A) is in the fibronectin type III repeat of Epha1 ectodomain involved in signal transduction and binding with ligands or protein-partners. The substitution Gly631Arg (c.1891G>A) is in the glycine-rich region of Epha1 tyrosine kinase domain responsible for ATP binding. We assumed the c.1475G>A and c.1891G>A mutations may cause changes in conformational flexibility and solubility of these Epha1 domains resulting in impaired Epha signal transduction. Predicted structural Epha1 changes and low c.1475G and c.1891A frequencies allow us to hypothesize that missense mutations in the EPHA1 gene may be responsible for autosomal recessive non-syndromic intellectual disability.

P08.26-M
Mutations in FOXP1 result in a recognizable mental disability phenotype
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Mental retardation with or without minor dysmorphic features represents a major challenge in clinical genetics. Over a decade ago, the introduction of arrayCGH analysis introduced the concept of reverse genetics in clinical practice identifying recurrent phenotypes in patients with similar molecular defects. With the increasing resolution of arrayCGH analysis, monogenic defects can sometimes be readily identified. A careful clinical description of the concurrent phenotype may therefore direct specific gene analyses in patients with similar phenotypes (“gestalt” diagnosis) or may prioritize variant analysis through next-generation sequencing of gene panels or exomes.

We present three novel patients with FOXP1 mutations. All patients presented with severe intellectual disability with nearly absent speech, patient 3 additionally had tonic-clonic seizures, horizontal nystagmus, spastic tetraparesis and agression. The facial features in these patients included a frontal upsweep, a broad forehead, broad and bent palpebral fissures, hypertelorism, a bulbous nasal tip, prominent nasolabial folds and a wide mouth. These striking features and the identification of a de novo 420kb intrinsic deletion on arrayCGH analysis in patient 1 enabled us to identify a FOXP1 mutation (p.R152X) by direct sanger sequencing in patient 2 and to prioritize data analysis in a large gene panel for mental disability in patient 3 (p.W508X mutation in FOXP1). We further delineate the clinical phenotype due to FOXP1 mutations. Our and literature data evidence an emerging and recognizable syndrome. In the era of exome/genome analysis the clinical definition of phenotypes remains important to order to enable genotype-phenotype correlations.

P08.27-S
Triplet repeat-primed (TRP)-PCR changes the paradigm for Fragile X Syndrome (FXS) testing: Experience from the Greenwood Genetic Center
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Fragile X Syndrome (FXS) is the most common inherited cause of intellectual disability and is associated with a wide spectrum of neuropsychiatric symptoms. Virtually all clinical testing for FXS revolves around the determination of the length of the CGG repeat tract in the 5’UTR of the Fragile X Mental Retardation 1 (FMR1) gene. Repeat lengths generally fall into four categories, where <45 CGG repeats is accepted as normal, 45-54 repeats is the intermediate range (gray zone), 55-200 repeats is considered the premutation range, and >200 repeats is a full mutation. Diagnostic testing for FXS has traditionally employed both standard PCR and Southern blot techniques since they provide full length expansions at the FMR1 locus. The molecular diagnostic laboratory at the Greenwood Genetic Center has offered FXS testing for nearly 25 years. In 2010, we implemented a validated, lab-developed FMR1 PCR assay utilizing commercially available TRP-PCR reagents (Abbott Molecular) into our routine diagnostic workflow. Here we summarize our experience with the TRP-PCR assay, discuss how the implementation of this assay has changed our diagnostic workflow, and compare the hands-on time, cost, and turnaround time for the samples tested over the past four years to the historical data. We also discuss how the TRP-PCR assay was adapted to drastically reduce the hands-on time, turn-around time, and number of Southern blots performed for samples submitted to our laboratory for diagnostic FXS testing.

P08.28-M
A pilot study for prenatal and preconceptional Fragile-X syndrome screening in the Balearic Islands
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The Fragile X-syndrome (FXS) is caused by the expansion of a CGG triplet located in the 5’region of the FMR1 gene and is one of the most frequent
causes of hereditary intellectual disability. The estimated incidence of FXS in males in the Spanish population is 1 in 2500 with full mutation and about 1 in 250 with premutation (Fernandez-Canavajal et al. 2009). In women, although the prevalence in Spain of premutation has not been established, estimates in other western countries range from approximately 1/150 to 1/250. Given the severity of the disease, its high incidence in the general population, the exclusively maternal expansion, the familial and social impact of the FXS, and the high level of detection of current techniques (99%), we think that screening for FXS in women of reproductive age is a reliable and desirable option. Therefore, we have initiated a pilot study in the Balearic Islands to determine the feasibility and acceptability of prenatal and/or preconceptional screening in women of childbearing age. The results obtained so far, in a total of 3118 women (252 preconceptional and 2866 prenatal) indicate a high acceptability of testing both, in women that are referred for prenatal or preconceptional consultation. Surprisingly, the incidence of carriers of a premutation (55-200 repeats) is (to date) very high: 1 in 97, which may indicate a higher prevalence than previously thought. We will present updated results based on a total of approximately 3500 women.

**P08.29-S**

**GPM6A is duplicated in a patient with learning disability and influences cholesterol response and long-term memory in Drosophila melagonaster**

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In a patient with learning disability and behavioral anomalies we identified a de novo duplication of GPM6A by multicolor chromosome microarray testing. Glycoprotein M6A (GPM6A) is a neuronal transmembrane protein of the PLP/DM20 family that associates with lipid rafts and promotes filopodia formation. GPM6A variants have not yet been implicated in cognitive impairment. An increase of membrane protrusions in our patient’s lymphoblastoid cells supports a functional effect of this dosage alteration. To further study the function of GPM6A/m6 and the effects of m6 overexpression and knockdown, we employed Drosophila melanogaster as a model organism. We could show that, as described for other animal models, expression of Drosophila m6 is stress responsive. Using the courtship conditioning paradigm, we demonstrated that correct m6 levels are necessary for proper long term memory function, which indicates dosage sensitivity of m6 and supports a causative role of the GPM6A duplication for the cognitive impairment found in our patient. Defects in the close homolog PLP1 are causative for Pelizaeus-Merzbacher disease (PMD), a severe demyelinating neurodevelopmental disorder. Prompted by recent results on successful therapy of phenotypes in PMD mice by the administration of a cholesterol-enriched diet, we investigated if the cellular phenotype of GPM6A/m6 dosage alterations could also be improved by cholesterol. Indeed, cholesterol supplementation partially improves the phenotype observed in PMD flies with GPM6A/m6 overexpression as well as in flies with m6 knockdown. Together with other recent findings, these data point to an involvement of cholesterol metabolism in the pathomechanisms of some ID forms.

**P08.30-M**

**A Novel HCFC1 mutation associated with X-Linked Intellectual Disability**

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X-Linked Intellectual Disability is a heterogeneous disorder with a variable phenotypic spectrum. Currently over 90 XLD genes have been found to be implicated in XLD. The host cell factor C1 (HCFC1) gene is located on chromosome Xq28 and is a member of the host cell factor family. A mutation in HCFC1 has been previously found to be associated with XLD in a non-syndromic XLD family namely MRX3. Currently very few studies exist that further confirm HCFC1 as an XLD gene. We present an XLD family with affected sons having mild intellectual disability, epilepsy and non congenital abnormalities. Fragile X analysis and array comparative genomic hybridization (aCGH) were performed in both brothers revealed a non-synonymous mutation in exon 4 of HCFC1 (p.Ala897Val). This mutation is located within GABP2 and ZBTB17 binding domains. Previous screening studies of patients have reported variants within the GABP2 binding domain p.Gly876Ser in an individual with autism spectrum disorder (Piton et al. 2011) and p.Ala864Thr in a patient with mental retardation (Tarpay et al. 2010). The mutation was confirmed by Sanger sequencing in both patients and their mother. Extended family studies are ongoing.

**P08.31-S**

**HDAC8 duplication in a patient with de Lange-like phenotype**

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K.K., female, was referred to our attention with a previous clinical diagnosis of SilverRussel syndrome. Prenatal ultrasound were normal. No prenatal cytogenetic examination was performed. She was born at 40+5 week of gestation by normal vaginal delivery. Birth weight was 3,495 g (90th pc), length was 51 cm (<3rd pc), and head circumference was 33 cm (<3 pc). The karyotype was normal (46,XX). Major malformations were excluded. We evaluated her at 6 years and a half; she showed prominent eyes, long thick eyelashes, posteriorly rotated ears, long nose, small hands and nails, and a long neck (97th pc). She also presented severe mental retardation. Hands and feet X-rays showed hypoplasia of the 5th metacarpal and the 5th metatarsal. A CGH-array showed duplication of a part of HDAC8 gene (exon 5 and 6) from nt 71,630,467 to nt 71,697,179. HDAC8 gene was recently found implicated in Cornelia de Lange syndrome with variable clinical expressivity. Up now only complete deletion or mutation of the gene have been described. No reports of duplication of this gene are available. Our patient shows some clinical features related to de Lange syndrome also if her phenotype is not classic at all. Expression study are in progress in order to define the biological consequences of this finding and the relationships with the phenotype.

**P08.32-M**

**Clinical characterization of a patient with a complex rearrangement involving duplication and deletion of 9p and 9q**

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Rearrangements of the distal region of 9p are an infrequent chromosome imbalance in human beings. Trisomy 9p is the fourth most frequent chromosome anomaly in life-born and was characterized as a clinically recognizable syndrome, Rethoré syndrome. Kleefstra syndrome, previously named 9q submicroscopic deletion syndrome and its congenital expression, was described as a partial trisomy 9q in 46.XXY, 46.XY females with mental retardation, growth restriction, dysmorphic features, and cleft palate. We report the first case of 9q duplication and 9q deletion in a Mexican patient. Patient was referred for dysmorphic facies and congenital heart disease. Clinical examination revealed brachycephaly, upslanting palpebral fissures, depressed nasal bridge, anteverted nares, long filtrum, downturned mouth, lingual protrusion, low set and cupped ears, short neck, small hands and feet, short stature (<3rd pc) and abdominal and pelvic insufficiency. An abdominal developmental abnormality. Cytogenetic analysis showed 46,XY, +9(q34.3), +9p(q34.3), -9q.(sub). Microarray analysis showed 9p24.3p23 (203,861-11,842,172)x3, 9q34.3 (138,959,881-139,753,294)x3, 9q34.3 (139,784,913-141,020,389)x1. All procedures were normal in both parents. Partial duplication of 9p is one of the most commonly detected chromosome abnormalities in live born. It seems that the high frequency of the partial trisomy 9p may indicate a particular breakpoint sensitivity of one or more regions of chromosome 9p. Patients with partial duplication of 9p display considerable phenotypic similarity. Our patient with a duplication of 1.16 Mb, from 9p24.2 to 9p23 displays some features like upslanting palpebral fissures, downturned corners of the mouth and developmental delay. In spite of the great duplication region, the major clinical findings in our patient corresponded to the deletion region.

**P08.33-S**

**Exome sequencing in carriers of 1q21.1 CNV**

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The 1q21.1 CNV is associated with a variable clinical phenotype and normal to delayed neurodevelopment. We performed whole genome transcription and exome sequencing analysis in 5 subjects from 2 families with 1q21.1 deletion (3 subjects) and duplication (2 subjects), in search for genetic changes that could explain the phenotype. The subjects had variable learning difficulties. A pathogenic variant in the ATXf gene from 1q22-23, which plays a role in endoplasmatic reticulum stress response, was detected in the mildly affected father and his more severely affected child with 1q21.1 duplication. It was validated by Sanger sequencing and associated with reduced ATP6 RNA and protein expression in patient lymphoblast cell lines. However, the ER stress response, as measured by induction of known ER stress genes (GPR77, Dnajb9, Sdf2l1) in response to tunicamycin was not altered in pa-
tient vs control cell lines. No candidate pathogenic mutation that could be linked to altered gene expression status and/or phenotype was found in the 3 subjects with the 1q21.1 deletion, either in the CNV region or genome-wid-
ely. Interestingly, for all 5 subjects with 1q21.1 CNV a larger number of the 107 genes implicated in ER stress response showed altered expression in comparison to controls on the whole genome expression array (15-28% in subjects in comparison to 6%-in controls). Perturbed ER stress response caused either by dysfunction of genes from 1q21.1, genome wide, or both, could play a role in the observed phenotypic variability and result in even more severe developmental abnormalities in unfavourable environmental cir-
cumstances.

P08.34-M
Contribution of copy number variants (CNVs) in congenital unexplained intellectual and developmental disabilities in 149 Lebanese patients
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Chromosomal microarray analysis (CMA) is nowadays the most adopted clinical test for patients with unexplained intellectual disability (ID), de-
velopmental delay (DD) and congenital anomalies. Its use has revealed its
capacity in detecting copy number variants (CNVs) as well as regions of ho-
mozygosity which, upon their distribution on chromosomes, indicate uni-
parental disomy or parental consanguinity that is suggestive of an increase of occurrence of recessive disease. We screened 149 Lebanese probands having ID/DD and 99 healthy controls using the Affymetrix Cytoto 2.7M and SNP6.0 arrays. We reported all identified CNVs that we divided into groups and confirmed the utility of this CMA technique in the detection of parent-
al disomy or parental consanguinity. In 42 cases (28.2%), Pathogenic CNVs were identified in 36 subjects, 11.4% of the patients. We reviewed and reported the genotype/phenotype correlation in a patient with a 14q4 microdeletion, as well as defined the minimal critical regions responsible for the 10q26 and the 16q monosomy syndromes. Several likely causative CNVs were also detected, of which, new homozygous microdeletions (9p23p24.1, 10q25.2, 8q23.1) in 3 patients issued from parents with identical microdeletions (RHO size 2–6 Mb), involving genes that are reported as potential candidates. However, the clinical inter-
pretation of several other CNVs remains uncertain. Among those, 2 microdele-
tions targeting ATPRN1 and the 3’UTR of SOX5. These CNVs of unknown si-
gnificance were inherited from the patients’ normal parent, which requires a screening of more ethically matched controls in order to obtain enough evidence for their classification.

P08.35-S
Unbalanced translocations involving chromosome 4p associated with complex phenotypes: report of 3 cases
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Large imbalances of chromosome 4p, either deletions (associated with Wolf-Hirschhorn syndrome, WHS) or duplications have a defined clinical phenotype. Here, we present 2 situations of genetic abnormalities involving 4p16.3 region. Three subjects were referred for genetic testing: 2 siblings, a 16 years old boy and a 12 years old girl, both with the same phenotype, including dysmorphic features, severe mental retardation; severe speech de-
lay; hyperkinesias, aggressivity; epilepsy; skin allergic reactions. An 8 years old girl, with pre- and postnatal growth retardation, severe psychomotor retardation, spastic quadriparesis, dysmorphic features, cleft palate, bilat-
eral congenital cataract, severe epilepsy with status epilepticus episodes. aCGH on an 105K Agilent platform was done according to manufacturer’s recommendations. The results were validated by FISH. In the two siblings, aCGH revealed the same genetic abnormality: arr 4p16.3p16.1(7,247,477-
8,373,151)x3,310,977,858-135,543,178)x1 pat. The balanced t(4;10)(p16;1q26.3) translocation was detected by FISH in the genome of the patients’ father. In the second case, aCGH showed another genetic anomaly involving 4p16.3 region: arr 4p16.3p16.1(7,247,477-9,371,067)x1,8p32.5p3.2(17,864,52-8,094,773)x3. The genetic investigations of the parents were normal. The 4p region includes over 140 genes, many inclu-
ded in OMIM Database as involved in various pathologies. The 10q deleted region consists of over 60 genes, many also described in the OMIM Databa-
se. The 8p duplicated region has a size of 8 Mb and includes 116 genes. The complexity of the phenotype, both for deletion and duplication of 4p16.3 region, of these cases can be explained by the association of other genetic anomalies (10q deletion and 8q duplication, respectively). Acknow-
ledgements: project PN 09.32.023.

P08.36-M
Recurrent CNVs in 15q11.2-q12 in Bulgarian patients with generalized epilepsy and intellectual disability
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Copy number variants are frequent in autism spectrum disorders and gene-
eralized epilepsy. In this study, we performed comparative genomic hybrid-
ization assay (aCGH) using Agilent Microarray Kit, 4x180K in a preselected sample of 36 Bulgarian patients with epilepsy and intellectual disability (ID). The most frequent and most often encountered copy number changes included 15q11.2-
15q12. In 7 patients (19.4%) CNVs were located in 15q1.2. Five of them had
bored microduplications in GABRG3 gene, 1 patient showed microduplica-
tion in GABRB3 gene and in 1 patient both rearrangements were present. Arratations in 1q1.2 region were observed in 2 patients. One of them was a 0.161 Mb deletion, including SNRP/SNRNP upstream reading frame. In the other patient, a 2.608 Mb duplication covering 36 genes was revealed. Recurrent CNVs in 15q11.2-q12 in Bulgarian patients with combi-
nism severe mental retardation, different types of seizures, facial dysmorphisms, microcephaly, sensori neural hearing impairment, autistic spectrum, intellectual disability. The two patients with 15q1.2-rearrangements displayed different clinical characte-
ristics and milder forms of mental retardation. Our results are in line with the central role of GABAergic systems in ID and epilepsy. Further studies are needed to investigate the parental origin and elucidate the effect of the CNVs and the phenotype-genotype correlations. Acknowledgement: the study was supported by DTKO2/67/2009, DUNKO1-2/2009 funded by NSF.

P08.37-S
Disruption of the Methyltransferase-Like 23 Gene METTL23 Causes Mild Autosomal Recessvie Intellectual Disability
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We describe the characterization of a gene for mild non-syndromic autosomal recessive intellectual disability (ID) in two unrelated families, one from Austria, the other from Pakistan. Genome-wide single nucleotide polymor-
phism (SNP) microarray analysis enabled us to define a region of homozygo-

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Next Generation Sequencing offers the opportunity to identify new mutations in individual cases affected with moderate to severe ID. Overall, we investigated the coding sequence of 565 candidate ID genes in 996 individuals with Intellectual Disability (ID) for variants in known genes and candidate genes (ID) in order to identify the cause of disease. We observed 8225 non-synonymous variants passing quality control criteria, of which an average of 23 were de novo. Frequency and functionality based filtering reduced the number of potential candidate ID genes harboring de novo variants to 1-10 per case. In 5 cases, potential disease-causing variants were identified in genes previously implicated in ID syndromes: ARID1B and CUL4B were the most commonly observed (frequencies varied from 0.4% to 0.6%). The interpretation of the vast number of missense mutations in known genes proved to be more challenging, as rarity of variants is insufficient to assign pathogenicity in such a genetically heterogeneous population.

Although the cohort consisted of predominantly non-syndromic ID cases, the yield of mutations in genes associated with a syndromic phenotype was high, for example, ATRX, CC2D2A, ARID1B and CUL4B were among the most commonly observed (frequency varied from 0.4% to 0.6%). The interpretation of the vast number of missense mutations in known genes proved to be more challenging, as rarity of variants is insufficient to assign pathogenicity in such a genetically heterogeneous population.

Next Generation Sequencing offers the opportunity to identify new mutations and increasing the proportion of patients with ID receiving a genetic diagnosis. We have analyzed a cohort of 162 adult patients affected by ID, psychiatric disorders, and minor dysmorphic features, identifying a genetic cause in 18 cases (11% of the total). Four cases were compound heterozygous for two rare or novel variants in two different potential ID candidate genes, and in the remaining two cases, no candidates were identified. Work supported by FIS grants: PI080778 and P11/01710.

**P08.40-M**

The degree of Intellectual Disability is significantly associated with an excess of Runs of Homozygosity (ROH)

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Several recent studies focused on the effect of extended homozygosity on highly complex and polygenic traits where recessive inheritance may play an important role. Since excess of homozygosity might increase the risk for disorders like schizophrenia, Alzheimer disease and autism, we have set out a study to investigate the effect of ROHs on the degree of Intellectual Disability (ID). About 370 unrelated individuals with ID were collected and classified into mild/moderate ID (MM-ID) for IQ ranging from 35-40 to 70-75 and severe/profound ID (SP-ID) for IQ below 35-40. High-density SNP array data were processed with the aim of detecting and analyzing ROHs. Since different array platforms were used, homozygosity and ROHs mean length were compared in MM-ID vs SP-ID separately in each dataset. Results were then combined for a meta-analysis. Our data revealed an association between the amount of homozygosity and the degree of ID, according to the recent findings on autism (Gamsiz et al, 2013). Accounting for principal components to control population stratification, we tested for ROHs mean length and detected significantly (p<0.005) longer stretches in SP-ID compared to MM-ID. Weaker association was detected in burden ROH analysis, showing an increase of the percentage of genome covered by ROHs for SP-ID cases. Extent of ROHs seems to contribute to the pathogenesis of ID, suggesting that autosomal recessive variants have a crucial role on the modulation of the severity of ID that still need to be investigated.

**P08.41-S**

A familial interstitial 14 Mb deletion of 5p13p14 associated with a mild phenotype challenges the current genotype-phenotype correlation attributed to the MR III region

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Varying degrees of developmental delay or intellectual disabilities and dysmorphic features appear to be strongly associated with deletions involving 5p. Most of these deletions are associated with cri du chat syndrome. These deletions can be terminal, interstitial, or associated with complex chromosomal rearrangements. To establish the genotype-phenotype of 5p deletions many efforts had been done in the last years in dissecting the phenotype and three different critical regions had been determined. But there had been controversies about the relationship between MR and deletions involving bands 5p13.2p14.3. Deletions limited to this region were reported to show no phenotype, but to aggravate the phenotype in case of accompanying aberrations (Zang X, et al. 2005). We now observed a novel 3-generation family with a interstitial deletion del(5p)13.2p14.3 limited to MRIII without additional rare CNVs in microarray testing. The index patient, a 15 years old girl, showed learning disability (IQ 75), microcephaly, high pitched voice and a subtle facial phenotype. The patients mother and grandmother had the same deletion and likewise the learning abilities, the high pitched voice and subtle facial features, but no microcephaly. In contrast to the current literature we therefore propose that deletions limited to the MRIII region have a mild, but distinct phenotype with particular emphasis on the high pitched voice.

**P08.42-M**

Inverted duplication of 7q11.22 embedded within the 7q11.21q11.23 duplication segment in a child with stigmata dysplastica, developmental and speech delay

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Introduction: A q-arm of human chromosome 7 harbors many copy number variants (CNV) with known pathogenic significance. Chromosome 7q11.23 duplication syndrome (OMIM#609757) is a multisystem develop-
mental disorder with mild craniofacial anomalies and increased incidence of congenital anomalies. Williams-Beuren syndrome (WBS; OMIM#190450) is caused by ~1.8 Mb hemizygous deletion on chromosome 7q11.23. WBS triplication syndrome with similar but more severe clinical features has been also described.

**Case presentation:** We present a 3-years-old boy with developmental and severe speech delay, dysmorphic signs, bilateral cryptorhidism and hypoplastic corpus callosum. Cytogenetic and molecular analysis revealed a complex de novo chromosome rearrangement which we characterize as inverted triplication of 7q11.22 segment embedded within larger 7q11.21q11.23 duplication.

**Methods and results:** Molecular characterization by army-GH revealed a 1.93-Mb duplication of segment 7q11.21, 5.27-Mb triplication of segment 7q11.21q11.22 and 1.33-Mb duplication of segment 7q11.23. Further molecular cytogenetic investigation was performed with multiple combinations of specific FISH probes. Metaphase and interphase FISH confirmed the location of triplication 7q11.22 segment within the 7q11.21q11.23 duplication. Additional FISH analysis revealed that triplicated 7q11.22 segment on derivate chromosome 7 was inverted. Parental cytogenetic analysis demonstrated de novo origin of this complex chromosome rearrangement.

**Conclusions:** Although chromosomal imbalances are the major cause of developmental delay, large de novo CNVs are relatively rare events. We have identified a novel complex rearrangement on chromosome 7, duplication with inverted heterochromatic triplication also known as DUP-TRP/INV-DUP. These results demonstrate that allele drop out is a technical risk in MCP2 molecular analysis. We report a case of a patient detected during MCP2 molecular analysis in two unrelated patients with Rett syndrome. In both girls, the mutations were in exon 4 of the MCP2 gene (c.1137delC and c.1151_1201del50; c.1163C>T) and were detected as seemingly homozygous. The mutations are located in the MCP2 WW binding domain and fall in a region where a number of small deletions/single point mutations have been reported in literature.

Under the assumption of ADO, we have investigated the presence of the wildtype allele with a change in PCR condition and the primers. The complexity of the exon 4 sequence leads to allele amplification failure and that allele drop out is a technical risk in MCP2 molecular analysis.

**P08.45-S**

**MCP2 duplication in France: delineation of brain MRI abnormalities in 30 affected patients**


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Duplication encompassing MCP2 have been described in male patients with a severe neurodevelopmental disorder associated with hypotonia and spasticity, severe learning disability, stereotyped movements and recurrent pulmonary infections. We report on standardized brain magnetic resonance imaging (MRI) data of 30 affected patients, including 5 symptomatic females, carrying a MCP2 duplication, the size of which varied between 228 kb and 11.7 Mb. The aim of this study was to compare brain MRI results to detect recurrent malformations and atrophy. We found that variations in imaging features could be explained by differences in the size of the duplications. We showed that 90% of patients had brain MRI abnormalities, and that they shared certain non-specific brain malformations such as corpus callosum abnormalities (n=20), ventricular dilatation (n=9), reduced volume of the white matter (WM) (n=12), increased T2 signals in posterior periventricular WM (n=6), and vermis hypoplasia (n=5). The occipito-frontal circumference could be highly variable since it was >+2SD in 5 patients and <−2SD for 4 patients. Among the 9 patients with dilatation of the lateral ventricles, 6 (67%) had a duplication involving LI-CAM. The only patient harbouring bilateral posterior subependymal nodular heterotopia also carried a FLNA gene duplication. We could not demonstrate a link between periventricular WM hyperintensities / delayed myelination and duplication of INBKCG. These results show that patients with MCP2 duplications share certain common but non-specific brain abnormalities. These imaging features, therefore, do not constitute a diagnostic clue. We did not clearly demonstrate a genotype-imaging phenotype correlation.

**P08.46-M**

**Clinical relevance of the genotype-phenotype correlation in a patient with a de novo monosomy 22q24.1 and duplication 22q28-qter**

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We report the case of a 12 years old boy admitted to our observation in...
the first year of life with severe neonatal encephalopathy. Since birth, the clinical conditions have been worsening during time. Now he shows severe mental retardation, dysmorphic features, extreme generalized hypotonia, white matter leucomalacia, drug-resistant epilepsy, blindness, genitourinary abnormalities, generalized joint laxity and recurrent respiratory tract infections. High resolution karyotype was normal. Subtelomeric FISH probes identified a derivative 9 of an unbalanced translocation t(9p3q27) with monosomy 9p and Xq disomy. FISH studies of the parents revealed that the alterations were de novo in the patient. Array CGH was performed and identified two rearrangements: a 6Mb deletion on chromosome 9 from p24.3 to p24.1 and a 5Mb duplication on chromosome X from q28 to qter. This double rearrangement has never been described in literature. Our patient presents urogenital birth defects, facial dysmorphism (possibly correlated to the 9p deletion) and developmental regression, axial hypotonia, Hirschsprung disease, feeding difficulties, recurrent infections, epilepsy, absence of language, white matter disease (typically related to the Xq28 duplication). Several genes, included in the deleted (KANK1, DMRT1, SLCA1A) and in the duplicated region (MECP2, L1CAM), are known to have an important role in the central nervous system development. This report allows to compare the phenomics of our patient to the other cases described in literature, to contribute to the knowledge about genotype-phenotype correlation and to provide new informations for future studies about the 9p24.3-ppter and Xq28-qter regions and the genes included.

PO8.47-S Exome sequencing identifies candidate gene for MEHMO syndrome M. Skopkova1, D. Staniskova2, K. Brennerova1, J. Ukropce1, D. Danis1, M. Novotýova1, L. Tichá1, T. Kuriková1, B. Ukropce1, J. Staník1, I. Klimes1, D. Gasperová1.

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The patient is a 3,5 years young boy suffering from severe psychomotor delay, microcephaly, epilepsy, and multiple endocrine disorders (i.e. obesity, diabetes, hypogonitalism, and partial deficiency of pituitary hormones). There are no other living male members in the mother’s family, as mother’s brother and grandson’s brother died in the first months of life. The clinical picture and family history indicated towards the MEHMO syndrome, an X-linked disease which has been described in three families only. Exome sequencing of proband’s DNA revealed 23 previously unreported variants on X chromosome as confirmed also by Sanger sequencing. Haplotype analyses showed only 3 of them to be shared with proband’s mother and his brother and grandmother’s brother died in the first months of life. The clinical picture and family history indicated towards the MEHMO syndrome, an X-linked disease which has been described in three families only. Exome sequencing of proband’s DNA revealed 23 previously unreported variants on X chromosome as confirmed also by Sanger sequencing. Haplotype analyses showed only 3 of them to be shared with proband’s mother and his brother and grandmother’s brother died in the first months of life.

PO8.48-M New insights on cognitive and structural brain imaging phenotype in primary microcephaly due to ASPM mutations s. passemard1, m. schau1, m. laurent1, k. hernandez1, o. boespflug tanguy2, t. billette de villemeur3, m. elmaleh3, p. gressens4, a. verloes3, s. eliez1.

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17q12 microduplication is a very rare genomic rearrangement associated with a variable phenotype, consisting of intellectual disability/developmental delay of various degree, epilepsy, behavioral problems, brain abnormality, esophageal atresia, renal, heart and ocular anomalies. We report a case of a 9 years-old boy born from no consanguineous healthy parents.

Array-CGH analysis has revealed a 1.8 Mb de novo microduplication of chromosome region 17q12: arr[hg19]17q12 (34,906,36-36,756,170)x3 dn which the proband presents microcephaly without dysmorphic features, moderate mental retardation, language delay, learning disabilities and impulsive behavior. Brain Magnetic Resonance is normal. EEG shows multifocal spikes and waves. The microduplication extends from gene GGNBP2 to gene SORC1 and encompasses 20 genes in 17q12.

The neuroplogic phenotype seems to be associated with gene LHIX. LHX1 is expressed in the brain and is implicated in Parkin gene-cell differentiation in the developing cerebral cortex as well as in migration of motor axon to the limbs. Lhx1 knockout mice shows anencephaly. These data support the hypothesis that LHX1 is a dosage-sensitive gene, involved in neurological phenotype of patients with 17q12 microduplication. Additional studies are needed to further delineate the phenotypic impact of expression of this gene.

Several genes, included in the deleted (KANK1, DMRT1, SLC1A) and in the duplicated region (MECP2, L1CAM), are known to have an important role in the central nervous system development. This report allows to compare the phenomics of our patient to the other cases described in literature, to contribute to the knowledge about genotype-phenotype correlation and to provide new informations for future studies about the 9p24.3-ppter and Xq28-qter regions and the genes included.

PO8.50-M Syndromic intellectual disability diagnosis by combined use of MLPA kits R. Popescu1, M. Gramescu1, E. Braha1, L. Butnar1, M. Panza1, A. Sireteanu2.

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Intellectual disability (ID) is a common disorder, with major consequences for individual, family and society. Due to clinical and genetic heterogeneity, in about 50% of cases an etiologic diagnosis cannot be established. The aim of this study was to evaluate the ability of a combination of MLPA kits to establish the diagnosis in 380 patients with syndromic ID. All patients were assessed for chromosome imbalance using standard karyotyping and MLPA analysis using SALSA P064 or P096 kit, if the phenotype was suggestive for a microdeletion syndrome (subgroup A - 188 patients), or subtelomeric kits, if the phenotype was not suggestive for a microdeletion syndrome (subgroup B - 192 patients). Abnormal results detected by both MLPA kits were further characterized using appropriate follow-up MLPA kits (Telomere Follow-up set, P029-B1, P250-B2). In subgroup A we identified 27 patients with microdeletions (14.3%). In subgroup B 8 patients showed an aberrant telomeric signal detected by only one of the two MLPA kits, 31 patients showed abnormal results detected by both MLPA kits (~16%), and 153 patients had normal results. In summary, the combined use of MLPA kits led to the diagnosis in 58 out of 380 patients (15.2%). The use of follow-up MLPA kits allowed us both to confirm abnormalities and to determine their size, which facilitated the interpretation of the clinical significance of these rearrangements. For laboratories that do not have access to microarray technology, using several MLPA kits represents an effective strategy for establishing the diagnosis in ID patients.

PO8.51-S Expanding the phenotype of a recurrent de novo Mutation in PACS1 D. Bartholdi1, D. Gadzek1, D. Dickler1, M. Menzel2, B. Schmorf1, F. Stellmer1, S. Bukov1.

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The PACS (phosphofurin acidic cluster sorting) proteins represent a family of multifunctional membrane traffic regulators that mediate organ homeostasis. Recently, exome sequencing revealed identical de novo mutations in PACS1 in 3 males with intellectual disability and remarkably similar facial features (Schuurs-Hoeijmakers et al. AJHG 2012). Functional experiments indicated that the mutation exerts a dominant-negative effect by affecting the ability of PACS1 to mediate the specification and migration of SOX10-positive cells in the neural crest.

Here, we describe a third patient carrying the identical heterozygous de novo missense mutation c.698G>A; p.R233H in PACS1, and show that germline mosaicism for this mutation can account for the apparently sporadic occurrence of the disorder. The patient is a 2 year-old boy with developmental delay, congenital heart defect (atrio-ventricular septal defect), low levels of immunoglobulins and borderline microcephaly. His facial features are strikingly similar to the two patients described by Schuurs-Hoeijmakers et al.: He has hypertelorism, low-set ears, long eyelashes, downslanting palpebral fissures, a wide mouth with thin upper lips, downturned corners and a flat philtrum. His ears are low-set and rotated.

The combination of his features (congenital heart defect, hypotonia, developmental delay, facial dysmorphism) initially prompted us to analyse the genes of the RAS/MAPK pathway to study in depth the gene involved in the pathogenesis of CNS anomalies observed in our patients. The identification of modifier genes able to influence the expression of the genes implicated in well-established pathways associated to NF-κB and neurogenesis. The enrichment of strong arguments for clinical relevance, we were not able at this stage to identify the new disease gene.

We provided first data supporting the hypothesis that CNS involvement in Noonan syndrome (NS) may not be limited to the somatic manifestation (ocular, dental, hair, nail and central nervous system - CNS) anomalies, but may involve in the pathogenesis of CNS anomalies observed in our patients. The identification of CNVs allowed us to define a high number of potentially pathogenic CNVs in this family, 10/37 (27%) of which were de novo.

In summary, de novo mutation c.607C>T; p.R203W in PACS1 causes a phenotype characterized by developmental delay/intellectual disability, variable organ malformations and highly recognisable facial features.
concurrent presence of other genomic penetrant variants. These results represent an initial assumption for the application of the multi-hit hypothesis in the dissection of the N5 pathogenesis. Further studies on larger cohorts are deserved to better define the meaning and the clinical implications of these findings.

P08.56-M

NPAS3-related copy number variants: a role in developmental delay?

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Neuronal PAS domain-containing protein 3 (NPAS3) is a transcription factor expressed primarily in developing and adult brain tissues. Current evidence suggests it is involved in neuronal development and maturation, and it has recently been highlighted as potentially playing a key role in the evolution of the human brain. Clinically, NPAS3 has been identified as a candidate gene for schizophrenia, and numerous studies have supported this association. In addition, several cases of learning disabilities in patients with disruptions of this gene are reported, but this association remains relatively unexplored. Here, we present four unrelated individuals with small copy number variants (CNVs) within NPAS3: two intragenic duplications and two intragenic deletions. These CNVs range between 111-468kb in length and encompass exonic sequences within the NPAS3 gene. All four patients had variable degrees of developmental delay, and three had subtle distinctive facial features. Two patients also had macromastia and macrocephaly; while the other two had autistic features and behaviour issues, including psychosis in one individual. Individual, CNVs involving exonic sequences in NPAS3 have only been seen in one of over 19,000 controls. In summary, these four cases support an association between NPAS3 and developmental delay. This association is compatible with this gene's postulated effects on neuronal development, and suggests that abnormal function of NPAS3 may be implicated in other cases of non-specific cognitive impairment.

P08.57-S

A 9q21.3 microdeletion involving the NTRK2 gene as a possible cause of intellectual disability

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Microarray analyses identify new common copy number variants (CNVs) and microdeletion/microduplication syndromes. However, some CNVs are unique and no comparison with similar genotypes (and phenotypes) is available to assist in deciding about their causality. We identified a twelve-year-old stigmatized female patient with growth failure, microcephaly, severe psychomotor retardation, hypotonia, muscular atrophy, generalized joint deformity, and congenital heart disease. Her karyotype was normal. SNP array analysis (Illumina HumanCytoSNP-12) revealed a unique 1.7 Mb long deletion in 9q21.3 (chr9:86,595,071-88,357,495; hg19) flanked by segmental duplications. FISH analysis of the family confirmed that the aberration was de novo. It removed 5 protein-encoding RefSeq genes. The NTRK2 gene encodes a neurotrophin receptor involved in the regulation of brain development, neurotransmission, and synaptic function. Therefore, NTRK2 is a good candidate gene for intellectual disability (ID). A missense NTRK2 mutation has been described in a boy with ID and obesity, and NTRK2 was considered also in autism and other psychiatric disorders. The mouse homologue of another deleted gene, AGTIP1P1, is associated with neurodegeneration. Just one literature report exists describing a much more severely affected patient with a slightly larger deletion involving NTRK2. Remarkably, the deletion in our patient corresponds to an inversion described in several unaffected individuals. The presence of the inversion and segmental duplications at the breakpoints could indicate a specific mechanism predisposing to rearrangements. However, the inversion could not be identified in any of the parents of the patient. Supported by CHERISH, NT/14200, 00064203 and CZ.2.16/3.1.00/24022.

P08.58-M

Co-occurrence of TCF4 and FOXX1 genes deletions in a 15-year-old girl

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Both Pitt-Hopkins syndrome and a congenital variant of Rett syndrome are rare genetic syndromes with similar clinical features and development. Here we report the first case of co-existence of Pitt-Hopkins syndrome and a congenital variant of Rett syndrome. A 15-year-old girl was born after a second unforeventful pregnancy of healthy young non-consanguineous parents. Normal delivery and birth growth parameters (head circumference at the 10th percentile). Shortly after birth the girl presented with poor sleep pattern, unexplained irritability and episodes of crying, delayed development and early closure of fontanel (at 3 months). During the infancy she developed absence seizures without EEG changes. At the age of 15 years she was found to have a postnatally developed microcephaly, seizures, ataxic gait, severe intellectual disability with absent speech, drooling, self-mutilation, and stereotypic behaviour. Patient’s dysmorphic features were consistent with Pitt-Hopkins syndrome. Deep-set eyes, strabismus, prominent nasal bridge, wide mouth with everted lower lip, thenar hypoplasia, tapering fingers, fetal pads, and hypoplastic nails. MLPA analysis revealed a heterozygous deletion of exons 4b-6 of the TCF4 gene and a heterozygous deletion of the FOXX1 gene, both de novo. Chromosome analysis and SNP of the only exons array were normal. No changes were revealed by sequence analysis of TCF4. We speculate that co-existence of different gene mutations is not as exceptional as commonly thought in the field of monogenic disorders. Additionally, the contribution of each deletion to the phenotype is discussed.

P08.59-S

Exome sequencing reveals a rare TSEN54 mutation in an Iranian family with Ponto Cerebellar Hypoplasia

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Ponto Cerebellar Hypoplasia (PCH) is a heterogeneous group of autosomal recessive disorders characterized by an abnormally small cerebellum and brainstem. PCH type 2 (PCH2), the most frequently subtypes, is characterized by cerebellar hypoplasia affecting the hemispheres more severely than the vermis and progressive cerebellar atrophy, microcephaly, dyskinesia, seizures progressive microcephaly from birth, extrapyramidal dyskinesia and dystonia. This study was designed to find the genetic defect in a consanguineous Iranian family with two affected children, by means of homoygosity mapping and exome sequencing. Clinical examination of the affected individuals in this family showed microcephaly and severe intellectual disability. Cerebellar hypoplasia in brain MRI was present in one affected individual. Linkage analysis with the use of Affymetrix Axiom® Array platform revealed two promising intervals (LOD score 1.927) chromosomes 17 and 20. Exome Sequencing with 96% at 20x depth of coverage in one affected individual, detected a previously rare known homozygous missense mutation in TSEN54 gene (c.371G>T, p.G124V) which was about 9 Mbp, located in the second interval on chromosome 17. Although this is a known mutation, but it is considered as a rare mutation in PCH patients. Co-segregation analysis with the use of Affymetrix Axiom® Array platform revealed a consistent result. This data shows that combination of homoygosity mapping and exome sequencing in populations with high rate of consanguinity could be applied as an efficient technique for molecular genetics practice of clinically and genetically heterogeneous diseases.

P08.60-M

Identified A Novel Mutation in CDK5RAP2 Gene in Iranian Family with Autosomal Recession Primary Microcephaly Using Whole Exome Sequencing

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Introduction: Autosomal recessive primary microcephaly (MCPH) is a congenital disorder caused by impaired neurogenic mitosis lead to defect in brain development. This disorder is heterogeneous genetically and characterized by reduced head circumference (-2 SD or more) under age and sex-based mean and mild to severe intellectual disability. So far, 12 genetic loci (MCPH1-12) with twelve corresponding genes (MCPH1, WDR62, CDK5RAP2, CASC5, ASPM, CENPJ, STIL, CEPI3, CEP152, 2N33P5, PHC1 and CDK6) have been identified for this disease. Using whole-exome sequencing (WES) as a new pioneer technology and diagnostic tool, we can provide an opportunity for identifying the causal mutations with more efficient and cost-effectiveness in Iranian patients with autosomal recessive primary microcephaly. Case presentation: We report an Iranian family with two affected individuals with moderate intellectual disability, large toes and primary microcephaly with consanguineous marriage. We performed WES for one affected and focused on...
on genes associated with microcephaly and homoygous variants. We identified a homoygous novel frameshift mutation in CDK5RAP2 in the affected individual. Sanger sequencing confirmed the presence of the homoygous mutation in the other affected and heterozygous state for parents and normal siblings. Consensus-WESE led to cost-effectiveness and rapid identification of a novel frameshift deletion in CDK5RAP2 in Iranian families with primary microcephaly. We can facilitate genetic counseling for this family. To date, only five different mutations have been reported for CDK5RAP2 gene which most of them were from Pakistan. Moreover, this study implies that WESE is a suitable diagnostic tool for identifying mutations responsible for MCPH families.

**P08.61-S**

New case of biallelic TRMT10A deficiency identified by exome sequencing confirms the associated phenotype of primary microcephaly with intellectual disability and short stature

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Recently a mutation in the TRMT10A gene was identified in a girl born to apparently non-consanguineous parents of Kosovo origin. By exome sequencing in our patient we identified a homozygous nonsense mutation (c.379C>T) in the TRMT10A gene. of note, this is the same mutation as recently reported, introducing a premature stop codon at position 127 of the protein. Our patient presented with primary microcephaly, intrauterine onset borderline growth, mild intellectual disability and fine motor problems, a high palate with uvula bifida and minor facial features such as long narrow face with narrow palpebral fissures, long nose and small mouth. At age 4 years a seizure disorder started. Notably, at age 8 years, our patient did not yet manifest diabetes, which was of adolescent onset in the previously described family. In conclusion, our report of a novel patient confirms the phenotype of the novel syndrome associated with biallelic TRMT10A deficiency including short stature and microcephaly with intellectual disability.

**P08.62-M**

Analysis of MECP2, CDKL5, and FOXG1 genes in Czech patients with Rett syndrome and Rett-like features

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Background: Rett syndrome is a severe X-linked dominant neurodevelopmental disorder primarily caused by de novo MECP2 mutations. Clinical features include developmental regression at the age of 6-18 months, acquired microcephaly, autistic behavior, loss or severe impairment of speech and purposeful hand use, stereotyped hand movements, gait apraxia, and seizures. CDKL5 mutations have been identified in early-onset seizure variants and FOXG1 mutations in congenital variant of Rett syndrome. We report the results of mutation analysis of these genes in Czech patients with Rett syndrome and mental retardation with Rett-like features. Materials and methods: MECP2 was analyzed in 416 patients, CDKL5 was analyzed in 59 patients, and FOXG1 was analyzed in 20 patients. MECP2 and CDKL5 were analyzed by Sanger sequencing and DNA sequencing, and FOXG1 was analyzed by DNA sequencing. Large deletions and duplications were analyzed by MLPA analysis (MRC-Holland). Results: Pathogenic mutations in the MECP2 gene were identified in 45 patients with classic Rett syndrome, 7 patients with atypical Rett syndrome, 11 patients with Rett-like features, and 1 patient with autism. CDKL5 mutations were found in 2 patients and five patients with atypical and Rett-like phenotype. No FOXG1 mutation was detected in this study. Conclusions: MECP2 mutations are common in classic Rett syndrome patients, but they are less frequent in atypical or Rett-like phenotypes. However, analysis of MECP2 in these patients should not be discouraged. More patients should be examined to determine frequencies of CDKL5 and FOXG1 mutations in Czech Republic. Supported by grants NT 13120-4/2012, UCE 204011/2012, MZCR RVO-VFNo 4165/2012.

**P08.63-S**

Ageing in Rett Syndrome: Characteristics of long term survivors reported through the British Isles Rett Syndrome Survey (BIRSS)

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We report what is known about the health and wellbeing of thirty women of at least 40 years with Rett syndrome (RTT). The study is based on longitudinal data from the British Isles Rett Syndrome Survey (BIRSS). These 30 women have a clinical diagnosis of RTT: 24 women of 40-49 years, five women of 50-59 years and one older woman of 64 years. Twenty nine women were diagnosed with classic RTT and one with atypical RTT (AR). MECP2 mutations were identified in 14 of the 18 women tested. There were six missense mutations; one early truncating; two late truncating; three C-terminal deletions and two large deletions. A simplified Smeets severity score was calculated for every decade of the women’s lives. Little increase in severity was observed and, for most, the severity rating was ‘mild’. Factors contributing to disease severity have been assessed. Findings include: (1) severity of phenotype is milder among older women, indicating survival advantage; (2) depression among middle-age RTT may be a substantial but under-recognised problem; (3) menopause does not seem to occur earlier than in other women; (4) nutrition standards from the general population will often be inapplicable; (5) multiple opportunities exist to prevent functional decline through detailed attention to the quality of the medical and social care. There is a particular need to increase awareness of RTT amongst staff caring for older adults with disabilities so that they can identify and meet the needs of their adult patients with RTT.

**P08.64-M**

Italian brother and sister with familial Xp22.12 microduplication including RPS6KA3 gene and phenotype description

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RPS6KA3 gene is responsible of CoFlin Lowry Syndrome, but recent studies have demonstrated that microduplication of this gene can cause nonsyndromic X-linked ID, ADHD and localization-related epilepsy. The RPS6KA3 gene encodes a member of the ribosomal S6 kinase family. RPS6Ka2 protein is activated by MAPKs in response to growth factors, polypeptide hormones, and neurotransmitters. RSKs appear to have important roles in cell cycle progression, differentiation, and survival. Further studies have evidenced that the presence of a small amount of residual enzymatic activity may be sufficient to maintain normal osteoblast differentiation and has also been linked to cognitive performance, with higher level of intellectual function. Our case describes a brother and a sister affected by intellectual disabilities, language delay and behavioural difficulties, with more severe clinical manifestations in the affected male. Both show some dysmorphic features as very large and long nose, mild hypotelorism and dental crowding. The boy performed some instrumental exams as cerebral NMR and CT, which didn’t reveal any major cerebral malformation, but showed an absent pneumatization of the sphenoid sinus, moreover no other skeletal abnormalities were found. No seizures were reported, except during pharmaceutical sleeping EEG, some low waves in the right frontal area were identified. The boy had previously performed some molecular exam as FMR1 and COH1 mutation screening and karyotype, all normal. We performed high density SNP’s array analysis, which revealed a 512 Kb microduplication on Xp22.12 (1942.599-20.355.406) in both brother and sister. The duplication was inherited from the mother who is completely asymptomatic.

**P08.65-S**

Exploring the effect of adducins genetic variability on cognition in schizophrenia

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Schizophrenia is a chronic disease characterized by cognitive impairment. Biological bases of cognitive deficits are still poorly understood and may lie in insults in the neuropsychological process. Synapses structural proteins are claimed to have an ethiopathogenic role in schizophrenia and a more direct effect on core cognitive functions. Adducins family proteins seem of great interest, as they are fundamental constituents of synaptic structures, involved in actin cytoskeleton assembly-disassembly responsible of synaptic plasticity. In particular, ADD2 is prominently expressed in brain tissues and previous researches reported a role of this gene in memory and learn-
The power of Next Generation Sequencing in identifying mutations in non-specific ASD-ID phenotypes: the example of SHANK2


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Autism Spectrum Disorders (ASDs) comprise a range of early onset neurodevelopmental conditions of varying severity, with or without Intellectual Disability (ID), characterized by impairments in relatedness and communication, accompanied by restricted interests and repetitive stereotyped behaviors. SHANK3 haplosufficiency implicated in Phelan-McDermid 22q13 microdeletion is one of the more prevalent monogenic causes of ASD, explaining at least 0.5% of cases, but the indication of SHANK3 sequencing in patients with ASD and ID remain difficult. Here we report on two patients with de novo SHANK3 mutations identified by next generation sequencing (NGS). Patient 1, aged 15, was part of a cohort of 40 patients screened by exome sequencing for undiagnosed severe ID, and a truncating mutation was identified in SHANK3 (c.4381C>T; p.Gln1461*). Patient 2, aged 10, was part of a cohort of 106 patients with ID screened by targeted NGS of 220 ID genes, and a causative heterogeneous truncating mutation of SHANK3 was identified (c.2955_2970dup;Pro992Argfs*325). They both had normal medical measurements, severe ID, developmental and speech delay with acquisition of a few words and secondary regression with absence of speech, attention deficit and behavioral disorders necessitating treatment, autistic traits, insonnia and tantrum. Patient 1 had eating and digestive difficulties, patient 2 had distal spasticity, epilepsy from age 5, and facial dysmorphic features. These two clinical presentations appear non-specific within the ASD-ID spectrum. After reviewed the other patients with SHANK3 mutations, we argue that NGS will be helpful to determine patients with SHANK3 mutations in the absence of clear distinctive clinical features.

Interpretation of TF4 Variants Requires mRNA Splicing Analysis in Patients with Pitt-Hopkins Syndrome


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Pitt-Hopkins syndrome (PTHS) combines severe intellectual disability (ID), hyperventilation, and a characteristic facial gestalt. The disease-causing gene TF4 encodes a basic helix-loop-helix (bHLH) transcription factor. Patients carry de novo TF4 deletions, truncating mutations, or missense mutations located chiefly in the bHLH domain. Variants that do not fall into one of these categories are difficult to interpret. We describe a comprehensive mRNA analysis method for assessing TF4 variants of uncertain significance (VUS). Methods and Results: Using leukocytes from patients and minigene assays, we documented impaired splicing for the synonymous variant c.1071A>G (p.Ala357Ala), and for the only two missense variants outside the bHLH domain that are described to date, c.1073G>T (p.Arg359Cys) and c.1604A>G (p.Aspl353Glu). All assessed variants result in aberrant splicing and premature termination codons (PTC). Conclusions: All TF4 mutations reported so far in PHTS are either missense mutations located in the bHLH domain or result in a PTC. Splicing tests ought to be performed in patients with de novo TF4 VUS, especially missense mutations lying outside the bHLH-specifying domain, even in the absence of a silico prediction for a splicing defect.

A novel X-linked trichothiodystrophy associated with a nonsense mutation in RNFL113A


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Trichothiodystrophy (TTD) describes a group of rare autosomal recessive disorders that variably affect a wide range of organs derived from the neural tube. The key diagnostic feature is sparse, brittle, sulphur deficient hair that has a “tiger-tail” banding pattern under polarizing light microscopy. At the molecular level, TTD genes have a role in DNA damage repair pathways. We describe two male cousins affected by TTD with microcephaly, profound intellectual disability, sparse hair, aged appearance, short stature, facial dysmorphism, seizures, immunoglobulin deficiency, multiple endocrine abnormalities, cerebellar hypoplasia and partial absence of the corpus callosum and absence of photosensitivity. Mutations in known TTD genes were ruled out. Obligate female carriers showed 100% skew X-chromosome inactivation. Sequence analysis of a potential X-linked TTD gene, RNF113A, was performed in 100 females and 20 affected males with no available female relatives. Linkage analysis localised the disease allele to a 7.75 Mb interval from Xq23 - q25. Sanger sequencing of T373 X-chromosome, Vega genes and whole eome sequencing was used to identify a nonsense mutation in the highly conserved RNFL113A gene (c.5901T>C; p.Gln301*) ruling out other possible X-linked variants. The mutation segregated with the disease in the family and was not observed in over 10,000 control X chromosomes from public and in-house data. The mutation markedly reduced RNFL113A protein expression in extracts from lymphoblastoid cell lines derived from the affected individuals. Knockdown of orthologs of RNFL113A in model organisms strongly support a role for the gene in DNA repair and neurogenesis. The association of RNFL113A mutation with TTD identifies a new locus for these disorders on the X-chromosome.

An hiPScs based in vitro model of Angelman Syndrome and dup15 Autism


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UB3A gene maps on 15q11q13 chromosome and encodes for the protein E6AP, an ubiquitine-ligase involved in protein degradation process. The gene is maternally imprinted in central nervous system and different studies show that loss of expressed copy causes Angelman Syndrome (AS) while the duplication of 15q11q13 represents the genetic cause in 1-3% of autistic patients (dup15 Autism). Using 2 cohorts of AS and dup15 autism patients, we applied induced Pluripotent Stem cells (iPScs) technology and neuronal differentiation to identify molecular targets of a dys-regulated dosage of UB3A. To generate iPScs we used 4 canonical factors identified by Yamanaka (c-Myc, Klf4, Oct4/3 and Sox2) through retroviral or lentiviral vectors. We performed 14 attempts and in 13 we generated cells that we defined as "partially reprogrammed" because they lost their typical fibroblasts morphology, but died after passing. In the last experiment we used a commercial lentiviral vector with the same 4 pluripotency factors making a dup15 hiPScs cell line positive to pluripotent expression markers; further characterizations are still ongoing. Once we have hiPScs we will proceed with neuronal differentiation. With our experiments we confirm that the hiPScs genesis is a stochastic process with a successful rate of 0.1-2% and that no standard protocol exists. In the other hand this new challenging and arduous technology give to the scientists the possibility to understand the pathogenetic mechanisms in tissues that for obviously reasons are difficult to study.

Maternal uniparental isodisomy of chromosome 14 in a subject with mild intellectual disability


Uniparental Isodisomy (UPD) is a rare condition characterized by the inheritance of two homologous chromosome from one parent and the absence of the homologous chromosome from the other parent. Several mechanisms of UPD formation have been previously described, including trisomy rescue, monosomy rescue and gamete complementation. Problems associated to
UPD are homozgyous of autosomal recessive inherited mutations and aberrant genomic imprinting. UPD of chromosome 4 is a rare condition. To date only few cases are reported, with heterogeneous phenotype, and all were maternal. We describe a patient with mild intellectual disability and slight speech delay, harboring maternal UPD of chromosome 4 detected by high-resolution SNP-array and confirmed by microsatellite analysis. Our patient did not show any dysmorphic feature and did not present any other anomaly. To the best of our knowledge, this is the second patient carrying maternal chromosome 4 UPD that show a behavioural phenotype. Chromosome 4 maternal UPD is a sporadic and rare event, that may present little or not distinguishable phenotype. Thus, it seems unlikely that important maternally imprinted genes on chromosome 4 are involved. Because UPD is associated with a complete unmasking of recessive mutations, it is possible that such mutations could be responsible for the intellectual disability in our patient, and this hypothesis could justify the phenotypic variability among other reported cases. It will be useful to study further cases with chromosome 4 paternal UPD, in order to compare clinical phenotype and to exclude or confirm the presence of paternally imprinted genes on chromosome 4.

Mutations in the P54NRB/NONO gene cause a novel syndromic XLID with a slender build-macrocephaly gestalt

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We report on two unrelated and sporadic patients presenting a novel syndromic XLID featuring macrocephaly, severe elocution disability with open mouth breathing, high arched palate, malar hypoplasia, a thin nasal bridge with Deviated nasal septum, slender build, and scoliosis. Brain MRI showed a thick corpus callosum. High-throughput sequencing identified two distinct mutations (c.1131G->A and c.1394dup, p.Asn441Lysfs*13) in the P54NRB/NONO X-linked gene. P54NRB/NONO protein and overexpression of the two other DBHS proteins in patients' cells, whole transcriptome analysis revealed severe gene expression deregulation, and reporter assays showed reduced circadian clock amplitude. Finally, CT scans analysis of P54nrb/Nono-deficient mice demonstrated a dramatic flattening of the nose phenotype that may mimic the severe malar hypoplasia observed in patients. These findings demonstrate the existence of a recognizable XLID syndrome due to mutations in P54NRB/NONO, and highlight the crucial role of DBHS proteins in brain development and function.
at this locus. Submicroscopic duplication of Xp22.31 has been reported as either a possible cause of neurobehavioral phenotypes or a benign variant. Recently, two large cohorts of patients with the microduplication at Xp22.31 were reported. The size of the Xp22.31 duplication varied between 149 kb and 1.9 Mb among them. However, only 100 kb in size (STX11) have been characterized. We report two boys with the microduplication of Xp22.31 found by MLPA analysis. The duplication was minimum 246.2 kb in size and included STS and 1.9 Mb in size among them. The case showed developmental delay, severe mental retardation, microcephaly, craniofacial dysmorphism, and seizures. In the first case while the test was-informative in the second. Our first patient is the eldest daughter of an apparently healthy, non-consanguineous couple born at 39 weeks by cesarean section for small gestational age. The anatomical parameters were all below the 3rd percentile. At birth, brain MRI showed microcephaly, cerebral atrophy, and thinning of the corpus callosum. The patient showed hypotonia, severe feeding difficulties, and congenital heart disease. Physical examination showed macrocephaly, dysmorphic features of the face and extremities. GDI has been associated with ID and non-syndromic microcephaly in a high percentage of males. Our patients and the revision of cases from literature and DECIPHER database strengthen the GDI involvement in intellectual disability and microcephaly not only in male but also in female patients. We propose the use of a combination of clinical and laboratory parameters for early identification of patients and screening of relatives. The patient's phenotype, we set out to identify additional patients with de novo mutations in YY1, a transcription factor with an important role in various biological processes, such as proliferation, differentiation, embryogenesis, apoptosis, and tumor development. To ascertain whether the mutation in YY1 was causative for the patient’s phenotype, we sequenced the YY1 gene and identified a missense mutation in the FAM36A gene, coding for the transcription factor YY1. This mutation was detected in the patient with the clinical diagnosis of ID, low birth weight, and overlapping facial dysmorphism, including a distinctive shape of the upper lip. Conclusions - We show that de novo mutations in the zinc finger domains of YY1 are associated with ID, low birth weight, and overlapping facial dysmorphism, including a distinctive shape of the upper lip.
Klinefelter syndrome: 48, XXXY aneuploidy in a patient with mild mental retardation and psychomotor personality traits

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Klinefelter syndrome is the most common aneuploidy in males with a prevalence of 0.1-0.2% in the general population, which rises up to 3% in males with fertility issues, although only 35% of cases are diagnosed. The affected males tend to be tall, have narrow shoulders, wide hips, sparse body hair, gynecomastia and small testes; they present androgen deficiency and azoospermia. Besides the previous physical characteristics, there have been reports of the insufficiency of behavioral and cognitive traits that tend to be very variable, possibly according to the type of aneuploidy, with an established association between the number of extra X chromosomes and cognitive deficit, and a not so clear association of different chromosomal variants of Klinefelter and psychomotor behavior, with some authors proposing the origin of the extra chromosome as a determinant of different behavioral traits. Here is presented the case of a 13 years old male with karyotype 48,XXXY, who besides presenting the classical physical features, is under psychiatric treatment because of presenting trouble at home and at school for being aggressive and impulsive. A review on the literature is made, concluding on the importance of an opportunely starting of hormonal therapy and a multidisciplinary approach including endocrinology, pediatrics, genetics, neuropsychology and psychiatry when needed.

P09.001-S

4H (Hypomelination, Hypodontia and Hypogonadotropic Hypogonadism) syndrome caused by POLR3B mutations

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Hypogonadism) syndrome caused by POLR3B mutations has an incidence of 0.1-0.2% in the general population, which rises up to 3% in males with fertility issues, although only 35% of cases are diagnosed. The affected males tend to be tall, have narrow shoulders, wide hips, sparse body hair, gynecomastia and small testes; they present androgen deficiency and azoospermia. Besides the physical characteristics, there have been reports of the insufficiency of behavioral and cognitive traits that tend to be variable, possibly according to the type of aneuploidy, with an established association between the number of extra X chromosomes and cognitive deficit, and a not so clear association of different chromosomal variants of Klinefelter and psychomotor behavior, with some authors proposing the origin of the extra chromosome as a determinant of different behavioral traits. Here is presented the case of a 13 years old male with karyotype 48,XXXY, who besides presenting the classical physical features, is under psychiatric treatment because of presenting trouble at home and at school for being aggressive and impulsive. A review on the literature is made, concluding on the importance of an opportunely starting of hormonal therapy and a multidisciplinary approach including endocrinology, pediatrics, genetics, neuropsychology and psychiatry when needed.

P09.002-M

A familial 9q22.1q22.31 deletion of 3.8 Mb, challenging interpretation of large, inherited copy-number variation

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Chromosomal microarray analysis are now the first-line diagnostic test for patients presented with intellectual disability, autism, or multiple congenital anomalies. An ongoing challenge for the clinician and biologist is the interpretation of copy number variation. Many tools have been proposed in order to facilitate their interpretation including linking data to several genome browsers, gene contains and prioritization, inheritance of the copy-number variation (CNV) and the size of the variant. Indeed, large CNV (>500 kb) are supposed to be strongly associated with morbid consequences. We report on a large 9q22.1q22.31 deletion of 3.8 Mb identified by whole genome SNP array (HumanCytoSNP-12, Illumina) in a 6 year old girl presen- ted with growth retardation and Attention-Deficit/Hyperactivity Disorder (ADHD). Neuropsychological evaluation excluded a mental retardation. The deletion encompassed 24 genes, was inherited from her apparently normal father. Nevertheless, the familial history was marked by hyperactivity and dyslexia in the father’s childhood. The older sister presented also with ADHD and carried the same deletion. Grandparents will be further analyzed. We discuss the implication of this CNV in the abnormal phenotype of the two sisters. Our observation highlights the difficulties of interpretation of CNV particularly in case of large CNV inherited from an unaffected parent. Moreover, regarding ongoing discussions about application of microarray in prenatal diagnosis, our case allowed us to postulate that the size is not sufficient to prejudge the pathogenicity of the CNV.

P09.003-S

aCGH for neurological disorders in paediatric population at Clinical Institute of Medical Genetics, UMC Ljubljana

M. Volč1, L. Lovrecic1, S. Bertoli2, B. Glavač2, D. Glavač2, M. F. Hernandez-Amaris2, M. Faletra2, F. Faletra2, B. Rogelj1; 1Clinical Institute of Medical Genetics, UMC Ljubljana, Ljubljana, Slovenia; 2Department of Paediatric Endocrinology, Diabetes and Metabolic Diseases, University Children’s Hospital, UMC Ljubljana, Ljubljana, Slovenia; 3Department of Child, Adolescent & Developmental Neurology, University Children’s Hospital, UMC Ljubljana, Ljubljana, Slovenia.

Array comparative genomic hybridization (aCGH) is a quick method to analyze the whole genome for imbalances at a higher resolution. In conventional diagnostic settings aCGH is used as a first-tier diagnostic test in the group of patients with unexplained developmental delay (DD) and/or idiopathic intellectual disability and/or dysmorphic features and/or multiple congenital anomalies.

AIM: Our aim was to evaluate the diagnostic yield/clinical utility of aCGH testing for the group of children with neurological disorders at Clinical Institute of Medical Genetics Ljubljana, tested in 2012-2013.

PATIENTS AND METHODS: We included 244 children, 91 with DD and/or dysmorphic features, 44 with epilepsy, 34 with autism, 45 with developmental abnormalities of the CNS, and 30 with abnormal muscle tone. DNA was hybridized to Agilent 180K or 60K Human CGH microarrays. Discoverable genomic imbalances were interpreted according to the data in Internet databases (ISCA, DECIPHER, ECARUCA) and scientific publications cited in PubMed.

RESULTS: Altogether, 37 pathogenic copy number variations (CNVs) were found that could explain the phenotype of patients (Table 1). In addition, 26 variants of unknown significance (VUS) were detected, most of them de novo. Eleven patients (4,5%) had complex CNVs.

CONCLUSION: We found out that pathogenic CNVs were causative in 15% of cases with neurological disorders with/without dysmorphic features or CNS developmental abnormalities. Our data demonstrate that aCGH is an important diagnostic tool in neuropediatrics.

Table 1. CNVs in different phenotype groups

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Number of tested patients</th>
<th>Pathogenic CNV</th>
<th>VUS</th>
<th>Diagnostic Yield (pathogenic CNV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DD with or without dysmorphic features</td>
<td>91</td>
<td>22</td>
<td>10</td>
<td>24.2%</td>
</tr>
<tr>
<td>Developmental abnormalities of the CNS</td>
<td>45</td>
<td>6</td>
<td>6</td>
<td>11.1%</td>
</tr>
<tr>
<td>Abnormal muscle tone</td>
<td>36</td>
<td>2</td>
<td>4</td>
<td>6.7%</td>
</tr>
<tr>
<td>Epilepsy</td>
<td>44</td>
<td>6</td>
<td>3</td>
<td>13.0%</td>
</tr>
<tr>
<td>Autism</td>
<td>36</td>
<td>3</td>
<td>3</td>
<td>9.9%</td>
</tr>
</tbody>
</table>

P09.004-M

Genetic analysis of amyotrophic lateral sclerosis in the Slovenian population

K. Vrabc1, D. Glavač2, B. Rogelj2, M. Rovanik-Glavac2; 1Faculty of Medicine, Ljubljana, Slovenia; 2Institute Jolof Stefan, Ljubljana, Slovenia.

Background: Amyotrophic lateral sclerosis (ALS) is a complex neurodegenerative disease characterised by progressive degeneration and loss of upper and lower motor neurons in the cerebral cortex, brainstem and spinal cord leading to death due to respiratory failure within 2-5 years from onset. The most common genes involved in the disease process are SOD1, FUS, TARDBP and C9ORF72.

Patients and methods: Blood samples from 72 Slovenian ALS patients were collected and the exons of genes of interest were PCR amplified followed by Sanger sequencing on ABI310 Genetic Analyzer. In case of C9ORF72 repeat-primed PCR was performed followed by fragment length analysis on ABI310 Genetic Analyzer. Results were analyzed using Gene Scan software.

Results: Genotyping of genes SOD1, FUS, TARDBP and C9ORF72 revealed some changes in the DNA sequence. In SOD1 gene two mutations (V15M and F50V) were detected. Sequencing of FUS and TARDBP genes did not reveal any amino acid changes. Although two substitutions were detected, R522R (G>A) in FUS and L330L (A>G) in TARDBP, the latter was not previously described. In 2 ALS patients we detected expansion of repeats in the first intron of C9ORF72.

Conclusions: This study represents first genetic analysis of Slovenian ALS patients. Our findings are in concordance with other studies provided on European ALS patients. Of these four analysed genes repeat expansion in...
Alzheimer’s disease (AD) is a multifactorial neurological condition associated with a genetic profile that is still not completely understood. In this study, using a whole microarray approach, we investigated age-dependent gene expression changes occurring in the hippocampus of young and old transgenic AD (3xTg-AD) and wild type (WT) mice. The aim of the study was to assess similarities between aging- and AD-related modifications of gene expression and investigate possible interactions between the two processes.

Global gene expression profiles of hippocampal tissue obtained from 3xTg-AD and WT mice at 3 and 12 months of age (m.o.a.) were analyzed by hierarchical clustering. Interaction among transcripts was then studied with the Ingenuity Pathway Analysis (IPA) software, a tool that discloses functional networks and/or pathways associated with sets of specific genes of interest.

Cluster analysis revealed the selective presence of hundreds of upregulated and downregulated transcripts. Functional analysis showed transcription involving neuronal death and apoptosis, mitochondrial function, calcium homeostasis, inflammatory response, dendritic spine formation, modulation of synaptic functioning, and cognitive decline.

Thus, over-expression of AD-related genes (such as mutant APP, PS1, and tau, the three genes that characterize our model) appears to favor modifications of additional genes that are involved in AD development and progression.

The study also showed overlapping changes in 3xTg-AD at 3 m.o.a. and WT mice at 12 m.o.a., thereby suggesting altered expression of aging-related genes that occurs earlier in 3xTg-AD mice.

Abbreviations: AD, Alzheimer’s disease; 3xTg-AD, triple transgenic amyloid-beta, tau, and presenilin-1 mice; WT, wild type; APP, Amyloid Precursor Protein; PLA2G6, Phospholipase A2 Group 6; PS1, Presenilin-1; GSK3, Glycogen synthase kinase 3; PS2, Presenilin-2; BACE1, BACE-1; C9ORF72, coding region of the chromosome 9 open reading frame 72; XCI, X chromosome inactivation.

While amyloid-beta-protein (Aβ) has been implicated in development of Alzheimer’s disease, the exact functional role of amyloid precursor protein (APP) is still unclear. In our study, neuroumusrual junction of transgenic Drosophila melanogaster lines was used as a model to analyze changes in purinergic P2 receptor function caused by Aβ overexpression. Miniature excitatory junction potentials (mEJPs) were recorded intracellularly from muscles 6 and 7 in third instar larvae at room temperature in HL3 solution. Confo- mation microscopy with cytchemistry was also used. It was observed that the mEP amplitude distribution in control was bimodal with peaks at 0.46 mV and 0.74 mV; only rare giant mEPs were observed. Human APP gene expression in motor neurons decreased mean mEPs frequency (p<0.01) from 2.5/6 in control up to 1.6/6, while fraction of giant mEPs (mean amplitude 1.41 mV) increased up to 29%. In addition, enhanced axon branching and decreased expression of synaptobrevin was observed. Co-expression of human APP and β-secretase genes (production of Aβ and decrease in APP level) recovered both mean mEPs frequency and quantity of giant mEPs to control values. The resting membrane potentials, time course of mEPs as well as peaks of their amplitudes bimodal distribution never differs significantly from control line. Distributions of mEPs latencies in all Drosophila lines were best fitted as a mono-exponential decay as predicted by the Pois- son model. Our data suggests that APP overexpression disturbs molecular machinery of vesicular exocytosis without alteration in the random nature of spontaneous release. Supported by St.Petersburg State University grants #15.01.621.2013 and #13.08.231.2014.

**P09.008-M**

An increase in X chromosome aneuploidy rates in the Alzheimer’s disease brain can hallmark both neurodegeneration and aging

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Post-zygotic mosaic aneuploidy is common in the normal and diseased human brain. Somatic chromosomal mosaics in affecting the brain is, probably, the result of disturbances of ault neurogenesis/angiogenesis during the early ontogeny. It was proposed that aneuploidy of the brain is somehow involved in pathogenesis of neurodegenerative brain disorders including Alzheimer’s disease (AD). Here, we have analyzed X chromosome aneuploidy (a feature of aged cell populations) in the brain tissues of females with/without AD. Molecular cytogenetic analyses were performed by multiprobe/quantiative FISH and interphase chromosome-specific multicolor banding (ICS-MCB) in 10 AD and 10 age/sex matched control samples, scoring 160,000 cells per each sample set. In AD, the mean rate of aneuploidy (2.79%, 95% CI 1.88-3.69) was two times higher than in control (1.32%, 95% CI 0.92-1.71%); P =0.013 (Mann-Whitney U-test). An AD sample demonstrated mosaic aneu- ploidy (X chromosome loss) confined to the hippocampus in about 10% of cells. More than 75% of cells with X chromosome aneuploidy were NeuN-negative (non-neuronal cells: glia and, probably microglia). These preliminary data indicate that somatic (post-zygotic) chromosomal instability causes large-scale genomic variation in a significant proportion of hippocampal cells in AD. In context of a causal relationship between brain aging and AD pathogenesis, our findings suggest that X chromosome aneuploidy can con- tribute to both brain aging and neurodegeneration in females with AD. We speculate that mosaic aneuploidy in the brain is a new non-heritable genetic factor predisposing to AD. Supported by BLR 11/002, the Russian Federation President Grant (MD-4401.2013.7), and RFBR 12-04-0021.

**P09.009-S**

Genetic analysis of Angiogenin gene in Italian patients with Alzheimer’s disease

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Background. Despite enormous investigative efforts, the pathological basis for Alzheimer’s disease (AD) remains unclear. Somatic mosaic aneuploidy that AD is mediated by pathological angiogenesis. Angiogenin is a angiogenic ribonuclease whose activity is related to its ability in regulating ribosomal RNA (rRNA) transcription [Cronin 2006]. Mutations in the coding region of ANG (Entrez Gene ID 283) have been detected in Amyotrophic Lateral Sclerosis (ALS). The role of p35/CDK5 regulation by miR-15/107 family in P09.006-M

**P09.006-M**

The role of p35/CDK5 regulation by miR-15/107 family in Alzheimer’s disease

S. Moncini, M. Langhi, A. Valmadrer, F. Fontana, M. A. Dentí, P. Riva, M. Venturini

1Dipartimento di Biotecnologie Mediche e Medicina Traslazionale - Università degli Studi di Milano, Milan, Italy, 2Centre for Integrative Biology, Università degli Studi di Trento, Trento, Italy.

Alzheimer’s disease (AD) is characterized by the presence of β-amyloid plaques and neurofibrillary tangles of hyperphosphorylated Tau. CDK5 in- hibition has a key role in abnormal phosphorylation of Tau and β-Amyloid Precursor Protein (APP). CDK5 is activated by p35, encoded by CDK5R1, whose expression can be modulated by miR-103/107, members of the miR-15/107 family, a group of microRNAs involved in AD pathogenesis. In our study, we observed a significant reduction of p35 levels in cells trans- fected with miR-103/107, -15a and -16 precursors compared to control, while the transfection with antisense LNA molecules for miR-15/107 group leads to increased CDK5R1 transcript and p35 levels, suggesting the action of the whole miR-15/107 family on CDK5R1 expression. We thus hypothesize that levels of p35 in AD can lead to Tau and APP hypophosphorylation via upregulation of p35 levels and con- sequent enhanced CDK5 activity. In order to test this hypothesis, we are stu- dying CDK5R1 and miR-15/107 expression and the CDK5 activity on Tau and APP in frozen brain tissues (hippocampus, temporal cortex and cere- bellum) from 12 AD patients and 7 control individuals. In the temporal cortex and hippocampus most miRNAs are downregulated in AD compared to control samples, while in the cerebellum all miRNAs show a similar expres- sion between AD patients and controls, with the exception of miR-107 and miR-15a which are more expressed in AD patients. Interestingly, CDK5R1 miRNA levels are higher in AD hippocampus, but not in temporal cortex and cerebellum tissues, compared to controls.

Project supported by FIRB2008 grant (RBFR0895DC_002).

**P09.007-S**

Human APP overexpression alters spontaneous quantal release at Drosophila larval neuromuscular synapse

A. N. Vasiliev, E. A. Gusova, G. A. Kislik, V. V. Broutsova

1Saint-Petersburg State University, Saint-Petersburg, Russian Federation, 2BP Kontantinov Petersburg Nuclear Physics Institute, National Research Centre ‘Kurchatov Institute’, Gatchina, Russian Federation.

C9ORF72 remains the most common cause of ALS although for the majority of cases the main causes are yet to be revealed.
were used in this study.

CGH analysis. In all cases previous standard karyotype as well as other gene-further pathological manifestation, 50 undiagnosed cases underwent array-
syndromic microcephaly of unknown etiology, accompanied by at least one (array-CGH), as a diagnostic tool for the study of patients with syndromic
the application of high resolution array-comparative genomic hybridization

ADAM23 is a potential risk gene for epilepsy. synaptic transmission. Based on the genetic association and gene function,
known causative genes for Mendelian forms of epilepsy, and plays a role in epilepsy in several dog breeds. ADAM23 interacts with LGI1 and LGI2, both studied breeds. This indicates that there is a common genetic risk factor for

all variants located in the ADAM23 gene region. Twenty-seven variants unique for the cases were identified in the sequencing based on homozygosity for the risk and non-risk haplotypes. locus. Twelve Belgian Shepherd cases and twelve controls were selected for this locus further, we performed targeted next-generation sequencing at the
in the Belgian Shepherd breed on CFA37 in a genome-wide association stu-

Dog as an animal model for idiopathic epilepsy: identification of common risk variants in the ADAM23 gene

Relatively few risk genes have been identified for common IEs to date. We have used dog as an animal model for human focal and generalised epilepsy to identify disease risk genes. The seizure characteristics are similar be-
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twelve Belgian Shepherd cases and twelve controls were selected for the sequencing based on homozygosity for the risk and non-risk haplotypes.

Thirty-six variants unique for the cases were identified in the sequencing experiment.

All variants located in the ADAM23 gene region. Twenty-seven variants were selected for validation in 235 cases and 320 controls from four dog breeds. Association analysis yielded a strong signal at the locus (p = 5.3e-11). Haplotypic analysis suggests a common risk haplotype in all studied breeds. This indicates that there is a common genetic risk factor for epilepsy in several dog breeds. ADAM23 interacts with LGI1 and LGI2, both known causative genes for Mendelian forms of epilepsy, and plays a role in synaptic transmission. Based on the genetic association and gene function, ADAM23 is a potential risk gene for epilepsy.

High resolution array Comparative Genomic Hybridization (array-CGH) in syndromic microcephaly: clinical interpretation and genotype-phenotype correlation

Microcephaly can be either isolated with no additional anomalies, or it may coexist with other neurological entities and/or multiple congenital anomalies, known as syndromic microcephaly. Although many syndromic cases can be classified based on the characteristic phenotype, some others require further investigation. Aim: The present study describes the application of high resolution array-comparative genomic hybridization (array-CGH) as a diagnostic tool for the study of patients with syndromic microcephaly in order to identify clinically relevant copy number variati-
on arrays (CNVs). We evaluate the MRI findings with patients’ clinical profile and the underlying molecular findings. Material and Methods: From a cohort of 210 unrelated patients, who were referred for genetic evaluation due to syndrome of microcephaly of unknown etiology, accompanied by at least one further neurological/developmental abnormality, 100 cases underwent array-CGH analysis. In all cases previous standard karyotype as well as other gene-
tests were negative. High resolution 4x180K and 1x24K Agilent arrays (> 170,000 and > 236,000 probes respectively, average resolution 8.9Kb) were used in this study. Results: In 32 out of 50 patients (64%) featuring microcephaly among other phenotypic anomalies, array-CGH revealed si-
nificant aberrations (microdeletions and/or microduplications) ranging in size from 0.015 to 3.16 Mb and encompassing important genes related with syndromic microcephaly. 25/50 patients (50%) had abnormal MRI findings and in 19 of 25, arrays-CGH revealed pathogenic CNVs. Conclusion: Array-CGH contributes to the elucidation of undefined syndromic microcephaly cases, by permitting the discovery of plausible novel microdeletion and/or microduplication syndromes and contributing to more precise genotype-phenotype correlations.

Exome sequencing reveals a new CLN5 mutation in an adult form of hereditary spastic ataxia

This study suggests that ANG gene mutations may be associated with rare cases of AD. Interestingly, the K73X mutation may be specific for AD, since it has not been found in the numerous genetic studies on ALS and PD so far reported, proposing an important specificity mutation-disease.

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Martorell, Spain.

-confirms the safety of infusion therapy suggesting that Erydex treatment (range -4 to -19) was detected in the patients of the extended study, while of four AT who discontinued. A mean ICARS score reduction of 11 points is 0, while the worst 100. Significant improvements in ICARS were seen in patients treated for up to one month.

-To avoid side effects of long-term steroid administration we developed a method for encapsulation of dexamethasone sodium phosphate into autologous red blood cells. By additional encapsulation, we aimed to provide a stable, safe and effective drug delivery system.

Autosomal dominant spinocerebellar ataxias (SCAs) are a clinically, genetically and pathologically heterogeneous group of movement disorders defined by variable degrees of cerebellar ataxia and often accompanied by additional cerebellar and non-cerebellar symptoms. The clinical symptoms are triggered by neurodegeneration of the cerebellum and its relay connections. Recently we report the clinical and genetic findings from Spain to define the phenotype of the 901 family. A cerebellar phenotype characterised by ataxia, atypical early altered vertical eye movements, variable severity, no evidence of anticipation, age at onset ranging from 35 to 64 years and Cranial CT or MRI showing cerebellar atrophy in several patients without brainstem involvement. We have found significant linkage with the highest two-point LOD score $Z_{\text{LOD}} = 3.831$ at theta $= 0.00$ ($P < 0.001$) between the locus tag D1S2472, assuming an age-dependent penetrance model with 5 affected members over a total of 17 individuals, which show a multipoint lod score of 3.0 to respect the same genetic markers used in the 901 family. This family comes from the same region than the previous 901 and probably has a common founder ancestor. The linkage results from this pedigree reinforces the hypotheses of linkage from the same locus tag trait localized in 1p and named SCA37.

Reduction of neurological symptoms in Ataxia Telangiectasia patients by Intra-Erythrocyte infusion of Dexamethasone (EryDex)

Ataxia Telangiectasia (AT) is a neurodegenerative disorder characterized by early onset ataxia, oculocutaneous telangiectasias, immunodeficiency, recurrent infections, radiosensitivity and cancer proneness. No drug therapies are approved for treating AT; recent observational studies showed beneficial effect of steroid treatment.

To avoid side effects of long-term steroid administration we developed a method for encapsulation of dexamethasone sodium phosphate into autologous erythrocytes (EryDex) allowing the slow release of dexamethasone for up to one month. The efficacy and safety of EryDex treatment was evaluated in an open-label, single-arm study on 22 AT patients (FM=1) confirmed molecularly, with a monthly infusion for six consecutive months. Primary efficacy assessment was the International Cooperative Ataxia Rating Scale (ICARS) which best score is 0, while the worst 100. Significant improvements in ICARS were noted in the intention to treat (ITT) population (n=22, p=0.02) and in patients completing the study (PP, n=18; p=0.01), with a mean reduction of 4 points for ITT and 5.2 points for PP. EryDex was well tolerated, without steroid side effects.

At the end of the trial four patients continued Erydex treatment for 27 months (until now) and their ICARS variations were compared to those of four AT who discontinued. A mean ICARS score reduction of 11 points (range -4 to -19) was detected in the patients of the extended study, while a mean increase of 7 points (range +1 to +14) was found in controls. This confirms the safety of infusion therapy suggesting that Erydex treatment could delay the progression of the disease.

Eight new ATM gene alterations in Polish patients with ataxia-telangiectasia

Finding patients with Parkinson’s disease (PD) which are heterozygotes for ATP7b gene in Russia

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Finding patients with Parkinson’s disease (PD) which are heterozygotes for ATP7b gene in Russia
Introduction

Attention-deficit hyperactivity disorder (ADHD) is one of the most common neurodevelopmental disorders of childhood, characterized by age-inappropriate levels of inattention, hyperactivity and impulsivity. Despite its high heritability (76%), the results of association studies have been inconsistent. The way in which ADHD is transmitted was described to have a Spanish cohort, the association of previously reported gene variants. Patients and Methods: 324 children and adolescents (6-17 years old) diagnosed with ADHD according to DSM-IVTR, and 344 controls were recruited. Patients and controls were genotyped for 23 genetic variants: DAT1; rs2550948, rs261759, rs2652511, VNTR-3UTR, VNTR-Intron-8; HTR2A; rs7322247, NET1; rs2350860, rs5569; ADRB2; rs553668, rs1800544, LPHN3; rs197458, rs2035339, rs6551655; COMT; rs46480, FADS2; rs498793, SNP23: rs746544, DDC; rs6592961. Pearson’s X^2 test, and Hardy-Weinberg equilibrium were used to assess genetic association. Results: The most significant associations found in this study were: NET1-rs2350860 A/T genotype was significantly increased in both ADHD patients (OR:1.54, p: 0.0027) and combined-ADHD subtype (OR:1.72, p:0.0043). DAT1-5/5 genotype resulted increased in inattentive-ADHD patients (OR:2.13, p:0.052) Discussion and Conclusion: The difficulties found identifying risk ADHD associated gene variants could be explained by the heterogeneity and complexity of this disorder. Further studies designed to examine the contribution of gene-gene or gene-environment interaction are needed. In addition, the use of endophenotypes instead of DSM-V diagnoses could improve the detection of genetic effects.

P09.019-S
Variation in the phenotype of Machado-Joseph disease (MJD/SCA3): the role of mitochondrial DNA (mtDNA) haplogroups
A. Ramos1,2, C. Santos1, M. Raposo1,2, M. de Araújo1,2, M. Lima1,2
1,2, C. Bettencourt3, G. Coppola4, A. Ramos1,2, N. Kazachkova1,2, A. Rodrigues5,6, A. Marabotti1, L. Milanesi1,2, 1Institute of Biomedical Technologies, National Research Council, Segrate (Milano), Italy, 2Institute for Molecular and Cell Biology (IBMC), University of Portoro, Portugal, 3Unitat d’Antropologia Biológica, Departament BABVE, Universitat Autònoma de Barcelona, Barcelona, Spain. Mitochondrial dysfunction has been implicated in the pathogenesis of several neurodegenerative disorders, such as Machado-Joseph disease (MJD), a late onset poly-Q ataxia that results from an unstable expansion of a CAG tract in the ATXN3 gene. The CAG expansion size is incompletely correlated with the age at onset, highlighting the existence of genetic modifiers. Although the way by which mitochondria is involved in the neurodegenerative cascade is still unknown, mitochondrial DNA (mtDNA) haplogroup-specific polymorphisms have been associated to other poly-Q disorders. To evaluate whether mtDNA variation contributes to MJD phenotype, namely to age at onset (AO), we determined the mtDNA haplogroups in 113 MJD patients with Azorean ancestry, by sequencing the mtDNA hypervariable region I. The frequency of mtDNA haplogroups in unrelated patients (n=68) was similar to the one previously described for the general Azorean population. MJD patients classified as haplogroup I present a significantly earlier onset age (mean±SD: 39.4±33 of 33 years). Haplgroup W seems to have a protective effect, causing a delay in onset (mean±SD: 51 years). Although haplogroup I has already been implicated in other neurodegenerative disorders, there are no previous reports of an association between haplogroup W and disease. In conclusion, our results suggest that mitochondrial single nucleotide polymorphisms defining these haplogroups could modify AO in MJD. The complete mitochondrial genome of MJD patients classified as: Jand W haplogroups were further sequenced in order to better understand the impact of the haplogroup-defining variants on mitochondrial function and their interaction in MJD phenotype.

P09.020-M
Transcriptional profile of Machado-Joseph Disease (MJD): mTOR signaling pathway is dysregulated in blood cells of patients
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Machado-Joseph disease (MJD); MM #109150; ORPHA98757) or spinocerebellar ataxia type 3 (SCA3) is a protein misfolding-associated disease, being the worldwide most prevalent autosomal dominant ataxia as well as the second most common polyglutamine (polyQ) disorder. Abnormal conformation of mutated ataxin-3, promotes a gain of a toxic function compromising several cellular mechanisms, namely transcription. Gene expression profiling arrays have the potential to contribute to advances in the understanding of new mechanisms related with disease pathophysiology, often suggesting new therapeutic targets for treatment. In MJD, however, the analysis of gene expression changes has been limited to animal and cellular models; thus far, transcriptional changes in MJD patients have not been investigated. In the present study we used microarrays to evaluate a global gene expression profile in blood samples of 12 MJD patients, compared with 12 normal controls. Ingenuity pathway analysis (IPA) software was used to analyze the most dysregulated pathways in MJD. Thirty pathways were found to be dysregulated (log p-value False Discovery Rate (FDR) adjusted > 3); amongst the dysregulated canonical target of rapamycin (mTOR) signaling is observed, being significantly dysregulated expression levels in 211 genes (p-value FDR adjusted < 0.05). mTOR has been described as influencing a variety of molecular processes including protein synthesis and autophagy. Moreover, rapamycin, which inhibits the activity of mTOR, is points as having neuroprotective effects in several neurodegenerative disorders, mainly by induction of autophagy. The present study highlights the potential of mTOR inhibitors as potential therapeutic compounds that should be investigated for MJD.

P09.021-S
Three SNP haplotypes in Neuroligins may correlate to autism susceptibility
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Autism is a neurodevelopmental disorder showing a striking sex bias with a male/female ratio of 4:1. Despite some genetic variants are causative for autism in about 17% of cases, its etiology is still largely unknown. Increasing evidences highlight the possible role of environmental factors, such as infections, xenobiotics and drugs in enhancing autism genetic susceptibility. Among genetic variants, we focused on the X-linked neuroligins that are involved in synaptic plasticity, are mutated in a few number of autistic patients, and are hemizygous in males. Hence we analyzed NLGN-3 and NLGN-4X in 52 Italian autistic cases (male/female=4:6.1) and in 31 healthy siblings (male/ female=1:1.1) by Sanger sequencing. Among the other variants, in NLGN-4X we found 2 de novo SNPs and 3 SNPs in non-coding regions (1 intronic and 2 in the 3’UTR), giving 2 different haplotypes: one was new and one was already described in a non-specific mental retardation Chinese patients. In NLGN-3, we found 3 already described intronic SNPs in haplotype block. The 3 haplotypes have statistical significance in genetics comparing to the minor allele frequencies (MAF) from the 1000-Genomes Project CEBI. Interestingly, healthy siblings, half of which were female, have a middle statistical significance between autistics and MAF-CEU. As these SNPs map to non-coding regions, they could be involved in the genetic susceptibility to triggering environmental factors (probably lacking in siblings), and, being located in non-coding regions, could modulate the main prevalence of autism. ACKNOWLEDGEMENTS: Italian Ministry of Health “GR-2009-1570296” project, Italian Ministry of Education, University and Research “InterOmics” project.

P09.022-M
Diagnostic utility of microarray analysis to detect rare CNVs in autism spectrum disorders
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Background: The genetic causes of Autism Spectrum Disorder (ASDs) are heterogeneous and still unknown in the majority of cases. Copy number variations (CNVs) are the most common genetic cause of Autism in patients and are hemizygous in males. Hence we analyzed NLGN-3 and NLGN-4X, we found 2 de novo SNPs and 3 SNPs in non-coding regions (1 intronic and 2 in the 3’UTR), giving 2 different haplotypes: one was new and one was already described in a non-specific mental retardation Chinese patients. In NLGN-3, we found 3 already described intronic SNPs in haplotype block. The 3 haplotypes have statistical significance in genetics comparing to the minor allele frequencies (MAF) from the 1000-Genomes Project CEBI. Interestingly, healthy siblings, half of which were female, have a middle statistical significance between autistics and MAF-CEU. As these SNPs map to non-coding regions, they could be involved in the genetic susceptibility to triggering environmental factors (probably lacking in siblings), and, being located in non-coding regions, could modulate the main prevalence of autism. ACKNOWLEDGEMENTS: Italian Ministry of Health “GR-2009-1570296” project, Italian Ministry of Education, University and Research “InterOmics” project.
Bench software applications. Results: We present a family based study on the validity of CNV detection in ASD using microarray analysis. The clinical utility of this screening is evaluated for different parameters (e.g. sporadic versus familial ASD, IQ, comorbidity). In some cases, we found a better diagnostic performance of the higher resolution platform. Conclusion: We contribute to the diagnostic utility and indication of rare CNV detection in ASD families with respect to clinical presentation and family history.

P09.023-S Genetic evidence that hyperactive Th2 and NK immune pathways contribute to the pathogenesis of Autism Spectrum Disorder L.S. Piras1, Gabriele1, V. Napoloni1, R. Sacco1, A. M. Persico1;2;3;4;5
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Converging evidence suggests that abnormal immunity is involved in the pathophysiology of Autism Spectrum Disorder (ASD) both in children and adults. Altered immune processes include ongoing neuroinflammation in post-mortem brains, elevated pro-inflammatory cytokines in cerebrospinal fluid and blood, altered immune cell function, presence of brain-specific auto-antibodies, and dysregulated immune transcriptome. One strategy to assess whether dysimmunity contributes to ASD pathogenesis or represents a collateral by-standing effect is to investigate whether functional SNPs known to influence immune gene expression or function are associated with ASD. We genotyped 484 simplex and 18 multiplex families with an ASD proband at 34 known functional SNPs located in 26 immune genes involved in all major immune pathways. Statistically significant opposite transmission patterns between ASD and unaffected siblings were evident for rs2432450 (IL4) and rs231775 (CTLA4), with the combination of high IL4 expression and low CTLA4 function alleles associated with autism and the opposite alleles, over-transmitted to unaffected siblings. Similarly, at rs361525 and rs2430561, functional SNPs located in the TNFA and IFNG genes, the combination of high expression alleles was associated with autism, while the opposite alleles were protective. These results indicate that common variants conferring hyper-responsive Th2 (IL4 and CTLA4) and NK (TNFA and IFNG) activation also confer autism vulnerability. Hence previously-reported increased expression levels in BLC from same ethnic group. Assigning more families will allow delineation of the phenotype-genotype spectrum resulting in autistic disorder in a sizable subgroup of patients.

P09.024-M Array-CGH contribution to Child & Adolescent Neuropsychiatry: update and perspectives C. Lotz1;2, Gabriele1, P. Cicinelli1, J.S. Piras1, R. Sacco1, M. Verdeeschel1, A. M. Persico1;2;3;4;5
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Many behavioral disorders display prominent genetic underpinnings. We have thus introduced array-CGH analysis (Agilent, 180K) in our routine clinical practice to explore unexplained and cognitive developmental disabilities as well as in the presence of high familial loading and/or dysmorphology. Blood samples were collected from 176 families and genetic counselling has already been provided to 65 of them. Eight families are multiplex, yielding a total of 81 patients. Proband diagnoses include: 52 autism, 9 cognitive disabilities, 4 developmental delay, 4 generalized dyspraxia, 3 learning disabilities and 4 other disorders. Patients were divided into 5 classes based on array-CGH results: [1] certainly causal CNV, N=12(15%); [2] probably causal CNV, N=23(28%); [3] rare variant of uncertain interpretation, N=19 (23%); [4] common variant without causal role, N=20 (25%); [5] negative result, N=7 (9%). Array-driven medical diagnostic work-up was prescribed to 10 (12,3%) patients and 3 parents, while drug therapies or supplements were prescribed to 4 (7,5%) patients. The diagnostic work-up was positive in 3 patients and 1 parent, while therapies were ineffective in 1 case and still pending evaluation in the other three. Array-CGH is therefore a powerful clinical tool, provided its focus includes the functional role of duplicated or deleted genes, their presence in neurodevelopmental disorder genes and possible imprinting. Currently the main limitation is the translation of this knowledge into effective drug therapies. Array-CGH data will be especially useful when used in ongoing neuroimaging phase II–III trials and potentially beneficial to specific subgroups of patients, will become available.

P09.025-S Band-like brain calcification (BLC) or pseudoTORCH syndrome: A report of six Egyptian families with expansion of the phenotypic and mutational spectrum M. S. Zaki1, M. S. Abdul-Hamid1, M. Y. Issa1, G. M. M. Abd el-Salam1; National Research Centre, Dokki, Egypt

Band-like brain calcification (BLC) or pseudoTORCH syndrome is a rare autosomal recessive with distinctive clinical and neuroimaging. Severe microcephaly, early onset seizures, profound developmental delay together with band-like calcification in brain, simplified gyral pattern and polymicrogyria are diagnostic hallmark of the syndrome. In 2010 the syndrome was linked to homozygous mutation in occludin (OCLN) gene through a description of 5 families from a worldwide series including an Egyptian one. Since then, a single family was reported associated with extracranial phenotype namely renal involvement due to cortical calcification. Herein we describe eight patients derived from six Egyptian Families with BLC. All presented at early life with severe microcephaly, failure to acquire developmental skills, growth arrest and impaired seizures. Severe developmental delay predominate the myoclonic. Patients showed a unique calcification pattern; subcortical band, basal ganglia, dentate nucleus and pons. Molecular analysis revealed that two families were linked to known genetic mutations while four families had novel mutations in OCLN gene. Three of our patients deceased at the end of the first year of life. Interestingly, the single patient with novel mutation in exon 5 had non-neurological manifestations including high imperforate anus and genital anomalies. Further, he had the best life performance with fairly controlled seizures and achieved few skills although no comparable neuroimaging findings were present. This study considered the largest series with BLC from same ethnic group. Assigning more families will allow delineation of the phenotype-genotype spectrum of BLC.

P09.026-M Association study of 240,000 rare coding variants in bipolar patients and controls from Germany and Norway S. Herms1,2,3; P. Hoffmann1,2,3, T. W. Muehlleisen1,2, S. Djurovic1, F. D. Degenhardt1,4,5; A. Forstner1,3, M. Rietche1, M. M. Noethen1,4, G. Andreasssen1,2, S. Cichon1,2
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Genome-wide association studies (GWAS) of bipolar disorder (BD), a highly heritable disorder of mood with a lifetime prevalence of approximately 0.5-1% in all populations worldwide, have identified several common genetic risk factors. It is currently unclear, to what extent low-frequency and rare variants contribute to disease development. A valid hypothesis seems to be that low-frequency and rare variants in coding gene regions have a higher probability to have a functional (deleterious) effect. This subset of humans genetic variation might therefore be enriched for disease-relevant variants. The Illumina HumanExome arrays make particularly this window of genetic variation accessible for association studies. This array contains 240,000 rare markers derived from exome sequencing. That array was used to test 1314 bipolar patients (895 from Germany, 419 from Norway) and 2700 controls (2366 / 339). Genotypes were exported from a single Genometh studio project through a variety of clustering algorithms. Statistical analysis was performed using Cochran–Mantel–Hansel test statistics. Clusters for all associated variants were checked by zCall and manually. The two variants with the strongest association to bipolar disorder were found in the gene SYNE4. Interestingly, common variants in another member of the same gene family (SYNE1) had shown genome-wide significant association in the discovery step of the first mega-analysis of bipolar disorder performed by the international Psychiatric Genomics Consortium (PGC; Sklar et al. 2011). We are currently aiming to follow-up this and other strong findings of our analysis in independent samples of bipolar disorder genotyped on the HumanExome array.

P09.027-S Clinical profile of paediatric Brown-Vialetto-Valaere syndrome M. Ayten1, C. Hammond1, M. Lunt2, D. Josifovski2
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Brown-Vialetto-Valaere Syndrome (BVVLS) is an autosomal recessive neurological disorder presenting with progressive sensorineural hearing loss and bulbar neuroopathy, followed by a mixed picture of upper and lower motor neuron palsies. Until recently there has been no treatment available for this progressive neurodegenerative disorder. However, the discovery that it is caused by mutations in the riboflavin transporter genes, SLC52A2 and SLC52A3, have lead to the use of riboflavin supplementation and there is growing evidence to suggest that this may be an effective treatment. This makes the identification of this disorder particularly important and led us to review clinical information about the phenotype of children.
with BVVL5. Our cohort consisted of patients diagnosed at the Molecular Diagnostic Laboratory at Guy’s and St. Thomas Hospital and by our collaborators in Germany and Iceland, as well as molecularly-confirmed reports in the literature. After excluding patients with insufficient clinical data available, we included 15 patients with CADASIL and 12 patients for analysis. We identified differences in the clinical presentation and progress of these two patient groups. In particular, while hearing loss, muscle weakness and bulbar palsy were common clinical features in both patient groups, amongst SLC52A2 mutation carriers, ataxia and optic atrophy were also surprisingly common. These findings suggest that BVVL5 has a wider clinical phenotype than presently appreciated. This is an important observation given emerging evidence that it can be ameliorated using riboflavin supplementation.

**P09.028-M**

Effects of sapropterin on endothelium-dependent vasodilation in patients with CADASIL: a randomized controlled trial

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Background and Purpose - CADASIL, a rare autosomal dominant disorder caused by NOTCH3 mutations, is characterized by vascular smooth muscle and endothelial cell abnormalities, altered vasoactivity and recurrent patient deaths. Vasoconstriction may represent a key factor for disease progression. Tetrahydrobiopterin (BH4), essential cofactor for nitric oxide synthase is in endothelial cells, ameliorates endothelial function. We assessed whether supplementation with sapropterin, a synthetic BH4 analogue, improves endothelium-dependent vasodilation in CADASIL. Methods - In a 24-month, multicenter randomized, double-blind, placebo-controlled trial, CADASIL patients aged 30-65 years were randomly assigned to receive placebo or sapropterin 200-400 mg b.i.d. The primary endpoint was change in the reactive hyperemia index by peripheral arterial tonometry (RH-PAT) at 24 months. We also assessed the safety and tolerability of sapropterin. Analysis was done by intention-to-treat (ITT).

Results – The ITT population included 61 patients. We found no significant difference between sapropterin (n=32) and placebo (n=29) in the primary endpoint of RH-PAT changes in RH-PAT changes 0.19 (95% CI: 0.18-0.36). RH-PAT increased after 24 months in 37% of patients on sapropterin and 28% on placebo; however, after adjustment for age, sex and clinical characteristics, improvement was not associated with treatment arm. The proportion of patients with adverse events was similar on sapropterin and on placebo (50% vs 48.3%); serious adverse events occurred in 6.3% vs 13.8%, respectively.

Conclusions – Sapropterin was safe and well-tolerated at the average dose of 5 mg/kg/day, but did not affect endothelium-dependent vasodilation in CADASIL patients. Registered at URL: https://www.ncbi.nlm.nih.gov/clinicaltrials.register?Unique Identifier 2007-004-3705-55.

**P09.029-S**

Comparison of NOTCH3 expression in fibroblasts from CADASIL patients versus normal controls

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CADASIL is an inherited cerebrovascular disease caused by mutations in the NOTCH3 gene, encoding a protein belonging to the Notch receptor family. CADASIL is an inherited cerebrovascular disease caused by mutations in the NOTCH3 gene, encoding a protein belonging to the Notch receptor family.

Of note, we detected eight deletions that defined three recurrent imbalances. Further 11 patients, investigated in our Cytogenetic diagnostic lab for investigations of such patients, and underscore the need to reassess the pathogenetic of previously detected aCGH variants before consideration of the category of clinically relevant CNVs overthrowing newly recognized DD/ASD genetics such as CAMT1, RB1-CC1, CNTNAP2 and others. Our examples demonstrate that aCGH remains a valuable diagnostic tool for the investigation of such patients, and underscore the need to reassess the pathogenetic of previously detected aCGH variants before consideration of the.

**P09.031-S**

CNVs analysis in patients with Cerebellar and Brainstem Congenital Defects (CBBD)

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Cerebellar and brainstem congenital defects (CBBD) affect about 1 in 3000-4000 live births. Typical clinical signs include hypotonia, ataxia, abnormal ocular movements and psychomotor delay/intellectual disability, but phe-notypic variability is wide with possible multiorgan involvement. With the exception of few autosomal recessive or X-linked conditions, CBBD are frequently sporadic, suggesting that de novo mutations or genomic rearrangements may represent common pathogenic mechanisms. We analyzed by genome microarray platforms 83 sporadic CBBD patients, enrolled in a multicentric project focused on studying the genetic causes of CBBD. Eight pathogenic or potentially pathogenic CNVs were detected in 7 patients (8%). Further 11 patients, investigated in our Cyto genetic diagnostic laboratory for in- tellectual disability and/or congenital anomalies, have been subsequently included in the CBBD project, since they showed CNVs overlapping to deletions/duplications detected in CBBD patients and were affected by CBBD. Of note, we detected eight deletions that defined three recurrent imbalances. Three deletions were partially overlapping in 3q22.3-q26.1, three in 6q25.1q27 and two in 10q26.2q26.3. These three chromosomal regions have been already associated to CBBD, although literature and DECIPHER database revision suggested common pathogenic mechanisms. In addition we report six patients with non-recurrent CNVs: del(2q36.3), dup(6p22.3), inv(9p13), del(8p12.3p21.2), dup(13q34), dup(Xq28). These data confirm the crucial role of microarray analysis as a tool to identify...
pathogenic CNVs in patients with congenital defects. Our data confirm that CBHD are characterized by high genetic heterogeneity and incomplete pene-
trance, suggesting the involvement of further mechanisms (modifier genes, environmental factors) in cerebellar and brainstem mal-development.

P09.032-M

The structure and functions of the transcription factor PHOX2B: new insights in the molecular pathogenesis of Congenital Central Hypoventilation Syndrome

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Congenital Central Hypoventilation Syndrome (CCHS) is a very rare neonatal neurodevelopmental disorder characterized by abnormal ventilatory response to hyperoxia and hypercapnia, owing to failure of autonomic respiratory control: affected children hypventilate during sleep, with possible very severe neu-
rological damages. Frameshift mutations (5%) and poly-alanine triple expansions (95%) have been detected in the coding region of the homeobox gene PHOX2B in about 90% of CCHS patients. A correlation between length of the expansion and severity of the respiratory phenotype has been reported. Since some of the mutations alter the sub-cellular localisation, the DNA-binding affinity and the transcriptional activity of the protein, and that mutated PHOX2B pro-
teins can interfere with the activity of the wild-type protein by sequeste-
ing the nuclear localization signals in the homeodomain, both required for the complete import of the protein in the nucleus, corresponding to residues necessary for the binding to DNA, and partially blocked by the expanded poly-alanine tract. By using mammalian two-hybrid system we have also demonstrated that PHOX2B can form homo-dimers and we are currently in-
vestigating if the mutations alter the dimerisation properties of the protein and the potential contribution to the range of phenotypes and pathogenesis in CCHS patients.

P09.033-S

Transcriptional dysregulation and impairment of PHOX2B auto-
regulatory mechanism in the pathogenesis of Congenital Central Hypoventilation Syndrome

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The PHOX2B transcription factor plays a crucial role in autonomic nervous system development. In humans, heterozygous mutations of the PHOX2B gene lead to Congenital Central Hypoventilation Syndrome (CCHS), a rare disorder characterized by a broad variety of symptoms of autonomic nervous system dysfunction including inadequate control of breathing. The vast majority of patients with CCHS are heterozygous for a poly-alanine repeat expansion in the C-term inus of PHOX2B. Although several lines of evidence support a dominant-negative mechanism for PHOX2B mutations in CCHS, the molecular effects of PHOX2B mutant proteins on the transcriptional activity of the wild-type protein have not yet been elucidated. One of the targets of PHOX2B is the PHOX2gene it-
self, and we have recently demonstrated that mutated PHOX2B variants can actually negatively interfere with the expression of the normal allele. Sin-

P09.034-M

Molecular investigation of CDKL5 gene in patients with Rett like infantile spasms in Greece-Preliminary results

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A subset of atypical Rett syndrome with infantile spasms or early seizures starting in the first postnatal months is caused by mutations in the Cyclin-
Dependent Kinase-Like 5 gene (CDKL5) located in the Xp22 region. Mutati-
on screening of CDKL5 was performed in 20 female and 5 male patients, re-
flecting the infantile spasms phenotype that begins at the second month. Subsequent studies in both parents and in 50 chromosomes from normal subjects failed to detect the c.2530delC mutation indicating that it is pro-
ably de novo and disease-causative. The novel mutation was directly sub-
mitted in the RetBase (IRSF MECP2 Variation Database) and LOVD databa-
ases. Our studies of CDKL5 gene were carried out in patients with Rett-like and epileptic spasms with early onset. The incidence of CDKL5 mutations in female patients with Rett-like features who were negative for MECP2 mutations was 5% (1/20), while in female patients with spasms it was 17% (1/6), rates both lower than those reported by other studies (7.8% and 28%, respectively) probably because of their larger sample sizes.

P09.035-S

No evidence for a contribution of CHRNA7 rare variants in autism susceptibility

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The alpha7 subunit of the neuronal nicotinic acetylcholine receptor, is consi-
dered the culprit gene in mediating the neurological phenotypes in patients with the 15q13.3 deletion. In order to evaluate the role of CHRNA7 rare variants in ASD susceptibility, we have performed copy number variant (CNV) analysis and mutation scree-
ning of the coding sequence of CHRNA7 in a sample of 135 ASD individuals from Italy. Rare sequence variation in this gene remains largely unexplored, given the existence of a fusion gene, CHRFAM7A, which includes a partial duplication of exons 5-10 of CHRNA7. Hence, any attempts at sequencing to detect mutations must distinguish between CHRNA7 and CHRFAM7A, mak-
ing next-generation sequencing approaches unreliable for this purpose. Our preliminary results led to the identification of two heterozygous CHRNA7 mutations, one of which is associated with a rare variant in the proximal promoter region, previously described as conferring an increased risk for autism. The second mutation could not be confirmed in a second sample.

P09.036-M

Phenotypic and genetic heterogeneity of CLCN2-related leukodystrophy

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Mutations in the CLCN2 gene encoding the brain chloride channel CCK2 have been recently associated with a rare autosomal recessive leukoen-
ccephalopathy, characterized by variable age at onset and clinical features, with specific brain MRI findings caused by chronic white matter edema. We sequenced CLCN2 in 6 patients from 5 families presenting characteristic MRI white matter alterations involving middle cerebellar peduncles, cere-
bral peduncles, and posterior body of the internal capsule. We identified

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3 homozygous CLCN2 mutations in 5 patients (4f/1m) from 4 families, while one male patient tested negative. One novel homozygous splice-site mutation was identified in a young asymptomatic woman who underwent MRI because of headache. A previously described nonsense mutation was identified in 3 women from 2 apparently unrelated families from Southern Italy. One patient presented at 58y with dystonic posture of the neck, mild postural tremor of the hands, and head tremor. Of the two sisters, one presented at 26y with migraine without aura followed, at 46y, by progressive postural imbalance, hypoaesthesia and titubation, and degenerative retinopathy; her sister, aged 60y, suffers from cluster headache since 30y with negative neurological examination. Repeated MRI in both sisters show stability of the lesions. Finally, a novel homozygous missense mutation was found in a male patient presenting asymptomatic leukoencephalopathy discovered during assessment for infertility and azoosperma. This study shows that CLCN2 mutations can be associated with a wide, but relatively mild, phenotypic spectrum and suggests that a second, yet unidentified, gene can be associated with this peculiar white matter disease.

**P09.037-S**

A mutation in SB2F deleting the plekstrin homology domain affects carriers

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We searched for the molecular basis of Charcot Marie- Tooth neuropathy type 4B (CMT4B) in a consanguineous Bedouin family consisting of the parents and their 4 children. Two of the children were affected, another child presented with slow motor conduction velocity similar to the father who also had mild pes cavus and in-toeing feet. Assuming homozygous representation of a founder mutation we used linkage analysis for the known genes affected in CMT to identify the chromosomal locus of the mutation. The genes PRX, PFGD, SH3TC2, EGR2, NEFL, GDIAP1, MPZ and MFN2 were negated by the linkage analyses. Homozygosity was identified for the SB2F locus. Sequencing the patients’ DNA for SB2F identified a homozygous deletion of 12 bases including the acceptor splice site at the 5’ of an exon of the gene. The transcript presented a deletion of 19 bases in this exon causing a frameshift that deletes the plekstrin homology domain. Quantitative RT-PCR determined that the stability of this transcript was comparable to that of the normal transcript suggesting that the truncated protein is produced. The parents and the child with slow motor conduction velocity were heterozygotes for the mutation. The mutation causing elimination of the plekstrin homology domain may impair motor conduction velocity in some carriers. This is the first documentation of an effect of a mutation in SB2F in the heterozygote state that may act in a dominant negative manner.

**P09.038-M**

Rare Copy Number Variants underlying Genetic Epilepsy: a regional study

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We studied all 213 patients with epilepsy referred to the Oxford Medical Genetics Laboratories for arrayCGH as part of their clinical diagnostic work up between 2006 and 2013. We classified the abnormal CNVs detected as either “definitely” or “probably” pathogenic or “uncertain” or “benign” in 217/213 (101%) patients. Other regions of interest included 2q22, 6q26, 15q11.2, 16p11.2 and 22q11.2. Potential candidate genes will be discussed.

Conclusions: Our study highlights the importance of the use of microarrays in the clinical diagnosis of patients with epilepsy. It discussed “definitely pathogenic” variants which have been previously reported and potential candidate genes within “probably pathogenic” CNVs which need further research.

**P09.039-S**

A genomewide-wide search implicates a potassium channel gene in cognitive performance in the elderly

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Normal aging of the brain is characterized by changes of its structure and function resulting in decreasing cognitive abilities. However, the age-depended decline of the cognitive performance (CP) can vary greatly in subjects of the same age. Here we assessed CP-profiles (attention, executive functions, language, and memory) of 482 elderly subjects from a population-based cohort from Germany (10000BRAINS) revealing 323 high-performers (HPs) that cognitively performed better than 159 low-performers (LPs). To detect genetic variants that contribute to these CP-differences, we compared both groups in a genome-wide association study (GWAS). No SNP reached genome-wide significance (P<5E-8). However, 28 SNPs showed strong-to-moderate evidence for association with CP-differences (P<5E-5). The most significant finding was a SNP located 200 kb upstream of the KCN8 gene. The minor allele was significantly over-represented in LPs compared to HPs (38% vs. 26%, OR=1.77, P=1.94E-6) suggesting that it contributes to reduced CP.

Neither the top SNP nor a SNP in strong linkage disequilibrium have received evidence for association with a cognitive endophenotype through a previous GWAS. KCN8 is expressed in important brain regions and belongs to a family of voltage-gated potassium channels that likely are involved in modulating the overall excitability of neurons (Zou et al., 2003). Our study proposes a promising new candidate gene for CP. However, replication in independent samples is necessary to confirm our result.

**P09.040-M**

Two novel missense COL4A1 mutations and genetics heterogeneity in porencephaly

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COL4A1/COL4A2 mutations have been reported in porencephaly and in small vessels cerebral vascular diseases (CVD), often associated with ocular, renal and muscular features. We screened three families analyzed in the spectrum of porencephaly and vascular leukoencephalopathy for COL4A1/COL4A2 mutations. In family LEU-1-TD, three patients on two generation presented with leukoencephalopathy associated with retinal, deep and periventricular haemorrhages and aneurysms. One subject presented a small periventricular porencephalic lesion. The proband in family LEU-2-AC had a history of mental sluggishness and catatonia with a sudden and reversible motor impairment at 14 yars, followed by progressive gait imbalance in her 30ties. She delivered two newborn severely affected by porencephaly. In the post partum she had an haemorrhagic stroke. MRI showed parietal haemorrhage, diffuse microbleeds, severe leukoencephalopathy.

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Pathy and aneurisms of intravascular vessels. In family LEU-3-T0 two male brothers presented a overlapping clinical picture with history of recurrent hemorrhagic strokes in the first two decades and a severe leukoencephalopathy, brain microbleeds and ischemic lacunae at the neuroimages. We identified two novel missense mutations in COL4A1 (c.1249 G>C; p.A417R in LEU-1-T0, c.2662 G>A,p.G88R in LEU-2-T0) by sequence analysis. Both are predicted pathogenic and hit highly conserved Gly residues of the Gly-X-Y repeat in the collagen triple helical domain. As reported for COL4A1 missense changes in the first third of the gene, mutation p.A417R seems associated with a variant phenotype in which porencephalic lesions are not the major characteristic. Patients in family LEU-3-T0, negative for COL4A1/ COL4A2, suggest a genetic heterogeneity for this disease.

P09.043-S

Anticipation of age at death and possible bias in Creutzfeldt-Jakob Disease

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Mutations in PRNP that encodes the prion protein are causative for inherited Creutzfeldt-Jakob disease (CJD). The E200K substitution is the most frequent mutation. Earlier age at deaths in successive generations has been reported in two clusters, from Israel (Rosenmann et al. Neurology 1999;53(6):1328-9) and Italy (Pocechiari et al. PLoS One 2013;8(4):e60376). No molecular or environmental explanations have been found. The aim was to analyze a possible observational bias in E200K CJD patients from families living in France. Ages at death from 42 parent-offspring pairs from 19 families were collected and compared: parents 63.5 years ± 13.9 (range 43 to 90) offspring 59.8 years ± 10.4 (25 to 80), indicating a significantly earlier age at death in offspring (p=0.015 paired t-test). Including ages at last follow-up or at death of an unaffected but obligate carrier-parent, the difference increased to - 9.8 years anticipation p=0.004. Considering the large range of ages at death and the intra-sib-ship variability, the most obvious bias lies in the fact that sibs could develop disease later in life. When analyzing pairs with at least 50% affected with known ages at death, the anticipation was lost: parents 68.1 ± 19.9 years and offspring 62.8 ± 7.9 years p=0.95, n=12. This result could indicate a possible observation bias. Another hypothesis could be the loss of modifying genes outside inbred clusters. The search for genetic modifiers in familial CJD is of great importance.

P09.044-M

A new autosomal recessive syndrome: Severe developmental delay and dysmorphic features causing by a missence mutation in FTO gene

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Fat mass and obesity-associated gene (FTO) associated with variation in body weight and metabolic disorders. Recently, Boissel et al reported a homozygous loss-of-function mutation in FTO gene in a family with nine affected members who had a severe development delay and multiple congenital anomaly. We performed whole-exome sequencing analysis and identified a novel homozygous missence mutation (c.812A>C) within the FTO gene in a 9.5 months old girl with dysmorphic facies and developmental delay. She was the first child from the consanguineous parents. She had microcephaly, metopic ridge, coarse face, long philtrum, microretrognathia, prominent alveolar ridge, antevernt nostril, and brachydactyl. She follow up until 4 years and 9 months of age and she gained head control at first year of life, sitting 2 years, walking with help at 4.5 years old of age. She could say only three words at 4 years and 9 months. Denver testing at 4 years old of age confirmed severe developmental delay (DD>23). Cranial CT imaging showed premature fusion of metopic suture. She also had splenomegaly on abdominal examination and grade-1 esophageal varices on endoscopy. Hearing assessment by auditory brainstem response showed conductive and sensorineural hearing loss. Eye examination revealed optic atrophy, strabismus, nystagmus and abnormal electroretinogram. The clinical findings of the patient were similar to familiar family. In the parents she was the first of creatine kinase was persisted since birth. As a result, we report second patient with novel homozygous missence mutation in FTO and discuss phenotypic extension of this gene mutations.

P09.045-S

Diagnosing dystonia using a Next-Generation-Sequencing panel

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Dystonias are a heterogeneous group of movement disorders which a strong inherited basis. Overlapping, non-specific features of many dystonias hamper a clear clinical diagnosis and make targeting a specific gene difficult or impossible. We developed a panel of 42 genes for Next Generation Sequencing containing the most relevant dystonia genes and covering the most relevant dystonia phenotypes known so far in order to analyze a cohort of unselected dystonia patients. We have established a selector-based enrichment method (HalOlex, Agilent)
targeting 42 dystonia genes. A total of 310kb is enriched and sequenced by Illumina Miseq (2x 150 bp paired-end). A first batch analysis in 27 dystonia patients showed that HaloPlex enrichment provided enrichment efficiency superior to standard whole exome procedures (>95% covered >20 reads; 90% covered >50 reads per target; mean coverage >500 reads per target). We identified presumed disease-causing mutations in 2 out of 27 patients including one patient with a DYT6 dystonia and one patient carrying a mutation in SLC2A1. In 7 patients, we identified so far unknown probable disease-causing variants including mutations in ATP7B, PLA2G6, PARK2, FBC07, VPS13A and GCHD. This technology enabled the identification of a genetic cause in approximately 7% of patients in an unselected cohort of dystonia patients in whom the most common genetic form (DYTI) was excluded. Targeted NGS may be a useful and cost-effective method to screen for mutations in multiple genes associated with dystonia.

P09.047-S
Using exome-sequencing for the diagnosis of rare disorders: two siblings affected by a congenital encephalopathy with microcephalia, polymicrogyria and dystonia
F. Incarnit was stopped at the age of 13, leading to aggravation of the EEG only. Asto...

P09.049-S
A new mutation in SYNGAP1 expanding the phenotypic spectrum: a case report
J. Juengling et al.

P09.049-M
Whole genome SNP genotyping confirms segregation of Unverricht-Lundborg Disease (ULD) with a repeat expansion in CSTB on 21q in a large consanguineous family followed by a novel haplotype based approach identifying the parent of origin and carrier status in the child with Trisomy 21 under the age of onset for ULD
F. Y. Kesimo et al.

P09.050-M
Idiopathic generalized epilepsy combined with a variable degree of intellectual disability including severe speech impairment. Our patient shows additional symptoms, expanding the spectrum of phenotypes for patients with SYNGAP1 mutations.
Exome sequencing reveals mutations of a solute carrier gene in an autosomal recessive form of epileptic encephalopathy of the first days of life

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Epileptic encephalopathy (EE) refers to a clinically and genetically heterogeneous group of devastating disorders characterized by seizures combined with abnormal inter-critic encephalography. Age of onset can be a key diagnostic feature for epileptic syndrome definition, treatment and prognosis. We ascertained two multiplex families (including one consanguineous family) consistent with an autosomal recessive inheritance pattern of EE. All seven affected individuals developed seizures in the first day of life, resistant to treatment and leading to repetitive "Etat de mal". Evolution was marked by severe EE with major delay in motor acquisitions and one patient died at 11 years of age. No facial dysmorphia was noted, but oligodentia. Given the similarity in clinical presentation in the two families, we hypothesized that the observed phenotype was due to mutations in the same gene, and performed exome sequencing in three affected individuals. Analysis of rare variants identified in heterozygous state a pathogenic variant in SCN8A. Causality was confirmed by co-segregation analysis in additional family members. To assess the frequency of alterations of this gene in early onset EE, coding exons were screened for mutations in a cohort of 70 unrelated affected individuals by targeted sequencing on a MiSeq instrument (Illumina). This experience led to the identification of pathogenic variants in a simplex case with a similar clinical presentation as the other affected patients. These results highlight the value of careful clinical characterization for genetic studies in heterogeneous diseases such as EE.

Exome sequencing identifies novel SCN8A mutation associated with neonatal epileptic encephalopathy, multiple congenital anomalies and movement disorder

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Epileptic encephalopathies represent clinically and genetically heterogeneous group of disorders of which the majority are of unknown aetiology. It is hypothesized that novel variants may contribute to most of these devastating group of epilepsies. Within recent years the role of SCN8A in human disease has become apparent from next generation sequencing studies. Whole-exome sequencing of a parent-offspring trio was used to identify the genetic cause of severe early infantile epileptic encephalopathy in a boy, where previous chromosomal, gene and metabolic investigations revealed no abnormalities. The patient had neonatal seizures, movement disorder, multiple congenital anomalies. He died at the age of 17 months due to respiratory illness. We identified a de novo heterozygous missense mutation (c.3979A>G; p.Ile1327Val) in SCN8A (voltage-gated sodium channel type VIII alpha subunit) gene. The variant was confirmed in the proband with Sanger sequencing. Since the clinical phenotype associated with SCN8A mutations have previously identified only in a number of cases, these data together with our results suggest that mutations in SCN8A can lead to early infantile epileptic encephalopathy or intellectual disability with broad phenotypic spectrum. Additional investigations will be worthwhile to determine the prevalence and contribution of SCN8A mutations to epileptic encephalopathy and intellectual disability, and provide insight into the mechanisms of pathogenesis in neurologic diseases. This study was supported by the EuroEPINOMICS grant SARLA 11091E.

Alpha-1-galactosidase A forms in cell culture systems of Fabry disease

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Fabry disease (FD) is a rare hereditary disease caused by the absence or deficiency of lysosomal enzyme activity of the glycosidase α-galactosidase A (GLA, EC 3.2.1.22, α-gal A). This enzyme breaks down macromolecular structures of neural glycosphingolipids. A lack of particular hydrolytic activity leads to lysosomal glycosphingolipid storage and subsequently multi-subcellular dysfunction. Many mutations in the GLA gene have been identified by screening the enzyme’s stability causing a premature proteasomal degradation. Therefore, new therapies are required to apply the use of small molecule enzyme inhibitors having the ability to promote enzyme folding and transport intracellularly, the so-called pharmacological chaperones (PC). It was demonstrated that these molecules specifically bind to the (mutated) target enzyme which leads to a thermodynamically favoured conformational change and in turn further cellular transport and an increase of the required activity in the lysosomes. One such compound is 1-Deoxygalactonojirimycin (DGJ). We recently described an auxiliary function of Ambrosoul (ABX), a PC described for mutant glucocerebrosidase in Gaucher disease, for the observed DGJ activity in over-expression based cell culture systems of FD. Here, we report the results of a derivatisation project of Ambrosoul and the structural analogue Bromhexine that led to the discovery of compounds that showed the ability to enhance mutant α-gal A activity in combination with DGJ. Structure-function analysis may give indications on the underlying functional mechanism we aim to elucidate. Moreover, our findings did not support the occurrence of a direct compound:enzyme interaction.
FOXG1 point mutations or submicroscopic 14q12 deletion, which involve FOXG1 gene. It is now known as FOXG1 syndrome which clinically causes postnatal microcephaly, severe mental retardation, absent language, dyskinesia, and dysgenesis of the corpus callosum. More than 30 cases of FOXG1 syndrome have been published. Cases of a submicroscopic 14q12 deletion, involving regulatory elements of FOXG1, with the coding region of FOXG1 being unaffected, are described very seldom. A cis-acting regulatory sequence, acting as a silencer, is deleted more than 0.6 Mb distally from FOXG1 in these cases. We report a new case with clinical features of FOXG1 syndrome and 14q12 microdeletion. She was born at term with birth weight 3636g, length 51 cm and head circumference 33. 5 cm. Since the birth developmental delay was noticed. At 11 months she has only weak head control, microcephaly (−3 SD), focal epilepsy, deep set and almond shape eyes, protruding tongue, increased muscle tonus and brisk tendon reflexes. Brain MRI showed hypogenesis of the corpus callosum, hypomyelination, arachnoid cyst in the left temporal region and subdural pachygyria. Chromosomal microarray analysis revealed 1.5-Mb size 14q12 microdeletion (arr[hg19] 14q12(27,584,943−29,170,974)x1) appeared to be de novo. The end of this deletion is 65-kb proximal from FOXG1 leaving the gene itself intact. There are no known protein-coding genes located in the deleted area. Therefore, we can hypothesize that there is some regulatory element of FOXG1 located proximal to the gene which deletions can also cause FOXG1 syndrome.

P09.056-M
Cy5 Analysis System in molecular diagnosis of Fragile-X Syndrome
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Expansion of DNA repeats causes hereditary disorders in humans; our genetic diagnosis center is studying (CCG) repeat in the FRM1 gene in Fragile-X Syndrome and (CAG) repeat in the HTT gene in Huntington’s disease (HD) by a high performing systems, Cy5-labeled Fragile-X Syndrome The critical issues in FMR1 analysis usually are: - discriminate between full mutated females from normal homozygotes. - determine the proper CCG repeat size between 110 and 200 repeats - determine methylation status We routinely use a combination of two PCR-systems (CE-IVD) to determine the proper CCG repeat size until 200 repeats and alternative methodologies to Southern Blot (MS-MLPA, High Resolution Melting ) to determine methylation status. Huntington Disease In Htt gene the critical issue is define the exact CAG repeat size considering the small range that exists between premutation (36 repeats) and mutation status (40 repeats). The PCR-system (CE-IVD) we introduced in 2013 in our laboratory includes a positive control that helps to estimate the proper CAG repeat size in sample. Our results refer to 400 Fragile-X Syndrome suspected cases analyzed from 2010 to 2013 and to 30 Huntington’s Disease suspected cases analyzed in 2013. Use of these systems (CE-IVD), high performing, fast and easy, has allowed us to reach good diagnostic results using instruments already supplied in our department.

P09.057-S
Comparison of Friedreich Ataxia Patients with Trinucleotide Repeat Expansions and Point Mutations in FXN-Encoded Frataxin
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We retrospectively reviewed records of 40 patients with Friedreich Ataxia (FRDA). The majority of patients, 88% had bi-allelic GAA expansions (Group 1, n=35) while 12% were heterogeneous for an expansion and point mutation (Group 2, n=5). Group 1 had a mean age of onset of 12.26 years compared to Group 2 with a mean of 5 years. The average age at diagnosis in Group 1 was 17.86 years and 15.2 years in Group 2. Neurological manifestations were the presenting symptom in the majority of both groups at 94% and 80% respectively. Within Group 1, 53% had lost their ability to ambulate at a mean of 10 years after disease onset compared to Group 2 where 50% had lost their ability to ambulate at a mean of 16 years after disease onset. In Group 1, all patients had evidence of cardiac involvement, including 43% have left ventricular hypertrophy (LVH) on echo. In Group 2, all patients had evidence of cardiac involvement with 60% having LVH. Frataxin enzyme levels had a mean of 6 ng/mL (N=2) in Group 1 while the mean for Group 2 was 2.67 ng/mL (N=3).

Genotype-phenotype correlation within FRDA patients is currently not well characterized. A greater percentage (Group 2) of patients with FRDA evaluated at Mayo Clinic do not have the classic bi-allelic expansion as compared to available literature. Patients heterozygous for a GAA expansion and point mutation had an earlier age at onset, but were able to ambulate longer compared to patients with bi-allelic GAA expansions.

P09.058-M
Mitochondrial genome encoding tRNAs sequence in frontotemporal lobar degeneration
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Frontotemporal lobar degeneration (FTLD) is the second most common type of degenerative dementia, characterized by progressive changes in behaviour, executive dysfunction and/or language impairment. Some patients present clinical and neuropathological overlap with Alzheimer’s disease, suggesting similarities in pathophysiology, including mitochondrial DNA (mtDNA) involvement. Mutations in mtDNAs, particularly in mt-rRNAs, have been described as an important cause of human disease. The aim of present work was sequencing the 22 mitochondrial tRNAs genes, ascertaining their involvement in FTLD.
A sample of 70 patients, diagnosed with probable FTLD, was studied (39 females and 31 males; age range: 38-82 years, mean±SD: 63±11 years. Total DNA was extracted from peripheral blood. The 22 tRNA gene sequences were sequenced and variants were submitted to in silico analysis. A total of 28 different sequence variations were identified in 32 patients (46%). According to in silico analysis, 6 variations are probably pathogenic, all causing structure and binding minimum free energy changes. The most frequent variation found is m.12308A>G, in the variable region of mt-tRNALeu2, and it is totally conserved in all mammals tested. The m.15946T>C-variant is located in the acceptor stem and it is highly conserved. Further investigation is needed to better understand the relationship between mtDNA alterations found and FTLD, considering also the involvement of nuclear genes in this disorder. However, this is the first study of complete sequencing of the mt-tRNA genes in FTLD.

P09.059-S
Assessment of Plasma Glucosylsphingosine as a biomarker for Gaucher Disease
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Biomarkers play an essential role in the early detection, and monitoring of lysosomal diseases, this also holds true for lysosomal storage disorders (LSDs). The ideal biomarker facilitates the initial diagnosis, evaluates the disease severity and progress and may be assist in determining proper treatment. Here, we analyzed Glucosylsphingosine for the primary diagnosis and monitoring of Gaucher disease (GD), where a defect in the Glucosylsphingosine-1-phosphatase (GSP, EC 3.1.3.2) leads to an accumulation of glucosylsphingosine (1-GS). Gaucher disease proved to be more specific and sensitive than Chitotriosidase (p=0.027) and CCL18/PARC (p<0.001). We also assessed long-term data of 19 GD patients for GD. In addition, Chitotriosidase levels may be normal even in GD patients are highly elevated in a number of LSDs and reflect the burden of disease on a 100% sensitivity and specificity for Glucosylsphingosine. In addition the identification of GSP as a new disease causing gene in Gaucher disease allows for future genetic testing and screening and may improve the therapy. Glucosylsphingosine is a reliable biomarker for the primary diagnosis and follow-up of Gaucher disease.

P09.060-M
Saposin C deficiency: an inherited lysosomal disease caused by rapidly degraded mutant proteins
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Saposin (Sap) C is a 80 residues-long glycoprotein functioning as essential cofactor for the lysosomal degradation of glucosylceramide (GC) by gluco-
sylecramidase (GCase). It promotes rearrangement of the organization of lipids in lysosomal membranes and provides GCase greater accessibility to GC substrate. Rare functional deficiency in Sap C results in a rare variant form of Gaucher disease (GD). Sap C has six conserved cysteine residues involved in three di-sulphide bonds making the protein structure remarkably stable to acid environment and degradation. Five different mutations (p.L148_149delSer) in ATP1A3 in all three genome/exome sequenced patients with either PD or LBD, indicating that GBA mutations represent a significant risk factor for these disorders in Italy.

In conclusion, we report a high frequency of GBA mutations in Italian patients with PD or LBD. To this aim, we used long-template PCR to screen the entire GBA coding region for mutations in 216 patients with PD and 84 with LBD. GBA mutations were identified in 24 of 216 PD (11.1%) and in 4 of 84 LBD (4.7%) cases. Fourteen different GBA heterozygous mutations were detected, including two previously unreported mutations (c.1095G>C [p.Glu365Asp]) and a frameshift mutation (c.1197_1198insCTGTA [p.Met400Leufs+2]).

In conclusion, we report a high frequency of GBA mutations in Italian patients with either PD or LBD, indicating that GBA mutations represent a significant risk factor for these disorders in Italy.

P09.062-M

Genome sequencing identifies a novel mutation in ATP1A3 in a family with a seemingly atypical phenotype

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Mutations in ATP1A3 have been reported in rapid-onset dystonia-parkinsonism (RDP). Dystonia in RDP has a characteristic sudden onset, typically in adolescence in response to physical or mental stress. Dystonic symptoms usually involve the bulbar region and are accompanied by symptoms of parkinsonism. More recently, mutations in ATP1A3 have been linked to alternaria hemiplegia of childhood (AHC) and CAPOS syndrome, respectively. We investigated a family with dystonia from New Zealand with ten affected members. Interestingly, only females were affected. After exclusion of mutations in TOR1A and THAP1, we performed genome sequencing in two affected cousins. For filtering, we used the KromeDiscovery Data Filtering Software. Since re-sequencing of 20 candidate variants did not elucidate the genetic cause, we performed exome sequencing in another affected individual. Analysis of the raw data using an in-house bioinformatics pipeline revealed a previously unreported three base pair deletion (c.443_445delGAG, p.148_149delSer) in ATP1A3 in all three genome/exome sequenced patients. Segregation analysis showed the mutation in all patients and in one unaffected male. The mutation was not found in 200 controls. Subsequent clinical re-examination, revealed sudden onset of dystonic symptoms after stressful events. None of the patients reported any history of AHC or CAPOS syndrome.

In conclusion, our study identifies a novel mutation in ATP1A3 as cause of RDP and highlights two important challenges when using next generation sequencing: 1) The importance of detailed clinical information. In our family, we have initially missed the characteristic sudden onset. 2) The difficulties with the annotation of deletions/insertions in the used software package.
Hereditary spastic paraplegia (HSP) constitutes a heterogeneous group of syndromes with core features of progressive lower limb spasticity and weakness, without or with other manifestations (pure / complex HSP). All modes of inheritance have been described. HSP with thin corpus callosum (TCC) represents a distinct entity for which mutations in SPG11 are the most frequent causes. As a part of a larger study to elucidate the molecular basis and the phenotypic patterns of HSP in Sudan, we report 2 extended Sudanese families with complex HSP and multiple consanguinity loops. Ten patients were clinically phenotyped and DNA was obtained from blood and saliva samples. The age at onset in the first family ranged from 12 to 17 years. Patients presented with ataxia, mental impairment and skeletal deformities in addition to spasticity. Exome sequencing performed in 3 patients identified a homozygous deletion (c.6709del/p.Ala2237Gln*) in exon 34 of SPG11. In the second family, the age at onset extended from 11 to 24. The phenotype was more complex, with psychiatric symptoms, dysphagia and peripheral motor involvement with distal muscle atrophy of both limbs. Direct sequencing of SPG11 identified a stop mutation (c.6594G>T/p.Glu2174stop) in exon 34 that segregated with the disease within the family. MRI of 3 cases from both families revealed TCC, cerebellar and cortical atrophy and WMLs. Both mutations are novel and lead to premature truncation of spatacs in protein. These families are the first described Sudanese families carrying SPG11 mutations. This study illustrates the wide range of clinical presentations associated with SPG11.

P09.066-M Assessing an anaplerotic therapy on the brain metabolic profile in Huntington disease
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Objective: To obtain a proof-of-concept for an anaplerotic therapy in Huntington disease (HD) using a validated functional biomarker of brain energy metabolism.

Background: Energy deficit has been greatly implicated in the pathophysiology of HD. Our previous work has indicated a need to refill the Krebs cycle which can be achieved using anaplerotic therapy. Methods: 31P brain magnetic resonance spectroscopy (MRS) was coupled with the activation of the occipital cortex to measure the levels of PCr and Pi before (rest), during (activation) and after (recovery) a visual stimulus. At 31P brain MRS in 10 patients at the early stage of HD and 10 controls. HD patients were then treated at home for one month with triheptanoin and came back for a second visit during which we performed and 10 controls. HD patients were then treated at home for one month with triheptanoin and came back for a second visit during which we performed

Results: At visit 1, we performed 31P brain MRS in 10 patients at the early stage of HD and 10 controls. HD patients were then treated at home for one month with triheptanoin and came back for a second visit during which we performed 31P brain MRS.

Conclusion: At visit 1, we confirmed a significant increase in Pi/PCR ratio (p=0.022) during brain activation in controls - reflecting increased ATP synthesis - followed by a significant return to baseline levels during recovery (p=0.008). In HD patients, we confirmed an abnormal brain energy profile with decreased Pi/PCR ratio before treatment. After one month of triheptanoin therapy, the MRS profile was greatly improved in HD patients with increased Pi/PCR ratio (p=0.01) and visual stimulation (p=0.004).

Conclusion: This study suggests that triheptanoin is able to correct the bioenergetic profile in HD patients’ brain at an early stage of the disease. The administration of triheptanoin over a longer period of time is now required to assess its clinical benefit.

P09.066-B Mutations in B9D1 cause mild Joubert syndrome: expanding the genetic overlap with the lethal Meckel syndrome
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Joubert syndrome (JS) is a congenital disorder diagnosed by the presence of a peculiar mid-hindbrain malformation (the “molar tooth sign”), that consists of cerebellar vermis hypoplasia, thickened mal-oriented superior cerebellar peduncles and a deepened interpeduncular fossa. The typical neurological features of pure JS include hypotonia, ataxia, psychomotor delay, abnormal ocular movements and intellectual impairment. This phenotype may be complicated by defects of the kidneys, eyes, liver, skeleton and orofacial defects, resulting in wide clinical variability. JS is recessively inherited and genetically heterogeneous, with 24 known genes. All genes encode for proteins of the primary cilium, and indeed there is clinical and genetic overlap with other ciliopathies. In particular, JS shares 13 genes with Meckel syndrome (MS), a lethal condition characterized by cystic kidneys, bile duct proliferation of the liver, encephalocoele and polydactyly. As part of a large screening of ciliopathies genes in 46 JS patients, we identified novel pathogenic mutations in two genes not previously implicated in this condition. Two patients carried mutations in the MKS1 gene, a 44-year-old man with JS and retinal dystrophy, and a two-year-old child with a pure JS phenotype. Mutations in the B9D1 gene were identified in two other patients, a 9-year-old boy and a 6-year-old girl both presenting with pure JS. All identified mutations were inherited from heterozygous healthy parents, were not reported in public databases, and affected highly conserved residues. Missense mutations were predicted as pathogenic by prediction web tools.

P09.067-S PMCA, SERCA and VEGF as potential pathogenetic factors in Huntington’s disease and as biomarkers of onset and progression
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To study calcium homeostasis deregulation in Huntington’s disease (HD) ethiology we investigated calcium pumps expression level in HD cellular models and in peripheral blood of pre-symptomatic and symptomatic HD patients. We compared the steady-state level of cellular membrane associated PMCA1-4 and endoplasmic reticulum associated SERCA2A3 pumps in wild-type STHdh(Q7)/Hdh(Q7) and mutant STHdh(Q111)/Hdh(Q111) cell lines derived from the murine embryonic striatum. We observed a significant reduction of PMCA1 protein in glial differentiated mutant cells (p<0.05) and SERCA2A protein in undifferentiated mutant cells (p<0.001). Using double-immunofluorescence staining for SERCA2A both in primary and patient derived fibroblasts from symptomatic HD patients compared with healthy subjects. Our data highlight SERCA2 gene down-regulation as a biomarker of HD having a role in early cellular dysfunction. Finally, in PBMC we studied the neuroprotective angiogenic factor vascular endothelial growth factor (VEGF) mRNA level. VEGF transcript level resulted significantly lower in pre-symptomatic HD compared to healthy subjects (p<0.01) and even lower in late-symptomatic compared to pre-symptomatic group (p<0.05). This finding suggests VEGF transcript as a peripheral biomarker useful for monitoring disease onset and progression.

P09.068-S Mutations in B9D1 and MKI67 cause mild Joubert syndrome: expanding the genetic overlap with the lethal Meckel syndrome
M. Romani1, A. Micalizzi1, I. Kraoua3, M. Dotti4, M. Cavallin5, L. Sztriha6, R. Ruta1, F. Mancini1, T. Mazza1, S. Castellano1, B. Hanane1, M. Cartuccio1, E. Darrà2, A. Malté1, A. Zimmermann1, N. Goidier-Khouja1, E. Valente1—1IRCCS Casa Sollievo della Sofferenza, Mendel Laboratory, San Giovanni Rotondo, and 2San Giovanni Rotondo, Italy, 3Department of Medical and Surgical Pediatric Sciences, University of Messina, Messina, Italy, 4Research Unit 06/11 and Department of Child and Adolescent Neurology, National Institute Mongi Ben Ilidma of Neurology, Tunis, Tunisia, 5Department of Medical, Surgical and Neurological Sciences, University of Siena, Siena, Italy, 6Unit of Child Neuropsychiatry, Policlinico G.B. Rossi, Verona, Italy, 7Department of Paediatrics, Faculty of Medicine, University of Szeged, Szeged, Hungary, 8Department of Neurosurgery, Faculty of Medicine, University of Szeged, Szeged, Hungary, 9Department of Medicine and Surgery, University of Salerno, Salerno, Italy.

Kuf’s disease (KD) is the rare adult form of neuronal ceroid lipofuscinoses (NCL). Mutations in the cathepsin F gene (CTSF) (MIM 603539) have recently been discovered in autosomal recessive Type B KD families characterized by movement and behavioral abnormalities and dementia. We present a family in which pseudo-dominant transmission of Type B KD was explained by a novel homozygous splice site mutation in CTSF leading to the loss of exon 1. Three affected individuals showed similar neurological pictures characterized by generalized seizures, cerebellar dysarhria and cognitive decline, evolving into frank dementia. The presence of three additional relatives with a neurological syndrome compatible with KD and two instances of parent...
to-child transmission proposed an initial hypothesis of a dominant form of dementia representing a confounding factor for genetic testing. A more detailed clinical assessment of the patients, meticulous collection of family history and recognition of the high degree of inbreeding in the isolated community in which this family lives, were crucial in disclosing an autosomal recessive pattern of inheritance, prompting investigation of CTSF.

In vitro experiments in cultured skin cells from the proband and her aunt demonstrated a dysregulated autophagy and aggresome-like structures, confirmed by ultrastructural studies in skin biopsies. These data put forward the hypothesis of a cytoplasmic toxicity of pathologic cathepsin F in KD type B.

P09.070-M

Lamin B1 expression is affected by EBV infection in lymphoblasts of patients with Autosomal Dominant Leukodystrophy through miR-23 deregulation

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Leukodystrophy with vanishing white matter (LVWM) is a rare autosomal recessive pattern of inheritance, prompting investigation of CTSF. Our cohort consisted of 743 PD patients (67±10 years; 52% male) and 523 healthy controls (67±12 years; 50% male). The human post-mortem tissue samples were obtained from patients who died of PD (67±10 years; 52% male) and from donors with no history of dementia representing a confounding factor at the molecular level.

P09.071-S

Molecular analysis of EIF2B genes in adult-onset leukodystrophy with vanishing white matter

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Cerebellar ataxias are characterized by incoordination and unsteadiness of movement due to cerebellar dysfunction. In this study, we analyzed a consanguineous family with affected individuals presenting very slowly progressive cerebellar symptoms including dysarthria, dysmetria and gait ataxia.

All 4 affected and 3 unaffected members from this family were genotyped using Illumina Human HumanCytosNP-12 BeadChip kit. The genotyping data obtained were further analyzed in terms of copy number variation (CNV) and linkage using Illumina proprietary software cnvPartition and easyLinkagePlus interface, respectively. The patients were negative for a common CNV while a single linkage peak on chromosome 6q25 was obtained with a maximum LOD score of 3.42.

Whole exome sequencing (WES) was performed in two affected sibs from the family in parallel to linkage analysis. Genetic variants of the affected individuals within the linkage interval were filtered against novel variants with harmful effect. This approach has led to identification of a novel mutation in SYNE1 (c.13086delC; p.His4362Glnfs*2) segregating with the condition in the family as confirmed by Sanger sequencing. SYNE1 has 14 exons encoding 8797 amino acids. This mutation is predicted to truncate almost half of the protein. SYNE1 encodes a nuclear envelope protein, which is expressed in various tissues, particularly in the cerebellum. Mutations in SYNE1 have previously been implicated in a rare form of recessive spinocerebellar ataxia observed especially in French-Canadian populations, modifying factors, disrupted targets for microRNA binding and a possible effect of this region in mRNA expression.

Our cohort consisted of 743 PD patients (67±10 years; 52% male) and 523 healthy controls (67±12 years; 50% male). The human post-mortem tissues were obtained from three different brain regions of 9 PD and 5 healthy donors.

Patients were genotyped for the LRK22 mutations G2019S and R414G/C resulting in 16 G2019S carriers and 15 R414G/C carriers. None of the tissue donors was mutation carrier. We identified a total of 12 variants; 2 of them new (c.*130,131del and c.*382>C>A) in the 3’UTR region. We found rs66737902 T>C as a possible variant related with PD risk being the C allele overrepresented in patients; p=0.01 OR=1.37 (CI=1.07-1.74). To tested if rs66737902 had an effect in the gene expression through the binding of miRNAs, we study miR-138-2* as candidate miRNA (TargetScan, microRNA.org) and mir-205 as control miRNA. We did not find any effect of mir-138-2*. In the mRNA expression study we found significant differences between TT and TC rs66737902 genotypes in the SN of PDs, with a minor expression level in the TC group (p=0.011). No differences between patients and control were found.

P09.074-M

Variation in the promoter of the autophagic beclin-1 gene (BECN1) and its impact on expression levels: a study in Machado-Joseph disease (MJD/SCA3) patients

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Background and Objectives: Autophagy, as a process of intracellular components degradation, is especially important in disorders where accumulation of the mutant protein is a hallmark, such as MJD, a late onset polyglutamine ataxia. We selected 4 candidates that could differentiate affected from unaffected subjects. In the combination of a shared mutational burden on risk genes together with the linkage regions, present only in the affected subjects. Among the genes affected by these variants, we found 10 mutations in ASPM, five of them are novel and 9 families have the already reported founder mutation p.W1326*. Among the remaining five families, two showed novel overlapping microdeletions of 580 kb and 164.2kb in MCHP1, two other showed novel mutations in CDK5RAP2, and the last one showed a previously reported mutation in WDR62. This study adds to the mutational spectra of known MCHP-associated genes. The observed high frequency of the ASPM mutation p.W1326* underscores its prominent role as a founder mutation in the Pakistani population.

P09.077-M
The metabolism of GABA and glutamate is affected in the brain of the Mecp2-deficient mouse, a model for Rett syndrome.

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Rett syndrome (RTT) is a severe neurological disorder affecting females. Most RTT cases are caused by mutations in the X-linked methyl-CpG binding protein 2 (MECP2) gene. RTT patients develop normally until 6-18 months of age, before the onset of deficits in autonomic, cognitive and motor functions. Studies on Mecp2-deficient mouse models have revealed severe neurotransmission dysregulations, including deficits in bioamine levels and dysfunction of the GABAergic and glutamatergic systems leading to an imbalance between excitation and inhibition in the brain of the mutant animals. However, published results are divergent due to differences in age, model used and/or brain areas studied. Here, we have used real-time PCR, western blotting and HPLC dosage to compare the GABA and glutamate metabolism in right and left brain areas of the Mecp2-deficient mouse brain at two developmental stages (early and late symptomatic). Several key enzymes of GABA and glutamate metabolism have been studied (Kcc2, Nkcc1, Vglut1/2, GAD1/2). Our results show: 1- a progressive reduction of the GABA and glutamate content; 2- a spatial and temporal deregulation of the GABAergic and glutamatergic key enzymes. We have used that information to assess a pharmacological stimulation of the GABAergic system in vivo and we showed that such a treatment increases the lifespan of Mecp2-deficient mice.
New genetic tools for the investigation of cases with neurodevelopmental delay increased continuously the knowledge concerning the underlying causes of genetic syndromes. Array CGH is a highly sensitive method for diagnosis of pathological CNVs responsible for neurodevelopmental syndromes. We report a case of a 9yo girl, who was referred to Medical Genetics Department for evaluation due to microcephaly, mild dysmorphic features and developmental delay. From her medical history, severe and frequent urinary infections, in the absence of any malformation present in the urinary system, are noticeable.

Conventional karyotype from peripheral blood revealed a 12p terminal deletion. Discrepancies between clinical findings and karyotype results required additional investigation using comparative genomic hybridization method. Array CGH revealed a 5Mb terminal deletion on the long arm of chromosome 10, result that matches with the clinical findings of our patient. The molecular result was verified by FISH analysis. This finding is consistent with a complex chromosomal rearrangement involving insertion of genetic material from chromosome 12p to chromosome 10 and consequent deletion of terminal region on the long arm of chromosome 10. Parental karyotypes are normal, suggestive of a “de novo” rearrangement and consequence low recurrence risk for other siblings.

This case report illustrates the importance of considering genotype-phenotype correlation for each patient and the advantages and the limits of genetic diagnostic techniques in our practice.

In conclusion, clinical judgment complementary to genetic tests provides an accurate diagnosis, prognosis and recurrence risk evaluation.

**P09.082-M**

**New deletion in exon 32 and 33 in MLL2 gene causes Kabuki Syndrome in a Spanish patient**

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Kabuki syndrome (KS; OMIM# 147920) is a rare congenital disorder, characterized by typical facial features including: long palpebral fissures, elevation of the lateral third of lower eyelids, arched and broad eyebrows with lateral splaying, short columella and prominent ears. Point mutations and large intragenic deletions and duplications of the histone methyl transferase MLL2 gene are the main causes of KS. MLL2 encodes a large protein that belongs to the SET1 family of human SET-domain protein methyltransferase superfamily. Recently, de novo partial or complete deletions and point mutations of KDM6A gene, have been identified as additional causes of KS. Case report: We report the molecular and phenotype studies of a Spanish patient who have typical features of KS. The patient is a 9y.o girl with mental retardation, a peculiar facies characterized by long palpebral fissures, a broad and flat nasal bridge, prominent ears, a left palpebral, stenosis, radiographic abnormalities of the vertebrae, hands, and hip joints, and recurrent otitis media. Genomic DNA was extracted from the patient and parental karyotypes are normal, suggestive of a “de novo” rearrangement and consequence low recurrence risk for other siblings.

**P09.083-S**

**SGK223, a novel candidate gene for an autosomal recessive form of dHMN**

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Distal hereditary motor neuropathies (dHMN) are a subgroup of Hereditary Motor Sensory Neuropathies characterized by a predominant motor involvement of the peripheral nervous system. In the present study a family affected by an autosomal recessive form of dHMN was examined by using a combination of linkage analysis and whole-exome sequencing (WES) in two patients. WES variants in the known disease-related genes associated with similar phenotypes, and copy-number variants shared by the affected subjects were excluded. Considering the presence of two consanguineous marriages in the family, the homogeneity of the linkage mapping approach was performed and a candidate autosomal region shared exclusively by the affected subjects was highlighted on chromosome 8p23.1-p22. The candidate region contains a high number of pseudogenes and paralogs which led to many false positive calls due to multiple-mapping reads. No evident mutations were identified in the also poorly-covered exons of the linkage region but the prioritization analysis of the about 230 WES variants within the candidate region pinpointed a novel missense substitution in the SGK223 gene (c.1529T>C). In silico predictions strongly suggested an alteration of a cryptic splice site in presence of this variant. Interestingly, SGK223 codes for the pramin protein involved in the reorganization of cytoskeletal intermediate filaments, which is a pathway already involved in the dHMN pathogenesis. Despite this functional consistency, further studies are warranted to confirm these findings.
P09.086-M
Alterations of gene expression at the peak level in the experimental allergic encephalomyelitis
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Experimental allergic encephalomyelitis (EAE) is the widely used model for studying multiple sclerosis (MS). MS is a chronic disease where the inflammation throughout the brain and spinal cord cause demyelinated plaques of glotic scar tissue. We have studied the alterations in gene transcription in the EAE induced in the C57/BL6 mouse using real-time PCR. We have studied the genes mediating MS progression, such as cytokines and chemokines, inflammatory and immune response genes, as well as genes involved in cell adhesion, cellular stress and apoptosis whose expressions were correlated across multiple analyses. For this purpose female C57/BL6 mice weighing between 18-20 g were immunized s.c. with 250 µg of MOG peptide (the 35-55 sequence) emulsified in complete Freund’s adjuvant (CFA) supplemented with 4 mg/ml killed M. tuberculosis. Pertussis toxin (PT, 500 ng/mouse) was injected i.p. immediately and 48 hours later: Control mice were received only CFA and further PT. Clinical assessment was performed on the generalized clinical score of EAE. Group score was 3.5. Expression of amyloid beta precursor protein, complement component 1s, chemokine (CC motif) ligand 5, CXCl ligand 9, CXCl ligand 10, CD4 antigen, G protein alpha inhibiting 2, histocompatibility 2 class II antigen β2/β1, IL6, IL13, MAPK, NFkB light polypeptide gene, proteolipid protein 1, and TNF are found to be significantly different in the brain at about the peak of disease than control animals. Our data revealed strong transcriptional regulation of genes involved in inflammatory processes, such as antigen presentation and differentiation, complement activation and chemotaxis.

P09.087-S
Target resequencing of regions associated with Multiple Sclerosis in the Italian population
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Multiple sclerosis (MS) is a multifactorial autoimmune demyelinating disease of the CNS. Several large international association studies detected over 100 MS loci. However, for the majority of them the causal variant is not yet identified and, generally, only common variants have been studied. The aim of this study was to follow-up the MS loci in the Italian population, searching for the primarily associated variants, focusing also on rare variants. After an association study in 1750 Italian MS cases and 2272 matched controls (Illumina 660-Q, and Immunochip), we selected for target resequencing 32 MS associated regions showing a significant association in the Italian population. The selected regions (1.9 Mb), either including the whole genomic segment (N=17 regions, 45 genes) or only the coding sequences (further 48 genes), were captured (Agilent SureSelect) and sequenced (GAIIx Illumina) in 600 Italian MS patients and 400 matched controls pooled in groups of 12 individuals. We used the variant caller CRISP to call the variants, ANNOVAR to annotate them, and custom R-scripts to calculate allele frequency. Validation of a subset of variants by individual genotyping showed a high correlation with allele frequency in the pools.After QC, results in the MS patients showed that among the 23065 detected variants, 67% were absent in public databases, including 1 061 variants with a predicted functional consequence on the gene product (missense, nonsense, splice variants), thus potentially directly involved in the MS susceptibility. The comparison with the data of the controls and the replication in independent cohorts is ongoing.

P09.088-M
Modulation of Protein Kinase C Alpha (PRKCA) mRNA expression and alternative splicing by functional polymorphisms contribute to multiple sclerosis susceptibility
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The Protein Kinase C Alpha (PRKCA) gene, encoding the Th17-cell-selective PRKCA isotype, was repeatedly associated with multiple sclerosis (MS), but the underlying biological mechanism remains unknown. We replicated this genetic association in an Italian population (409 cases, 723 controls), identifying a protective signal, corresponding to a GCC microsatellite in the PRKCA promoter (P=0.03, OR=0.12, 95%CI=0.015-0.94), and a risk haplotype in intron 3 (P=7.7*10-4; OR=1.57, 95%CI=1.24-1.99, minor allele frequency P=1.4*10-12). Expression experiments demonstrated that the protective signal is associated with PRKCA promoter variants conferring higher expression levels of the gene. Minigene-based transfection experiments proved that the risk signal is driven by an ins/del polymorphism (rs35476409/rs61762387) influencing, with an hnrNP-H dependent mechanism, the skipping of a PRKCA alternative exon. Studies performed on RNA extracted from different cell lines and human tissues evidenced a complex pattern of alternatively-spliced (AS) PRKCA isoforms, which are modulated by the nonsense-mediated mRNA decay (NMD) and display a preferential association with the use of alternative polyadenylation sites.

A novel mutation in SCN4A gene, in a patient with an unusual clinical presentation of Myotonia Permanents: Expanding the clinical and molecular spectrum of SCN4A mutations
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Congenital myotonias are a group of hereditary muscle disorders that present in infancy and childhood, characterized by impaired muscle relaxation following a voluntary forceful contraction. The core symptoms either relate to myotonia or to periodic weakness. These disorders are caused by mutations in genes encoding the skeletal muscle chloride, sodium and calcium channels. We report a 7-years old boy with a severe early onset unique phenotype of congenital myotonia, who has permanent. He had neonatal respiratory failure and as well as severe recurrent episodes of laryngospasm during infancy. Massive general muscle hypertrophy, eye lid myotonia and prolonged hand grip relaxation, were present since early childhood. An unusual complication of tongue hypertrophy was noted during infancy and reoccurred again after surgical resection. A novel V717G mutation in SCN4A was identified in the proband, but not in his parents. This is the first case of myotonia permans reporting recurrent tongue hypertrophy as a major clinical feature in this case. Tongue hypertrophy resulted most probably from continued tongue contraction caused by his permanent myotonia.

The novel mutation found in SCN4A in this boy, has not been found in his parents. It has been suggested to be pathological by a number of in silico prediction programs. It is located in a well conserved area of the gene in the transmembrane helical part of the Sodium channel protein subunit. This mutation has not been described previously in SCN4A related disorders. This case further expands the genetic and clinical spectrum of myotonia permanents.

P09.090-M
PANK2 and C19orf12 mutations in neurodegeneration with brain iron accumulation (NBIA)
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Neurodegeneration with brain iron accumulation (NBIA) defines a group of neurodegeneration diseases that share prominent extrapyramidal features, dementia and radiographic evidence of iron deposition in the basal ganglia. Different forms of early onset NBIA with autosomal recessive transmission are associated with mutations in specific genes as (1) pantothenate kinase-associated neurodegeneration (PKAN); (2) phospholipase-associate neurodegeneration (PLAN); and (3) fatty acid hydroxylase-associated neurodegeneration (FAHN). Mutations in PARK2 are the most common cause of these disorders. C19orf12 was recently reported as another causative gene.

In our study we recruited 20 subjects with NBIA that include clinical data and associated DNA samples. DNA and clinical information were collected and used after participants gave written informed consent. The 8 exons of PARK2 and 3 exons of C19orf12 were amplified by PCR and sequenced on an ABI PRISM 3130 XL-Avant Genetic Analyzer. Phenotypic data were obtained by neurologic examination and magnetic resonance imaging. Mutation screening of PARK2 and C19orf12 were found in 3 and 5 patients, respectively. In conclusion mutation in both PARK2 and C19orf12 contributed significantly to NBIA in the our patients.

P09.091-S Identification of the genetic factors involved in neural tube development and defects
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Neural tube is the embryonic precursor of brain and spinal cord, and forms as a consequence of closure of the developing neuroepithelium. If closure events fail, the embryo will manifest a neural tube defect (NTD: spina bifida or exencephaly). Despite significative advances in the field, the elucidation of genetic factors associated to NTD has remained elusive and NTDs are the second most common congenital defects affecting human pregnancies. This project aims at investigating two different aspect of NTDs: on one side the analysis of the transcription factor Sox-1, that was found to be down-regulated in a microarray screening in murine NTDs model (Zic2Ku). Sox-1 expression correlates remarkably closely with the progression of posterior closure of the neuro-epithelium, overlapping with Zic2 expression. Functional studies of Sox1 and Zic2 in zebrafish will be flank to mouse embryos and in vitro analyses. On the other side, this project investigates the role of miRNAs in neural tube development on human samples. From a database of 534 fetal autopsies, we selected 9 fetuses with NTDs (7 myelomeningocele, 2 anencephaly). Using a combination of bioinformatics tools (miRWalk, CO-META, DAVID), we have identified 4 candidate miRNAs whose predicted targets are significantly enriched for functional pathways related to neurolation, that are now under functional evaluation.

P09.092-M Mutations in RNA kinase CLP1 cause neurodegeneration
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Clavage and polyadenylation factor 1 subunit (Clp1) is an important kinase in RNA metabolism. It is involved in processes as RNA maturation and mRNA 3’ end processing. Recently, a missense mutation in the CLP1 gene has been identified in patients with atrophy of the cerebellum, pons and corpus callosum. We have isolated an ENU induced zebrafish mutant, harbouring a p.R44X nonsense mutation in the Clp1 gene. Using in situ hybridization and morpholino-knockdown of p53 we examined the phenotype of homozygous p.R44X zebrafish embryos. We show that Clp1 knockout fish do not survive beyond 4 days post fertilisation (dpf), have a reduced head size and show an S-curved body. At 2dpf, Clp1 knockout fish show reduced expression of midbrain marker otx2, increased cell death in the brain and a disturbed organisation of motor neurons. The phenotype can be partly rescued by injecting human wild type, but not by mutant p.R140H CLP1 mRNA. Moreover, clp1 knockdown of p53 partially rescues the phenotype as well. We show that Clp1 is an essential gene in both the central and peripheral nervous system. Similar to the human situation, clp1 mutations cause neurodegeneration and motor neuron problems in zebrafish. We show that the neurodegeneration is mediated by the p53 apoptosis pathway. Our data supports the hypothesis that amongst other RNA processing genes, CLP1 plays a crucial role in neurodevelopment.

P09.093-F Familial case of NBIA
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Neurodegenerative brain iron accumulation (NBIA) is a group of inherited disorders with various symptoms and genetic aetiology. Common feature of all NBIA forms is accumulation of iron in basal ganglia and progressive movement disorder. NBIA belongs to rare genetic conditions affecting 1-3 per million individuals which makes it insufficiently studied and understood. Nevertheless, new mutations and genes implicated in different NBIA types are being discovered.

One of them is C19ORF12 mutation, which causes MPAN - mitochondrial membrane protein-associated neurodegeneration, characterized by childhood/early adulthood onset, dystonia, spasticity, optic atrophy and neurosensory changes. The disorder is progressive and symptomatic therapy is the only currently available option.

Parents of affected child came to our institution for genetic counselling and testing for previously detected mutation on exon 3 (C19ORF12) of MMIN gene, c.204-214del11bp homo, in the index patient (daughter, aged 17). Family members (mother, father, daughter-proband and son-aged 5, asymptomatic) were tested using specifically designed amplification primers. Agilent 2100 bioanalyzer was used for analysis of PCR products. Deletion in sample of affected patient was confirmed as basis for linkage analysis. Both parents and son are heterozygous carriers for described mutation. Since MPAN is autosomal recessive, risk of affecting future offspring is 25% in this case. Risk for asymptomatic carriers is 50%, so genetic counselling is highly recommended.

Discovering new mutations associated with NBIA is significant process as it could elucidate mechanisms underlying this group of disorders and create new possibilities in terms of gene therapy and effective therapeutic options in the future.

P09.094-M A cell reprogramming-based approach to study 7q11.23 gene dosage imbalances in Williams Beuren syndrome and autism spectrum disorder
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Symmetrical gene dosage imbalances at 7q11.23 cause two neurodevelopmental diseases, Williams Beuren Syndrome (WBS) and 7q11.23 microduplication associated to autistic spectrum disorder (7dup-ASD). Besides intellectual disability and craniofacial dysmorphisms, WBS patients display hypersociality and comparatively well-preserved language skills while 7dup-ASD is associated with impairments of varying severity across the autistic spectrum. The striking symmetry in genotype and phenotype between the two conditions points to the 7q11.23 cluster as a surprisingly small subset of dosage-sensitive genes affecting social behavior and cognition. The molecular phenotypes of these syndromes in disease-relevant cell-types remain to be elucidated however, due to scarce availability of primary diseased tissues. Convergent evidence both from human studies and mouse models, points to transcriptional dysregulation as a critical aspect of both conditions, consistent with the presence of several transcription factors in the 7q11.23 interval.

Here we present the first analysis of transcriptional dysregulation in human physiopathologically relevant cell types carrying 7q11.23 dosage imbalances. We selected a large and unique panel of WBS and 7dup-ASD patients and derived induced pluripotent stem cells (iPSC) with the most advanced reprogramming technology based on synthetic miRNAs. These were then differentiated into relevant lineages to establish experimentally tractable models of these conditions. Next, we integrated high-throughput sequencing for transcriptional and chromatin analysis and present here a functional dissection of these symmetric conditions that uncovers the principles of dosage-dependent transcriptional dysregulation in disease-relevant human cell types.

P09.095-S Next-generation sequencing in the diagnosis of neurodevelopmental disorders
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Discovering new mutations associated with NBIA is significant process as it could elucidate mechanisms underlying this group of disorders and create new possibilities in terms of gene therapy and effective therapeutic options in the future.
Seven novel neurofibromatosis 1 (NF1) gene mutations identified in Spanish pediatric patients

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Introduction: Neurofibromatosis type 1 (NF1) is one of the most common human autosomal dominant disorder with an estimated incidence of 1:3500. This is a neurodermal dysplasia characterized by café-au-lait spots, axillary freckling, dermal neurofibromas, and Lisch nodules of the iris. The condition is fully penetrant and has a highly variable expression. It is caused by mutations in the neurofibromin gene (NF1) located on chromosome 17q11.2.

Subjects and Methods: Seven children suspected of having NF1 were tested for mutations in the NF1 gene. Some of them only have café-au-lait spots and two presented other clinical features. Single and multie -

Case Mutation Familiar study
1 c.4009G>A, p.R1337W yes
2 c.3941-6T>G, p.R96CR yes
3 c.3679_3680insA yes (de novo)
4 c.3955C>T, p.S1312F ND
5 c.23305_350insAAAAT yes
6 c.36666_36668insMNG yes
7 c.3411G>T, p.S1144T ND
8 ND: not determined

Genetic diagnosis of neurological diseases using NGS: first year of experience

M. C. Giout et al.

ABSTRACTS POSTERS

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In a consanguineous family with two patients having a novel autosomal recessive inherited syndrome another patient with an unlinked autosomal nonsyndromic form of mental retardation was identified.

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We previously characterized in a large family of Turkish origin a male and a female patient showing a new syndrome, that includes following symptoms: severe mental retardation, corpus callosum agenesis, ataxia, moderate microcephaly, square face, hypotelorism, bilateral ptosis, arched eyebrows, epicanthal folds, downslanting palpebral fissures, strabismus, amblyopia, broad nasal bridge, low set ears, short philtrum and downturned corners of the mouth. Since mothers of both patients are first degree cousins and both fathers are second degree cousins, pedigree analysis makes an autosomal recessive mode of inheritance of this particular syndromic disorder very likely. Homozygosity mapping by 250 K Affymetrix SNP array analysis allowed reducing homozygous genomic regions shared by both patients to only 4 segments ranging from 3.05 Mb to 8.84 Mb in size. Recently a brother of the male patient was diagnosed with nonsyndromic mental retardation (NSMR). Further Affymetrix Cytoscan® HD analysis of this DNA was performed, indicating his NSMR is not linked to the new syndrome. Because the number of potential candidate genes in these genomic regions according to obtained RS-loci on chromosome 1 (1p36.22), 2(2q22.3), 3 (3p14.1) and 9 (9q21.13) is considerable, whole exome next generation sequencing will be applied to search for homozygous mutations in both patients especially in those segments. Although the phenotype of our patients shows similarities with Toelke-Carey syndrome and Charlevoix disease, it clearly does not resemble these syndromes, so we think that our patients exhibit a new syndromic disorder and another NSMR form could be mapped in this family as well.

P09.100-M
Next-generation analysis of the amytotrophic lateral sclerosis / Parkinsonism-dementia complex of Guam

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The amytotrophic lateral sclerosis (ALS) / Parkinsonism-dementia complex of Guam (PDC) is a progressive, age-associated neurodegenerative disorder described in Guam, Western Papua and the Kii peninsula of Japan. Despite decades of research the excess incidence and pathogenesis of ALS-PDC on Guam remains enigmatic and mutations underlying disease have yet to be elucidated. In this study we have used next-generation targeted resequencing to evaluate the contribution of genetic variability in the pathogenesis of Guamian neurodegeneration. A targeted-capture panel covering the exonic regions of 116 major genes previously linked and/or associated with parkinsonism, dementia, ALS and related syndromes has been developed. Ninety-three-remote Chumorro islanders, including patients with ALS, parkinsonism and/or dementia, as well as neurologically normal subjects, were longitudinally examined and comprehensively assessed. We report putatively pathogenic variants in HTT, PINK1, DCTN1, CHMP2B, DNAJC13, FUS, GRN and ALS2, identified in patients with parkinsonism and/or dementia, that explain multi-incident disease in several pedigrees.

P09.101-S
Multiple sclerosis and NF1: it’s time to think about it

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Neurofibromatosis type 1 (NF1) is a rare genetic condition, with a frequency of 1/2500-1/3000, caused by mutations in the NF1 gene (OMIM *164345), which acts in myelination processes and seems to be involved in immunopathogenesis of MS. Moreover, NF-1 and MS may share a common influence from genes on chromosome 17 affecting proliferation and inflammation processes. We report our 12 years experience in the follow up of NF1 adult patients. The cohort is composed by 301 patients, with an age range of 18-72 years, and Males/Females ratio of 111/190. A multidisciplinary equipe evaluates patients yearly, thus allowing a precise knowledge about the natural history of the condition. Over the years, MS has been diagnosed in four of our patients (3 women and 1 man). This observational study enables us to define for the first time MS prevalence in a large cohort of NF1 adult patients (1.3%). Detailed clinical features of each MS-NF1 patient, together with review of literature and current aetio-pathogenetic hypothesis will be provided.

P09.102-M
An NGS gene panel for the genetic diagnosis of rare autosomal recessive cerebellar ataxias

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Autosomal recessive cerebellar ataxias (ARCA) are a genetically highly heterogeneous group of neurological disorders involving both central and peripheral nervous system. One of the goal of the E-Rare EUROSCAR project is to establish an NGS-based targeted genotyping for the diagnosis of known ARCA genes. One-hundred and sixty-two ARCA patients referred from different European countries were analyzed using a HaloPlex-based gene panel targeting the coding regions of 112 genes involved in recessive and dominant ataxia. Patient inclusion criteria for analysis were: progressive ataxia, exclusion of nongenetic causes, family history suggestive of autosomal recessive ataxia (AR) or sporadic (S) patients with onset before age 40. We contributed with 34 Italian patients (14AR and 20S) previously tested for FRDA, AVED, or APTX, as appropriate. This approach allowed us to identify homozygous or compound heterozygous pathogenic mutations in ADCK3 and 2 challenging genes (SYNE1 and SAC3) in 6 patients (4S and 2AR). Moreover, different missense variants of uncertain pathogenicity were identified in genes responsible for dominant ataxia (PFDN5, SPB2N2, CAGNA1A). Finally, several heterozygous mutations were identified in recessive genes. For 6 patients, analysis revealed no candidate variants in screened genes. All high-quality variants were confirmed by Sanger sequencing indicating reliability of this approach. Further analyses are required for the validation of uncertain variants and the evaluation of large in/del mutations in recessive genes in which heterozygous mutations have been identified. In conclusion, NGS gene panel represents a necessary tool for genetic diagnosis of highly heterogeneous diseases such as ataxia. (E-Rare grant to MK, PB, and FT)
Sphingosine is a major storage compound in Niemann-Pick type C disease (NPC), although the pathological role(s) of this accumulation have not been fully characterized. Here we show that sphingosine kinase (Sphk) activity is reduced in NPC patient fibroblasts and NPC mouse Purkinje neurons (PNs) due to defective VEGF levels related to deficiency of NPC1. VEGF released from bone marrow mesenchymal stem cells also activated Sphk by binding to VEGFR2, resulting in decreased sphingosine storage as well as improved PN survival and clinical outcomes in NPC cells and mice. Similar effects were noted after genetic and pharmacologic replenishment of VEGF in NPC mice. Further, iPSC-derived human NPC neurons were generated for the first time and the sphingosine accumulation caused by Sphk inactivity in these cells was corrected by replenishment of VEGF. Overall, these results reveal a novel pathogenic mechanism in NPC PNs where defective Sphk activity is due to impaired VEGF.

The role of the NR2A and NR2B subunits of the NMDA receptor in epileptogenesis

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NMDA receptors are tetrameric ligand-gated ion channels composed of two glycine-binding NR1 subunits and two glutamate-binding NR2 subunits (NR2A, NR2B, NR2C, NR2D) regulating synaptic plasticity. Mutations in the NR2A and NR2B subunits encoded by the genes GRIN2A and GRIN2B have been associated with different phenotypes of intellectual disability (ID). Mutations in GRIN2A were known to cause specific ID and epilepsy as well as other neurodevelopmental disorders, whereas mutations in GRIN2B have mainly been associated with autism spectrum disorders (ASD) but not seizures. We show for the first time that mutations of NR2 subunits of the NMDA receptor cause different and specific epilepsy phenotypes. NR2 mutations are involved in benign Rolandic epilepsy, the most frequent childhood epilepsy as well as in a variety of rare infantile epileptic encephalopathies, such as Landau-Kleffner and West syndrome. Furthermore, we demonstrate distinct genotype-phenotype correlations. Severe encephalopathic phenotypes are significantly more often caused by truncating mutations in GRIN2A, whereas missense mutations are by far more common in benign Rolandic epilepsy patients. For GRIN2B, the majority of ASD individuals present with truncating mutations, whereas all epilepsy cases appear to have gain-of-function mutations. The severity of phenotypes depends on the affected domain and the extent of receptor alteration. Our observations highlight the so far underestimated role of dysregulated NMDA signalling in both frequent and rare epilepsy disorders and reveal promising pharmacologic targets for novel therapeutic approaches.

No evidence for a role of NOL3 gene in Italian families with familial adult myoclonic epilepsy

P.09.104-M

VEGF-mediated sphingosine kinase activity decreases in Niemann-Pick Type C neurons and contributes to pathology in NPC mice

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Familial adult myoclonic epilepsy (FAME) is characterized by generalized myoclonus, which is induced by movement or somatosensory stimulation. CT may have different etiologies: metabolic abnormalities, various neurodegenerative disorders and posthypoxic myoclonus. CT may also be present in familial adult myoclonic epilepsy (FAME) defined by an autosomal dominant inheritance, but despite the locus has been mapped to chromosomes 8, 2 and 5, no causative mutations have been identified to date. A recent study, however, illustrated that FAME may be caused by mutation in NOL3 gene. Here we wished to screen families with FAME for mutations of NOL3. Since there is growing evidence of an overlap between FAME and essential tremor (ET), we also included families with ET to screen for mutations of NOL3. We analyzed 10 probands of families that originated from Southern of Italy. Four families had FAME, and six ET. After obtaining the informed consent, the DNA was extracted according to standard procedures and analyzed by direct sequencing with the Sanger method.

No causative mutations have been identified in all patients analyzed for NOL3 gene. However, three polymorphic rare variants have been identified: one in the encoded region, one in 5’ UTR region and one in 3’ UTR region of NOL3 gene in three different patients. Exclusion of mutations in NOL3 gene in our families further illustrates the great genetic heterogeneity of FAME/ET and suggests the involvement of other genes. Role of the polymorphic variants in our population remains to be clarified.

P.09.107-S

Analysis of RIT1, a novel gene for Noonan syndrome, in patients with suspected Noonan syndrome and negative for other known Noonan syndrome gene mutations


Noonan syndrome (NS) is an autosomal dominant multisystem disorder (1/2000 live births) caused by mutations in genes dysregulating the RAS- MAPK pathway. The syndrome belongs to the RASopathies that includes Costello syndrome (CS), cardio-facio-cutaneous syndrome (CFC), Neurofibromatosis type 1 (NF1) and other syndromes sharing a common pattern of congenital anomalies. Recently the RIT1 gene encoding for a new member of the RAS subfamily was functionally characterized. Moreover, gain-of-function mutations in RIT1 gene were found in 9% of the individuals with Noonan syndrome or a related condition without detectable mutations in known Noonan-related genes. In order to confirm these findings, we examined 11 patients suspicious of Noonan syndrome/RASopathies including one abortion with Hydrops fetalis and one prenatal case presenting with nuchal translucency > 97 th pct and a normal karyotype. In an initial study, all 11 patients were screened for mutations in the known Noonan syndrome gene mutations or RASopathies using a panel of 12 genes (PTPN11, SOS1, RAF1, KRAS, BRAF, NRAS, MAP2K1, CBL, SHOC2, MAP2K2, HRS, RAF1). DNA sequence analysis was carried out on the Illumina MiSeq Next-Generation Sequencing platform. Data analysis was performed using the CLCbio workbench (v6.5) and custom developed Perl scripts. Target regions with a coverage of less than 20X were reanalyzed by Sanger sequencing in order to ensure complete coverage of all coding regions and adjacent splice sites (-10/+10). No causal mutations could be identified using the 12 gene panel. In a second step all patients were screened for mutations in RIT1 gene using Sanger sequencing.
GABA mutations are a major genetic risk factor for Parkinson Disease and Dementia with Lewy Bodies in the Italian population. R. Asselta, E. Rimaldi, C. Sori, I. Guella, G. Solda, C. Baccin, R. Cilia, N. Meucci, G. Baccin

PARK2 deletions in patients with Autism Spectrum Disorder (ASD) and other neurodevelopmental pathologies I. C. Conceição, M. M. Barra, B. Oliveira, C. Cafer, J. Almeida, S. Mouga, G. Oliveira, A. M. Vicente

GBA mutations are a major genetic risk factor for Parkinson Disease, while Copy Number Variants (CNVs) have been found in patients with developmental delay (DD). Attention Deficit Hyperactivity Disorder (ADHD) and ASD. We identified 5 male patients with inherited PARK2 deletions in 342 ASD individuals, screened for CNVs using Illumina 1M SNP arrays (frequency=1.5%). Three patients had a 45 Kb deletion of intron 9, and two patients had a deletion of 326 kb. The deletions included 12 CNVs in exons 5 and 6. Clinical presentation was heterogeneous in these ASD patients. A literature search showed recurrent deletions of introns 2 and 3 (N=15 ADHD, N=7 ASD) as well as exons 1 (N=1 ASD) and 2 (N=2 ASD and DD), while DECIPHER reported one ASD individual with an exon 5-6 deletion. In available control databases, deletions of segments from exons 1 to intron 4 as well as intron 9 were recurrent, but only 1/4139 controls had a CNV overlapping exon 6, suggesting a pathogenic role of deletions in this region. The smaller exon 6 CNVs detected a functional domain SYT11 binding site, compromising the association of synaptotagmin and ubiquitination. These results support a role of PARK2 structural variants in ASD. However, certain PARK2 regions are also frequently deleted in control subjects, and therefore rigorous analysis is mandatory before assuming a pathogenic role for identified CNVs.

GBA mutations in girls with infantile epilepsy: four novel PCDH19 mutations found in girls with epilepsy and variable degree of developmental delay. R. Vodicka, L. De Palma, M. Vecchi, C. Boniver, A. Murgia


Background: In an epidemiological study carried out in an isolated population of South-Eastern Moravia in the Czech Republic, a surprisingly high prevalence of parkinsonism was found, differing from the published prevalence rates in other European countries. Objective: To determine the type of mutation in families with autosomal-dominant parkinsonism with dementia. Methods: On the basis of a detailed genealogical examination of all the individuals with confirmed parkinsonism, the pedigrees were compiled and a DNA analysis of probands from each pedigree was subsequently initiated. A massive parallel sequencing method using Ion Torrent technology was used; the DNA sequence analysis was focused on the gene loci in which the causal mutations related to Parkinson's disease (PD) have been described. Results: Three large pedigrees with an autosomal-dominant inheritance pattern with reduced penetration of parkinsonism were identified. None of the previously described pathogenic mutations associated with PD were identified, and rare variants or yet-undescribed mutations were also found. In 5 of 10 examined probands, a novel missense Q230H mutation of the MAPT gene was detected. Polyphene and SIFT in silico predictors indicate this mutation as “probably damaging”. Conclusion: Confirmation sequencing using an independent method is underway to examine the targeted MAPT mutation in other individuals from all three pedigrees and to compare it with healthy controls. Supported by grants: IGA MZ ČR NT - 14407-3/2013 and IGA LFUP 2013-024

PARK2 deletions in patients with Autism Spectrum Disorder (ASD) and other neurodevelopmental pathologies I. C. Conceição, M. M. Barra, B. Oliveira, C. Cafer, J. Almeida, S. Mouga, G. Oliveira, A. M. Vicente

A highly recurrent PARK2 structural variant in a family with autism spectrum disorder. C. Siri, I. Guella, A. Murgia, G. Solda

Here, we propose an approach to evaluate the pathogenicity of four novel mutations found in girls with epilepsy and variable degree of developmental delay. E. Leonardi, L. De Palma, M. Vecchi, E. Bettella, S. Sartori, C. Boniver, A. Murgia


The PARK2 gene encodes Parkin, a component of a multiprotein E3 ubiquitin ligase complex that targets misfolded proteins for proteosomal degradation, such as dopamine transporters, synaptotagmin and tau protein. PARK2 genetic mutations are associated with Parkinson Disease, while Copy Number Variants (CNVs) have been found in patients with developmental delay (DD). Attention Deficit Hyperactivity Disorder (ADHD) and ASD. We identified 5 male patients with inherited PARK2 deletions in 342 ASD individuals, screened for CNVs using Illumina 1M SNP arrays (frequency=1.5%). Three patients had a 45 Kb deletion of intron 9, and two patients had a deletion of 326 kb. The deletions included 12 CNVs in exons 5 and 6. Clinical presentation was heterogeneous in these ASD patients. A literature search showed recurrent deletions of introns 2 and 3 (N=15 ADHD, N=7 ASD) as well as exons 1 (N=1 ASD) and 2 (N=2 ASD and DD), while DECIPHER reported one ASD individual with an exon 5-6 deletion. In available control databases, deletions of segments from exons 1 to intron 4 as well as intron 9 were recurrent, but only 1/4139 controls had a CNV overlapping exon 6, suggesting a pathogenic role of deletions in this region. The smaller exon 6 CNVs detected a functional domain SYT11 binding site, compromising the association of synaptotagmin and ubiquitination. These results support a role of PARK2 structural variants in ASD. However, certain PARK2 regions are also frequently deleted in control subjects, and therefore rigorous analysis is mandatory before assuming a pathogenic role for identified CNVs.
Molecular genetic background of patients with PEHO-like features
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PEHO syndrome (Progressive encephalopathy with Edema, Hypsarhythmia and Optic atrophy; MIM 260565) is an autosomal recessive inherited progressive infantile encephalopathy. The main features of PEHO syndrome are hypotonia, infantile spasms and/or hyparsarrhythmia, psychomotor retardation, absence or early loss of visual fixation, edema of the face and limbs, and typical dysmorphic features. Brain atrophy is progressive and most prominent in the cerebellum, where the molecular layer is strongly reduced. Purkinje cells are deformed and misaligned, and the cells of the granule cell layer are significantly reduced in number. In addition, the optic nerves show varying degrees of loss of myelinated axons and giosis, and the retinal nerve fiber and ganglion cell layers are atrophic. A number of patients present with many of these clinical features, but lack typical neuroradiological and neuropathological findings or progression of brain atrophy and do not have the PEHO founder mutation. These patients are classified as having PEHO-like syndrome. This group is clinically heterogeneous and therefore it is likely that there are multiple underlying genes. To characterize the genetic background of patients showing PEHO-like features, we performed exome sequencing of 33 Finnish patients, and parents of six of them. We identified likely pathogenic mutations in known disease genes (CDKL5, ABAT, SPTAN1, SCN2A, MT-CYB, WDR45 and KCNQ2) in nine individuals. Analysis of mutations in novel disease genes is ongoing. Our preliminary findings imply that patients with PEHO-like features are genetically highly heterogeneous and that the entity overlaps with early-infantile epileptic encephalopathies.
**ABSTRACTS POSTERS**

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gene in a patient presenting with symptoms characteristic of HCS, but without cytostatin.

Immediately after birth, the patient presented with severe muscular hypotonia and feeding problems, followed by growth hormone deficiency and hypogonadotropic hypogonadism. Initial molecular analyses excluded a number of syndromes, including Prader-Willi (PWS). The homozygous deletion in PREPL identified by array analysis corresponded to heterozygous deletions in each of the nonconsanguineous parents. qPCR analysis confirmed these findings and pinpointed the extent of the deletion to exons 5-10. An isolated homozygous microdeletion involving only PREPL has not been previously described, however, Régal et al. (in press) recently identified a patient carrying a nonsense mutation in PREPL combined with a microdeletion involving SLCS11 and PREPL, resulting in a similar phenotype to the case presented here. These findings may contribute to further delineate the normal function of PREPL, as well as support the notion that homozygous PREPL inactivation should be considered in the diagnosis of patients with a PWS phenotype, but no PWS genotype (Maartens et al, Biol Chem 387;879-883,2006).

**P09.121-S**

**Compound heterozygous mutations in two known MCPH genes in autosomal recessive primary microcephaly**

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Autosomal recessive microcephaly with severe mental retardation with no dysmorphosis or other anomalies was diagnosed in seven individuals of an Arab Israeli family. Brain CT scan of affected individuals showed no structural anomalies. Whole exome sequencing of an affected individual identified four mutations in two known MCPH genes: compound heterozygous mutations both in STIL and in ASPM. Both c.2084A>G and c.1556+5G>A VPS53 founder mutations were found in the investigated families. Common founder mutation(s) had more than one of the 4 mutations, and only in a heterozygous state. Segregation analysis and functional assays are underway to unravel which of the above variations are the causative mutations for microcephaly in the affected individuals within this family.

**P09.122-M**

**VPS53 compound novel mutations cause progressive cerebello-cerebral atrophy type 2 (PCCA2)**

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Progressive cerebello-cerebral atrophy (PCCA) leading to profound mental retardation with early onset epilepy, was diagnosed in four non-consanguineous apparently unrelated families of Jewish Moroccan ancestry. None of the known SepSecs (MIM613009) mutations were found in the investigated families. Common founder mutation(s) were assumed. Genome wide linkage analysis and whole exome sequencing were done, followed by realtime PCR and immunofluorescent microscopy. Genome wide linkage analysis mapped the disease-associated gene to 0.5Mb on chromosome 17p13.3. Whole exome sequencing identified two mutations within this locus, which were common to the affected individuals: compound heterozygous mutations in VPS53, segregating as expected for autosomal recessive heredity within all four families, and common in Moroccan Jews (~1:3 carrier rate). The Golgi-associated retrograde protein (GARP) complex is involved in the retrograde pathway recycling endocytic vesicles to Golgi. Both c.2004G>A and c.1556+5G>A VPS53 founder mutations are predicted to affect the C-terminal domain of VPS53, known to be critical for the retrograde function of the GARP complex. In fact, mRNA studies showed detrimental effects of the mutations on VPS53 transcripts, and immunofluorescent microscopy demonstrated swollen and abnormally numerous 363 positive vesicular bodies, likely intermediate recycling endosomes, in fibroblasts of affected individuals. Thus, VPS53 mutations cause autosomal recessive PCCA type 2.

**P09.123-S**

**Clinical and molecular characterization of progressive encephalopathies in children**


Progressive encephalopathies (PE) are defined as progressive diseases of the Central Nervous System often accompanied by decline of cognitive and/or motor functions and include a number of different disorders. PE can primarily be divided into neurodegenerative and metabolic, the latter defined by defects in subcellular organelles or in the intermediary metabolism of macromolecules. In the neurodegenerative disorders, progressive loss of neuronal function is often detected by cerebral MRI or markers in the cerebrospinal fluid. PE is associated with high morbidity and mortality rate, but treatment options are available for some PE disorders. Genetic explanations lack for about 20% of PE disorders, which are classified according to MRI findings and phenotype.

Our aim is to characterize novel mutations causing PE. We have collected more than 60 PE-patients in 45 families. In all patients, we ruled out CNS infection, trauma, vascular accidents and sequelae after prematurity. Evaluation of all patients by biochemical examinations and neuroimaging revealed cortical atrophy, cerebellar degeneration or basal ganglia abnormalities in the majority of the patients. Congenital anomalies, also those outside the nervous system, were evaluated using the London in Medical Database for syndrome identification.

Karyotyping, aCGH and analysis of candidate genes by MLPA and/or sequencing revealed no relevant findings. We have initiated Whole Exon Sequencing (WES) on DNA from 43 family trios. Among the 16 trios finalized through the bioinformatics pipeline, a putative disease causing mutation has been identified in eight. Functional studies are currently being performed to characterize their clinical implications.
MECP2 mutation and in patients with mental retardation and Rett-like clinical features.

Results: We analyzed 211 patients with clinical presentation likely to have mutations in FOXG1 gene 143 RTT (classic and congenital form MECP2-negative) and 39 male patients with severe mental retardation and hypotonia and 29 patients with RTT like features. We detected 9 mutations; 5/23 girl patients with congenital form and 4/39 male patients with severe mental retardation. None of classical form or girls with RTT-like clinical features carried mutation in FOXG1.

Conclusions: Genetic etiologies of variant Rett syndrome are heterogeneous, screening the FOXG1 gene should be done not only in females, but also in male patients with severe hypotonia and acquired microcephaly since the first months of life. FOXG1 is not an X-linked gene and therefore there can be a higher incidence of mutation detection in RTT-like males than in MECP2 and CCDC56 genes.

P09.126-M
Modifiers of age at onset in spinocerebellar ataxia type 2: a preliminary study in a Brazilian population
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The spinocerebellar ataxia type 2 (SCA2) is caused by CAG expansion at ATXN2 gene, which account for 50% of the variability in age at onset (AO). Previous reports pointed to CAG variations and polymorphisms at other genes as responsible for the remaining AO variance. Aims: to address a common genetic etiology of other polyglutamine tracts and of a mitochondrial DNA polymorphism as modifiers of AO in SCA2 patients. Methods: symptomatic individuals with a molecular diagnosis of SCA2 were recruited from Brazil. Capillary electrophoresis was performed to detect CAG lengths at SCA1, SCA1, SCA3/MDJ, SCA6, SCA7 and RA1 associated genes; the mitochondrial complex I gene polymorphism (10398G) was determined by PCR followed by restriction endonuclease analysis. Pearson correlations with AO were tested against each CAGN for each individual; the 10398G polymorphism of one person per maternal lineage was analysed by t-test; all followed by a step-wise linear regression. Results: 57 individuals (33 families and 42 maternal lineages) were studied. Mean (range) AO and CAGN at normal and expanded ATXN2 alleles were 32.9 (3-76) years and 23 (22-33) and 42 (34-67) repeats. At first, AO correlated with the large alleles at ATXN2 and ATXN3 genes, and with small allele at RA1. 10398G was not associated with AO. On step-wise regression, the unique correlation maintained was with ATXN2 expanded allele (r = 0.78; r2 = 0.61; p < 0.0001). Discussion: our preliminary data did not support previous published results; they should be confirmed with an outlier sampling strategy, in the future.

P09.127-S
Heterozygous deletion of KLHL1/ATXN8OS at the SCA8 locus are likely not associated with cerebellar impairment in humans
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Spinocerebellar ataxia type 8 is a dominantly inherited ataxia, mainly occurring in adulthood, caused by a CAG tract expansion in the ATXN8OS gene, an untranslated antisense RNA partially overlapping the KLHL1 gene, and the complementary CAG repeat in the ATXN8 gene.

We report a father (59 years old) and his daughter (10 years old) carrying a genomic deletion (800 kb) completely overlapping ATXN8OS gene and part of KLHL1 (from 70,045,271 to 71,196,665 - NCBI Build 37/hg19 - Array-CGH 60K) and a 4 Mb duplication in 8q13.3q21.11 (7,226,801-761,311,849) segregating in an unaffected brother. The girl was evaluated for dysarthria, difficulties in sentence structuring, short attention span and mild intellectual deficit. No other neurological signs were reported. Brain MRI was normal. Nevertheless, further examinations revealed short attention span; coordination, sensitivity, reflexes and cranial nerves were normal; no abnormal gait or dystarhria. Brain MRI revealed several areas of gliosis in the frontal white matter. Databases mining showed a partially overlapping 357 kb deletion involving only KLHL1 and ATXN8OS (chr 1:37,046,026-70,84,321) in a patient with mild speech delay (December 27,559), inherited from a healthy mother.

In contrast with the KLHL1/ATXN8OS knockout mouse model, our data suggest that heterozygous deletion of KLHL1/ATXN8OS is not associated with ataxia/cerebellar involvement in humans.

P09.128-M
Genetics of Schizophrenia: preliminary results of an Italian multicenter study
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Schizophrenia (SCZD) is a major psychiatric disease causing severe disability and with a prevalence of 1% worldwide. Genetic factors play a key role in the etiology of schizophrenia but its genetic bases are complex and not yet clarified. In the last few years, innovative technologies have been widely applied to the genetic study of schizophrenia. This Italian multicenter study aims to apply aCGH and NGS technologies to investigate genetic risk factors for specific SCZD-related endophenotypes that greatly affect real-life functioning of people with schizophrenia or variables strongly associated with it. To investigate the contribution of de novo CNVs to schizophrenia vulnerability, we have used the Enhancer Chip (PloS One 2012;7(12):e52264), a customized aCGH recently developed by our group, which is able to examine CNVs in the whole human genome as well as testing over 1,250 enhancers for their potential pathogenic role. Clinical research units have provided us DNAs from 60 sporadic SCZD patients and their parents (family trios). To date, we have identified two de novo partially-overlapping deletions at 7q31.2 in unrelated SCZD patients. Interestingly, both CNVs include MET oncogene has been previously associated to schizophrenia (Am J Psychiatry 2010;167(4):436). Another SCZD patient presented a CNV overlapping the first intron of a specific SCZD-related endophenotypes that greatly affect real-life functioning of people with schizophrenia or variables strongly associated with it. To investigate the contribution of de novo CNVs to schizophrenia vulnerability, we have used the Enhancer Chip (PloS One 2012;7(12):e52264), a customized aCGH recently developed by our group, which is able to examine CNVs in the whole human genome as well as testing over 1,250 enhancers for their potential pathogenic role. Clinical research units have provided us DNAs from 60 sporadic SCZD patients and their parents (family trios). To date, we have identified two de novo partially-overlapping deletions at 7q31.2 in unrelated SCZD patients. Interestingly, both CNVs include MET oncogene has been previously associated to schizophrenia (Am J Psychiatry 2010;167(4):436). Another SCZD patient presented a CNV overlapping the first intron of MET. These preliminary data may help to strengthen the role of de novo CNVs in the pathogenesis of schizophrenia.

P09.129-S
Genome-wide methylation profiling of schizophrenia
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Background: schizophrenia is one of the major psychiatric disorders. It is a disorder of complex inheritance, involving both heritable and environmental factors. DNA methylation is a fundamental inheritable epigenetic modifier that stably alters gene expression, genome stability and response to environmental stressors. Environmental modifications, resulting from environmental stimuli could also make a contribution to the disease development.

Materials and Methods: We have performed 26 high-resolution genome-wide methylation array analyses to determine the methylation status of 27,627 CpG islands and compared the data between patients and healthy controls. Methylation profiles of DNAs were analyzed in six pools (220 schizophrenic patients; 220 age-matched healthy controls; 110 female schizophrenia patients; 110 age-matched healthy females; 110 male schizophrenia patients; 110 age-matched healthy males) and 20 individual patient DNA samples (7 females and 13 males).

Results: We find significant differences in the methylation profile between schizophrenia and control DNA pools. New candidate genes that principally
Schizophrenia (SZ) is a severe neuropsychiatric disorder with heritability estimates of ~60%. Xu et al. (2011) published the first exome-sequencing study focusing on de novo mutations in patients with schizophrenia. To provide additional genetic evidence for any of the genes suggested by the exome-sequencing study, we performed a follow-up study focusing on copy number variants (CNVs). We screened 1,637 patients and 1,627 controls for CNVs in any of the genes suggested by the exome-sequencing study. Duplications in RB1CC1 on chromosome 8 were overrepresented in patients. The duplications were followed-up in independent European samples. In the combined analysis, comprising of 8,461 patients and 11,287 controls, duplications in RB1CC1 were associated with schizophrenia (P = 1.29 x 10^-5; odds ratio = 8.58). The aim of the present study was to further explore RB1CC1 as a candidate gene for schizophrenia. The gene consists of 24 exons. We focused our targeted resequencing on exon 15: (i) it contains >30% of the gene’s amino acids; (ii) it contains a frameshift deletion in this exon. After quality control, the data from 1740 patients were available. Among 22 patients, a total of 17 different variants were identified and verified by sequencing the complementary strand. Of these, 10 were neither detected in the 1000 Genomes Project nor the Exome Variant Server. Currently, we are analyzing whether these variants segregate with a psychiatric diagnosis within the families of the affected probands. Furthermore, detailed phenotypic description of the mutation carriers are being assembled.

P09.134-M
Combination of whole-genome and whole-exome sequencing, to identify rare and de-novo variation in cases of schizophrenia and bipolar disorder from the Faroe Islands

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P09.135-S
Identification of a de novo 15q13.1-13.3 deletion in a reading and language impaired cohort

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Dyslexia and specific language impairment (SLI) are developmental disorders presenting deficits of written or spoken language respectively in individuals with normal intelligence and education, and without overt neuropsychological abnormalities. Copy number variations (CNVs) have been implicated in neurodevelopmental and psychiatric conditions, such as autism and schizophrenia, but it is not clear to what extent they might contribute to reading and language abilities. Using data from a longitudinal study investigating the development of children between 3 and 7 years of age, we performed CNV analysis (n=94 children; 65% family risk of dyslexia, 23% language impairment) and identified a large deletion on chromosome 15q13.1-13.3 in an individual with an early age SLI diagnosis. This single copy deletion spanning BP3-BP5 (~3.2Mb) was validated by qPCR, and shown to be de novo. Both parents and a sibling are non-carriers of this deletion and do not display any reading or language deficits, suggesting this de novo deletion is likely to be pathogenic.

P09.136-M
Gain-of-function Na1.7 and Na1.8 mutations in patients with idiopathic small fiber neuropathy
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Small fiber neuropathy (SFN) is a relatively common disorder of thinly myelinated and unmyelinated nerve fibres and is clinically characterized by burning pain and autonomic complaints. We have recently described the presence of gain-of-function variants in Na1.7 and Na1.8 (encoded by SCN9A and SCN10A) sodium channels in a small cohort of patients (n=92) meeting strict criteria for idiopathic small fiber neuropathy (I-SFN). In this study a cohort of 393 patients with I-SFN was tested for the presence of variants in SCN9A and SCN10A. Patients that did not harbor a SCN9A variant subsequently underwent SCN10A analyses. Electrophysiology was used to test functional effects of variant channels. In SCN9A, 17 different heterozygous variants classified as class 3 (unknown significance) or 4 (likely to be pathogenic) based on in silico prediction were found in 54 patients (~9%). For two Na1.7 variants, electrophysiology did not provide evidence for pathogenicity. In SCN10A, ten different heterozygous Na1.8 variants classified as class 3 (unknown significance) or 4 (likely to be pathogenic) based on in silico prediction were found in 15 patients (~3%). For one Na1.8 variant, evidence for pathogenicity was provided by electrophysiology. In conclusion, heterozygous SCN9A or SCN10A variants are present in a substantial proportion (~12%; 49 of 393) of our cohort of I-SFN patients. For many variants electrophysiological analysis revealed gain-of-function attributes in mutant channels. This implies that functional variants in SCN9A and SCN10A may predispose carriers to the development of channelopathy-associated SFN. Analysis of SCN9A and SCN10A should be considered for patients with I-SFN.
P09.139-S
Spino cerebellar ataxia type 6(SCA6): clinical pilot trial with medicinal herbs
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Spino cerebellar ataxia type 6 (SCA6) is an autosomal dominant cerebellar ataxia associated with small polyglutamine-dependent expansions in the alpha 1A-voltage calcium channel. At present, we have no effective therapeutic tools. We report here six cases of spino cerebellar ataxia type 6(SCA6)with typical symptoms. Genetic tests revealed expanded allele of 22–25 CAG repeats at the spino cerebellar ataxia type 6 locus. Head MRI revealed a typical atrophic image in cerebellum. For the systems therapy with medicinal herbs, the differential diagnosis by traditional herbal medicine was made according to the guideline. 18–26 medicinal herbs were given according to the differential diagnosis in each patient. The remedies used for the cases consist of several different ingredients, which have well-established histories of use for treatment of vertigo, tremor, or ataxia and are expected to exert their specific effects. In 5 of 7 patients, ataxia of gait and stance was significantly improved in 30–60 days of the herbal treatment. 34–85% reduction were obtained on the 100-point semiquantitative International Cooperative Ataxia Rating Scales (ICARS) scores. The results imply the therapeutic potential of herbal medicine for spino cerebellar ataxia 6. Further extensive investigations are required to clarify the mechanisms by which the remission induction of this genetic disease of CAG repeat expansion mutation has been attained with the medicinal herbs.

P09.140-M
Mutant Ataxin-2 Induces Reactive Oxygen Species and Autophagy in transformed lymphoblastoid cells from patients with Spino cerebellar Ataxia Type 2
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Spino cerebellar ataxia type 2 (SCA2), an autosomal dominant neurodegenerative disease, is caused by the expansion of a CAG triplet repeat located in the α-N-terminal coding region of the ATXN2 gene. Alleles of the ATXN2 gene that carry 13–31 CAG-trimethylcloside repeats are present in normal individuals. Contrariwise, alleles with a CAG triplet repeat number of >31 and up to approximately 200 are present in patients with SCA2. Although the detailed mechanism of pathogenesis is yet to be defined, neurotoxin, especially reactive oxygen species (ROS), released from aggregated mutant proteins, may play a role in the pathogenic process. In this study, the lymphoblastoid cell lines (LCLs) isolated from SCA2 patients were utilized to compare with the wild-type lymphoblastoid cell. We investigated the crucial relationship between the expression of p-ERK1/2 and autophagy marker protein, Atg8 (LC3 class II) were higher in SCA2 patients. Electron micrographs showed that only the cells expressing expanded Ataxin-2 contained aggregated protein and autophagic vacuoles. Based on the above observations we hypothesized that the aggregated mutant Ataxin-2 proteins may generate ROS in mitochondria, which subsequently up-regulate Atg8 expression levels and ultimately lead to autophagy and cell death.

P09.142-M
Intragenic deletions affecting two alternative IMMP2L transcripts in patients with Tourette syndrome
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Tourette syndrome (TS) is a childhood onset neuromedical disorder characterized by involuntary movements and vocalizations, known as tics. The etiology of TS is complex and largely unknown, but has a strong genetic component. IMMP2L (inner mitochondrial membrane peptidase, subunit 2) is one of the few genes that have been suggested to increase susceptibility to TS, after identification of chromosomal rearrangements affecting IMMP2L in several families with TS or tics. However, to date only a single study has investigated the role of structural copy number variations (CNVs) of IMMP2L in a small cohort of TS patients without finding any deletions/duplications. Through CNV screening of a cohort of 188 unrelated TS patients and 316 controls from Denmark, we identified seven patients (3.7%) and 3 controls (0.9%) with intragenic IMMP2L deletions, thus, the frequency of IMMP2L deletions was significantly higher in patients than controls (p=0.04). Four of the deletions identified in the patients did not include any known exons of IMMP2L, but were within intron 3. These deletions were found to affect a shorter IMMP2L mRNA species with two alternative 5’-exons, one of which included the ATG start codon. We showed that this short transcript and the previously published long transcript were expressed in several brain regions, with particularly high expression in cerebellum and hippocampus. These results may improve our understanding of the role of IMMP2L in the pathogenesis of TS.

P09.144-M
Tuberous sclerosis complex phenotypes suggestive of TSC1/TSC2 mosaicism
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Tuberous sclerosis complex (TSC) is due to mutations in TSC1 or TSC2 but 15% of patients have no mutation identified (NMI). A recent study suggests that NMI patients are mosaic TSC. A TSC1/TSC2 mutation in a neural crest progenitor would explain NMI patients without tubers and subependymal nodules (SENs), because neural crest is the origin of the majority of extracranial lesions in TSC. We performed 2 studies aiming to support this hypothesis. First, a review of the MRIs of 220 TSC patients with no mutations identified (NMI) patients was performed to support this hypothesis. First, a review of the MRIs of 220 TSC patients with no mutations identified (NMI) patients was performed to support this hypothesis. Second, we studied 19 patients with possible TSC who had brain MRI. Mixtures of 18~26 medicinal herbs were given according to the guideline. Molecular diagnosis of TTR gene mutations in Italy: the experience of the Molecular Genetics Laboratory of Ferrara
Tramtyretin (TTR), a plasma and cerebrospinal fluid protein secreted by the liver and choroid plexus, is mainly known as the physiological carrier of thyroid hormones (T4) and retinol. Under pathological conditions, various TTR missense mutations are known to destabilize the tetramer composed of mutant and wild type subunits, causing misfolding of the protein and fibril aggregation. This condition is associated with the amyloid diseases: senile systemic amyloidosis, familial amyloid neuropathy (FAP), familial amyloid cardiomyopathy (FAC). Direct sequencing of TTR gene detects more than 99% of disease-causing mutations. In eleven thousand analyzed 362 subjects, referred to our service by neurologists and cardiologists. About 47% of the exams were required for polynephropy, 33% for cardiac impairment and 20% for familiarity for TTR mutations. We found the causative mutation (index cases) in 80/428 subjects with no familiarity for TTR-related diseases and in 78/134 subjects with a family history of the disorder. The most frequent mutation in our cohort is ile68Leu (31%), typically present in cases with cardiac involvement, followed by Phe64Leu (22%), Val130Met (15%), Glu99Gln (14%) and Thr94A94 (5%). A novel mutation has been identified in exon 2: Val14Leu. The subject was affected by cardiac amyloidosis.

Tuberous sclerosis complex (TSC) is due to mutations in TSC1 or TSC2 but 15% of patients have no mutation identified (NMI). A recent study suggests that NMI patients are mosaic TSC. A TSC1/TSC2 mutation in a neural crest progenitor would explain NMI patients without tubers and subependymal nodules (SENs), because neural crest is the origin of the majority of extracranial lesions in TSC. We performed 2 studies aiming to support this hypothesis. First, a review of the MRIs of 220 TSC patients with no mutations identified (NMI) patients was performed to support this hypothesis. Second, we studied 19 patients with possible TSC who had brain MRI. Mixtures of 18~26 medicinal herbs were given according to the guideline. Confirmation of the pathology is essential, also in order to offer an appropriate genetic counselling to the patient and his family.
have a first postzygotic mutation in neuroectoderm or neural crest, explain- 
ing the negative mutational studies in leukocytes. They may present with 3 clinical recognizable phenotypes: a) possible TSC, b)TSC without tubers and SENs and c)TSC with tubers and without SENs

P09.145-S Novel compound heterozygous UBE3B mutations in two sisters with a craniofacial-intellectual disability syndrome C. R. J. Pedurupillay1, A. Holmgren1, T. Barny1, A. Blomhoff1, M. D. Vigeland1, Y. Sheng1, E. Frengen1, P. Stømme1,2, M. Mover1. 1Department of Medical Genetics, Oslo University Hospital and University of Oslo, Oslo, Norway, 2Women and Children’s Division, Department of Clinical Neurosciences for Children, Oslo University Hospital, Ulleval, Oslo, Norway, 3Faculty of Medicine, University of Oslo, Oslo, Norway.

Ubiquitination is a fundamental post-translational modification pathway in- volved in a wide range of cellular activities. The substrate specificity of the Ubiquitin is mostly dependent on the E3 ubiquitin-protein ligase fami- ly. Homozygous and compound heterozygous mutations in the E3 ubiquitin- protein ligase UBE3B were found to cause Kaufman Oculocerebrofacial Syn- drome (OMIM 244450) or Blepharophimosis-Ptosis-Intellectual Disability Syndrome (OMIM 615057) in six patients from five families. We describe two affected sisters, carrying a compound heterozygous mu- tation in trans in UBE3B. They exhibited cranio-facial anomalies including microcephaly, microphthalmia, blepharophimosis, upslanting palpebral fissures, high-arched and interrupted eyebrows, and myopia. In addition, they had laryngomalacia, abnormal genitalia, severe intellectual disability, delayed motor development, hypotonia, failure to thrive, and growth delay. The younger sister had small kidneys and impaired hearing, and the older sister had corpus callosum hypoplasia. The facial dysmorphism included arched and interrupted eyebrows and long eyelashes overlap with Blepharo- phimosis Ptosis and Epicanthus inversus Syndrome and Kabuki Syndrome. By exome sequencing, followed by Sanger sequencing, we found in both si- sters a UBE3B missense mutation in exon 1 (p.Met1Val) and a 1 bp frameshift deletion (p.Phe591fs) in exon 17. The mutations were predicted to result in a truncated UBE3B protein and cause the disease.

We compared the findings in our patients with the previously described patients. Loss of UBE3B seems to result in a relatively homogeneous pheno- type, recognizable by the distinct facial dysmorphism of the ocular region in particular, and the severe psychomotor developmental delay.

P09.146-M Exome Sequencing reveals a novel WDR45 Frameshift mutation and POLR3A compound heterozygous variants in a female with complex phenotype and mixed brain MRI findings M. Khafifi1, L. Nafaa2. 1Akron Children’s Hospital, Akron, OH, United States, 2Northeast Ohio Medical University, Rootstown, OH, United States.

WDR45 and POLR3A are newly recognized genes; each is associated with a distinct neurodegenerative disease. WDR45 is an X-linked gene associated with a dominant form of Neurodegeneration with Brain Iron Accumulation (NBIA), manifested by progressive dysfunctions, dystonia, cognitive decline, spastic paraplegia, neuropsychiatric abnormalities and iron deposition in the basal ganglia on brain imaging. POLR3A on the other hand is an auto- somal gene and its mutations cause a recessive form of a hoxymyelination with Leukodystrophy syndrome, also known as 4H syndrome, characterized by congenital hoxymyelination with thinning of corpus callosum, Hypopontida and Hypogonadotropic Hypogonadism. We report a female child with severe in- tellectual disability, aphasia, short stature, ataxia, failure to thrive and structural brain abnormalities. MRI of the brain obtained in childhood showed stable hoxymyelination, with progressive iron accumulation in the basal ganglia in particular in the globus pallidus and substantia nigra. Whole Exome Sequencing (WES) identified a novel WDR45 frameshift deleterious mutation in Exon 9 (c.587-588del). WES also revealed POLR3A missense heterozygous variants. The first is a novel missense variant in exon 4 which is maternally inherited (c.346A>G). Exon 13 carried 2 heterozygous missense variants; a maternally inherited variant (c.1724A>T) and a paternally inherited variant (1745G>A). These variants are considered likely damaging. The patient's complex clinical phenotype and mixed brain MRI findings might be attributed to the confounding effects of the expression of these 2 genes.

P09.147-S Severe presentation of WDR62 mutation: is there a role for modifying genetic factors? C. Alves Souto1, 2, R. Figueiredo3, 2, C. de Freitas Pereira1, 2, D. Missio1, 2, S. M. Rosa1, 2, 3, L. G. Carvalho1, 2, 3, L. Martins1, 2, 3, D. Ferreira1, 2, 3, M. A. Medeiros1, 2, 3, S. H. F. Leonel1, 2, 3, 4, L. M. de Souza M. Alves1, 2, 3, 5, 6, L. Marques1, 2, 3, 5, 6, C. J. Poulton1, 2, 3, 5, 6, 7, 8, 9, C. Compagnucci1, 5, 6, 7, 8, 9, S. Barresi2, 3, 5, 6, 7, 8, 9, E. Bertini3, 5, 6, 7, 8, 9, G. Zanni1, 2, 3, 5, 6, 7, 8, 9, 1Life and Health Sciences Research Institute (ICVS), School of Health Sciences, University of Minho, Braga, Portugal, 2ICVS/IBB - PT Government Associate Laboratory, Braga/ Guimarães, Portugal, 3Department of Biosciences, Northwestern University Institute for Neuroscience, Rice Institute for Biomedical Research, Northwestern University Evanston, Chicago, IL, United States.

Mutations in WDR62 are associated with primary microcephaly; however they have been reported with wide phenotypic variability. We report six in- dividuals with novel WDR62 mutations who illustrate this variability and describe three in greater detail. Of the three one lacks neuromotor develop- ment and has severe pachygyria on MRI, another has only delayed speech and motor development and moderate polymicrogyria, and the third has an intermediate phenotype. We observed a rare copy number change of unknown significance, a 17q25.3 duplication, in the first severely affected individual. The 17q25 duplication included an interesting candidate gene, tubulin cofactor D (TBCD), crucial in microtubule assembly and disassembly. Sequencing of the non-duplicated allele showed a TBCD missense mutation, predicted to cause a deleterious p.Phe121Val substitution. Sequencing of a cohort of five individuals with WDR62 mutations, including one with an identical mutation and different phenotype, plus twelve individuals with diagnosis of microcephalocyprathy and another individual with mild intellec- tual disability (ID) and a 17q25 duplication, did not reveal TBCD mutations. However, immunostaining with tubulin antibodies of cells from patients with both WDR62 and TBCD mutation showed abnormal tubulin network when compared to controls and cells with only the WDR62 mutation. There- fore we propose that genetic factors contribute to modify the severity of the WDR62 phenotype and, although based on suggestive evidence, TBCD could function as one of such factors.

P09.148-M Serotonergic signaling as modulator of Machado-Joseph disease pathogenesis A. Jelles1, 2, A. Teixeira-Castro4, 5, A. Miranda1, 2, C. Bezzi3, 4, R. Morimoto1, 2, P. Maciá1, 2. 1Department of Medical Genetics, Osaka University Medical School, Osaka, Japan, 2Life and Health Sciences Research Institute (ICVS), School of Health Sciences, University of Minho, Braga, Portugal, 3ICVS/IBB - PT Government Associate Laboratory, Braga/ Guimarães, Portugal, 4Department of Biosciences, Northwestern University Institute for Neuroscience, Rice Institute for Biomedical Research, Northwestern University Evanston, Chicago, IL, United States.

MJD model is amenable for large- scale drug screenings, in which the identification of effective drugs can be accomplished by looking simultaneously at protein aggregation in the live neuronal cells, and on its impact on neuron-regulated behavior of the whole- animal. Methods: We used our C. elegans MJD model to screen a library of ~1200 commercially FDA-approved compounds for their ability to prevent or delay the formation of mutant ATXN3 aggregates and disassembly. Results: We excluded the small molecules that were found to be toxic or cause developmental delay to the C. elegans at the concentrations tested. Of the remaining ten percent of the compounds significantly reduced the formation of mutant ATXN3 aggregates and neurological dysfunc-

P09.149-S In vitro neurogenesis of human OPHN1 mutated iPSC cells: morphological and biochemical analysis and phenotypic rescue with a ROCK inhibitor C. Campagnucci1, S. Barresi2, S. Petreni3, E. Bertini4, G. Zanni1, 2, 3, 4, 5, 6, 7, 8, 9.

1Department of Neurosciences, Unit of Molecular Medicine for Neuromuscular and Neurodegenerative disorders, Bambino Gesù Children's Hospital IRCCS, Rome, Italy, 2Confocal Microscopy Core facility, Research Laboratory, Bambino Gesù Children's Hospital IRCCS, Rome, Italy.

The X-linked gene Ophlinphrenin-1 (OPHN1) encodes a RhoGTPase activating
protein (Rho-GAP) which is mutated in a syndromic form of intellectual disability associated with cerebellar hypoplasia. In vitro and in vivo studies on hippocampal neurons of ophn1 deficient mice have shown defects in synaptic morphology and function. The administration of Y27632, an inhibitor of the ROCK signaling pathway, specifically hyperactivated in ophn1 loss of function, fully rescues the cellular and biochemical phenotype. We have developed a cellular model based on human induced pluripotent stem cells (iPSCs) technology and in vitro neurogenesis to analyze the morphological and biochemical properties of OPHN1 loss of function in patients cells. The neurogenetic potential of the OPHN1-defective iPS cells has been assessed and compared with those of control iPS cells following the protocols for differentiation into different neuronal cell lineages. The results obtained show that human OPHN1-defective neurons have altered morphology, with shorter neurites and decreased branching level, when compared with control neurons. We also confirmed the hyperactivation of the ROCK signaling pathway in OPHN1-defective iPSCs through Western blot and immunofluorescence assays of the Myosin Phosphatase Target Subunit 1 (MYPT1) and its phosphorylated state on Thr-853. We treated control iPSCs with ROCK inhibitors and observed a phenotypic rescue in terms of ROCK activity and neuronal morphology. We are currently exploring the molecular mechanisms underlying these processes and analyzing in detail the phenotype of OPHN1 defective cells at different stages of neuronal differentiation, before and after treatment.

P09.150-M
Rett syndrome: current situation in Algérie
Rett syndrome is a progressive neurological disease that affects mainly girls. It is characterized by severe developmental disorder of the central nervous system. It is caused by mutations in the MEC2P (methyl CpG-binding protein 2) in Xq28, comprising four exons, the first of which is non-coding. In this study, we analyzed the entire coding sequence of the MEC2P gene in 55 patients Algerians. 8 different mutations were identified in exon 4, 2 missense mutations (p.E394K, p.K135E), 3 nonsense mutations (R255X, R294X), and 4 frameshift mutations (a deletion of one base pair: c.806 G; c.750 C).

P09.151-S
The association analysis of CDH10 gene (rs4307059 and rs4327572) with autism in a South African population
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Autism spectrum disorders encompass a group of childhood neurodevelopmental and neuropsychiatric disorders characterized by deficits in verbal communication and impairment of social interaction. Numerous researches have pointed out that strong genetics components are involved in susceptibility to autism. Genome wide association studies have revealed strong association signal for CDH10 gene in SA population. In this study, we aimed to investigate the association of two SNPs (rs4307059 and rs4327572) of CDH10 gene of autism in the South African (SA) population. Aim: In this study we aimed to investigate the association of two SNPs with autism in the South African population. Aim: In this study we aimed to investigate the association of two SNPs with autism in the South African population. The association analysis of SNPs from genes ABHD11 and ABHD13 revealed with higher risk for autism in the South African population. A total number of 600 cases and 600 controls were compared with significant association signal for SNP rs4307059 and rs4327572 with autism in the South African (SA) population. Conclusion: The association of two SNPs with autism in the South African population is significant and the odds of 3.47 were observed in the South African population. The risk of autism increases in the presence of these SNPs in the South African population.
Hospital Bellvitge, Hospitalet de Llobregat, Spain.

Deletions involving the 17-22 repeats in the rod domain of dystrophin

P10.03-S
In Spanish population compared to the Italy cohort of patients previously

Our mutational screening reveals a higher prevalence of

populations, the most frequent

single mutation. In our cohort of patients, c.191dupA is, as in the majority of

Among patients presenting two mutations, six of them carried the c.191dupA

1, suggesting symptomatic carrier status.

We present data from an

Europe, in contrast to the low prevalence (2%) that has been reported in the

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ABSTRACTS POSTERS

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We investigated the percentage of patients sharing mutations in the rod
domain in a cohort of 160 BMD patients with gene deletions, followed at the
Cardiomyology and Medical Genetics of Naples Second University. We found
that 20 of them (12.5%) shared a deletion involving exons from 45 to 57, at
the 17/18 repeats level.

From a clinical point of view, based on muscular, cardiac and respiratory as-

sessment, 8 (40%) were asymptomatic, 11 (55%) had a mild phenotype and
only 1 (5%) had dilated cardiomyopathy, as the first manifestation.

Compared with data reported by Kaspar et al. (2009), the percentage of
asymptomatic patients in our cohort was higher (40 vs 8%) while that of
patients with mild phenotype was lower (49 vs 11%). The difference prob-

ably lies in a younger mean age of our patients in both groups (30.5 vs 20.2
and 24.3 vs 30.5, respectively).

Data here reported confirm that mutations involving repeats 17-22 of the
rod domain result in a late-onset mild Becker Dystrophinopathy.

P10.04-M
Mutations in the Charcot-Marie-Tooth disease-associated GDAP1 gene
does to calcium homeostasis dysfunction and endoplasmic reticulum stress

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Charcot-Marie-Tooth (CMT) disease is the most common inherited neuro-

muscular disorder and is characterized by large host gene heterogeneity. Mutations
in the GDAP1 gene show phenotypic and Mendelian heterogeneity in

CMT patients. GDAP1 is a mitochondrial outer membrane protein related
to mitochondrial dynamics network and different effects on the fusion and

pathways have been reported for recessive and dominant mutations, respectively.

Recently we have described the role of GDAP1 in the regulation of

store-operated calcium entry (SOCE), a complex process needed to fill endoplasmic reticulum (ER) after Ca2+ release. Dysregulation of calcium

and ER stress represent a common pathway in neurodegenerative
diseases. Here we present how missense mutations of GDAP1 expressed
in the human neuroblastoma SH-SYSY cells also affect calcium homeostasis
depending of their mode of inheritance and its relative position in the pro-

tein. Recessive mutations within the protein interaction domain of GDAP1
blocks Ca2+ influx during SOCE avoiding ER-Ca2+ refilling, while dominant
mutations show an exacerbated Ca2+ influx with high resting Ca2+ levels.

These mechanisms may affect the proper function of ER, which produce ER
stress that eventually could lead to neurodegeneration.

P10.05-S
Diagnostic time trend and genetic analysis of Charcot-Marie-Tooth
Disease in Denmark

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Copenhagen University Hospital, Copenhagen, Denmark.

Aim: To classify Danish patients diagnosed with CMT in the period 1977-
2012 according to genetic analysis, sex, and age at diagnosis.

Background: Charcot-Marie-Tooth Disease (CMT) is the most common in-
herited neurological disease. More than 70 genes associate with a CMT phe-

notype, but mutations in only four genes accounts for the vast majority of

cases. Little is known about the epidemiology of CMT in Denmark.

Material and Methods: Records with diagnostic codes IC8 33009 (atro-
phia mm. neuropathica, Charcot-Marie-Tooth) and ICD 10 G60.0 (hereditary
motor sensory neuropathy) from 1977 to 2012 were retrieved from The Da-
nish National Patient Register (DNPR). These data were linked with data on

genetic analysis for CMT between 1990 and 2012 at Department of Clinical
Genetics, Aarhus University Hospital and Copenhagen University Hospital.

Results: A total of 2084 patients with a CMT diagnosis were identified in
DNPR. Of these 712 patients (34%) had genetic testing, a genetically con-

firmed CMT diagnosis was obtained in 50%. In total, 17% of the patients
diagnosed with CMT since 1977 had a genetically confirmed CMT
diagnosis. This percentage was largest in the 0-9 years age group (35%). In
the 2008-12 cohort, 573 patients received a CMT diagnosis, 246 (43%) had a
genetic analysis for CMT, of which 124 (50%) confirmed the diagnosis.
The majority of confirmed CMT cases where caused by duplication of the
PMP22 gene (50%).

Conclusion: Only half of the newly clinically diagnosed CMT patients have
been genetically tested. Fifty percent of the patients had a genetically
defined diagnosis.
P10.06-M
Combined 12q24.3del and 17p12dup in a patient with truncal hypotonia and psychomotor delay
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1Department of Neurodegenerative Disorders, Mossakowski Medical Research Centre, Warsaw, Poland, 2Department of Biochemistry, Nencki Institute of Experimental Biology, Warsaw, Poland

The use of substantially improves the diagnosis of chromosomal abnormalities that are not evident by conventional karyotype. We report the clinical and molecular findings of a 2-year-old boy presented with truncal hypotonia, absent deep tendon reflexes and psychomotor delay, but no major malformations or dysmorphic features. Cytogenetic analysis was normal and DNA analysis for SMA revealed a triplication of SMN1. Subsequently an array CGH analysis with high resolution 4X180K Agilent arrays (~236,000 probes, average resolution of 8.9 Kb) showed a combination of the following: 1) 12q24.33 deletion [del(12)q24.33: 1.5 Mb]; 132,302-325,133,735,528; hg19] containing the genes FBRSL1, P2RX2, POLA, PXMP2, PGAMS, ANK2LE2, GOLGA3, CHR, ZNF505, ZNF26, ZNF94, ZNF140, ZNF10, ZNF262 2) 17p12 duplication [dup (17)p12.13.3MB; 14,111,772-15,379,208; hg19] involving the Charcot Marie Tooth syndrome type 1A (CMT1A) critical region within which PMP22 gene is contained. To our knowledge this is the first report of combined 12q24.3 deletion/17p12 duplication in a patient whose phenotype is typical of either the described 12q24.3 deletion or CMT1A, perhaps the combination of dosage sensitive OMIM genes in the aberrant regions contribute to the specific phenotype. Niyyazov et al., 2007; Keher et al., 2013; Choi et al., 2011;

P10.07-S
Whole exome sequencing in patients with congenital myopathies
I. Zarhavrić1,2, J. Colombo1,2, M. Sframeli1, J. Sigurðsson3, L. Feng1, R. Phadke1, C. A. Sewry1, J. E. Morgan1, F. Muntoni1;
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Mutations involving the sarcomeric glycoprotein desmin (DES) are responsible for at least 20% of cases of congenital myopathies, cardiomyopathies and related phenotypes. Molecular mechanisms of the changes leading to the disease remain ambiguous. Here we describe DES mutations found in the Polish population. The study group comprised 22 individuals representing 7 families with clinical diagnosis of desminopathy. DNA was extracted from peripheral blood using standard methods. Sanger sequencing identified one novel mutation (Q348P) and one previously described (A357_E359del) in the Polish population. Both mutations were predicted pathogenic using PolyPhen-2, SIFT and PROOVEAN software. A common ancestry of A357_E359del found in the Polish population, which additionally displays numerous centrally localized nuclei in the aberrant muscle fibers. The increased expression of desmin was confirmed using immunostaining. Abnormal localization of desmin with aggregates within the fibers was also observed. Additional staining for M-cadherin, α-actinin and MHCs confirmed severe disruption of myofibril organization. Abnormal alleles were more prominent for the Q348P muscle which additionally displays numerous centrally localized nuclei in the aberrant muscle fibers. Based on the in silico and ex vivo analyses, Q348P and A357_E359del mutations may be assumed pathogenic. It could be speculated that abnormal structure of mutated desmin results in aberrant folding and aggregation, triggering myofibril disruption.

P10.09-S
Haplotypology analysis and age of mutation in DMPK gene in Yakutia. M. Svarovskaïa1, S. Stepanova1, A. Marsavin1, A. Sukhiaimovskaï1, M. Nakimovaï1, V. Oikonomoïkaï1, S. Kitisso-Tsê1, E. Ramankuï1;
1Institute of Medical Genetics, Tomsk, Russian Federation, 2Siberian State Medical University, Tomsk, Russian Federation, 3Yakut Scientific Center complex medical problems, Yakutsk, Russian Federation

We studied the genetic variability DMPK locus in patients with MD and healthy population of Yakutia. The objectives of the study included the extetion of homozygous and heterozygous mutation in the Yakuts. In this work we used six SNP-markers in DMPK gene and six STR-markers which flanking DMPK locus. It was shown significant differences from patients MD and Yakut's population. The frequencies of alleles in three loci: rs572634, rs527221, rs919159 were significantly different. 29 haplotypes were found in patients with MD, and 37 haplotypes were found in Yakut's population. Major haplotype TTTCTC had 40% patients. Haplotypology GTCTCT was typical only for patients Yakuts. The haplotypology by microsatellite markers of MD patients was identified 14 alleles and 114 haplotype (frequencies from 0.6 to 8.6%). It was found the founder haplotype. Average number of generations were found 158.95 ± 192.51. The age of mutation was estimated 3179 years. This period before the formation of ethnic Yakuts.

P10.10-M
Mutational analysis of DMD gene reveals two novel small deletions in patients bearing no large deletions or duplications Z. Fattahi1, G. Zamani2, M. Fadaee1, M. Abkar1, H. Najmabadi2;
1Genetics Research Centre, University of Social Welfare and Rehabilitation Sciences, Tehran, Islamic Republic of Iran, 2Kariminejad - Najmabadi Pathology & Genetics Center, Tehran, Islamic Republic of Iran, 3Department of Pediatric Neurology, Children’s Medical Center, Tehran University of Medical Sciences, Tehran, Islamic Republic of Iran, 4Women’s College Research Institute, University of Toronto, Toronto, ON, Canada

Dystrophinopathies are inherited muscular dystrophies with mutations in DMD gene which contains 79 exons and is the largest human gene. DMD encodes Dystrophin protein and is the only gene responsible for the spectrum of dystrophinopathies. Genotype analysis has indicated that deletions of one or more exons account for ~65% of all cases, while 5-10% are in-frame or out-frame duplications and the remaining 30% of affected individuals may have point mutations, small deletions or insertions within the gene. In the present study, two patients diagnosed with Duchenne muscular dystrophy (DMD) are investigated. Multiple ligation-dependent probe amplification (MLPA) analysis which detects up to 98% of all deletions and duplications in DMD gene did not reveal any large rearrangement in these patients. Further investigation with application of two different techniques led to identification of two novel small deletions in DMD gene. In the first patient, all coding as well as flanking intronic regions of the dystrophin gene were PCR-amplified followed by sequencing and a hemizygous novel deletion, c.650-16_653del10 (p.D217V fs11X), was detected. Next, whole exome sequencing was applied to investigate the causative variant in the second patient that revealed another novel homozgyous deletion defined as c.8297delT (p.Leu2766Arg fsX27) in the DMD gene. The age of mutation in the Yakuts. In this work we used six SNP-markers in DMD gene and six STR-markers which flanking DMD locus. It was shown significant differences from patients MD and Yakut's population. The frequencies of alleles in three loci: rs572634, rs527221, rs919159 were significantly different. 29 haplotypes were found in patients with MD, and 37 haplotypes were found in Yakut's population. Major haplotype TTTCTC had 40% patients. Haplotypology GTCTCT was typical only for patients Yakuts. The haplotypology by microsatellite markers of MD patients was identified 14 alleles and 114 haplotype (frequencies from 0.6 to 8.6%). It was found the founder haplotype. Average number of generations were found 158.95 ± 192.51. The age of mutation was estimated 3179 years. This period before the formation of ethnic Yakuts.

P10.11-S
Next Generation Sequencing in facioscapulohumeral muscular dystrophy patients supports the idea that FSHD is a complex genetic disease
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The most common form of distal myopathy, facioscapulohumeral muscular dystrophy (FSHD), is a complex genetic disease that is caused by anomalous paternally inherited D4Z4 arrays on chromosome 4q35. FSHD is a heterogeneous disease with at least two different genetic types, FSHD1 and FSHD2. The majority of FSHD1 cases are caused by a deletion of the D4Z4 arrays on chromosome 4q35, while FSHD2 cases are caused by a different genetic mechanism. In this study, we used Next Generation Sequencing (NGS) to identify new genetic variations in FSHD patients. The study included 20 FSHD1 patients and 5 FSHD2 patients. NGS was performed using the MiSeq platform. The results showed that the majority of FSHD1 patients had deletions of the D4Z4 arrays, while the FSHD2 patients had no deletions. The study also identified several new genetic variations that were not previously known. These findings highlight the complexity of the FSHD genetic landscape and suggest that additional genetic factors may contribute to the development of FSHD.
Importantly we observed that earliest onset is not always associated with a disease.

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Muscular weakness in newborns and early infancy is a challenging clinical feature which may reflect either a neuromuscular disorder or only a secondary feature associated with a primary disorder for example of the central nervous system. Although the first signs may be present in first days of life, the full clinical picture might not be evident in newborns. An earlier and accurate diagnosis in those cases currently depends on a careful clinical assessment followed by the appropriate investigations. Recently developed research tools as next generation sequencing provide a distinct advantage to investigate the disease-related mutations in 11 infants with unknown conditions presenting with muscle involvement. With a mean average coverage of 394 and at least 20-fold coverage in 98% of the targeted region, we have identified genetic defects in 3 of 7 patients analyzed so far (43%): a previously reported homozygous splice site mutation in the IGHMBP2 gene known to be altered in spinal muscular atrophy with respiratory distress 1, a novel compound heterozygous mutations in the MUSK gene recently associated with congenital myasthenic syndrome, and novel de novo missense mutation in the TPMP3 gene involved in congenital fibro-type disproportionate myopathy.

Our data suggest that clinical exome sequencing is an efficient tool for timely and accurate diagnosis of clinically and genetically heterogeneous disorders in infants with undiagnosed muscular weakness.

P10.14-M Exome sequencing as a highly efficient diagnostic approach in muscular weakness in newborns and early infancy

P10.15-S Interaction among folate/homocysteine metabolism genes and endothelial nitric oxide synthase gene polymorphisms predicts the severity of Duchenne muscular dystrophy in Moldovan patients

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Introduction: We try to modeling gene-gene interaction between 5 mutations in 4 genes, involved in folate/homocysteine metabolism (FHMG) and eNOS- play a pivotal role in vascular homeostasis and endothelial function, that may alter the severity (in our case on the age at wheelchair dependency - 9 or 12 years) of this genetically simple disease.

Methods: A retrospective single institution long-term follow-up study was carried out in 148 corticosteroids-free DMD patients. The genotyping of
dystrophin gene were performed by the PCR to detect the deletion in DMD gene and the PCR-RFLP to identify the MTHFRG677Tand A1298C, MTRAR2756G, eNOS polymorphisms. Gene-gene interactions were analyzed using entropy-based multifactor dimensionality reduction (MDR).

Results: We evaluated single-site allelic and genotypic associations, geno-type equilibrium and multifocus genotype associations, using MDR, which failed to show a genetic model of severity of myopathic process. Have been adopted the MDR method to explore the synergistic effects of the studied polymorphisms on modifying to myopathic process. We selected the best model, which included the MTHFRG677T and A1298C, MTRAR2756G polymorphisms, cross-validation consistency is 9/10 (χ2=542.2, p<0.001) for case of wheelchair up to 12yrs). Since the selected polymorphisms were not associated with DMD there is evidence for the existence of epistasis between the two polymorphisms MTHFRG677T and eNOS (CVC10/10, χ2=5,3, p=0.02) in case of wheelchair up to 9yrs.

Conclusion: Our results indicate that the MTHFR, MTR and eNOS genes are modifying loci and presence of the high-risk alleles may associate with an increase in the severity of DMD.

P1.10.16-M
Limb girdle muscular dystrophy in the Czech Republic
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Limb-girdle muscular dystrophy (LGMD) is defined as a muscular dystrophy with predominantly proximal distribution of muscle weakness. It includes a number of disorders with heterogeneous etiology. We determined the frequency of recessive LGMD subtypes (LGMD2A, LGMD2B, LGMD2G, LGMD2J and LGMD2L) within a cohort of Czech LGMD2 patients using mutation analysis of the calpain3 (CAPN3), dystrophin (DMD), fukutin-related protein (Fukr), α-sarcoglycan (SCAα) and anancetomin (ANOS) genes. Last year we introduced next-gene sequencing to accelerate patient diagnosis and to widen spectrum of analysed genes. We designed capture library to target the coding exons of genes responsible for all known types of LGMD and genes responsible for muscular dystrophy with similar phenotype to LGMD. We observed that mutations of the CAPN3 gene are the most common cause of LGMD2. The frequency of particular forms of LGMD2 was 32.6% for LGMD2A, 41.1% for LGMD2D, 2.8% for LGMD2D, and 4.1% for LGMD2L. Using next-generation sequencing, we identified two patients with mutations in the gene encoding dystferlin (DYSF) - LGMD2B and a patient with mutations in the gene encoding β-sarcoglycan (SCGB) - LGMD2E. In total, we determined mutations in 41 % of Czech LGMD2 patients.

This work was supported by research grants CEITEC-CZ.1.05/1.1.00/02.0068, SuPReMMe - CZ.1.07/2.3.00/20.0045.

P1.10.17-S
Missense variations in ACADVL catalytic domain identified by the next generation sequencing of nonspecific LGMD patients
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One of the main problems of the research of rare autosomal recessive limb-girdle muscular dystrophy patients is the lack of mutations in the ARMD genes so far identified. The possible explanations are that a specific genetic cause of LGMD cannot be discovered by traditional DNA sequencing tests, or that the genetic heterogeneity is greater than expected with many other genes that should be analyzed. However, the present genetic testing is long, expensive and ineffective to cope with this second possibility. New powerful approaches for DNA analysis, like next-generation sequencing (NGS) and array CGH are revolutionizing the field with the human genome that can be properly analyzed. We combined SNP array-based linkage analysis and NGS technology to discover “orphan” LGMD mutations.

The patients to be studied were selected according to the following criteria: a) clinical diagnosis of LGMD; b) inconclusive molecular testing after the complete study of the known LGMD genes. The severity of disease was assessed by myo-pathology.

A number of mutations were identified. In particular in a LGMD family with an autosomal recessive inheritance, we have recently identified a new homozgyous mutation in the ACADVL gene, Asey-CoA Dehydrogenase - Very Long Chain, that is shared by all the affected family members and by another patient from the same town. In other two family a heterozygous compound mutations and homozgyous mutation in the ACADVL gene was also found. This demonstrates that specific amino acid change in the catalytic domain of the ACADVL gene may be associated with an LGMD-like phenotype and this should be considered in the differential diagnosis.

P1.10.18-M
Exome sequencing identifies mutations in a gene coding for the LIM-domain protein N-RAP in a BAG3 myofibrillar myopathy-affected patient
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Myofibrillar myopathies are neuromuscular disorders with disorganized myofibril structures at the Z-disk and accumulation of protein aggregates. BAGs-related myopathy represents a subgroup of myopathies caused by a mutation in the Bcl-2-associated athanogene 3 (BAG3), a co-chaperone with no direct role in muscle function. In order to investigate the possible involvement of other genes in BAG3 myopathy, we performed whole exome sequencing in an Italian family: a BAG3 myopathy proband affected by muscle weakness, respiratory insufficiency, cardiac arrhythmia, rigid spine, her unaffected parents and brother. Genomic DNAs from peripheral blood were exome enriched using Agilent SureSelectXT Human All Exon 50Mb kit.

All samples were sequenced in paired-end with 72 bp length reads on Illumina Genome Analyser IIx. In accordance to a compound heterozygous model of inheritance, we identified in the proband three non-synonymous heterozygous variants in N-RAP gene: one predicted extremely damaging was inherited from her father and the other two less damaging from her mother. The mutation inherited from her father is very rare (MAF=0.008%) and carries a aminoacidic substitution in a domain essential for the interaction of a non-canonical antitoxin filaments during myofibrillary assembly. The mutations from her mother cause aminoacidic substitutions in a domain critical for the Z-line assembly. Immunohistochemistry showed reduced N-RAP expression in the proband’s muscle biopsy compared to a control. The muscle specific N-RAP protein scaffolds 1-ZI assembly during myofibrillogenesis and has a LIM-domain. This is the first exome approach to a BAG3 myopathy and suggests a contribution of a LIM-domain protein to the complex phenotype of the proband.
P10.20-M  Childhood onset tubular aggregate myopathy associated with de novo STIM1 mutations. M. Niceta1, C. Meden1, F. Fattori1, B. Lindwall1, A. Ciofi1, A. D’Amico1, G. Tassoni1, S. Petretta1, M. Tulimius1, M. Tartaglia1, A. Oldfors1, E. Bertini1; 1Laboratory of Molecular Medicine, Bambino Gesù’ Children’s Research Hospital, Rome, Italy, 2Pathology, University of Gothenburg, Gothenburg, Sweden, 3Department of Neurology, Örebro University Hospital, Örebro, Sweden, 4Department of Pediatrics, University of Gothenburg, The Queen Silvia Children’s Hospital, Sahlgrenska University Hospital, Gothenburg, Sweden.

We investigated on three unrelated patients with tubular-aggregate myopathy and slowly progressive muscle weakness manifesting in the first years of life. All patients showed type 1 muscle fiber predominance and hypotrophy of type 2 fibers. Tubular aggregates of the sarcoplasmatic reticulum were abundant. In all three patients, de novo heterozygous mutations were identified in the STIM1 gene. In one of the patients, the mutation was identified by exome analysis, coupled to a hypothesis-driven filtering strategy. Two patients harbored the previously described c.326A>G p.His109Arg change while the third patient carried a previously unreported mutation (c.343A>T, p.Ile115Phe). All mutations affected the EF-hand motif, which is required for Ca2+ ions binding. Besides, the tubular aggregates, autophagic debris and myophosphorylase deficiency were documented in patients’ muscle cells. Consistent with previous findings, the c.326A>G change represent a recurrent mutation specifically related to early onset muscle weakness.

P10.21-S  Myotonia congenital type Becker in an endemic Bulgarian region S. Tintcheva1, A. Todorova2, T. Todorov2, I. Litvintseva1, V. Gergelcheva1, I. Tournev1, V. Miez1; 1Department of Medical Chemistry and Biochemistry, Sofia Medical University, Sofia, Bulgaria, 2Genetic Medical-Diagnostic Laboratory Genica, Sofia, Bulgaria, 3Department of Neurology, University Pediatric Hospital, Medical University, Sofia, Bulgaria, 4Department of Neurology, University Hospital ‘Alexandrovska’, Sofia, Bulgaria.

Myotonia congenita type Becker is an autosomal recessive nonystrophic skeletal muscle disorder primarily affecting lower limb muscles and later progressing to the arms, neck, and facial muscles. The disease is characterized by muscle stiffness and an inability of the muscle to relax after voluntary contraction. Sometimes muscle weakness may be transient. The autosomal recessive Becker type is caused by mutations in the CLCN1 gene, localized on chromosome 7q34 and encoding skeletal muscle chloride channel-1. Here we report 5 Bulgarian families genetically proved to carry mutations in the CLCN1 gene. The following mutations were detected: nonsense (p.Arg984*) splice-site (c.1471+1G>A), missense (p.Val273Met; p.Tyr524Cys). Two additional nucleotide changes were detected in an asymptomatic individual (c.328+4SC>T possible splice-site change and p.Phe167Leu). It was not possible to clarify, whether these changes are located on a single allele or affect both alleles. Two of the detected mutations are interesting from population point of view. The missense substitution p.Val273Met was detected in a large Roma family with a high proportion of endogamous marriages. Secondly, the novel missense substitution p.Tyr524Cys was detected in a large Bulgarian family with a number of affected individuals both in vertical and horizontal pedigree direction. The latter family originates from a small Bulgarian village and the citizens traditionally marry within the village, which is very surprising for the Bulgarian habits. Most probably there is an endemic region for myotonia congenital type Becker in Bulgaria, caused by the missense mutation p.Tyr524Cys.

P10.22-M  Targeted second generation resequencing reveals a homozygous CLCN1 mutation in a patient with an ambiguous myotonic dystrophy type 2 grey zone allele J. Radvansky2,2, E. Nagyova2,2, L. Kasadi1, G. Minarik2,2; 1Institute of Molecular Physiology and Genetics, Slovak Academy of Sciences, Bratislava, Slovakia, 2Genetics of Human Diseases, Comenius University, Bratislava, Slovakia.

In our previous study focused on the analysis of variability of the repetitive CCT tract in the CNBP gene in patients with myotonic dystrophy symptoms and healthy population, we reported a patient harboring an uninterrupted allele containing 34 CCT repeats. Although there are several indications that it is not a causative allele in that patient, it was not possible to exclude its role as a candidate gene associated with causing myotonia. Therefore the patient’s DNA was sequenced by targeted resequencing approach using a custom panel and Ion Torrent PGM. After alignment to the GRCh37/hg19 reference sequence three homozygous single nucleotide variations (SNVs) were identified. A missense variation p.S524G in the SCN4A gene (mutations of the gene are associated with paramyotonia congenita) and a missense variation p.G118W in the CLCN1 gene (mutations of the gene are associated with myotonia congenita) were found to be missense variations identified as variations according to the HapMap and 1000 Genomes Project data, the alleles harboured by our patient are in both cases the most common alleles and the reference sequence contains the rare variants (MAF=0.059 and MAF=0.009, respectively). The third SNV was a homozygous nonsense mutation p.P894* which is a known pathogenic mutation leading to myotonia congenita (Thomson’s disease). Identification of a known homozygous CLCN1 mutation found by targeted resequencing led to conclusion that the previously identified possible DM2 grey zone allele is not a disease causing allele in the case of the reported patient with consequence in the genetic testing in this family.

P10.23-S  Rapid High-Sensitivity Single-Step Screen for CTG Repeat Expansion Mutations in Myotonic Dystrophy Type 1 S. S. Chong1, M. Mullar1, K. Singh1, L. Rajan-Babu2, H. Law1, C. G. Lee3; 1National University of Singapore, Singapore, Singapore, 2National University Hospital, Singapore, Singapore, 3Manipal University, Manipal, India, 4Kerving Women’s and Children’s Hospital, Singapore, Singapore, 5Duke-NUS Graduate Medical School, Singapore, Singapore.

Myotonic dystrophy type 1 (DM1), the most common adult muscular dystrophy, is caused by expansion of a CTG repeat in the 3’UTR of the DMPK gene. Repeat size is correlated positively with phenotypic severity and negatively with age-of-onset, with affected individuals classified as mild (50-150 CTGs), classic (100-1000 CTGs), or congenital (>1000 CTGs) DM1. Southern analysis reliably detects all expansions, but requires micrograms of DNA, yields approximate allele size, and is labeled as laborious, time consuming, and costly. PCR across the repeat accurately sizes all normal and also expanded alleles, although the upper limit of detection is unknown. Newer triplet-primed PCR (TP-PCR) assays will detect all normal and expanded alleles, but product analysis by capillary electrophoresis (CE) is still costly when applied in high throughput screening situations where a majority of tested samples may be screen-negative. We have now adapted the 5’ and 3’ TP-PCR assays for single-step detection of DM1 expansions by melting curve analysis (MCA), wherein melt peak profiles from normal samples were distinctly different from samples carrying an expansion. In a blinded validation of 60 clinical samples enriched for affected individuals, a 48-repeat placental clone was used to establish a cut-off temperature separating normal from affected samples, and 100% concordance with their known genotypes was achieved. We conclude that TP-PCR MCA is an accurate yet simple, rapid and inexpensive screen useful for identifying DM1 expansion mutations. Follow-up confirmation and sizing can be accomplished by capillary electrophoresis following a quick 5-cycle extension-labeling of the positive TP-PCR product.

P10.24-M  Elevated miRNA levels in serum of myotonic dystrophy patients relate to disease progression A. Koutsioudiou1, T. Kyriakides2, Y. Christou1, E. Zamba Papanicolaou1, L. Phylactou1; 1The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus, 2Yale Center for Analytical Sciences at Yale School of Public Health, University of Yale, New Haven, CT, United States.

Myotonic dystrophy type 1 (DM1) is the most common form of adult-onset muscular dystrophy. Clinically, DM1 is a highly variable multisystemic disorder that primarily affects skeletal muscle and is characterized by progressive skeletal muscle weakness, wasting and myotonia. DM1 patients are diagnosed using genetic tests and their muscle wasting progression is currently monitored through electromyography and regular physical examinations. Many scientific reports emphasize the importance for the discovery of non-invasive serum-based biomarkers for the diagnosis and therapy of diseases, since they are easily accessible and convenient. The identification of blood-based biomarkers for DM1 would provide added value for the monitoring of the progressive muscle wasting. Scientific reports showed that circulating miRNAs are stably present within blood circulation and have the potential to be used as clinical biomarkers for many diseases. The aim of this pilot study was to detect and evaluate the usefulness of miRNAs as biomarkers for DM1. DM1 patients and healthy participants were recruited and miRNA analysis was performed on RNA isolated from serum samples. Results show that the levels of particular miRNAs are elevated in the serum from DM1 patients compared to controls, presumably, as a consequence of the degradation of muscle tissue during muscle wasting. Moreover, the miRNA serum levels correlate with the progression of muscle wasting observed in the patients. Based on these results, we propose that miRNAs can be used as potential serum-based molecular biomarkers for monitoring the progress of muscle wasting in DM1 patients.
Recent large changes have been identified in the nebulin gene (NEB) causing nemaline myopathy (NM). NM constitutes a heterogeneous group of disorders among the congenital myopathies, and mutations in NEB are a main cause. Next generation sequencing (NGS) for NM constitutes a first-line test and it includes a 32 kb triplicite region (TRI) where eight exons are repeated three times. We have designed a custom NM-NEB microarray to detect copy number variations in the currently known nine NM genes and one unpublished gene. To date, 230 samples from 170 families have been run with the NEB-GM microarray and we have identified NEB-TRI variation in approximately 14% of the NM families in this study cohort. The results suggest that the adjacent intronic repeat elements may predispose to the recurrent TRI variations. The possibilities of the TRI variation warrants further studies and elucidation of the exact breakpoints in each family. Moreover, we have identified seven different, novel, large disease-causing aberrations in NEB in seven different families. The size of the aberrations varies greatly, from only a part of one exon (0.9 kb) to more than half of the gene (133 kb). The NM-NEB microarray method is currently available for mutation analysis in our laboratory. Additionally, we have analyzed ten samples with exome sequencing and identified causative mutations for half of the families. We believe that the combination of the NM-NEB microarray followed by exome sequencing will accelerate mutation detection and improve the diagnostics of NM and related disorders.

P10.26-M

A novel homozygous deletion in SGCB identified by a targeted next generation sequencing approach and the Motor Chip

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Different forms of muscular dystrophy are caused by mutation in genes coding for proteins of sarcoglycan complex, resulting in severe childhood autosomal recessive muscular dystrophies (SCARMD). Point mutations but also copy number variations (CNVs) have been found in sarcoglycan genes. Here we report one patient with SCARMD phenotype resulting from a new homozygous deletion of the last 12 codons in the exon 6 and 3'UTR of beta-sarcoglycan gene (SGCB). For the genetic diagnosis of this heterogeneous condition, we have performed the next generation sequencing. In particular, we have used the Motor Haloplex, a customized target enrichment of regions related to muscular disease, recently developed by our group. We targeted all the exons and the ten flanking bases of 89 genes involved in muscular dystrophy, in no point mutations or ins/del were reported by bioinformatics analysis of the data, but a homozgyous deletion in the exon 6 of SGCB has been identified by IGV analysis of sarcoglycan genes. To confirm the result and map the breakpoint, we have used the Motor Chip (Clinical Chemistry, 2011 Nov;57(11):S84–96), a customized aCGH developed by our group, which is able to identify CNVs in 425 muscular genes. This data suggests that Motor Haloplex may be appropriate for routine diagnoses. It is able to investigate point mutations but also deletions and duplications so that is easier, less time-consuming than traditional genetic by gene approach. Finally, the combination between Motor Haloplex and Motor Chip is a strong strategy for the diagnosis of muscular dystrophies.

P10.27-S

Trio-based study of neuromuscular dysfunction using next-generation sequencing under a diagnostic setting

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We evaluated the use of next-generation sequencing to provide genetic diagnoses using a family trio consisting of healthy parents and an affected fetus which died intrauterine. The phenotype of the fetus was consistent with early onset neuromuscular disease. Suspected seizures, arthrogryposis multiplex congenita and suspected intrauterine eplepsy were detected in a sonography. We compared clinical gene panel analysis vs. whole exome sequencing (WES) for the family trio in order to identify candidate variants that account for the disease phenotype of the fetus. We used the TrueSight™ One Sequencing Panel, which targets 4813 genes associated with known clinical phenotypes, and the Nexters Exome Enrichment Kit (62Mb). Data was analyzed using an in-house bioinformatics pipeline. We analyzed the 4813 disease-associated genes in both sequencing approaches and found two compound heterozygous non-synonymous substitutions in a gene that is involved in neuromuscular functioning. Extending the analysis to the whole exome did not provide any new candidate variants. Gene panel sequencing yielded higher quality and coverage results compared to the WES, which showed a higher number of uncovered nucleotides within the clinical genes. In addition, WES revealed many variants in genes which cannot be directed towards answering questions related to the medical conditions. As a diagnostic test, clinical gene panel sequencing including all relevant disease-associated genes was more efficient than WES by means of quality, costs, time, and reporting. Although WES might not always lead to a direct diagnosis test, it is relevant for research to uncover new gene-disease associations.
P10.30-M Genetic testing of inherited muscular dystrophies and myopathies using Sequence capture and targeted resequencing

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The inherited muscular dystrophies and myopathies comprise a heterogeneous group of muscle diseases that share similar clinical features as progressive skeletal muscle weakness and wasting. Mutations in several genes that encode proteins of extracellular matrix, endoplasmic reticulum, nuclear envelope and sarcolemmal proteins are known to be responsible for muscular dystrophies and myopathies. The large overlap of phenotypic manifestations resulting from different gene mutations poses a challenge for determination exact type muscular disease. Targeted resequencing using next-generation sequencing technology is a cost-effective strategy to accelerate patient diagnosis. We designed capture library to target the coding and all flanking intron regions of 42 genes associated with group of disease as muscular dystrophies, congenital muscular dystrophies, congenital myopathies, distal myopathies and other myopathies. We performed targeted capture combined with next-generation sequencing using NimbleGen SeqCap EZ Choice library and Roche 454 sequencing platform in 33 Czech probands. Mutations associated with muscular dystrophies or myopathies were identified in 16 of them. Mutations were detected in AC-TAP1, CAPN3, COL4A1, DMD, DMP1, DMP2, LAMA2, LRPS, SEPN1 and SERYN gene. This work was supported by grants IGA MZ CR NT14574-3.

P10.31-S Swiss Cheese localization and function in the nervous system of Drosophila melanogaster third instar larvae

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Neuropathy target esterase (NTE) is the 6th member of 5-proteins of patatin-like phospholipase domain-containing proteins, PNPLA-1-9. Mutation in the catalytic domain of NTE (PNPLA6) can lead to the slowly developed diseases, known as organophosphorus compound-induced delayed neuropathy (OPIDN) and hereditary spastic paraplegia called NTE-related motor neuron disorder (NTE-MND). The Swiss Cheese (SWS) protein is an ortholog of NTE in Drosophila and shares 39% sequence identity with human NTE. The Drosophila sws genes are characterized by progressive degeneration of adult nervous system, gait hyperreflexia, and neuronal apoptosis. Drosophila sws mutants thus provide an experimental model for functional studies of the SWS/NTE role in maintaining neural integrity. There is an evidence of SWS role in the age-dependent brain changes. However, possible disturbance caused by point mutations in sws gene in Drosophila larvae remained unknown.

The swo-point mutants were used to investigate SWS localization and function in the nervous system of D.melanogaster larvae. We were shown SWS localization mainly in glial tissue of larval brain as well as in glial cells, wrapped axons in all investigated lines. Presence of SWS was also observed in the postmigratory membranes of the larval NMJ. SWS protein in small amount was also detected in larvae neurons. Quantitative and qualitative parameters of NMJ in all investigated sws-mutants also were changed comparing with wild type flies.

Thus, SWS dysfunction in earlier stages of Drosophila ontogenesis can be important for nervous system development and aging in D.melanogaster adults.

P10.32-M Central bradypnoea and hypotonia as diagnostic feature for male Rett syndrome

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Mutations in the MECP2 gene causing the X-linked dominant Rett syndrome are of autosomal recessive nature in the majority of patients. Although MECP2 mutations in males are usually considered lethal, leading to the prenatal death of the fetuses, few observations of boys with severe congenital encephalopathy were reported. We present here a novel case of a newborn boy with Rett syndrome caused by the de novo MECP2 frameshift mutation c.806delG. The clinical suspicion of male Rett syndrome was triggered by the rare combination of neonatal hypotonia and central bradypnoea. At the age of four days the newborn was transferred to the neonatal unit of a tertiary children’s hospital because of poor suck, dysregulation of muscle tone and a pathological breathing pattern. On admission, the neonate was lethargic and the clinical examination showed multiple abnormalities, such as a severe brady-/hypopnea with apneas of up to 30 seconds, followed by an arrest reaction, frequent yawning, axial hypotonia and hypertonatia of the extremities. The cerebral magnetic resonance (MR) image revealed bilateral T2 signal hyperintensities of the white matter, especially in the temporal and parietal areas, which raised the question of perinatal asphyxia. In the spectroscopy the lactate was elevated and the n-acetylaspartate level was low. The electroencephalogram (EEG) showed an immature activity with too many multifocal sharp transients. At discharge from the hospital, at the age of 1.5 months, the patient was more alert and was no longer dependent on nasogastric feeding.

P10.33-S Evaluation of AR genetic polymorphisms influencing spinal and bulbar muscular atrophy phenotype

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Spinal and bulbar muscular atrophy (SBMA) is caused by a pathological expansion over 38 of a CAG repeat in the first exon of the androgen receptor (AR) gene on chromosome X. AR interacts with a polyQ tract (La Spada et al., 1991). SBMA is an androgen-dependent disorder, with males with full disease manifestations, and females showing only mild symptoms even if homogygous for the mutation. While a correlation between expansion size of polyQ tract and disease severity has been reported, patients with the same number of CAG repeats have different age at onset and disease progression even if relatives. Human AR exon 1 encodes further amino acid stretches. The effect of these sequences on SBMA phenotype has not been studied yet. In order to further characterize the effect of AR coding repeated sequences on SBMA phenotype, we genotyped AR exon1 polymorphisms in 132 molecularly defined SBMA patients, referring to the Motor Neuron Clinic of the University of Padua and to Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan. Among AR exon1-1 trinucleotide stretches, a polymorphic GGN quadruplet encoding poly-G (1-4) stretch should be higher in patients with an earlier onset. Our study confirms that the length of SBMA-causing poly-G tract does not fully explain the disease phenotype and point to a polyG stretch within AR exon 1 that is a potential disease modifier in SBMA: REFERENCES: La Spada AR, Wilson EM, Lubahn DB, Harding AE, Fischbeck KH. Androgen receptor gene mutations in X-linked spinal and bulbar muscular atrophy. Nature. 1991;352:77-9.

P10.34-M Mutation analysis of SCN1A gene in Slovak patients with various types of childhood epilepsy

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Mutations in SCN1A gene are by far the most frequent cause of Dravet syndrome (DS) worldwide, with mutation prevalence approximately between 70 and 80%. One of the main features of DS is the fact that many patients do not respond well to the treatment with anticonvulsive drugs. Furthermore, there have been observations that some drugs, which function as sodium channel blockers, may induce an aggravation of seizures in patient, thus worsening their symptoms. As most of the DS SCN1A mutation result in truncation of the protein, the use of antiepileptic drugs would further decrease the inhibitory function in central nervous system. The aim of this study was to identify mutations in SCN1A gene in a broad testing group of patients with phenotypes ranging from DS to milder phenotypes such as genetic epilepsy with Febrile Seizures Plus (GEFS+). Completing mutation screening in 37 unrelated patients, 4 causative mutations located in the coding region were identified. All mutations were found in DS patients and in accordance with previously identified disease variants, found mutations, of which three are novel, result in creation of a premature stop codon, either by substitution or an insertion and thus to a possible loss of function of the altered protein. In summary, our findings contribute to the broadening of the mutation spectrum of SCN1A gene and enrich the clinical data of DS. Genetic testing may prevent the need for unnecessary clinical examinations and provide genetic counseling for families.
P10.35-S
De novo deletion or uniparental disomy as SMA determining cause: a case report
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We report a 3-months-old female with type I spinal muscular atrophy (SMA) born to a young and non-consanguineous couple. Molecular analysis confirmed the diagnosis of SMA with an homozygous deletion of SMN1. Parents analysis revealed that only the mother was a carrier of the SMA causative deletion. It is important to find out if the father, that carries two SMN1 copies, has a CIS-duplication of the gene on one chromosome bearing to a 2/0 genotype or the deletion occurred de-novo during gametogenesis. Indeed, the risk for future pregnancies could be reduced from 25%, usually estimated for SMA couple, to residual risk arising from recurrent de-novo mutation (2% of the affected individual). Alternatively, the uniparental disomy (UPD) of the maternal-deleted-chromosome, with an estimated incidence of about 1:3500 live birth, must be taken into account. Gene dosage analysis of the father’s relatives does not support a paternal 2/0 genotype. To discriminate between a paternal de-novo mutation and a maternal isodisomy we analyzed in the affected infant and parents a total of nine microsatellite markers in a region spanning 3.0 Mb at the SMN locus. Five microsatellites at 5’/3’-ends of that region demonstrate a maternal and paternal inheritance but could not definitively exclude the maternal segmental UPD. Finally, using a NGS approach, we analyzed three paternal and maternal genomic segments in the SMN2 genes of the infant. All together our data support for a de-novo mutation of paternal chromosome 5 as could result by unequal crossing-over during gametogenesis.

P10.37-S
Prevalence of SMN1 gene duplication in different ethnic groups: implication for carrier testing
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Spinal muscular atrophy (SMA) is an autosomal recessive disorder, characterized by symmetrical muscular weakness and atrophy. The incidence is variable from 1 in 6000 to 1 in 10000 live births in different geographic areas. A homozgyous deletion involving exon 7 in SMN1 gene is present in more than 95% of the cases. Carrier testing in parents and relatives of SMA patients is complicated by the occurrence of SMN1 gene duplication (4-5%).

Here we report on the SMN1 genetic test results obtained in our lab in the last two years. The most frequent clinical indication is represented by positive family history for SMA in 90% of the cases, carrier testing in relatives of SMA patients is complicated by the occurrence of SMN1 gene duplication (4-5%).

We calculated the frequency of different SMN1 genotypes in 326 tested subjects. We found a percentage of SMN1 gene duplications (subjects with 3 copies of SMN1) a bit higher than expected, ranging from 6 to 6.5%. The 55% of the subjects with the duplication is represented by North and West Europeans that have been tested because of consanguinity with the partner, 54% of the subjects with the duplication is represented by North and West Europeans that have been tested because of consanguinity with the partner, the remaining are Italians, 25% with SMA positive family history and 20% with parents heterozygous for the deletion.

Our results highlight once again the importance of taking into account the occurrence of SMN1 gene duplication when performing carrier testing and suggest that the prevalence varies in different ethnic groups, therefore affecting the recurrence risk assessment.

P10.38-M
Methylation level of SLC23A2, NCO2R and CDK2AP1 genes regulatory regions correlates with spinal muscular atrophy severity
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We analyzed in 24 IN patient and 23 III type and 29 IV type SMA patients with bisulfite sequencing. The methylation level of target CpG site and one nearby CpG site between 5’UTR of SLC23A2 was significantly higher in IV type compared to I type SMA patients. Moreover, a significantly increased allele A frequency of polymorphism rs1279683 (g.G>A) situated in target CpG site was found in III-IV type SMA patients. IV-III type comparison to I type SMA patients demonstrated decreased methylation level by 9-16% of target CpG site and nearest CpG site belonged to 5’UTR of NCO2R. Significant difference in the methylation level between different types SMA patients was revealed for three CpG sites located in promoters of 15-198 bp of TSS of CDK2AP1. Thus this study confirms that DNA methylation changes of SLC23A2, NCO2R, and CDK2AP1 might be associated with spinal muscular atrophy severity.

P10.39-S
Molecular analysis of common mutation associated with SMA in Romanian population
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Spinal muscular atrophy (SMA) is characterized by degeneration of motor neurons that cause progressive muscle weakness and muscle atrophy. SMN1 homozygous gene deletion is present in most patients with SMA (~95%). The objective of this study was to evaluate the SMN1, SMN2 and NAIP gene mutational status in SMA Romanian patients. In this study, we analyzed by PCR-RFLP 90 patients with suspected SMA and 52 relatives. We also performed three prenatal test feta with DNA obtained by amniocentesis from mother, who had prior history of another child diagnosed with SMA. We identified homozygous deletion of exons 7 and 8 of SMN1 and SMN2 genes in 35 patients, and only deletion of exon 7 at 2 patients. Homozygous deletion of exon 7 and 8 of SMN2 gene was identified in 2 patients and one relative of a patient. NAIP gene deletion was identified in 8 patients and 3 relatives. For prenatal testing, we identified homozygous deletion of exon 7 and 8 of SMN1 gene. Molecular diagnosis of SMA by identifying of SMN1 gene deletion is a useful tool for the diagnosis of SMA in Romania, being necessary determination of SMN copies for a better matching phenotype - genotype. This work was supported by CNCSIS-UEFISCDI project number PN II - IDEI 2152/2008 and Program 8, the project „Intervention for diagnosis and management of spinal amiotrophy and muscular dystrophy type Duchenne and Baker, and the prevention of their hereditary transmission“ 2011.

P10.40-M
Exon skipping mutation in collagen VI detected in a patient from Lithuania
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We report on a patient who presented with clinical features of Ullrich congenital muscular dystrophy. The patient is a girl, four years of age, the first child of healthy non-consanguineous Lithuanian parents. The proband was born at 40 weeks gestation in normal delivery. Hip dysplasia was noted from birth. The girl has chronic constipation from 2 months of age. Her intelligence is in normal range, but motor development is delayed. She could sit at age of 6 months, stand up with support at age of 21/2 years. She can walk only with support. Clinical examination at the age of 4 years revealed the contractures in elbows and knees but striking dystal phalanx hyperlaxity. The concentration of CK was mildly elevated (315 U/L). Spine CT and MRT investigations, cardiology were normal. The clinical diagnosis of Ullrich congenital muscular dystrophy was suspected and molecular genetic testing of COL6A1, COL6A2 and COL6A3 genes was performed. The mutation c.6210+5G>A, IVS16+5G>A, in intron 16 of the COL6A3 gene is a heterozygous state was detected. The analysis of parents confirmed de novo origin of the mutation. The protein immunohistochemistry analysis of the muscular biopsy concluded that the finding is compatible with dominant in frame deletion in exon 16. The mutation c.6210+5G>A, IVS16+5G>A, has already been described as causing Ullrich congenital muscular dystrophy. The molecular and clinical characterization of our patient provides additional information for genotype-phenotype correlation and confirms dominant acting mutation in collagen VI gene as a cause of Ullrich disease.
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14q32.2 Microdeletion and Thyroid carcinoma

P11.001-S

We describe two cases with opposite rearrangements (deletion and duplication) in 15q region including Insulin-like Growth Factor 1 receptor (IGF1R) gene. The IGF1R is involved in growth, insulin-related phenotypes, and long-chain fatty acid oxidation. Deletions of the 15q11.2-q13 region are associated with the Prader-Willi and Angelman syndromes, but also with several isolated phenotypes. Duplications lead to the Prader-Willi-like phenotype. We report two cases of combined short stature and obesity.

P11.002-M

Cardiac MRI in the evaluation of congenital heart disease

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Cardiac magnetic resonance imaging (MRI) has become an increasingly important tool in the evaluation of congenital heart disease. The aim of our study was to evaluate the diagnostic role of cardiac MRI in congenital heart diseases.

Cardiac MRI was performed in 100 consecutive patients, ranging from 1 week to 60 years of age, with congenital heart disease. The imaging protocol included breath-hold cine sequences, late gadolinium enhancement, and black blood imaging with different echo times (T1 and T2 weighted). The images were analyzed by an experienced cardiologist and a pediatric cardiologist.

The results showed that cardiac MRI is a valuable tool in the evaluation of congenital heart disease, providing detailed information about the anatomy and function of the heart. The diagnostic accuracy of cardiac MRI was comparable to or better than that of other imaging techniques, such as echocardiography and computed tomography.

P11.003-S

Two children with triplication of 16p11.2 associated with global developmental delay and dysmorphic features

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We report two patients with de novo triplication of 16p11.2. This region is relatively well characterized, and associated with global developmental delay, behavioural problems, dysmorphic facial features, seizures, abnormal head size, and variable congenital anomalies. This is the first report of 16p11.2 triplications and the associated phenotypes.

P11.004-M

Familial cases of 2q37.12 Deletion syndrome in Belarus: phenotype's variability, genetic counseling, prenatal diagnostics

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We describe two familial cases of 2q37.12 deletion syndrome, which are relatively well characterized, and associated with global developmental delay, behavioural problems, dysmorphic facial features, seizures, abnormal head size, and variable congenital anomalies. This is the first report of 16p11.2 triplications and the associated phenotypes.
The 22q11.2 Deletion syndrome (Del22S: #188400; #192430) manifested by distinct phenotype is characterized high (~10%) incidence of inherited cases (InhC). Disorder is displayed variability of phenotype’s expression for heart defect (HD) and mental delay (MD). Adult’s del22q11.2 carriers may exaggerate fidg signs. We present InhC del22q11.2 (4 families, 9 patients) confirmed by FISH and the scheme of genetic counseling in Belarus. InhC incidence comprises 10.5% (#4/38). Two families (F1, F2) underwent through genetic counseling due to affected children aged 1.5 and 3.5 months with prenatal hypoplasia, dysmorphic signs, retardation, hypocalcemia, thymus agenesis, immunodeficiency, HD (F1: multiple VSD+ASD; F2: VSD+ASD+PDA). Body weight was 1790g (F1) and 2100g (F2) at 3 and 3.5 weeks gestation accordingly. Affected newborns showed borderline intellect, facial dysmorphism; HD: ASD (F1); hearing impairment, unilateral renal agenesis (F2). In F3, F4 Del22S was firstly revealed at pregnant counseled due to fetus’ heart malformation. F3 presented 3 affected persons: pregnant with dysmorphism, cleft palate, hypertension; fetus with HD (VSD+aortic coarctation) and normal thymus was born at 35 weeks gestation (weigh 2400g), died in 2 days; 11 years old child with MD, VSD, P4: pregnant operated for tetralogy Fallot (TF) with dysmorphism, myopia, scoliosis, renal hypoplasia; fetus with TF. Both couples refused pregnancy’s termination. Patient’s management: cardiac surgery; follow-up for heart, mentality, immunological status, age-dependent assessment. Counseling del22q11.2 carriers: discussion for etiology, diagnostics, variability, impossibility to predict phenotype impairment-genetic risk (50%)-prenatal del22q11.2 testing for FISH helps for pregnancy’s counseling. Our data illustrate wide phenotype’s variability and importance of early Del22S carriers detection.

P11.005-S
Phenotypic characterization of a recurrent 22q11.2 deletion spanning low copy repeats (LCRs) C-E
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The well characterized 22q11.2 deletion syndrome is associated with a wide range of clinical phenotypes including the velo-cardio-facial syndrome (MIM 192430) or DiGeorge syndrome (MIM 188400). About 90% of patients have a common 3Mb deletion (typical deletion), between LCR22-A and LCR22-D, while a recurrent 2.1Mb deletion is found spanning LCR22-D and LCR22-H (distal deletion). We describe 2 patients (ages 4 and 2 years) with a 1.3Mb 22q11 deletion (1963229 bp-20788469 bp, Hg18) detected by a-CHH, that spans the distal end of the typical deletion and the proximal end of the distal deletion (between LCR22-C and LCR22-E).

We compared their clinical findings with those described in patients with similar deletions. We attempt to further delineate the associated phenotype. Patients present a quite homogeneous phenotype which seems to overlap with those described in Russel-Silver and Goldenhar syndromes. It is characterized by prematurity, pre and postnatal growth retardation, microcephaly, cranio-facial dysmorphic features, heart defects, ear and renal anomalies, and mild developmental delay. Cleft palate, skeletal defects and behavioural problems were also present. Genotype-phenotype correlation supports the hypothesis that haploinsufficiency of the CRKL (Crk-like) gene is the major candidate gene for the cardiac anomalies associated with the 22q11.2 deletion involving LCRs C-E.

P11.006-M
MLPA reveals broader spectrum of abnormalities in patients with 22q11.2DS phenotype
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MLPA analysis is considered to be a reliable, accurate and cost-effective method with high sensitivity and specificity for detecting 22q11.2 microdeletion, both typical and distal. MLPA analysis with SALSA MLPA P250-B1 DiGeorge (MRC Holland) kit was performed on the group of 78 probands with phenotype suggestive of 22q11.2 deletion syndrome (22q11.2DS) (CHD, facial anomalies, cleft palate, hypocalcemia). Different-sized deletion 22q11.2 was revealed in 28 cases. Most patients had common recurrent 3 Mb 22q11.2 deletion (79%). In our study MLPA test didn’t reveal deletions in 49 patients. One patient was found to have deletions of probes within 4q32.4 region included in the probe set. The phenotype of patient presented with hyperplastic right ventricle, hypoplastic left heart, hypocalcemia, narrow fontanelles, dysplastic ears, antverted nares, broad face, small lower jaw, double-sided cryptorchidism, chiasm of hand digits, seizures, cleft of soft palate. The patient’s karyotype showed derivate 4q terminal deletion of maternal origin (#4X,Yder(4)q(48)q35:q22)mat).

Previously we evaluated a group of 140 probands with phenotype typical for 22q11.2DS. Forty three patients were diagnosed with 22q11.2 deletion by FISH and MLPA techniques (31%). Three patients with highly suggestive 22q11.2DS phenotype were found to have chromosomal abnormalities and three patients in non-deleted group presented with other syndromes detected afterwards. Thus patients with negative 22q11.2 deletion test results showed quite a vast etiological heterogeneity and may have been undiagnosed for some genomic disorders. Array CGH should be advised in cases where discernibly suggestive phenotypes aren’t confirmed by FISH and MLPA methods.

P11.007-S
3p deletion syndrome: clinical presentation and molecular description of array CGH
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Deletion 3p syndrome is a rare contiguous-gene disorder involving the loss of 3p25-p26 delineated by array CGH and associated with characteristic dysmorphic features, microcephaly, developmental delay (DD) and growth retardation. It was suggested that a 1.5 Mb minimal terminal deletion causes the syndrome, but there is a strong connection between the severity of the disease and the size of the deletion (Gunnarsson and Foyen Brunn 2010, Pelteko et al 2012, Reiss et al 2012). We present three patients with normal karyotype and DD, various congenital anomalies and dysmorphic features. For array CGH analysis Agilent arrays 4x180k and 1x24k (>170.000 and > 236.000 probes respectively, average resolution 8.9kb) were used. An interstitial 3p25-p26 microdeletion was detected in all patients, ranging in size from 1.9Mb to 11.3Mb and comprising the genes CRBN, CNTN4, CHL1, LRRN1 and SRGAP3 (Ellery PM et al 2014). Additional pathogenetic aberrations which were also observed in two of our patients (dup 8q24.23-q24.3, dup 22q11.21) might contribute to the severe phenotype, presumably acting as modifiers of their clinical manifestations. Further studies of patients with application of array-CGH, will probably expand our knowledge on the phenotypic and genotypic spectrum of 3p deletion syndrome.

P11.008-M
3q27.1-q27.3 microdeletion syndrome: description of a pediatric case with previously undescribed features
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Here we describe a case of a 5 year old boy who came to our genetic service due to small stature in association with hypoplasdias and psychomotor retardation. Conventional karyotype was normal, while a microarray-CGH detected a 2.8Mb deletion spanning from 3q27.1 to 3q27.3: 46,XY,del(3)(q27.1q27.3). Recently a new 3q27.3 microdeletion syndrome has been described, overlapping molecular features with our case, but only partially coinciding regarding clinical phenotype. Our patient exhibit a genitourinary malformation previously unreported and a borderline IQ, opposed to reported severe mental impairment. IGOR and subsequent small stature and peculiar dysmorphisms are also seen. It is remarkable that all cases described before are adults, and as we concern this is the first case described at young age. This case expands the phenotypic and clinical features of the 3q27.3 microdeletion syndrome and offers new insights about genotype-phenotype correlation.

P11.009-S

In this report, we describe two girls with severe growth and developmental delay with polycystic kidney disease. Long arm of chromosome 4was consistently deleted in almost same positions. The common locus in the chromosome is 4q21-22. One patient had a simple 4q contiguous gene deletion, whereas the other patient had a complicated chromosomal rearrangement. In the first patient, a smaller part of the 4q was inserted to 3p. In both patients, abdominal ultrasonography revealed renal cysts. In the deletions PKD2, which causes autosomal dominant polycystic kidney disease (MIM 613095) was mapped. The common 52 genes deleted in both patients. Typical phenotypes, were severe growth and developmental retardations, and a characteristic facial appearance consisting of fronto bossing, thin broad
eyebrows, epicantal folds, missing, missing teeth. And mild hand and foot anomalies. Although these patients carry different chromosomal aberration, the common deletion causes the quite similar phenotype, suggesting a new syndrome due to 4q21-22 deletion.

P11.010-M
9p deletion syndrome-like in a girl with a 9p insertion on chromosome 2 without 9p deletion
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Partial deletions of 9p have been reported as a clinically recognizable syndrome characterised by the variable association of intellectual disability, speech delay, hypotonia, cardiac anomalies and dysmorphism.
We report a 3 years old girl with midface hypoplasia, downsloping palpebral fissures, teeth agenesis and teeth fusions. Language was not acquired. At birth, severe neonatal hypotonia and patent ductus arteriosus were present. Phenotype was consistent with the clinical diagnosis of “9p deletion syndrome” (OMIM#158170), with uncommon features such as bilateral cataract (diagnosed at 5 months), chronic constipation, high pain threshold and poor temperature control.
Karyotype analysis showed an apparent 9p deletion. Array-CGH (60K Agilent) demonstrated a 9q22.1p24.3 insertion in chromosome 2q [46,XX,ins(2;9)[q24.3;p22.1p24.3:35]]. The 9q microdeletion involved only two genes: SCNA9 and SCNA7. SCNA9 recessive mutations cause congenital insensitivity to pain. SCNA7 is not associated with human disease, and in mouse is likely to be a sodium-level sensor of body fluids in the brain. SCNA9 haplinsufficiency may support the clinical observation of the patient high pain threshold and the poor temperature control. We are verifying the presence of a second mutation on the remaining allele. Assuming that the 2q deletion cannot account for the whole phenotype, we speculate that a critical gene/regulatory region key for the “9p deletion syndrome” is located in the 9p breakpoint(s). This patient deserves future studies that may help to refine 9p critical region.

P11.011-S
Identification of de novo 2,62Mb deletion in chromosome 15q26.1 in a boy with stigmata dysplastica, developmental delay, short stature, microcephria, nasal polyposis, hypermetropia
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We report a 14 years old boy with developmental delay, stigmata dysplastica and multiple organ involvement. History: no familiar congenital anomalies or consanguinity; born on 38th gestational week. IUGR. Developmental delay: sitting at the age of 8 months, walking at 16 months; at the age of three years with speech with only some words. Short stature, microcephria, stigmata dysplastica, nasal polyposis and hypermetropia. Head MR showed suspect hypothalamic abnormalities. We are verifying the presence of a second mutation on the remaining allele. Assuming that the 2q deletion cannot account for the whole phenotype, we speculate that a critical gene/regulatory region key for the “9p deletion syndrome” is located in the 9p breakpoint(s). This patient deserves future studies that may help to refine 9p critical region.

P11.012-M
A novel mutation in the AAAS-gene in a Bulgarian patient with Triple-A syndrome
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Triple A (Achalasia-Addisonism-Alacrima, Allgrove) syndrome is an inherited condition characterized by three specific features: achalasia, Addison disease, and alacrima. Allgrove syndrome is inherited in an autosomal recessive fashion and is caused by mutations in the AAAS gene located at 12q13.13. The gene encodes a protein called ALADIN. It localizes to nuclear pore complexes, large multiprotein assemblies that are the sole sites of nuclear-cytoplasmic transport. Achalasia-Addisonism-Alacrima syndrome is a rare condition, incidence is unknown, and only scattered family and case reports are noted in the literature. Here we report a female patient with Triple A syndrome born to non-consanguineous parents from Bulgarian origin. She was diagnosed as Triple A at the age of 17. The patient expresses a typical phenotype: achalasia, Addison disease, and alacrima. The affected patient also has muscle hypotonia, recurrent infections with skin pigmentation, optic atrophy and papilla atrophy. Many of the features of triple A syndrome are caused by dysfunction of the autonomic nervous system and the neurological symptoms have worsen over time. Molecular genetic testing of the patient showed 2 heterozygous mutations in the AAAS-gene: an already reported nonsense mutation c.1024A>C p.(Arg342*) and a novel mutation c.1331-2dupT. The one basepair duplication, located 2 basepairs upstream exon 14, disrupts the donor splice site of intron 14 of the AAAS-gene. Based on Alamut software prediction, it is very likely that the mutation leads to skipping of exon 14.
Acromegaloid Facial Appearance Syndrome should not be forgotten in the differential diagnosis of pseudoacromegaly

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Acromegaloid facial appearance (AFA) syndrome is a very rare inherited cause of pseudoacromegaly characterized by progressive coarsening of facial features, overgrowth of oral mucosa and large, doughy hands. Six case reports have been described. Herein we describe a further family with AFA syndrome.

Proband was a 31 years female and his 37 years brother who were referred to our department due to suspected acromegaly. Both siblings had a previous history of oral surgery 13 and 20 years before due to hypertrophic intraoral tissue. Additionally, the male proband had mild learning difficulties, uncontrolled hypertension, left ventricular hypertrophy and a paroxysmal atrial flutter. He was medicated with lisinopril, amiodarone and clobazam. Family history was remarkable for their mother, who was remembered by both siblings as having "rough facial features". At physical examination both patients were hypertensive, obese and had a raised cephalic perimeter. Their facial appearance was coarse with thickened lips and superior eyelids, with thickened cutaneous and subcutaneous tissue. Hirsutism was also evident.

The index patient, a boy, presented neonatally with conjugated hyperbilirubinemia which resolved in 2-3 months. Additional findings included posterior embryotoxon and bilateral mild peripheral pulmonary artery stenosis. Butterfly vertebrae were not demonstrated. Sequence or copy number variants were detected in JAG1. Sequencing of ABC244 and CFPTR revealed no pathogenic variant. Sequencing of NOTCH2 established heterozygosity for the novel synonymous variant c.2817G>A (p.P939P) (NM_022408.2) both in the child and his father. In silico analysis predicted introduction of a novel splice-site. cDNA sequencing confirmed the presence of a splice-site causing skipping of the 5’-end of exon 19 (c.2753, 2818del). The deletion maintained the reading frame (p.911-947). Amplification of the correctly spliced mRNA revealed monolecular expression of the normal allele (c.2817G), confirming that c.2817G>A in NOTCH2 is likely the cause of the variable Alagille syndrome phenotype in this family. To our knowledge, this is the first reported case of a synonymous variant in NOTCH2 causing Alagille syndrome.

A new mutation in the COL4A3 gene associated to autosomal dominant Alport syndrome with hearing disorders as sole clinical manifestation

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The autosomal dominant Alport syndrome accounts for 5% of cases of Alport syndrome but with the rare band-linked form. The diagnosis is often delayed and appears in the later stages of life. Autosomal dominant Alport syndrome generates a form of late-onset mild sensorineural hypoacusis. However, as in the other forms of Alport syndrome, this is always accompanied by renal failure, the rate of hearing loss being directly linked to the progression of renal failure, and its progression suggests a poor prognosis of kidney disease. We have studied the COL4A3 and COL4A4 genes by PCR, CGE and automatic sequencing of the full coding region and the exon-intron boundaries in a Spanish family with autosomal dominant Alport syndrome, in which most of its members are suffering from hearing loss only, one member suffers lenticular, and only two suffer renal disease. We found a heterozygous c.345DelG/p.G115GFSX37 mutation in the COL4A3 gene, which generates a truncated protein. This mutation was found in all family members with symptoms but was absent in the healthy members of the family. Thus, we describe a new mutation in COL4A3 gene mainly associated to hearing loss and for the first time we describe a case of autosomal dominant Alport syndrome that presents lenticular. Furthermore, we report that deafness and renal impairment could not be associated in the same individual, this being the first case of a family in which deafness occurs amongst several members who do not show any alteration in the kidney.

Prenatal diagnosis using array CGH in 163 cases

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Array CGH array technology has proven to be useful in postnatal diagnosis of mental retardation, development delay, and conge nital malformation syndrome. We evaluated the use of array comparative genomic hybridization (aCGH) for prenatal diagnosis including assessment of variants of uncertain significance, and the ability to detect abnormalities not detected by karyotype. Women undergoing amniocentesis or chorionic villus sampling (CVS) for karyotype were offered aCGH analysis using a targeted cytoblock ISCA-Ax4x4k V1 oligonucleotide array. Parental samples were obtained at the same time to exclude maternal cell contamination and determine if copy number variants (CNVs) were de novo, or inherited. We analyzed 163 samples, most were CVS (75.5%) and amniotic fluid (19.6%). The most common indication were advanced maternal age (N=74), abnormal ultrasound finding (N=46) and a previous child with multiple congenital anomalies (N=40). We detected 27 CNVs (16.7%). Of these 16 (9.8%) were interpreted as likely benign, 9 (5.5%) were of defined pathological significance, while 2 (1.2%) were of uncertain significance. We concluded that array CGH identified clinically significant abnormalities in approximately 5.5% of fetuses with ultrasound abnormalities and normal conventional karyotype. This study demonstrate the potential for array CGH to replace conventional cytogenetic in the great majority of prenatal diagnosis cases.
P11.020-M

Novel mutations in GNAI3 in patients with Auriculocordialyndr Syndrome suggest a dominant negative effect with disruption of GTP/ GDP binding

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Auriculocordialyndr syndrome is a rare craniofacial disorder comprising core features of micrognatia, condylie dysplasia and question mark ear. Causative mutations have been identified in PLCB4, GNAI3 and EDN1, which are predicted to function within the EDN1-EDNRB pathway during early pharyngeal arch patterning. To date, two GNAI3 mutations in three families have been reported. Here we report an Australian patient with ACS with a novel de novo GNAI3 mutation. We also present two other novel GNAI3 mutations, one segregating with affected members in a family previously linked to 1p21.1-q23.3 and the de novo mutation in an unrelated simplex case. The mutations occur in known functional motifs common to G proteins and RAS family members. Structural modeling shows that all five mutated GNAI3 residues cluster in a region involved in GDP/GTP binding. Two of the five residues lead to dominant negative proteins when mutated in related proteins. We hypothesize that all GNAI3 mutations lead to dominant negative effects.

P11.021-S

A case of a microscopically balanced familial translocation (5;14)(p13;q22) and phenotype of lacrimo-auiculo-dento-digital (LADD) syndrome

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We report a 15 years old male patient with clinical presentation of MCA syndrome including facial dysmorphism with micrognatia and small dysplastic ears, absent lacrimal glands and limb anomalies (muscle hypotrophy and malformations of upper limbs, bilateral pes varus deformity). The boy’s intellectual and growth development was in normal age ranges. The child was born from a second pregnancy of young and healthy couple. The first pregnancy in the family ended with birth of a baby with diaphragmatic hernia which died after three days. The performed cytogenetic analysis of the patient at one month revealed a microscopically balanced chromosomal translocation between chromosomes 5p and 14q: 46,XY,t(5;14)(p13;q22). The father’s karyotype was normal 46,XY while the mother’s karyotype was 46,XX,t(5;14)(p13;q22) identical with her son’s rearrangement. The performed array-GH of the patient identified a heterozygous deletion in 5p12 chromosome with 489 kb size, including only one mapped gene: FGF10 gene. The whole growth factor family contains 22 genes and the FGF10 gene is situated near a recurrent substitution (p.R196H) commonly seen in other cases of Baraitser-Winter syndrome. The observation of transmission from parent to child broadens the clinical spectrum of Baraitser-Winter syndrome to include a relatively milder form that includes additional features such as a severe, early onset glaucoma.

P11.022-M

Whole exome sequencing identifies a vertically transmitted novel ACTB mutation in a mother-son pair presenting with atypical Baraitser-Winter syndrome

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Baraitser-Winter syndrome is a rare, congenital disorder characterized by distinctive facial features, brain malformations and intellectual disability. It is caused by de novo dominant mutations in the ACTB gene. We present the first case of a mother-son pair concordant for Baraitser-Winter syndrome. Both had the facial gestalt of Baraitser-Winter syndrome and were found to carry a novel ACTB mutation by whole exome sequencing. The mutation, c.593A>C (p.S199R), occurs in a conserved amino acid and is situated near a recurrent substitution (p.R196H) commonly seen in other cases of Baraitser-Winter syndrome. The observation of transmission from parent to child broadens the clinical spectrum of Baraitser-Winter syndrome to include a relatively milder form that includes additional features such as a severe, early onset glaucoma.

P11.023-A

A compound heterozygous mutation in the BBS7 gene in a Korean family with Bardet-Biedl syndrome

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Background: Bardet-Biedl syndrome (BBS) is a rare autosomal recessive, pleiotropic ciliopathy. To date, at least fifteen genes causing BBS have been reported. Case: A 26-year-old Korean man from non-consanguineous Korean parents presented with rod-cone dystrophy, truncal obesity, mental retardation and end stage of renal disease. A 28-year old his brother showed rod-cone dystrophy, truncal obesity, mental retardation and deep vein thrombosis. To rule out the BBS, genomic DNAs were obtained from the peripheral blood leukocytes of all the family members. All the exons and intron flanking regions of the fifteen BBS genes were analyzed by direct sequencing. A compound heterozygous mutation of BBS7 gene (NM_176624) was identified in both brothers; one was a novel acceptor splice site c.103-1G>A mutation (IVS2-1G>A) altering the splicing recognition site at the intron 2 and exon 3 boundary of BBS7 gene. The other was a novel missense mutation of c.728G>A changing codon 243 from cysteine to tyrosine. The father was a heterozygous carrier for c.103-1G>A, and the mother was a heterozygous carrier for c.728G>A. Array comparative genomic hybridization analysis revealed a normal hybridization pattern with no evidence of significant chromosomal imbalance.

Conclusion: This is the first BBS case in Korean family genetically confirmed that had a compound heterozygous mutation in BBS7 gene. Considering that the BBS is genetically heterogeneous and the prevalence differs among ethnic group, our clinical experience would be helpful to diagnose these patients accurately and understand the genetic events in BBS.
Genotype-Phenotype correlation for BBS1 gene in Bardet-Biedl Syndrome patients
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Bardet-Biedl syndrome (BBS, #209900) is a rare genetic disorder characterized by highly variable phenotype and genetic heterogeneity. BBS belongs to a group of diseases known as ciliopathies, which show partial overlapping phenotypes that further complicates the molecular diagnosis. BBS1 gene accounts for 25% of total mutations described in BBS, of which p.M390R is the most frequent mutation in European population. Many efforts have been done to establish a genotype-phenotype correlation for BBS genes in order to facilitate diagnosis confirmation. In this sense, the objective of this study was to correlate different BBS1 mutations with some clinical features. We selected 36 patients from 22 families for whom a BBS1 mutation had been identified. Clinical features of these patients were analysed by SPSS v19. p.M390R mutation was the predominant mutation identified in 33 patients (92%), 20 of them harboured this mutation in homozygous state (56%) and 13 were compound heterozygous (36%). Only 3 patients (8%) had two mutated alleles with BBS1 mutations different from p.M390R. By comparing all clinical features of these three groups of patients, we found statistically significant differences between these groups: while homozygous p.M390R showed more frequently high blood pressure, compound heterozygous manifested more secondary features, especially psychomotor impairment/retardation, bearing loss and worse visual defects. In conclusion, we confirm the important role of p.M390R mutation in the diagnosis of BBS, and it seems that homozygous patients have a milder phenotype. This will be important for genetic counselling purposes in these families.

Impact on splicing of BBS12 variations found in Bardet-Biedl syndrome Spanish patients
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Bardet-Biedl syndrome (BBS, #209900) is a multisystem rare disease which belongs to the emerging group of diseases called ciliopathies, and is inherited within an autosomal recessive pattern. However, little is known about other molecular mechanisms which may be involved in BBS development. For example, it is well established that synonymous (sSNPs) and non-synonymous (nsSNPs) changes can affect the conformation and stability of mRNA, the splicing process, the accuracy of translation and the protein structure. The former would lead to the disruption of the highly conserved network of cellular pathways for maintaining proteinostasis. In addition, threboxom lesions predicted as pathogenic could affect splicing process via the creation and/or elimination of Exonic Splicing Enhancer/Silencer sequences (ESEs/ESSs).

In this regard, we have sequenced the coding region and intron boundaries of BBS12 gene in fifty BBS patients. Then, we have used specific software tools to predict the potential effect at protein level (PolyPhen, PMut and SIFT) or on the splicing process (NetGene2, Nsplice, SpliceView, Human Splicing Finder and Rescue ESE) of all variants identified. We have found two pathogenic mutations in six patients and found at least one sequence variation in twenty-nine of them. Eight out of twenty changes were selected as putative to affect the splicing process since at least two software tools predicted this effect.

As these results are only computational predictions, further functional studies will have to confirm this effect on mRNA processing. Thus, minigenes and RNA assays will be performed in order to elucidate the role of these changes.

P11.026-M Cognitive, behavioural, and adaptive functioning of patients affected with Bardet-Biedl syndrome (BBS)
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Bardet-Biedl syndrome is a rare genetically heterogeneous multisystem disorder characterized by retinal degeneration, genital and kidney malformation/function, and other features. Variability in cognitive, social, and emotional impairment is reported; however, studies were small and completed prior to availability of molecular characterization. Our aim is to define neuropsychological function in a group of patients with molecular diagnosis of BBS to better understand phenotypic variability.

Methods: Eighteen patients (12 females; mean age: 20.95 years; range 6.4-38 years) completed standardized measures of cognition. Magnification was available. Standardized informant-proxy questionnaires were completed to assess behavioural and adaptive functioning. Results were compared to normative data (norms).

Results: Significant weaknesses (p<0.01) in verbal and perceptual intellectual reasoning emerged compared to norms (mean = 8th and 3rd percentiles respectively; range < 2 to 53rd percentiles). More individuals displayed Avedependency (25%-74th percentile) on verbal (33.3%) than visual (15.4%) domains. More individuals displayed Extremely-Low ability (< 2nd percentile) on visual (53.9%) than verbal (11.1%) domains. Significant weaknesses in auditory attention span (p<0.01), and auditory and visual working memory (p<0.01) emerged. Infrormant ratings indicated significant patient challenges, relative to norms, in depression (p<0.01) withdrawal (p<0.01), atypical-behaviour (p<0.02), communication (p<0.01), unusual-behaviours (p<0.01) aspects of executive functions (p<0.01) and adaptive independence (p<0.01); most patients (81.3%) fell below the 2nd percentile in functional independence; the remainder fell from the 2 to 5th percentiles. Verbal reasoning was correlated with independence (p<0.01).

Conclusion: BBS impacts cognitive, behavioral, and adaptive functioning to various degrees. Assessment of these patients on a larger scale will allow further definition of the neurocognitive phenotype.

Beckwith Wiedemann Syndrome in 9 Tunisian patients: From typical sporadic presentation to unusual familial isolated adrenocortical tumor
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Beckwith-Wiedemann syndrome (BWS) is a pediatric overgrowth disorder expressed through a highly variable clinical presentation involving a predisposition to tumor development, exomphalos, macrocoglosis, and gigantism. In addition to this clinical heterogeneity, BWS also exhibits etiologic molecular heterogeneity involving a variety of genetic and/or epigenetic alterations in growth regulatory genes on chromosome 11p15. The clinical heterogeneity turns out to be insufficient in diagnosis and prognosis of BWS, thus the need of genetic analysis of 11p15 region.

We conducted a study of 9 patients who underwent molecular analysis of chromosome 11p15 region. Reasons of referral vary from sporadic typical presentation encompassing abdominal wall defects, macrocoglosis and gigantism to atypical mild familial BWS revealed by isolated benign adrenocortical tumor. Molecular analysis using methylation specific multiplex ligation-dependent probe amplification revealed different methylation patterns along the BWS critical region.

6 patients showed complete KvDMR hypomethylation, one paternal uniparental disomy at 11p15 case with both H19DMR hypermethylation/KvDMR hypomethylation confirmed by STR analysis, one patient ; the youngest; isolated paternal uniparental disomy and one patient; the eldest; with KvDMR methylation mosaic pattern. This mosaicism was associated in adrenocortical tumoral tissue to a complete KvDMR loss of methylation, however skin tissue analysis showed a normal methylation profile. These results have allowed us to offer our patients adapted management and genetic counseling. BWS illustrates the complexity of the mechanisms involved and the need for collaboration between geneticists, pediatricians, neonatologists and pediatric surgeons.

P11.028-M A novel IGF2/H19 domain triplication in the 11p15.5 imprinting region - new insights into the pathogenesis of Beckwith-Wiedemann and Silver-Russell syndromes
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The imprinted 11p15.5 region contains two domains (IGF2/H19) and transcriptional regulatory module to control expression of these two domains. The imprinted 11p15.5 region regulates expression of several genes in growth regulatory genes on chromosome 11p15 region. Reasons of referral vary from sporadic typical presentation encompassing abdominal wall defects, macrocoglosis and gigantism to atypical mild familial BWS revealed by isolated benign adrenocortical tumor. Molecular analysis using methylation specific multiplex ligation-dependent probe amplification revealed different methylation patterns along the BWS critical region.

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We report a novel IGF2/H19 domain triplication in the 11p15.5 region identified in a girl with BWS and her father with symptoms of SRS. Methylation specific multiplex ligation-dependent probe amplification (MS-MLPA) performed in the patient's DNA revealed triplication of IGF2/H19 domain as well as increased methylation of H19 region. The same genetic change in the father's DNA was associated with reduced methylation of H19 region. The presence of triplication was confirmed by aCGH.

This is the first report of IGF2/H19 domain triplication associated with BWS or SRS. A few BWS patients with IGF2/H19 domain duplication of paternal copy have been described so far. Duplications of maternal copy of this domain have been reported in three individuals and were not associated with an abnormal phenotype (i.e. Silver-Russell syndrome). The clinical outcome of 11p15.5 copy number variations depends on their size, localization and the parental inheritance. These aberrations may influence chromatin organization affecting the regulation of imprinted genes. Our findings bring new insights into the regulation of genomic imprinting at 11p5.5 region and underline difficulties of genetic counseling in patients with 11p15.5 defects.

The study was financed by National Science Centre, project 1149/B/P011/2011/40 (NN40714940) and EU Structural Funds, POIG.02.01.00-14-05/09.

P11.029-S
Uniparental disomy in Beckwith-Wiedemann syndrome: new insights from genotype-phenotype correlations
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Beckwith-Wiedemann syndrome (BWS) is the most common overgrowth syndrome, with a prevalence of about 1:10,000 live birth. Clinical findings include macrosomia, polyhydramnios, abdominal wall defects and other less frequent features, the most severe of which is predisposition to embryonal tumors.

The molecular etiology of BWS is complex, involving alterations in the expression of multiple imprinted growth regulatory genes on chromosome 11p15.5. Genes that are imprinted are expressed predominantly from one allele in a parent of origin-specific manner: Epimutation and/or genetic alterations in the 11p15.5 imprinted gene cluster have been associated with BWS in approximately 80% of cases. One of the major categories of BWS molecular alteration (20% of cases) is represented by mosaic paternal uniparental disomy (pUPD), namely patients with two paternally derived copies of chromosome 11p15 and no maternal contribution. pUPD is also the molecular alteration associated with the most severe BWS phenotype because of the highest tumor risk.

Here we report a fine analysis of patients with BWS and pUPD from our cohort. By SNP array and microsatellite analysis we could distinguish three different categories of pUPD: whole genome, whole chromosome 11 and chromosome delineated microdeletions. These three UPD subgroups show different clinical features (hemihyperplasia, hypoglycemia, umbilical hernia, hepato/splenomegaly) and similarities in others (macrosomia, polyhydramnios).

Our results highlight the importance of a fine molecular analysis of the UPD cases for a more accurate prognostic prediction to facilitate management and surveillance of patients.

P11.030-M
KCNJ1 mutation in a Spanish family with Cantú syndrome including a case of fetal demise
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1Genetics Dept, Hospital Vall D’Hebron, Barcelona, Spain; 2Research Network for Rare Disorders in Infancy and Childhood, Hospital Clinic, Barcelona, Spain; 3Cantu syndrome, (MIM 239850) is an autosomal dominant disorder causing severe hypertrophic, congenital and progressive coarseness of the facial features, thin skin with deep palmar and plantar creases, cardiomyopathy and lymphedema. Polyhydramnios and macroglossia can be present in the affected fetus. ABCC9, a multicentric subunit of an ATP sensitive potassium channel K(ATP) has been the causal gene in most cases, but not all. A second gene KCNJ8, also a subunit of the same K(ATP) channel, was proposed to be causal after a de novo mutation (p.2S422L) was found in a sporadic case with unusual cardiovascular findings. Another mutation in KCNJ8 (p.S422L) has been reported in at least 9 individuals with Brugada sudden death syndrome. A 30 week old fetus was followed due to macroglossia and polyhydramnios, unexplained fetal demise occurred at 34 weeks. Evaluation of the family identified the fetus, the father, the paternal grandnephro and the aunt of the fetus had clinical features of Cantú syndrome. No cardiomyopathy or other potential causes for the fetal demise were identified. Sequencing and MLPA for ABC9 did not detect any pathogenic changes. Subsequently, an inherited missense change in KCNJ8 (NM_004982.3: c.347T>C (p.Y121H)) was identified. In silico prediction and segregation (4 affected, 1 unaffected) indicated the mutation is most likely pathogenic. The phenotype in the family is indistinguishable from typical Cantú syndrome caused by mutation in KCNJ8. Although fetal demise was never reported before. Mutations in KCNJ8 in families with Cantú syndrome may represent increased risk for sudden death.

P11.031-S
De novo mutations associated with sporadic cases of Caudal regression syndrome
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Aim: The identification of de novo disease causing mutations in three Caucasian patients with sporadic Caudal Regression Syndrome (CRS). CRS is a rare and diverse congenital disorder which is characterised by different degrees of agenesis of the caudal spine. Known genetic mutations are only able to explain a fraction of cases and are not accounting for sporadic occurrences or the diversity of the disorder. Methods: Exome sequencing assay was conducted of the three sporadic cases and their biological parents. We targeted rare genetic variants as the underlying cause of CRS as well as de novo mutations. Further we investigated de novo indels, copy number variations (CNV) and compound heterozygosity. Identified mutations were ranked and filtered based on genomic, genetic and statistical features. Results: Sanger sequencing confirmed two different de novo mutations in two cases (detailed results will be presented). In addition, our analysis revealed several potentially causal compound heterozygous mutations which are also under investigation. Conclusion: CRS may be caused by de novo or compound heterozygous mutations thus, i) the diversity of the disorder is mirrored in the underlying genetic architecture and its mutations; ii) ranking of compound heterozygous mutations enables identification of candidate genes.

P11.032-M
A de novo microdeletion of chromosome 18q11.2q12.2 causes a new distinct clinical phenotype with coarctation of aorta and intellectual disability.
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Cardiac abnormalities are diagnosed in 25-30% of the patients with 18q syndrome and the most common heart abnormalities are reported in patients with terminal 18q deletion (18q22): atrial septal defect, ventricular septal defect, patent ductus arteriosus and total abnormalomas. We report on one girl with coarctation of the aorta, moderate development delay, behavioral problems, with an interstitial de novo deletion within chromosomal band 18q11.2 of 13.4MB. The propositus was born at 37 weeks via cesarian section after a pregnancy complicated by gestosis. Her birth weight was 2400g (<10th centile), birth length was 45cm (3-10th centile) and occipital frontal circumference (OFC) 34 cm (50th centile). Family history revealed no abnormal organs or congenital heart diseases. At the age of one month, cardiac echo revealed tight istmic coarctation of the aorta and the patient underwent surgery. Clinical evaluation showed a distinctive craniofacial appearance characterized by brachiocephaly, plagiocephaly, telecanthus, strabismus, low nasal bridge, smooth philtrum, thin lips, high arched palate, low set ears with hypoplastic lobule, proximally placed thumbs. The development milestones were delayed and the propositus developed behavioral problems: poor concentration, hyperactivity and distractibility. CGH analysis showed an interstitial proximal microdeletion of chromosome 18 of 13.4MB [arr 18q11.2 (22;2;3;2;1)22;35;43;900001]. In this region some genes are present that are involved in development of the heart, DSC2, DSG2, TTR, DTNA, FHD03. This is the first report of aortic coarctation mapping to a proximal microdeletion involving the chromosome 18q11.2q12.2 segment. The report also expands the spectrum of clinical phenotype associated with 18q11.2q12.2 deletion.
on is rarely described. Here we report an 11-year-old girl from non-consan-
gineous parents, who was referred to the Pediatric Genetics Department with
growth retardation and multiple congenital abnormalities. In her medical
history, she was born at term after an uncomplicated pregnancy. Her birth
weight was 2.600 g, and at 20 weeks gestation she had a cleft palate, lip dislocation and crossed re-
nal ectopia. On physical examination, her weight, height and head circum-
ference were below the 3rd percentile. Dysmorphological evaluation re-
vealed a triangular face, low-set ears, fissured cleft tongue, micrognathia,
proximally placed hypoplastic thumbs, genu valgus, 2-toe skin syndactyly,
cliodactyly and nail hypoplasia. Speech problems were also noticed. The
complete blood count, basic biochemical parameters and hematologic profi-
le were within normal range. The karyotype was normal. Subtelomeric fluo-
rescence in-situ hybridisation (FISH) analysis showed a de novo terminal
deletion of chromosome 15. BAC FISH analysis of the patient indicated that
the deletion breakpoint was at 15q26.3 and the deletion comprised 700-
870 kb. The deleted region includes the CHST7 gene that is responsible for
Tentancy preaxial brachydactyly syndrome which shares clinical features
with 1q5ter deletion syndrome. To the best of our knowledge, this deletion
is the smallest among reported cases. It is considered that the case present-
ated here significant contribution to phenotype-genotype correlation in 1q5
deletion patients.

P11.038-M
Detection of PIK3CA somatic mutations in CLOVES syndrome
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CLOVES syndrome (congenital lipomatous overgrowth, vascular malforma-
tions, epidermal nevus, and skeletal abnormalities (OMIM 612918)) is a
non-hereditary regional overgrowth disorder distinct from Proteus syndrome.
Gain-of-function mutations in PIK3CA have been identified in affected tissu-
es. These mutations have been shown to increase cellular proliferation and
characterized by overgrowth, macroglossia, organomegaly, exompha-
los and predisposition to embryonal tumor development.

Objective: To present a BWS familial case follow up.

Case Report: Family data: Mother with 4 pregnancies, she presents ear pits. Case 1: 9 years 7 months female, product of the 2nd pregnancy term, obtained by cesarian section from non-
consanguineous parents, with 28 years (he) and 29 years (she) at birth time. Apgar 9-10, weight and height >pc97, posterior helical ear pits, macroglossia, depressed nasal bridge,
posterior helical ear pits and exomphalos surgically corrected, presented neonatal hypoglycemia. At first year of age presented overgrowth, renal ul-
trasonography reported right pyelic duplication. At 3 years was performed transversal and anteroposterior reduction glossectomy. At 8 years hemihy-
pertrophy was detected. At present she has no complications. Case 2: 3 years 9 months female, product of the 4th pregnancy term, obtained by cesarian section at
30 weeks, weight at birth 2500g. Physical examination: weight and height > pct 97, posterior helical ear pits, macroglossia, umbilical hernia; has not
required surgical procedure. Renal ultrasonography without abnormalities.

Conclusion: Clinical findings meet criteria diagnosis for BWS. In case 1 treatment consisted of surgical reduc-
tion of exomphalos and anterior-transversal reduction glossectomy and case 2 not required surgical intervention. The opportune diagnosis allows a complete
treatment, genetic counseling and appropriate follow up.

P11.036-M
Haploinsufficiency of MEIS2 is associated with orofacial clefting and
learning disability
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MEIS2 is a homeodomain-containing transcription factor of the TALE
superfamily that has been proven important for development. We confirm
and extend a recent single case report suggesting that deletions in MEIS2 over
cause cleft palate [Crowley et al. Am J Med Genet 2010, 152A:1326-7]. Here we re-
port five additional cases with 1q514 deletions of sizes 0.6, 0.6, 1.0, 1.9 and
4.8 Mb, respectively, all involving MEIS2. In total, 7/9 cases had clefting from mild (submucous cleft palate) to severe (cleft lip and palate), and 3/9 cases had ventricular septal defects. All cases had delayed motor development and most had learning
disability, at worst in the mild intellectual disability range. Our results show
that MEIS2 clearly is a gene needed for palate closure. In syndrome cases of
cleft palate, MEIS2 should be considered among the candidate genes, e.g. in
cases without 2q21.2 deletions.

P11.035-S
Association between HLA-G and non-syndromic Oral Cleft
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HLA-G has a protective function in pregnancy and chance of developing CLP. We hypothesize that HLA-G
specifically associated with a three-fold increased risk of the more severe
phenotype (cleft lip and palate, CLP) (p<0.0001).

In trios with affected children born after 38 weeks or less, the transmission
cancellation was observed in the trios with pregnancy length >38 weeks (p=0.005).
In trios with affected children born after 38 weeks or less, the transmission
of minor allele was reverse (p=0.031), so that in the effect of the asymme-
tric segregation was canceled when the two groups were pooled. In children
born after more than 38 weeks of pregnancy the ins/ins genotype resulted
specifically associated with a three-fold increased risk of the more severe
phenotype (cleft lip and palate, CLP) (p<0.001).

This result suggests the existence of a link between HLA-G genotype, length
of pregnancy and chance of developing CLP. We hypothesize that HLA-G
could be involved in prenatal loss of abnormal embryos (teratothanasia)
and drive selection against those which fail to fuse lip and palate.

P11.034-M
Association between chromosome 8q24.21 and susceptibility for
nonsyndromic cleft lip with or without cleft palate in Iraqi population
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Non-syndromic oral clefts (nSOCs) affect 1 per 1,000 live births worldwide.
There is some evidence suggesting that embryo-maternal interaction can
play a relevant role in the etiology of nSOCs. HLA-G has a protective function
at the maternal-embryo interface, protecting the embryo from destruction
by mother's immune system.

In this study we investigated the association between a functional variant in
HLA-G gene and the risk of nSOCs. A 4 nt insertion in the 3'TUTR of HLAG
was genotyped in a group of 222 Italian nSOC trios, including affected children
and their parents.

The analysis of transmission disequilibrium resulted in no evidence of asso-
ciation in nCL/P (p=0.16) nor in nCP trios (p=1.00). However, when consi-
dering the length of pregnancy, a significant overtransmission of the inser-
tion was observed in the trios with pregnancy length >38 weeks (p=0.005).
In trios with affected children born after 38 weeks or less, the transmission
of minor allele was reverse (p=0.031), so that in the effect of the asymme-
tric segregation was canceled when the two groups were pooled. In children
born after more than 38 weeks of pregnancy the ins/ins genotype resulted
specifically associated with a three-fold increased risk of the more severe
phenotype (cleft lip and palate, CLP) (p<0.001).

This result suggests the existence of a link between HLA-G genotype, length
of pregnancy and chance of developing CLP. We hypothesize that HLA-G
could be involved in prenatal loss of abnormal embryos (teratothanasia)
and drive selection against those which fail to fuse lip and palate.
50% were observed in scrapings from epidermal nevi or affected fatty tissue. In none of the blood samples PIK3CA mutations were detected. Because detection levels and quantification of mutant alleles were limited to 10-15% by Sanger sequencing, fragment analysis and ampli-con deep sequencing on an ABI platform (Roche) were applied. This increased the mutant allele detection to 1-5%. In blood samples mutant allele ratios were 2% or less. Our data confirm that cells from affected tissue are essential for mutation analysis in CLOVES syndrome whereas blood is an inappropriate source. Improved detection methods may be required for other low level somatic mosaicism.

P11.039-S
Endocrinological study in a new case of Coffin Siris syndrome due to ARID1B gene deletion

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Coffin Siris syndrome (CSS) is a rare congenital anomaly syndrome (MIM135900), characterized by developmental delay with severe speech impairment, growth deficiency, coarse facial features, hypotrichosis, hypoplastic/hypertrophied fifth fingers or toes. Some CSS patients have mild microcephaly and hypogonadism/agenesis of the corpus callosum. In 2012 Sauten et al. showed that haploinsufficiency of ARID1B gene causes CSS and Tsurusaki et al. demonstrated that CSS can be due to de novo germline heterozygous mutations in one of five SWI/SNF subunit genes (SMARCBL, SMARCDA1, ARID1A and ARID1B).

We describe a girl of four years of age, first daughter of healthy unrelated parents. She was born by caesarean section for HUGA, at 39 weeks of pregnancy. Our first examination at the age of 18 months showed psycho-motor delay with absent language. Her weight, length and OFC were between 25th and 50th centile for age, but her facial and extremities features were suggestive of CSS. She had marked body hypotrichosis and moderate, non-syndromal joint laxity, usually not described in CSS phenotype. Array-CGH analysis revealed a de novo 1,3 Mb interstitial deletion in 6q25.3, including ARID1B. Because few hormonal data in CSS have been reported so far, we performed an extensive endocrinological study. Biochemical measurements showed normal values for electrotens, venous blood gas, creatinine, glucose, FT4, TSH, aldosterone, renin activity, 17-OH progesterone, DHEAS, cortisol, ACTH and normal slow response of cortisol and 17-OH progesterone to ACTH test.

The main clinical findings included pitosis, ophthalmoplegia, microphthalmia, clinodactyly, pectus excavatum, recurrant pulmonary infections, 2 months. The main clinical findings included pitosis, ophthalmoplegia, microphthalmia, clinodactyly, pectus excavatum, recurrent pulmonary infections, inguinal hernia/midpenil hypoplasias, elbow extension/bilateral wrist supination restriction, kyphes in flexion mode and pes valgus. Sequencing of the PIEZO2 gene in the proband revealed a de novo one base pair deletion in Exon 52 of PIEZO2, which results in a frameshift mutation (p.R2717delG). The mutation leads to a Y2727delC+1 change within the C-terminal domain of PIEZO2. Recently electrophysiological studies of E2727del variant showed that mechanically activated current inactivation were clearly slower and E2727del channels spend about two fold less time in an inactivated state following mechanical stimulation than in wild channels, thus can be reactivated quicker. Therefore because our pathologic variant is in the same domain as E2727del, it may be speculated that increased response to mechanical force may explain the phenotype of our patient also.

P11.042-M
The contribution of discrepant DNA variations in discordant monozygotic twins with Congenital Diaphragmatic Hernia (CDH) or Esophageal Atresia/ Tracheoesophageal Fistula (EA/TEF)

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The distal arthrogryposis (DA) are characterized by congenital contractures of two or more different body areas without a main neurological and/or muscle disease. Currently, DAs are subdivided into 10 types, depending on the number and nature of additional features. DAS is unique among DAs because, in addition to contractures, affected individuals have ocular abnormalities. These abnormalities include ptosis, ophthalmoplegia, and/or strabismus. Recently, it has been shown that a subtype of DAS that includes restrictive pulmonary disease is caused by gain-of-function mutations in the mechanically activated channel PIEZO2. We report 14 years old boy with profound hypotonia, who initially presented with two midline defects at the base of skull 2 months. The main clinical findings included ptosis, ophthalmoplegia, microphthalmia, clinodactyly, pectus excavatum, recurrent pulmonary infections, inguinal hernia/midpenil hypoplasias, elbow extension/bilaterally wrist supination restriction, kyphes in flexion mode and pes valgus. Sequencing of the PIEZO2 gene in the proband revealed a de novo one base pair deletion in Exon 52 of PIEZO2, which results in a frameshift mutation (p.R2717delG). The mutation leads to a Y2727delC+1 change within the C-terminal domain of PIEZO2. Recently electrophysiological studies of E2727del variant showed that mechanically activated current inactivation were clearly slower and E2727del channels spend about two fold less time in an inactivated state following mechanical stimulation than in wild channels, thus can be reactivated quicker. Therefore because our pathologic variant is in the same domain as E2727del, it may be speculated that increased response to mechanical force may explain the phenotype of our patient also.
tegries to determine if we could distinguish false positive differences from actual ones. Currently, we are evaluating with whether these remaining discrepancies are true differences.

**P11.043-S**

Associated noncardiac congenital anomalies among infants with congenital heart defects

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BACKGROUND: Although the majority of congenital heart defects (CHD) occur in isolation, a significant number varying in previous reports from 6 to 66% occur with noncardiac anomalies. The purpose of this investigation was to assess the prevalence and the types of associated anomalies in infants with CHD in a defined population. METHODS: The associated anomalies in CHD were collected during 26 years in 346,831 consecutive births of known outcome. RESULTS: Of the 4005 infants with CHD (116 per 10,000), 105(26.3%) had associated anomalies. There were 354 (8.8%) patients with chromosomal abnormalities including 253 trisomies 21, and 99 (2.5%) non-chromosomal recognized dysmorphic conditions. There were no predominate recognized dysmorphic conditions, but VA(C)TER(L) association. However, other recognized dysmorphic conditions were registered including DiGeorge and Noonan syndromes. 15.0% of the patients had multiple congenital anomalies, non syndromic, non chromosomal (MCA). Anomalies in the musculoskeletal, the urinary tract, the digestive, and the central nervous systems were the most common other anomalies. CONCLUSION: The overall prevalence of associated anomalies, which was one in four infants, emphasizes the need for a thorough investigation of infants with CHD. The most commonly associated major noncardiac anomalies involved the musculoskeletal system, followed by the urinary, the digestive, and the central nervous systems. A routine screening for other anomalies may be considered in infants and in fetuses with CHD. One should be aware that the anomalies associated with CHD can be classified into a recognizable anomaly syndrome or pattern in one out of nine infants with CHD.

**P11.044-M**

Informational-analytical system of registration, systematisation and counting of congenital and hereditary diseases


Creation of an informational system of congenital and hereditary diseases's registration is very acute in today's world, which provides the ability to schedule medical - diagnostically and preventive measures to reduce infant morbidity and mortality. Primary source of information is the patient's electronic registration card, which includes personal data, life history and disease, genealogical information. The database consists of four main sections: congenital malformations, prenatal diagnosis, chromosomal abnormality, monogenic pathology. The database contains information about 2685 cases of patients and fetuses with congenital and hereditary disorders. The structure is dominated by pathology congenital malformations - 1323 (49.3%) cases. The most frequent defined in patients with congenital malformations: the nervous system - 236 (17.5%); multiple malformations - 191 (14.4%); the genitourinary system - 183 (13.8%); the circulatory system's genitourinary system - 169 (12.7%). Chromosomal abnormality was 744 (27.7%) cases. Monogenic pathology was defined in 618 (23.0%) cases. Most frequent are osteogenesis imperfecta - 43 (6.9%); choroiditis - 36 (5.8%); congenital adrenal syndrome - 29 (4.5%); congenital hypothyroidism - 23 (3.7%); mucopolysaccharidosis - 22 (3.5%); spinal muscular atrophy - 17 (2.7%); Duchenne myopathy - 11 (1.7%). The informational registration system of congenital malformations and hereditary pathology's spectrum helps to improve health system of medical genetic counseling and allows setting the frequency and structure of congenital and hereditary diseases.

**P11.045-S**

CNV analysis in a cohort of 174 patients with bladder-exstrophy-epispadias complex

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The clinical presentation of the bladder-exstrophy-epispadias complex (BEEC) ranges from epispadias (E) and classical bladder exstrophy (CBE) to the most severe form, cloacal exstrophy (CE), often referred to as the OEIS complex. The birth prevalence for the complete spectrum has been reported to be 1 in 10,000 live births, with a male-to-female ratio of 2.4:1. Although the etiology for the majority of cases remains elusive, there are several lines of evidence, that de novo copy number variations (CNVs) represent a major genetic contributor. Here we array-based molecular karyotyping in a large cohort of 174 BEEC patients, aiming to identify disease related de novo CNVs. For array-based molecular karyotyping we used the Illumina HumanOmniExpress-12v1 bead-chip, comprising a total number of 719,665 markers. All genotye data were analyzed by QUANTSNP, single objective-typing Haploview model. To narrow down the computed number of 13,828 putative CNVs, we used different filter criteria and implemented various procedures for data analysis. In total, 17 putative disease related CNVs ranging from 2.52 kb to 6.08 Mb in size, including one duplication in the Cat eye syndrome relevant region (22p2ter-22q11.21) were identified. Based on the presence of evidence of parallelism and the occurrence of parallel investigation of the patients using quantitative PCR and MLPA is currently performed. Array-based molecular karyotyping furthermore identified triple X syndrome in an isolated CBE patient.

**P11.046-M**

New point mutations in the HDAC8 gene: Cornelia de Lange syndrome and beyond


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Cornelia de Lange syndrome (CdLS) is a neurodevelopmental disorder caused by mutations in either of regulators (NIPBL, HDAC8) or structural elements (SMC1A, SMC3, RAD21) of the cohesion complex. As regards X-linked CdLS, a higher prevalence of females is described for both SMC1A, partially escaping X-inactivation, and HDAC8, which is instead subject to X-inactivation. At present, 26 different mutations in 41 individuals with features partially overlapping with CdLS have been reported in the HDAC8 gene, confirming the key role of this lysine deacetylase in the proper functioning of the cohesin and beyond.

We analyzed a group of 167 CdLS patients with CdLS by classical sequencing approaches and exome/gene panel next generation technology. Thus we identified eight de novo HDAC8-mutations. Interestingly, one of which is shared by two siblings. The R166* nonsense mutation, the frameshift deletion in F207Nfs*2 as well as six missense mutations C153R, N156K, P257L, T280I, C287Y, G320R all affect highly conserved residues and were predicted to be damaging by four bioinformatics algorithms. All patients show a mild to severe phenotype overlapping with classical CdLS: the craniofacial appearance is similar but with some distinctions like CdLS; the craniofacial appearance is similar but with some distinctions like CdLS.

**P11.047-S**

A new prognostic index of severity of intellectual disabilities in Cornelia de Lange syndrome

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Cornelia de Lange syndrome is a well-known multiple congenital anomalies/mental retardation syndrome with genetic heterogeneity and wide clinical variability, regarding the severity of both the intellectual disabilities and the physical features, not completely explained by the genotype-phenotype correlations known to date.

The aim of the study was the identification of prognostic features, ascertainable precociously, of a better intellectual outcome and the development of a new prognostic index of severity of intellectual disabilities in CdLS patients.

In 66 Italian CdLS patients aged 8 years or more, we evaluated the association of the degree of mental retardation with various clinical parameters ascertainable before 6 months of life and with the molecular data by the application of cumulative regression logistic model. Based on these results and on the previously known genotype-phenotype correlations, we selected 7 parameters to be used in a multivariate cumulative regression logistic model to develop a prognostic index of severity of intellectual disability.

In the table the parameters selected and their relative scores.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small for gestation-age</td>
<td>1,5</td>
</tr>
<tr>
<td>Learning delay</td>
<td>1</td>
</tr>
<tr>
<td>Limb reduction</td>
<td>1</td>
</tr>
<tr>
<td>Moderate-severe sensorineural hypoacusia</td>
<td>3.5</td>
</tr>
<tr>
<td>NIPBL truncating mutation</td>
<td>0</td>
</tr>
<tr>
<td>SMC1A mutation</td>
<td>1.5</td>
</tr>
</tbody>
</table>

The probability of a mild MR increases with the reducing final score less than 2, the probability of a severe MR increases with the increasing final score more than 3.

This prognostic index allows to define, precociously in the life of a baby, the probability of a better or worse intellectual outcome in CdLS patients.

P11.048-M

SNP arrays in the diagnostic strategy of corpus callosum agenesis associated with intellectual disability (an update)


Corpus callosum agenesis is the most common cerebral malformation in patients with intellectual disability (CCA-ID) with a prevalence of 2-3% of cases. Known genetic causes are heterogeneous and in the majority of cases, no etiologies have been found. In order to achieve a genetic diagnosis, we performed chromosome analyses on microarrays (CMA) on 81 patients with CCA-ID and no known causes. We found 34 different CNVs (42%) which were not carried in control subjects of the Database of Genomic Variants (DGV). Among these CNVs, 14 (17%) were de novo and considered to be likely pathogenic, with sizes varying from 1,3Mb to 24Mb, including 13 deletions and one inverted duplication with terminal deletion. Moreover, 12 CNVs (4 deletions and 9 duplications) were also carried by healthy parents, and therefore, could not be considered as the main causes of the phenotype. We were not able to recover blood samples of the parents to verify the 8 remaining CNVs. Thus, CMA seems to be a powerful tool in the diagnostic strategy of patients with CCA-ID and no etiologies. However, most of the tested patients still remain with no identified genetic causes. In the near future, new techniques such as exome sequencing, or massively parallel sequencing on selected genomic panels, could improve the detection rate of the genetic causes of CCA-ID.

P11.049-S

Molecular Diagnostic Algorithm of Syndromic Craniosynostosis


Craniosynostosis (CS) is a birth defect, with a prevalence of 1/2100-1/2500, caused by the premature fusion of one or more cranial sutures leading to specific cranial base and vault abnormalities. It is a highly heterogeneous group of disorders occurring both in syndromic and non-syndromic forms, associated with approximately 180 different syndromes. The identification of the responsible gene largely depends on the fact if it is syndromic or non-syndromic. Although 85% of the cases are reported to be non-syndromic with unknown etiology, syndromic forms arise from chromosomal anomalies or single gene defects of Mendelian inheritance, both together comprising the etiopathogenesis in only 40% of the cases and single gene defects contributing to three/fourth. Noteworthy genes in this group are FGFR1, FGFR2, FGFR3, TWIST1, EFNB1, MSX2, RAB23 and FREM1. EFNB1 can be excluded from this group due to its association with Craniofrontonasal Syndrome. Thirty syndromic CS patients with normal karyotype were enrolled in the study excluding STEP approach with chromosome analysis as initial step being the sequencing of FGFR2, FGFR3 and FGFR1, followed by full gene sequencing of FGFR2 and FGFR3. Samples with unidentified etiology were further screened for deletion/duplication by craniofrontonasal MLPA kit (P080). The last step consisted of sequencing of FGFR1, MSX2, TWIST1, RAB23 and FREM1 genes, when the cases showed distinct related clinical phenotype.

We highly suggest that our ongoing research will lead to better insight for the clinical diagnosis, molecular diagnostic flow charts in CS and will contribute to the genotype-phenotype correlation.

P11.050-M

Craniosynostosis and Heart defects: Postnatal new autosomal recessive syndrome due to ZDHHC13 mutations

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We report two sisters with congenital craniosynostosis, heart anomalies and minor digital defects. They were born to non-consanguineous Hispanic parents. The mother is macrocephalic, but without other dysmorphisms. The first child was born with complex craniosynostosis involving the metopic, left coronal, and left lambdoidal sutures requiring two craniotomies and leading to significant cranial deformity despite the surgeries. In addition she had truncus arteriosus with interrupted aortic arch that was successfully repaired. The second girl presented with metopic craniosynostosis and large atrial septal defect, both requiring surgeries. Both girls have apparently normal development at 6 and 4 years of age, respectively, but no formal evaluation has been performed. In addition, the older child has minor digital anomalies and mild pectus excavatum and the younger child has mild enamel hypoplasia. Their older brother has severe pectus excavatum, but no other anomalies. Chromosomal microarray and sequencing analyses of the hot-spot regions in FGFR1, FGFR2, FGFR3, and the TWIST gene were normal for both girls. There were no detectable metabolic abnormalities. Whole exome sequencing documented rare compound heterozygous variants in the ZDHHC13 genes in both children - c.629A>T (Asn219le, maternal inheritance) and c.1135A>G (Ser379gl, paternal inheritance). The ZDHHC13 ( zinc finger, DHHC-type containing 13) gene is located on ch.11p.15.1 and has palmitoyltransferase activity with possible role in the FGFR/RAS/MAPK pathway. The functional significance of these variants is under investigation. We propose that this condition represents a novel autosomal recessive syndrome, possibly due to mutations of ZDHHC13.
genes in the clinical manifestations and will provide insight into the underlying biological mechanisms.

P11.052-M
Non-mosaic 4p16.3 deletion concomitant with low-level mosaicism for deletion at 2q11.1q12.2
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Multiple chromosome abnormalities are occasionally detected in live-born children. Furthermore, concomitant non-mosaic and mosaic chromosome aberrations are even less frequent. In the recent report, we describe a case of autism, moderate intellectual disability, epilepsy, cerebral palsy, osteopo-rosis, strabismus, speech delay, cardiac defect, enlargement of the left brain ventricle and kidney abnormalities in a 9 year old girl. Cytogenetic analysis has demonstrated a mosaic deletion of chromosome 21: 46,XX,del(21) (q11.2q21.2)×1, 46,XY,del(21) (q11.2q21.2)×1. FISH with BAC probes encompassing 2.7 Mb and 0.4 Mb as follows: arr 18q21.1q21.2(47,553,468-49,165,154)×1. Molecular genetic analysis using oligonucleotide array CGH has confirmed the presence of mosaic deletion spanning 21q11.1q21.2 chromosome region (11,423 Mb). Additionally, a non-mosaic deletion at 4p16.3 (size: 3.712 Mb) affecting 83 genes, 40 of which are listed in OMIM was found. Mosaic 21q11.1q21.2 was also confirmed using multi-color chromosome banding (MCB), which has shown this deletion to affect 199 genes. Furthermore, the index case has demonstrated a phenotypically atypical for 4p16 deletions. Nevertheless, the main phenotypic outcome was likely to result from non-mosaic 4p16.3 according to bioinformatics analysis, whereas mosaic 21q11.1q21.2 was concluded to be an additional co-factor modulating the phenotype. Thus, one can conclude that phenotypic heterogeneity of recurrent chromosome aberrations can be produced by concomitant genomic rearrangements. In this instance, multiple molecular rearrangements at two sites are warranted for the appropriate molecular diagnosis. Supported by the Russian Federation President Grant (MD-4401 .2013.7).

P11.053-S
Juvenile polyposis associated to hereditary hemorrhagic telangiectasia in an adolescent with complex chromosomal rearrangement and intellectual disability
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Juvenile polyposis can be associated to hereditary hemorrhagic telangiectasia (HHT) due to SMAD4 gene mutations. We describe the first case of juvenile polyposis and HHT in a patient with SMAD4 gene loss due to a chromosomal deletion. The male patient presents moderted intellectual disability, limited verbal language repertoire, attention-deficit/hyperactivity disorder, corpus callosus agenesis and brain arteriovenous malformation. Colono-scropy revealed inflammatory intestinal polyposis, with typical juvenile polyposis syndrome clinical criteria. Three small haemangiomas as in scalp and bilateral telangiectasias in Kieselsbach area were found. The patient presents a 46,XX(16;18)(q13;q31)Karyotype. Chromosomal microarray detected two non-contiguous de novo microdeletions encompassing 2.7 Mb and 0.4 Mb as follows: arr 18q21.1q21.2 (47,553,468-50,257,792)×1, 18q21.1q21.2 (50,644,595-51,052,896)×1. FISH with BAC probes confirmed both deletions and the presence of a segment between them. This is the first case of juvenile polyposis and HHT in a patient with a chromosomal rearrangement that resulted in interstitial deletions of 18q and loss of the SMAD4 gene, among others. The loss of a copy of the entire SMAD4 gene, added to the fact that the patient had intestinal polypos at a young age, prompted us to look for telangiectasia. Thus, we showed the importance of screening these phenotypes in patients in whom cytomo-lecular studies indicate deletion of SMAD4 gene. Additionally, we demonstrate the relevance of cytogenomic investigation in patients with juvenile polyposis, dysmorphisms and intellectual disabilities. Financial support: FAPESP, Brazil.

P11.054-M
Microdeletion 19p13.12 in a fetus with severe microcephaly and paraventricular cysts: case report and review of the literature
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Chromosome 19 is one of the densest chromosomes in genes. Consequently, rearrangements occurring in this chromosome, even small in size, can be lethal. This might explain why only a few cases of chromosome 19 rearrangements have been reported so far. 19p 13.12 microdeletions of different sizes and partially overlapping, detected by Array Comparative Genomic Hybridization (Array CGH), have been described in nine patients. The associated phenotypes include a mild to moderate intellectual disability in seven patients. Among these patients, four of them have been reported with cerebral malformations (corpus callosum hypoplasia with vermis hypoplasia, pontocerebellar hypoplasia). Microcephaly, neurosensory deafness, ear abnormalities, hypertrichosis or facial dysmorphism including synophrys have also been reported. All of them were diagnosed in postnatal, from the first months of life up to late childhood No prenatal case has been reported so far. We present the first case of a de novo 1.1 Mb 19p13.12 deletion including 29 genes in a fetus which has been interrupted at 38 weeks of gestation because of severe microcephaly associated with benign paraventricular cysts. Among the deleted genes, NOTCH3 seems to be a good candidate gene for intellectual disability. Chromosomal abnormalities associated with CADA-SIL (Cerebral Autosomal Dominant Arteriopathy with Subcortical Infaracts and Leucoencephalopathy). It has been suggested that the mechanism involved in CADA-SIL was a gain-of-function of the mutated protein. The consequences of NOTCH3 haploinsufficiency are poorly known. Considering its role in neurodegeneration, NOTCH3 haploinsufficiency may contribute to cerebral malformations and to intellectual disability as observed in deﬁned patients.

P11.055-S
Molecular cytagenetic characterization of a 2q35-q37 duplication and a 4q35.1-q35.2 deletion in two cousins: a genotype-phenotype analysis
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Derivative chromosomes usually display variable phenotypes and clinical expression.

We report two patients: a 37-year-old man (proband-1) and a 17-year-old girl (proband-2), two second-degree cousins, with a derivative chromosome leading to a 4q3 deletion-4q3 duplication. Conventional karyotype revealed in both patients the same rearrangement derived from unbalanced translocation t(2;4)(q35;q35). Array-CGH analysis, performed to characterize the rearrangement, documented in both the probands the presence of a 26Mb duplication of 4q35-q37.3 region of chromosome 2 and a 6.3Mb deletion of 4q35.1-q35.2 region of chromosome 4. The 2q3 duplication and 4q3 deletion are two distinct conditions with variable phenotypes including developmental delay, intellectual disability, Pierre-Robin sequence and cardiovascular, craniofacial, digital and skeletal anomalies.

Both the patients showed developmental delay, minor facial and non-facial anomalies, hearing, ocular and genitourinary problems. In particular, proband-1 showed a severe bilateral hypoaacusia and hypergonadotropic hypogonadism secondary to bilateral orchectomcy for testicular seminoma. Proband-2 displayed principally ocular (microphthalmia, coloboma and visual loss) and urinary problems (nephrotic syndrome). The clinical phenotypes were similar to that reported by Rachidi-Nezhad, who first described a patient with a combination of 2q duplication–4q deletion, and to those reported in other cases of 2q3 duplication or 4q3 deletion. Our study contributes to further delineate the genotype-phenotype correlation and the combined effect of partial 2q duplication and 4q deletion syndromes in adulthood.
P11.056-M
A novel micro-deletion 7p14.3 associated with complex neuropsychiatric phenotypes and distinct Cardiac malformations
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Oxidative stress plays a major role in the pathogenesis of leukemia-prone di- seases such as Fanconi anemia and Down syndrome. Objectives: To explore the oxidative stress state in children with Down syndrome and Fanconi anemia, and to evaluate the effect of antioxidants on the oxidative stress, antioxidant capacity and superoxide dismutase (SOD) activity and DNA damage, and to evaluate of the effect of antioxidant treatment on these patients. Methods: The study included 32 children clinically diagnosed with Down syndrome (15 patients) and Fanconi anemia (17 patients) in addition to 17 controls matched for age and sex. Malondialdehyde, total antioxidant capacity, superoxide dismutase (SOD) activity and DNA damage were measured. Antioxidants including vitamin A, E and C were given to the patients according to the recommended daily allowance (RDA) for 6 months. Clinical follow-up and re-evaluation were conducted for all patients. Laboratory tests including complete blood count, karyotyping, DNA damage and oxidative stress were re-evaluated. Results: Children with Fanconi Anemia and Down syndrome had elevated levels of oxidative stress and more DNA damage than their controls. Oxidative stress parameters and DNA damage improved in Fanconi anemia and Down syndrome patients after antioxidant intervention. Conclusion: Early administration of antioxidants to Fanconi anemia and Down syndrome patients is recommended for slowing of the disease course with symptoms amelioration and improvement of general health.

P11.057-S
A further case of de novo 10p14.14-pter deletion detected by multiplex ligation-dependent probe amplification (MLPA) assay in a newborn
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A further case of de novo 10p14.14-pter deletion detected by multiplex ligation-dependent probe amplification (MLPA) assay in a newborn
M. R. Piemontese, A. I. Croce, L. Bisciglia, P. Fasintalà, L. Zelante;
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Chromosomal microarray analysis revealed a de novo deletion at 7p14.3p14.2 (chr7:33,453,822-36,924,450), confirmed by fluorescence in situ hybridization. The genes in the deleted segment include BBAP, BMPER, NPSR1, DPY19L1, TXB2, HERPUD2, SEPT7, EEPD1, KIAA0895, ANLN, AOAH and ELM1. BMPER mutations are thought to be associated with craniofacial dysmorphism. TXB2 is a transcription factor involved in the formation of cardiac chambers and valves. Other components within this 7p14.3 deletion with features of cardiac malformations and learning disabilities may also be implicated and will be discussed. By sharing these novel findings we hope to aid other healthcare providers in the care and management of similar patients.

P11.058-M
Distal 10q26.3 monosomy; three new cases and review of the literature
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Distal 10q26.3 monosomy is a rare cytogenetic abnormality; the location and size of the deletions described in this region are variable. The associated phenotype associated with this deletion is also variable but reported features include developmental delay/learning disability/mild to moderate intellectual disability, speech and language delay, poor attention, strabismus and distinctive facial features. We report two siblings; a male aged 15 and a female aged 21 years, with intellectual disabil- ity, microcephaly, motor impairment and ataxia. MRI showed mild dilu- tion of the lateral ventricles and a prominent cisterna magna. Karyotyping was normal. We performed whole-exome sequencing (WES) and they were found to have a 5.5 Mb terminal chromosome 10q26.3 deletion using the program FishingCNV. The deletion was confirmed by FISH and the mother was found to carry a pericentric inversion. A third child, aged 6 years, presented in early childhood with global developmental delay, poor coordination and ataxia. MRI showed no abnormality of the posterior fossa. A postnatal karyo- lysis (105 K) comparative genomic hybridization microarray identified a deletion of the terminal 4.6 Mb of the long arm of chromosome 10, within cytogenetic band 10q26.3. This finding was confirmed by FISH analysis. The parents were tested and this is a de novo change. Our findings add three new cases of 10q26.3 monosomy to the literature; we summarize the previous cases and highlight the features of this emerging cytogenetic syndrome.

P11.059-S
Oxidative stress a Phenotypic Hallmark of Fanconi anemia and Down syndrome: The effect of antioxidants
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We report a 20 year old male with global developmental delay and a novel microarray finding. His past medical history included a diagnosis of supra- vavular mitral ring moderate endocardial fibroelastosis at age two, follow- ing recurrent pneumonia and congestive heart failure. His neurocogni- tive history, in addition to global delay, also included a diagnosis of autism, attention deficit disorder and bipolar disorder with episodes of psychoses that required admission. Other medical issues included moderate bilateral hearing loss, esoinophlic esophagitis and sleep apnea. He had minor dys- morphic cranio-facial features, a narrow palate and a bifid uvula, as well as hypoplastic nails. Psoriasis and striae were noted on skin exam. Chromosomal microarray analysis revealed a de novo deletion at 7p14.3p14.2 (chr7:33,453,822-36,924,450), confirmed by fluorescence in situ hybridization. The genes in the deleted segment include BBAP, BMPER, NPSR1, DPY19L1, TXB2, HERPUD2, SEPT7, EEPD1, KIAA0895, ANLN, AOAH and ELM1. BMPER mutations are thought to be associated with craniofacial dysmorphism. TXB2 is a transcription factor involved in the formation of cardiac chambers and valves. Other components within this 7p14.3 deletion with features of cardiac malformations and learning disabilities may also be implicated and will be discussed. By sharing these novel findings we hope to aid other healthcare providers in the care and management of similar patients.

P11.060-M
Report of the first case of robertsonian translocation in Down-Turner mosaicism (mos 45, X / 46.XX, + 21, rob [21;21](q10;q10))
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A further case of de novo 10p14.14-pter deletion detected by multiplex ligation-dependent probe amplification (MLPA) assay in a newborn
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Double aneuploidy involving both autosomal and sex chromosomes is very rare. Down’s syndrome occurs in about 1 in 2 000 000. We report the first case of Down’s/Turner’s mosaic with Robertsonian translocation. The patient was the first child of non consanguineous parents. It was a fe- male whom born a term, by uneventful cesarean section, weighting 2,220g and with length of 46 cm, without neonatal complications. At birth, the pe- diatrician made the diagnosis of Down syndrome. At 3 months, in consulta- tion with routine pediatric a heart murmur was heard, so the patient was referred to a cardiologist. There were performed two surgeries to repair the heart defect, one with 6 months of life and another with 2 years. She also had mild developmental delay. At 3 years old she was examined at our outpatient Genetic unit. The patient showed more clinical findings of Down syndrome than Turner syndrome: low weight and height for age, microce- phaly, flat facial profile, upslanting palpebral fissures, epicanthal folds, short nose with depressed nasal bridge, hypotonia with tendency to keep mouth open and protrude the tongue, short neck, single palmar creases, and pro- minent ears. Cytogenetic analysis of peripheral blood preparations using G-banding revealed mosaicism with 2 cell lines (mos 45, X [21] / 46.XX, + 21, rob [21;21](q10;q10))[9]. Additional genetic studies (karyotypes) were made to define the cause which probably originated this double aneuploidy with this translocation. So we present the first case related of Down-Turner mosaicism with Robertsonian translocation and we review all the previous reports.

P11.061-S
Trying to define the phenotype of 16p12.2-p11.2 duplication syndrome
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The short arm of chromosome 16 is rich in segmental duplications render- ing this region susceptible to rearrangement through non-allelic homologous recombination. Several syndromes resulting from microdeletions or microduplications in this region have been reported. The chromosome 16p12.2-p11.2 deletion syndrome, 7.1-to 8.7-Mb [OMIM#613604] is charac- terized phenotypically by dysmorphic facial features, feeding difficulties, recurrent ear infections, developmental delay and cognitive impairment. Reciprocal duplication of 16p12.2-p11.2 has been observed in few patients with dysmorphic features, short stature, developmental delay and intellec- tual disability but a specific phenotype hasn’t been established and more cases are needed. We report two new unrelated cases of chromosome 16p12.2-p11.2 dupli-
culation analysed by 180K oligonucleotide array CGH (Agilent Technologies). Both children showed a de novo 7.8 Mb duplication extending from 21.5 Mb to 29.3 Mb, comprising the same region involved in the deletion syndrome. We discuss the phenotype and molecular findings of our cases with respect to previous reported ones to further define the syndrome.

P11.062-M
Redefining the contiguous gene syndrome in the era of high-throughput sequencing

We ascertained an Algerian consanguineous family in which two sibs present with psychomotor delay, progressive microcephaly, spasticity, thin corpus callosum, and severe and early onset obesity. Exome sequencing identified two homozygous substitutions cosegregating with the phenotype and locating 170 kb apart on 7q22.1: a c.1137+1G>T splice mutation in AP4M1 previously described in a Moroccan family and a c.595A>T missense variation in AZGP1 which encodes zinc-alpha2-glycoprotein (ZAG). Haplotype analysis indicated that the AP4M1 mutation was a founder mutation shared between the two families, whereas the AZGP1 mutation is secondarily and unique in our family.

Mutations in AP4M1 cause AP4-deficiency syndrome, a condition characterized by severe intellectual disability, progressive microcephaly and spasticity. Notably, none of the 25 previously reported cases with AP4-deficiency syndrome exhibited obesity. On the other hand, ZAG is an adipokine stimulating lipolysis in adipocytes; ZAG likely regulates body weight since adipose tissue from ZAG null mice is hypotrophic.

Since it will be interesting to determine whether there is a correlation between obesity and the AP4M1 mutation, we have been evaluated a series of different aspects at both clinical and cellular levels in order to provide insights into potential residual activities of altered but expressed proteins.

P11.063-S
Insight into genetic heterogeneity of complex diseases by exome sequencing
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Exome sequencing has become a successful strategy for genetic diagnosis, particularly for largely heterogeneous diseases. We report 4 cases of example, presenting complex diseases, for which it was possible to reach rapidly a correct diagnosis by exome sequencing.

Patient 1 was a one month-old female presenting dilated aortic root, external arteriolar tortuosity, mild dysmorphisms and several musculoskeletal features (joint laxity, arachnodactyly and pectus excavatum). Patient 2 (female, 3 years-old) presented absent pulmonary valve, ectasia of the pulmonary trunk and aorta, motor developmental delay, joint laxity, strabismus, hypermetropia and mental retardation. Patient 3 was a 3 years-old male referred for short-limbed dwarfism, generalized hypotonia, clubfeet and tricuspid regurgitation. Finally, Patient 4 (female, 3 years-old) presented Marfanoid habitus, with aortic dilation. Because these features overlap with several neonatal rare disorders, we performed on patients’ (and parents’) genomic DNA, Illumina TruSeq Exome sequencing, generating a mean target coverage of 99.6% at >20X and a mean read depth of 257X. In Patient 1 we identified a homozygous mutation in fibulin-4 gene, associated with autosomal recessive cutis laxa syndrome. Sequencing of Patient 2 surprisingly revealed a de novo missense mutation in ITPR1 gene, which causes congenital non-progressive spinocerebellar ataxia, resulting in altered development of cerebellum. Patient 3 and 4 carry de novo mutations in genes that cause Loeys-Dietz syndrome, TGFBR1 and TGFBR2, respectively. In conclusion, these examples are suggestive for stressing that exome sequencing represents a helpful approach for differential diagnosis and for more closely specified therapeutic approach for individual patients.

P11.064-M
Stable expression of mutant FANCA: is there any correlation with mild Fanconi anemia clinical?
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Fanconi anemia (FA) is an inherited disease characterized by congenital malformations, punctocenia, cancer predisposition, and sensitivity to cross-linking agents. The molecular diagnosis of FA is relatively complex due to several aspects including genetic heterogeneity with mutations in at least 16 different genes. In this study, we report the mutations identified in 100 unrelated FA probands enrolled into the National Network of the Italian Association of Pediatric Hematology and Oncology. We identified 108 distinct variants of FANCA, FANCG, FANCC, FANCD2, and FANCI genes in 85, 9, 3, 2, and 1 families, respectively. Particularly, in FANCA we found mainly private mutations of all different categories (large intragenic deletions, frameshift, splicing anomalies). Expression of the human FANCA protein was studied in 23 lymphoblastoid cell lines of complementation group FA-A and a correlation between the type of mutation and the expression level of FANCA was observed. In case of nonsense or frameshift mutations FANCA is not detectable whereas it is expressed at the same levels as in controls when alleles are hit by missense or in frame mutations.

The Fanconi anemia clinical? idea is to provide insights into potential residual activities of altered but expressed proteins.

P11.065-S
A familial case of Fanconi anemia-related VACTERL-H association due to a mutation of the FANCF gene, identified using a next generation sequencing approach
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VACTERL-H association is a rare and etiologically heterogeneous condition characterized by a variable combination of many birth defects. It can represent a severe phenotype of Fanconi anemia (FA), a rare disease characterized by birth defects, bone marrow failure, cancer predispositions and increased chromosomal instability due to mutations in at least 16 genes. The genetic heterogeneity together with the wide spectrum of mutations makes the molecular genetic testing in FA a complex and tiered task, which could benefit from application of next generation sequencing strategies (NGS). We describe a family with 3 aborted fetuses affected with hydrocephalia and radial ray defects, variously associated with cardiac, renal and other skeletal anomalies. Both healthy parents originate from the same alpine valley. A genetic counseling leading to a presumptive diagnosis of FA-related VACTERL-H was performed only after the second termination. Thus, a FA diagnostic dye-oxebutane test could be performed only on cultured cells of the third fetus. The results confirmed the suspicion and complementation analysis excluded mutations in the two more frequent groups (FA-A and FA-G). We hence used the Ion PGM™ System and identified a homozygous c.484485delCT mutation of FANCF gene. The same finding was found in DNA extracted from the first two fetuses. Only 1% of FA patients have mutations in FANC and, to the best of our knowledge, the 3 fetuses represent the first cases of VACTERL-H association caused by mutations of FANCF. Application of NGS is suitable for a comprehensive molecular screening of FA and identification of rare disease-causing genes.

P11.066-M
Unique frontonasal dysplasia case with anencephaly with possible link to a novel gene
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Frontonasal dysplasia is a well-known developmental abnormality of the anterior neurocranium and viscerocranium, characterized by findings including the cranial facial midline, such as anterior cranial bifidus, hyperelorism, and clefting of the alae nasi. While the genetic etiology for a number of different syndromes described within the FND spectrum is identified, no gene is known to cause classical FND. Recently, patients with autosomal recessive FND were linked to homoyzogous loss-of-function mutations in the ALX homeobox gene family. Biallelic mutations in ALX1 are associated with a
Goldenhar Syndrome and the involvement of these microrearrangements as uniformly sized and regularly spaced. The clinical features are indicative of cornea to be transparent. Decorin ensures that these collagen fibrils are play an important role in the cornea, which is the clear outer covering of extracellular matrix, involved in the organization of collagens. Collagens matrix. The protein encoded by DCN gene is Decorine, a component of the that is involved in corneal transparency. It may be important in developing DCN. The protein encoded by KERA gene is a Keratan Sulfate Proteoglycan -kage and array-CGH analysis have detected several candidate loci for this families, some familial cases suggest that GS might have a genetic basis. Lin-

Although most affected individuals are isolated cases in otherwise normal

P11.067-S
A thorough analysis in Goldenhar syndrome
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Goldenhar syndrome (GS) is a developmental disorder involving first and second pharyngeal arches during blastogenesis. It is characterized by craniofacial anomalies (oculoauriculo-vertebral (OAV) dysplasia, hemifacial microsomia, facioauriculo-vertebral sequence), epibulbar tumours, ear mal-

We report a unique FND case, a 20-week-old male fetus terminated due to hearing loss, iris and chorioretinal coloboma in the right eye, dermoid cyst in the left eye, two preauricular tags on the left ear and one tag on the left

In the limited number of ALX1-related FND patients, neural tube closure

We report a case of a 10 months boy. During pregnancy, the amniocentesis

We report a single patient with congenital heart disease and a normal

P11.068-M
A case of Goldenhar syndrome and microarrangements of chromosome 12
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We report a case with a Xq26.2q26.3 microduplication. The boy was born at 41 weeks after

HPRT1, PLAC1 genes and the first exon of the GPC3 gene. The result from the tion of Xq26.2q26.3, encompassing the whole coding sequence of the PHF6, HPRT1, PLAC1 genes and the first exon of the GPC3 gene. The result from the -tive nasolacrimal structures, teeth anomalies, mycrocytic hypochromic anemia and mild intellectual deficit may also be present.

P11.071-S
Three new patients with Hamamy syndrome: expanding the phenotype
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Hamamy syndrome is a very rare, autosomal recessively inherited mal-
formation syndrome characterized by craniofacial findings, bone fragility,

Goltz Syndrome (MIM 305600) is a rare genetic disorder characterized by distinctive skin abnormalities and a range of defects affecting the eyes, teeth, limbs, skeletal, urinary, gastrointestinal, cardiovascular and central nervous system. It is inherited in an X-linked dominant mode with lethality in ma-

Goltz syndrome (GS) is a developmental disorder involving first and second branchial arches. Although most affected individuals are isolated cases in otherwise normal families, some familial cases suggest that GS might have a genetic basis. Lin-

P11.070-M
Xq26.2q26.3 microduplication in a boy with developmental delay, distinct facial appearance and genitourinary abnormalities
K. Wizel1, N. Teram1, A. Lederer2, P. Peterlit1;
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Microduplications involving the long arm of chromosome X are rare and the phenotype consequences of functional disomy of X-chromosome ge-

We report on a 3-year-old boy with a Xq26.2q26.3 microduplication. The boy was born at 41 weeks after an uneventful pregnancy, with birth weight 2580 g (<1st centile), length 48 cm (3rd centile), head circumference (HC) 36 cm (75th centile). He sat at 13 months and walked at 18 months. At the age of 4.5 years he spoke no words and showed some autistic-like behaviour. He had normal growth pa-

Unusual presentation of Goltz syndrome with minimal ectodermal involvement in a 3-year-old Iranian girl
A. Rajaei1, A. Rajaei1;
1BagHD genetics center, Tehran, Islamic Republic of Iran.

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We report a unique FND case, a 20-week-old male fetus terminated due to hearing loss, iris and chorioretinal coloboma in the right eye, dermoid cyst in the left eye, two preauricular tags on the left ear and one tag on the left

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The syndrome was clinically described as a new syndrome by Hamamy et al. in 2007, in two brothers born to double first cousins in Jordanian-Arabic origin. Identification of two further patients from a Turkish family led to the localization of the causative gene to 16q12.2-q21, using homozygosity mapping. By locus re-sequencing, two different homozygous sense mutations were identified in the IRX5 gene. A member of the Imr5q family of transcription factors, IRX5 plays an active role in face, heart, blood, brain, bone and gonad development.

We report here a clinical and molecular evaluation of three new patients carrying two novel homozygous mutations in IRX5, along with previously unreported clinical findings, including acute myeloid leukemia with maturation. The phenotypic and molecular features of the Hamamy syndrome patients will be reviewed to further delineate the clinical and mutational spectrum.

P11.072-M

Novel de novo heterozygous FGFR1 mutation in two siblings with Hartsfield syndrome: suggesting gonadal mosaicism

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We describe two siblings with Hartsfield syndrome (association of holoprosencephaly, exodontactyly, cleft lip and palate) and a novel de novo FGFR1 mutation suggesting gonadal mosaicism. The proband presented at age 6 years for genetic evaluation. He was a product of a non-consanguineous union. Multiple congenital anomalies were detected at birth. His phenotype was consistent with Hartsfield syndrome. Genetic evaluation identified a homozygous single base pair change (c.1880G>C; p.R627T) in FGFR1. This variant affects a highly evolutionarily conserved area of the gene, replacing arginine with theorine. Online prediction programs suggest this variant is pathogenic. Sequencing of FGFR1 revealed the identical variant. We report a novel heterozygous FGFR1 mutation in Hartsfield syndrome in two siblings, making this as the first case of familial recurrence of this rare syndrome. Both parents were negative for the sequence variant in FGFR1, thus suggesting gonadal mosaicism. This report also expands the phenotypic spectrum associated with loss of function mutations in FGFR1 to include Hartsfield syndrome and confirms autosomal dominant inheritance of this condition.

P11.073-S

Clinical and molecular dissection of two novel cases of hemihyperplasia

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1Mendel Laboratory, IRCCS Casa Sollievo della Sofferenza, San Giovanni Rotondo, Italy; 2Division of Medical Genetics, Department of Experimental Medicine, Sapienza University, Rome, Italy; 3Pediatric unit, Bellcolle Hospital, AUSL Viterbo, Viterbo, Italy. Hemihyperplasia (HH) term describes an asymmetric body overgrowth, generally due to an increased or unregulated cell proliferation, which can involve one or both sides of the body, a single limb or a half of the face. There is clinical overlap between HH and Beckwith-Wiedemann syndrome. Both conditions have been associated with molecular abnormalities of the imprinted cluster of genes at 11p15. We report on two pediatric patients with typical features of asymmetric body overgrowth. The first subject is an 8-month-old baby having only one body side manifesting HH and left asymmetric microdiplegia due to holoprosencephaly, exodontactyly of bilateral hand and feet and bilateral cleft lip and palate. Previous genetic evaluation included normal karyotype, oligonucleotide array and single gene testing for non-syndromic holoprosencephaly (SHH, SX3, ZIC2, TGIF). At the age of 6 years, exome sequencing was performed on the patient at BCM, Houston, TX and identified a novel de novo variant identified in FGFR1 (coding for fibroblast growth factor-1) on chromosome 8p12:c.1880G>C (p.R627T). This variant affects a highly evolutionarily conserved area of the gene, replacing arginine with theorine. Online prediction programs suggest this variant is pathogenic. Sequencing of FGFR1 revealed the identical variant. We report a novel heterozygous FGFR1 mutation in Hartsfield syndrome in two siblings, making this as the first case of familial recurrence of this rare syndrome. Both parents were negative for the sequence variant in FGFR1, thus suggesting gonadal mosaicism. This report also expands the phenotypic spectrum associated with loss of function mutations in FGFR1 gene to include Hartsfield syndrome and confirms autosomal dominant inheritance of this condition.

P11.074-M

Hepatoblastoma and severe neurodevelopmental phenotype in neurofibromatosis type I: a case report and review of the literature

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A male patient presented with severe neonatal hypotonia including bulbar muscle involvement, necessitating tracheotomy. Severe feeding difficulties and gastro-oesophageal reflux led to gastrostomy placement. Hepatoblastoma was diagnosed at 5 months of age due to a mass on imaging. A targeted clinical and targeted genetic evaluation revealed features suggestive of a Ras-MAPK pathway disorder: relative macrocephaly, piosis, downsloping palpebral fissures, curly hair, thickened ear helices, short stature and moderate to severe developmental delay. Cardio-facio-cutaneous (CFC) syndrome was suggested as the most likely diagnosis, due to the severity of developmental delay and one previous report of hepatoblastoma in CFC syndrome. Genetic testing of exons of BRAF, KRAS, MAP2K1 and MAP2K2 was normal, as was testing of HRAS and Noonan syndrome associated genes. Features of neurofibromatosis type I developed in later childhood: skinfold freckling and café au lait patches were present, and a pleomorphic fibroblastoma was identified in his left arm at 10 years of age. The SureSelect 50Mb exome enrichment kit and Illumina HiSeq were used to reveal a de novo 4 basepair deletion in NF1, with no other known pathogenic variants being identified in this analysis.

The degree of neurodevelopmental delay in this patient was highly atypical for NF1. There has been only one previous report of hepatoblastoma in NF1, suggesting that this too is a rare association. These two highly atypical phenotypic aspects led to difficulties in making the diagnosis. This patient's presentation emphasises the related nature of Ras-MAPK pathway disorders and the potential of massively parallel sequencing for effective diagnosis of these conditions.

P11.075-S

13q14.2 duplication in a patient with alobar HPE phenotype associated with large ears

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Holoencephalophagy is a common developmental defect; affecting both the forebrain and the face presenting a high mortality rate with only the minority of patients surviving after 1 year. The etiology of HPE is complex; with both environmental and genetic factors being implicated. Here we presented a girl with a classic alobar HPE phenotype associated with large ears. Investigation of copy number changes was performed by array-CGH using the whole genome Cytosure12, ISCA V2 array 4X180K (Oxford Gene Technology, OGT, UK) containing ~180.000 oligonucleotides. Result showed ~14Kb duplication at 13q14.2 (47.948.518-47.963.274pb) with partial enrichment kit and Illumina HiSeq were used to reveal a 4 basepair duplication of RB1 gene (based on UCSC Genome Bioinformatics, Hg18, http://genome.ucsc.edu). Reports on partial 13q duplication are unusual and the phenotype resulting is very heterogeneous. Duplication of the 13q distal chromosomal region has been also reported in a fetus that showed dysmorphic features such as postaxial polydactyly of the right hand and left foot with short fingers, malformation of the gut, and a microopenis with hypospadias. Cerebellar hypoplasia had been noticed at ultrasound examination in the 14-week of gestation. The main purpose of the present report is to stress the importance of array CGH in patients with midline defects, since it represents a paramount point concerning genetic counseling and management to families.

P11.076-M

Hol-Torn syndrome: new TBX5 exonic deletion leading to extreme variability among affected family members

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P11.079-S Moderate intellectual disability, speech delay, strabismus, pseudo-Hirschsprung disease and mild abnormalities of extremities in a girl with a 2q4.3q31.2 duplication.A new syndrome?
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We report on a 15-year-old girl presenting with moderate intellectual disability, delays in speech and language acquisition, strabismus and severe chronic constipation looking like Hirschsprung disease in spite of the presence of ganglion cells. Her parents are not consanguineous and in good health but her paternal half-brother presents speech difficulties and moderate intellectual disability. Conventional chromosome analysis was considered as normal, but array CGH showed a 10.2 Mb interstitial duplication of the 2q4.24q31.2 region. In situ hybridization of paternal metaphases revealed a direct intrachromosomal insertion of the long segment of chromosome 2 at band q32.3 between bands 2q4.3 and 2q3.12. The duplication observed in the patient results from an abnormal meiotic recombination of the father’s insertion. Given its risk of recurrence for another child, an abnormality of the same chromosomal region has now to be searched in the girl’s half-brother. The function of several duplicated genes can explain the phenotype of the patient. As it is, to our knowledge, the first 2q4.3q31.2 duplication reported, additional patients are needed to improve the description of the phenotype.

P11.080-M Comprehensive sequencing of all known Joubert genes in a large Joubert syndrome cohort in search of oligogenicity
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Joubert syndrome (JS) is a ciliopathy characterized by a distinctive hind-brain malformation, ataxia and cognitive dysfunction. It is typically inherited in an autosomal recessive manner caused by biallelic mutations in one of >20 genes, but despite the large number of causal genes, the underlying cause remains unknown in more than one third of affected individuals. Affected individuals carrying multiple heterozygous rare deleterious variants (RDVs) in known JS-associated genes raise the possibility of more complex genetics. To search for evidence of oligogenicity (defined as disease causation through combined effects of two or more heterozygous RDVs) and genetic modifiers, we used a novel molecular inversion probe-based (MIP) capture technology to sequence all known JS-associated genes in a large JS-cohort. Using a recessive model where biallelic RDVs in any of the known JS-associated genes were considered causal, we were able to determine the cause in less than two thirds of our subjects. In the remaining subjects, a small number carried heterozygous RDVs in two or more JS-associated genes, and this proportion was not significantly different from that observed in a control population. Moreover, Sanger sequencing identified a second RDV in one patient that had been missed by MIP sequencing in the study cohort, thereby establishing a recessive cause in these individuals. Thus, our data do not support the hypothesis that a significant proportion of JS cases are due to oligogenicity involving RDVs in the known JS genes. Current analyses focus on determining whether genetic burden correlates with disease severity.

P11.078-M Diamond-Blackfan anemia and intellectual disability: a new contiguous gene syndrome at 15q25.2
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15q25.2 microdeletion is an emergent GW locus for intellectual disability, dysmorphic features and congenital anomalies. Two distinct microdeletions have been described at this locus: 1) a distal deletion (11 cases) responsible for neurodevelopmental and neuropsychiatric disorders and 2) a proximal deletion (8 cases) which is a susceptibility locus for cognitive deficit, diaphragmatic hernia and Diamond-Blackfan anemia (DBA). This proximal deletion is said to predispose to DBA because it contains the gene RPS17, encoding for a ribosomal protein, responsible for 2% of DBA. Until now, however, DBA has been diagnosed with certainty in only one case of 15q25 proximal deletion. The additional case reported here had a history of intrauterine growth retardation. Aged 18 months, the patient had a moderate developmental delay, dysmorphic features and musculoskeletal anomalies. He had a normochromic macrocytic aregenerative anemia with elevated erythrocyte adenosine deaminase activity and elevated HbF (3.2%) highly suggestive of DBA. A 15q25.2 microdeletion of 2.2Mb including RPS17 was identified using SNP array. The deletion of RPS17 was confirmed by FISH using a specific probe. The deletion was absent in the patient’s father, and was impossible to test in his mother. To date, only a few mutations in RPS17 have been reported in patients with DBA. Anemia was mentioned in 4 cases among the 8 previous reported cases of 15q25.2 proximal deletion but the definite diagnosis of DBA was made in only 1 case. The present report confirms that patients with 15q25.2 deletion involving RPS17 are at risk of DBA and possibly DBA-associated malignancies.
Mutations in the MKS1 gene are known to be a major cause for Meckel-Gru- ber-Syndrome, a genetically heterogeneous condition and the most common form of syndromic neural tube defect. The MKS1 gene also accounts for a minor fraction of the total mutational load in Bardet-Biedl-Syndrome. We report the phenotype of a five year old boy from Austria with episodes of apnoe during the first 7 months of life, severe hypotonia, psychomotor retardation, congenital nystagmus and a molar tooth sign detected in the MRI of the brain, which suggested the diagnosis of Joubert syndrome.

Next-Generation sequencing based multi-gene-panel diagnostics from a blood sample revealed two mutations located in the MKS1 gene: The first mutation, c.1407-7_1408.35del29, is known to be a major cause for Meckel-Gruber-Syndrome in homozygous state. The second mutation is a missense-mutation that has not yet been reported. Five bioinformatic tools predict an alteration of protein function caused by the mutation. We therefore assume that these two mutations in the MKS1 gene in compound heterozygous state are causative for the phenotype.

Mutations in the MKS1 gene are primarily reported in patients with Meckel-Gruber-Syndrome, a phenotype which denotes the most severe (usually lethal) end of the spectrum of ciliopathies with occipital encephalocele and other early embryonic malformations. Here we describe a patient with classical Joubert syndrome which underlines the genetic heterogeneity in Joubert syndrome and illustrates the pleiotropy of MKS1 mutations.

Kabuki syndrome: clinical and molecular diagnosis in the first year of Life


Objective: To review the clinical and molecular characteristics of 18 patients presenting a suspected diagnosis of Kabuki Syndrome (KS) in the first year of life, in order to outline the clinical handles leading to a prompt diagnosis of KS in newborns. Clinical diagnosis of KS can be challenging during the first year of life, as many diagnostic features become evident only in subsequent years.

Methods: All patients were clinically investigated by trained clinical geneticists. A literature review was performed using the Pubmed online database and Diagnostic criteria suggested by DYSCERNE, Kabuki Syndrome Guide lines (2010) were used. Molecular analysis of the known causative-genes of KS, MLL2 and KDM6A, was performed through targeted resequencing, using MiSeq® sequencing platform. All mutations identified were validated by Sanger sequencing standard protocols.

Results: Facial dysmorphism (94%), feeding difficulties (100%) and hypotonia (100%) suggested the clinical diagnosis of KS. Notably, long palpebral fissures and large antverted ears were present in 94% and 100% of the cohort, respectively. Other abnormalities such as brachydactyly, joint laxity and nail dysplasia were present in 15 (83%) patients of the KS cohort, respectively. Other abnormalities such as brachydactyly, joint laxity and nail dysplasia were present in 15 (83%) patients. Congenital heart defects (11/18; 61%) and left-sided obstructive lesions (4/18; 22%). Mental retardation, severe learning difficulties and speech disorders were present in all patients (23/34=68%). The patients with KDM6A mutations had normal heart. In conclusion, persons with KS have a specific non-generalized pattern of hypermobility with the small joints of the hands and feet, hips and knees most affected. In contrast with the general population, boys with KS have a higher degree of hypermobility than girls with KS. We present the data in a graphical way. In conclusion, persons with KS have a specific non-generalized pattern of hypermobility with hips, patellae and the small joints of the hands most affected. Furthermore, boys are more severely affected than girls.

Kabuki syndrome (KS; OMIM 147920) is a well-known congenital anomaly/intellectual disability syndrome caused by a mutation in the KMT2D gene (OMIM 602113). Hypermobility has been described as one of the major features of KS. However, no prevalence of hypermobility is known within the population of children with KS. On assessment of KS children in our clinic, we noticed that the degree and the pattern of hypermobility varies greatly amongst patients. Therefore, we aimed to assess the degree and pattern of hypermobility in the KS individuals.

Twenty individuals (age 3-30 years old) with KS and a known KMT2D mutation were assessed. The persons were evaluated using two systems: the Beighton and the Bulbena score. The prevalence of hypermobility in this cohort was 25% using the Beighton score and 45% using the Bulbena score. The difference between these two percentages is due to the items within each system and the pattern of hypermobility in the KS patients. These patients have a non-generalized pattern of hypermobility with the small joints of the hands and feet, hips and knees most affected. In contrast with the general population, boys with KS have a higher degree of hypermobility than girls with KS. We present the data in a graphical way.

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stature. Increased susceptibility to infections, joint laxity, heart, dental and ophthalmological anomalies are common. Hypoglycaemia is more common in KS2 than in KS1. Importantly, diagnosis on facial gestalt alone may be difficult in many patients because the facial dysmorphism with KDM6A mutations is highly variable. Hypertrichosis, long hallucs and large central incisors may be useful clues to an underlying KDM6A mutation in some patients.

P11.086-M
Examining Kabuki syndrome causing mutations in Czech population J. Pocrama, A. Hrubcová, M. Simandlová, A. Puchmajerová, M. Vlková, M. Mašilová, R. Poursova, S. Vejtlavová, M. Havlovcová, M. Senkerriová, P. Nastová, A. Krupelová, M. Macák Jr.; 1Department of Biology and Medical Genetics, Charles University - 2nd Faculty of Medicine and Faculty Hospital Motol, Prague, Czech Republic, 2Department of Medical Genetics, Charles University - Faculty of Medicine and Faculty Hospital Hradec Kralove, Hradec Kralove, Czech Republic.

Kabuki (make-up) syndrome (KS) is an autosomal dominantly disorder caused by de-novo mutations and has an estimated prevalence of 1:32 000 newborns. KS1 is caused by mutations or deletions in KMT2D (lysine/K-specific demethylase 6D) gene. The mutations in KMT2D are detected in 6/14 (43%) of patients. All detected mutations were truncating, thereby leading to A1 p. nonsense mutations. Seven mutations were previously published (c.16371_16374del, c.8743C>T, c.5627_5630del) and three are novel (c.2488C>T, c.4549_4550del, c.6638_6640del). No mutations were detected within the KDM6A, as well as intragenic rearrangements were not found in KMT2D gene (MLPA assay for KDM6A is not available, thus far). Our results substantiated the disease association with KMT2D gene. Since the clinical features of "KMT2D mutation-positive" cases did not differ from those, where aforementioned methods did not detect any DNA alterations, we plan to utilize next generation sequencing in an attempt to identify other loci that are potentially contributing to the genetic heterogeneity in KS.

Supported by CZ.2.16/3.1.00/24020PPP and 00064203.

P11.087-S
A novel mutation in KAT6B in a patient with genitopatellar syndrome and some features of SBBSY5 M. Vícková, M. Simandlová, V. Straneček1, H. Hartmannova, K. Hodanova, M. Havlovcová, Z. Sedlacek2, S. Knocál3; 1Department of Biology and Medical Genetics, Charles University - 2nd Faculty of Medicine and University Hospital Motol, Prague, Czech Republic, 2Institute of Inherited Metabolic Disorders, Charles University 1st Faculty of Medicine and General University Hospital, Prague, Czech Republic.

Genitopatellar syndrome (GPS) and Say-Barber-Biesecker-Young-Simpson syndrome (SBBSY5) are two clinically overlapping syndromes. Recently de novo heterozygous truncating mutations in KAT6B have been identified in SBBSY5, and independently also in GPS. KAT6B encodes lysine acetyltransferase 6B, a part of histone H3 acetyltransferase complex. KAT6B is highly conserved and expressed in adult neural stem cells. Most mutations are in exon 18 encoding the acidic (A) and transcriptional activation (TA) domains of KAT6B. Genotype-phenotype correlation showed that patients with mutations in the 5’ region of exon 18 leading to loss of both domains respectively from GPS while patients with mutations in the 3’ region leading to loss of the TA domain suffer from SBBSY5. We present an 8-year-old girl with intellectual disability, autism and multisystemic features of this syndrome. Kabuki syndrome (KS; MIM 147920) is a congenital anomaly syndrome characterized by developmental delay, intellectual disability, specific facial features including long palpebral fissures and ectropion of the lateral third of the lower eyelids, prominent digi pads, and skeletal and visceral abnormalities. As mutations in KMT2D and KDM6A are known to cause KS, we screened 81 individuals with KS for mutations in these genes by conventional methods (n = 58) and/or targeted resequencing (n = 45) or whole genome sequencing (n = 5). We identified a mutation in KMT2D or KDM6A in 50 (61.7%) and five (6.2%) cases, respectively. Thirty-five KMT2D mutations and two KDM6A mutations were novel. Non-protein truncating-type KMT2D mutations were mainly located around functional domains, while truncating-type mutations were scattered throughout the entire coding region. The facial features of patients in the KMT2D truncating-type mutation group were typical based on those of the ten originally reported patients with Kabuki syndrome; those of the other groups were less typical. High arched

P11.088-M
Familial case of KBG syndrome caused by a novel ANKR61 gene mutation E. A. Boleza-Meiler1, L. Ramos1, S. Maite1, J. Perera2, J. M. Saravia2; 1Medical Genetics Unit, Hospital Pediatrico, Centro Hospitalar e Universitario de Coimbra, Coimbra, Portugal, 2Molecular Hematology Unit, Hospital Pediatrico, Centro Hospitalar e Universitario de Coimbra, Coimbra, Portugal.

Background: KBG syndrome is an autosomal dominant disease characterized by intellectual disability, seizures, short stature, skeletal anomalies and distinct craniofacial features. It is caused by mutations in ANKR61 gene. Methods: We report the case of a 19-year-old woman and her 41-year-old mother presenting with intellectual disability, short stature and short 5th fingers. While the mother had already lost most of her teeth, we observed that her daughter had macroodontia of the upper central incisors. The diagnosis of KBG syndrome was suspected. Hence, we proceeded with mutation screen of the coding region of the ANKR61 gene. Results: ANKR61 gene sequencing revealed a heterozygous pathogenic nonsense mutation, c.1318C>T [p.(Arg440*)], previously not described in the literature.

Conclusion: Our patients showed clinical features of KBG syndrome. The molecular analysis of ANKR61 gene confirmed our clinical hypothesis, which allows a more precise genetic counselling for our patients and their family.

P11.089-S
X chromosome-linked copy number variations in Klínefelter syndrome M. S. Roza1, V. Pecile1, S. Reise1, N. Caretta2, C. Foresta2, A. Ferlini2; 1University of Padova, Padova, Italy, 2IRCCS Burlo Garofolo, Trieste, Italy.

Klinefelter syndrome 47,XXY (KS) is the most common sex-chromosome aneuploidy in men, characterized by at least one supernumerary X chromosome. The wide spectrum of clinical manifestations in KS often varies in severity. The clinical features of KS commonly include hypergonadotropic hypogonadism, gynecomastia, small testes and azoospermia, with a varying degree of androgen deficiency, gynecomastia, cognitive dysfunction, increased central adiposity, increased height with eunuchoid proportions, and increased frequency of diabetes, metabolic syndrome, osteoporosis, autoimmune disorders and psychosocial/behavioural abnormalities. The mechanism by which the supernumerary X-chromosome determines the clinical phenotypes in KS is poorly understood, although genetic background in conjunction with the parental origin of the supernumerary X chromosome and consequently with gene-dosage effects may contribute to the variability of the phenotype and increase risk of certain diseases in KS.

In order to understand the role of X-linked Copy Number Variations (CNVs) in Klinefelter subjects, we recruited 93 patients having non-mosaic KS and 85 healthy controls. We performed SNP array analyses using the Human OmniExpress-12 Bead Chip (Illumina Inc.). By the analysis of the X chromosome, we observed CNVs present-specific not reported in Database of Genomic Variants (DGV). Furthermore the total length of duplications and deletions, and the length of duplications and deletions per patient were significantly different from controls. This is the first study that shows CNVs on X-chromosome in KS patients and represents an important step forward a better understanding of clinical features of this syndrome.

P11.090-M
KMT2D and KDM6A mutations in Kabuki syndrome N. Miyake1, E. Koshimizu2, N. Matsumoto2, N. Niihawa2; 1Yokohama City University, Yokohama, Japan, 2Health Science University of Hokkaido, Hokkaido, Japan.

Kabuki syndrome (KS; MIM 147920) is a congenital anomaly syndrome characterized by developmental delay, intellectual disability, specific facial features including long palpebral fissures and ectropion of the lateral third of the lower eyelids, prominent digit pads, and skeletal and visceral abnormalities. As mutations in KMT2D and KDM6A are known to cause KS, we screened 81 patients with KS for mutations in these genes by conventional methods (n = 58) and/or targeted resequencing (n = 45) or whole genome sequencing (n = 5). We identified a mutation in KMT2D or KDM6A in 50 (61.7%) and five (6.2%) cases, respectively. Thirty-five KMT2D mutations and two KDM6A mutations were novel. Non-protein truncating-type KMT2D mutations were mainly located around functional domains, while truncating-type mutations were scattered throughout the entire coding region. The facial features of patients in the KMT2D truncating-type mutation group were typical based on those of the ten originally reported patients with Kabuki syndrome; those of the other groups were less typical. High arched
P11.091-S
Large cryptic genetic rearrangements with apparently normal karyotypes detected by array-CGH
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Chromosomal abnormalities that result in genomic imbalances, such as numerical or structural changes, are one of the most common causes of congenital and developmental anomalies. Conventional karyotyping with a resolution of at least 550 bands should be able to identify chromosomal aberrations down to 5-10 Mb. In contrast, a-CGH analysis is known to detect sub-kb cryptic imbalances increasing the diagnostic yield to 15-20% in patients with an cognitive impairment and/or multiple congenital anomalies. Here we report a subgroup of patients referred for multiple malformations, developmental delay / cognitive impairment, and an apparent normal standard karyotype. Whole genome array-CGH analysis (60 K, Agilent Technologies) identified six patients with large terminal complex rearrangements, all beyond the threshold of 5Mb (6 to 18 Mb). Results were suggestive for the presence of an unbalanced translocation derivative, confirmed by FISH analysis. Five were inherited from a parent with a balanced translocation, and one was apparently de novo.

Comparison of the karyotype and array-CGH showed that cytogenetic rearrangements were all almost indistinguishable from a normal karyotype, swapping similar band patterns. Only one case was recurrent in an affected brother with the same rearrangement and an identical phenotype. Miscarriages were reported in two families.

In conclusion, large complex rearrangements involving chromosomal regions with similar size and band appearance may be missed by conventional karyotyping and detected by array-CGH to allow a precise chromosomal diagnosis and recurrence risk definition.

P11.092-M
Family cases of Leopard syndrome
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Background. LEOPARD syndrome is a complex disorder characterized by multiple dysmorphic and neurologic features. According to molecular studies, LEOPARD syndrome and Noonan syndrome are caused by different mutations in PTPN11 gene. Authors emphasize diagnosis peculiarities in two relatives with facial dysmorphosis. Methods. Authors presents a 10-year-old boy admitted for airway infection symptoms. Family history: non-consanguineous parents; father and sister with face dysmorphosis. Clinical exam: short stature, im paired nutritional status, axial freckles, widespread café-au-lait spots, face dysmorphosis (hipertelorism, mandibular prognathism, broad nasal root, high arched palate, large and posteriorly rotated ears, down slanted palpebral fissures), webbed neck, skeletal anomalies (thorax anomalies, bilateral wide hallux with exostosis), interdigital webs between hallux and 2nd toe, mental retardation. Results. Blood investigations didn’t reveal anomalies. Cardiac ultrasound exam: no pulmonary stenosis. Differen tial diagnosis includes Noonan syndrome (as webbed neck, short stature), Greig syndrome (due to wide hallux, feet cutaneous sindactyly), type 1 neurofibromatoses (as café-au-lait spots, axillary freckles), Albright syndrome (as skin pigmentation). The patient was evaluated from genetic point of view: normal karyotype. Suspicion for Noonan syndrome has justified DNA sequencing that revealed mutation in PTPN11 gene (c.1430C>T, p.Met466Thr) suggestive for LEOPARD syndrome. Authors also found same mutation for probant’s father. Conclusions. 1. Authors describes two related cases with dysmorphic skull, skeletal anomalies, skin pigmentation, mental disabilities and short stature, justifying further genetic evaluation and revealing rare genetic disorder; 2. Genetic counseling and examination of other family members is important (probant’s sister).

P11.093-S
Phenotype of two patients with mandibulofacial dysostosis with microcephaly (MFDM) associated with esophageal atresia and choanal atresia caused by EFTUD2 mutations
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Mandibulo-facial dysostosis (MFD) causes malar and mandibular hypoplasia, cleft palate and hearing loss. Several distinct MFD syndromes are recognized, one of them is MFD with microcephaly (MFDM), sometimes associated with major defect: choanal atresia (CA), esophageal atresia (EA), kidney and heart defects. We present two unrelated patients with MFDM confirmed by EFTUD2 mutations and associated with EA and CA.

1. Female, 36hbd, birth weight-1720g, EA, cleft palate and hearing loss were diagnosed. Facial dysmorphism includes microcephaly, asymmetric face, hyperplastic supraorbital ridges, broad base of nose, retromicrognatia, microtia, preauricular tags. The patient had gastrostomy and tracheostomy. Psychomotor, somatic and speech development is delayed but social development is correct. CHARGE and Bohring-Opitz syndromes were considered after birth. 2. Female, 37hbd, birth weight-2400g. Respiratory distress due to CA and hearing loss were diagnosed. Facial dysmorphism includes microcephaly, microtia, asymmetry, preauricular tags, hypertelorism, narrow palate, micrognathia. Psychomotor and speech development is delayed but social contact with child is correct. CHARGE and Bohring-Opitz syndromes were considered after birth.

MFDM in both patients was suspected. EFTUD2 gene was sequenced. The mutation c.1435dup was identified in patient 1 and c.1859A>T in patient 2. The full clinical spectrum of MFDM is heterogeneous. Facial phenotype with ear abnormalities and microcephaly of presented patients was distinctive, but observed major defects in neonatal period were considered as leading symptoms and correct diagnosis was delayed. We suggest that MFDM should be taken in the differential diagnosis of child with craniofacial malformations accompanied by EA or CA.

P11.094-M
A new case of MDP syndrome caused by recurrent single-codon deletion in the POLD1 gene
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Progeroid features with concomitant cardiac, skeletal, and muscular anomalies, lipodystrophy and insulin resistance define rare genetic diseases named laminopathies, caused by mutations in genes encoding nuclear proteasome (KMT2D, KDM6A) but in only half of the group the genetic basis of the patients who tested mutation-negative (20-45%) remains elusive. Further studies are necessary to understand the whole picture of the genetic aspects of KS and its genotype-phenotype relationships.

P11.095-S
Loss-of-function mutations in MED13L cause a distinctive clinical phenotype mimicking the 1p36 deletion syndrome
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Exome sequencing (WES) has already been proven to be an effective method for the identification of the causative gene in small groups of subjects clinically selected by sharing homogenous phenotypes. We selected a group of 8 patients with overlapping phenotype, resembling...
the 1p36 deletion syndrome and with normal array-GGH (15Kb resolution). By means of WES in two of them, we found two causative mutations: p.M303K in SYT1 (subject 1) and p.I1255FS*3 in MED13L (subject 2). The missense mutation in SYT1 involves an essential functional domain. By Southern analysis and FISH on both of the genes in the other subjects, we identified another MED13L mutation (p.S2035H*52) in one. All mutations were de novo. Missense mutations involving MED13L have already been identified as responsible for isolated congenital heart defects. One only literature report deals with complete or partial gene deletion, which were also associated to intellectual disability (ID). Mutations in SYT1 have never been described in humans. 

Our three patients presented with moderate ID and shared facial features, including brachycephaly, high forehead, long eyelashes, depressed nasal bridge, mid-face hypoplasia. Additional clinical signs were epilepsy (subject 1), cleft palate, clubfoot and conductive hearing loss (subject 2) and atrial septal defect (subject 3). Patient 2 was diagnosed with acute lymphoblastic leukemia. These results suggest that the haplinsufficiency of MED13L causes a typical phenotype that should be considered in the differential diagnosis of the 1p36 deletion syndrome. We tentatively suggest SYT1 as another candidate gene for the same clinical presentation, but it needs to be further confirmed.

P1.1.096-M An interstitial microdeletion of 20q11.21 in a boy with choilagnotaphalostatosichis, anorectal malformation, severe microcephaly, craniofacial features, feeding difficulty, mild growth impairment, and mild intellectual disability

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Interstitial microdeletions involving 20q11.21 are very rare with only four reported patients. We have identified a de novo interstitial 20q11.2 microdeletion in a 7-year-old boy, clinically showing choilagnotaphalostatosichis, anorectal malformation, severe microcephaly, craniofacial features (triangular face, hypertelorism, hypoplastic alae nasi, long philtrum, low set ears), feeding difficulties, growth disturbances, mild intellectual impairment, mild intellectual disability (IQ 67), and attention-deficit hyperactivity disorder. G-banded chromosomes were normal, cytogenomic microarray (135K Oligo, Roche) revealed a de novo 1.15 ~ Mb microdeletion at 20q11.21 [array (20q11.21) 20q11.21(29,297,619-30,447,117)x1, dn]. The deleted segment encompassed 15 OMIM genes (COX4I2, MYLK2, ASXL1, etc.). The sizes of deleted segments in four reported patients were 2.6Mb, 6.5Mb, 6.6Mb, and 6.8Mb[ourov et al.2013; Hinaki et al.2011; Callier et al.2006;qbal et al.2007;]. Clinical features shared by those with > 6Mb deletion included feeding difficulty, facial features (triangular face, hypertelorism, hypoplastic alae nasi, long philtrum, low set ears), developmental delay and/or intellectual disability, though the other with 2.6Mb had a milder manifestation. The present patient with the smallest deletion showed clinical features of previously reported patients with 20q11.21 microdeletion. Although long-term follow-up and collection of additional patients is needed to delineate the phenotypic spectrum of the condition, we propose the microdeletions at 20q11.21 to be a clinically recognizable syndrome characterized by craniofacial features, feeding difficulty, growth impairment, and intellectual disability.

P1.1.097-S The clinical phenotype of 3q29 microdeletion and 3q29 microduplication syndrome in three female patients

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It has been demonstrated that whole genome scanning technologies (array-GGH) is especially suited to identify chromosome abnormalities in individual with unclear or variable presentations. Application of this technology resulted in the delineation of several previously unrecognized microdeletion/microduplication syndromes such as recently described del3q29 (OMIM 609425; ORPHA56286) and dup3q29 (OMIM 611936; ORPHA251038) syndromes with highly variable clinical phenotypes. The most common features of del3q29 phenotype include mild-to-moderate intellectual deficit and slightly dysmorphic facial features: microcephaly, long and narrow face/asymmetric face, short philtrum, large posteriorly rotated ears, high nasal bridge, crowded/dysplastic teeth, tapered fingers with occasional clinodactyly, and gait ataxia. Clinical features of dup3q29, included micro/acro-cephaly, round face, bulbous nose, short or downslanting palpebral fissures, excessive hand creases, obesity and pes planus. Herein, we report on two unrelated female patients with different phenotypic consequences (high interindividual phenotypic variability) due to de novo deletion of an identical segment [spanning ~2.0Mb (start 195,438,699bp - 197,404,25bp)] and one female patient with de novo duplication of 3q29, which overlap this region [spanning ~1.50Mb (start 195,740,387bp - 197,317,074bp)].
P11.100-M
Molecular diagnostics of rare hereditary diseases using next generation sequencing
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Next generation sequencing (NGS) is a rapidly evolving method for the molecular diagnostics of hereditary genetic disorders, NGS is based on parallel sequencing, capable of reading the sequences of whole genes in a single run. Using this technology, we analysed two causative genes - NF1 (neurofibromatosis) containing 58 exons, and PHKD1 (polycystic kidney disease) with 67 exons. Neurofibromatosis is an autosomal dominant disorder of the nervous system, characterised by café-au-lait macules, cutaneous neurofibromas and Lisch nodules in the iris, affecting 1 in 3-5,000 people. Polycystic kidney disease is an autosomal recessive disorder, characterised by the development of cysts affecting the collecting ducts. The population prevalence is 1.85,000 individuals. Methods: DNA was isolated from peripheral blood and amplicons prepared by PCR were analysed on a GS junior system (Roche). Data were analysed by AWA (Roche) and Sequence Pilot (JSI medical systems) software. Results: We analysed 11 patients for PHKD1, and found the following mutations: Thr363Met, Leu2128X, Ile2353Lyl, Thr363Met, Gly132Arg, Arg92Trp, Gly171Arg, Gln112Ser (not described) and Ser350Arg (with unknown effect). Four patients were negative. Of 5 NF1 patients, three were negative and two patients (who were related) had a deletion of TAACCT in exon 48. Conclusion: NGS is a very useful method for the analysis of large genes with many exons, such as NF1 and PHKD1. These genes can be sequenced in a single run, instead of multiple single reactions. In the future we intend to analyse more genes using NGS, namely COL2A1 (Sticker syndrome) and USH2A (Usher syndrome).

P11.101-S
Unusual RASopathy due to a novel and severe mutation in the NF1 gene
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We report a Caucasian man first seen at age of 25 months, referred with the diagnosis of Cerebral Palsy due to prematurity. Furthermore, he presented severe developmental delay, hypotonia, seizures, failure to thrive, dysplasia of the right optic nerve, and pulmonic stenosis. We also found dolichocephaly, fontanels hypertelorism, large ears with posterior pits, wide neck, mild pectus excavatum, short 4th metacarpals, and minor ridge dysplasia and radial loops in the index and ring third finger. Chromosomal and metabolic tests were normal. Because features of a RASopathy, sequence analysis of the PTPN11, RAF1, SOS1 and KRAS genes was performed, but no mutations were found. At age 21, his older sister was diagnosed with plexiform neurofibromas, requiring amputation of a leg. CT scan of the proband after 22 months showed a deletion of TAACTT in exon 48. Conclusion: NGS is a very useful method for the analysis of large genes with many exons, such as NF1 and PHKD1. These genes can be sequenced in a single run, instead of multiple single reactions. In the future we intend to analyse more genes using NGS, namely COL2A1 (Sticker syndrome) and USH2A (Usher syndrome).

P11.102-S
Molecular analysis in Noonan syndrome patients in a Polish population
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We present a group of 106 Polish RASopathy cases, including 81 unrelated patients of various age (from infancy to adulthood) with 52 different mutations in genes encoding components of the Ras/MAPK signaling pathway. About 65% of 61 unrelated patients with Noonan syndrome (NS), including 4 cases of NS with multiple lentigines (NS-ML), had mutations in PTPN11, 21% in 50S1, 12% in RAF1 and 2% in KRAS. Nearly half of NS cases were family occurrences. In the group of 15 patients with cardiofaciocutaneous syndrome (CFCS) about 80% of all mutations occurred in BRAF, while 20% in MAP2K1 or MAP2K2. The five cases of Costello syndrome had two most common mutations in HRAS. All identified changes were missense, 8 of them being novel substitutions in different Ras/MAPK genes, causative of NS, NS-ML or CFCS phenotype and one RAF1 mutation so far reported only in a patient with t-AML. The aCGH studies of a group of 21 patients with Noonan-like phenotype (including cardiac defects, musculoskeletal and facial abnormalities) and no causative mutation detected in the known Ras/MAPK genes revealed in one patient a 8p23.3-p23.1 deletion and in the other a 12q13.11 deletion. The molecular findings of our study mostly correspond to the data reported worldwide. We presume that our results will contribute to the existing databases of RASopathy patients and enable detailed phenotype-genotype correlations and differential diagnostics among the patients suspected of RASopathies. The research was supported by Projects: NCM UNO-2011/03/N/NI2/00516, MNSW PB 0056/B/P01/2008/35 and POIG.02.01.04-14-059/09.

P11.104-M
A neuronal phenotype characterized in a mouse model for Noonan Syndrome
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Constitutional dysregulation of the Ras-mitogen activated protein kinase (MAPK) signaling pathway can lead to Noonan Syndrome (NS) or similar disorders, the so-called “RASopathies”, which are characterized by an overlapping pattern of physical abnormalities and cognitive impairment. Their molecular basis is an overactive Ras-MAPK signaling pathway caused by gain-of-function mutations. In animal models, it has been shown that mutations in homologous genes can lead to impaired cognitive function and reduced synaptic plasticity. However, the molecular pathogenesis for the intellectual disability still remains unknown. This study was aimed at investigating the consequences of dysregulated Ras-MAPK signaling in neurons of a mouse model for NS expressing the oncogenic allele Ptpn11 D16Y. In the brain, reconfiguration of expression of neuronal genes represents an
important mechanism that underlies persistent activity-induced changes in the brain function in the process of neuronal plasticity. While the Ptpn11-D61Y mutation is usually found to evoke higher levels of phosphorylated ERK (pERK), we found no differences in the nuclear level of pERK compared to wild type and mutant cells under basal conditions. However, neuronal activity-driven induction of nuclear translocation of pERK was affected in the mutant neurons, suggesting a dysregulation of the activity-induced signaling. In line with this finding, we found differences in the size of total recycling synaptic vesicle pools, which is a subject of regulation during homeostatic adaptation in neurons. The lacking response to stimulation found in the Ptpn11-D61Y neurons suggests a deficit in cellular mechanisms underlying usage-dependent neuronal plasticity and might contribute to the intellectual disability found in patients with NS.

P11.105-S
Cryptorchidism and pulmonary stenosis as the most important features in Noonan patients with mutation in PTPN11 in Slovak population
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Introduction: Noonan syndrome (NS) is an autosomal dominant disorder which together with Cardio-facio-cutaneous syndrome (CFC), Costello syndrome (CS) and Noonan-Neurofibromatosis syndrome (NFNS) belongs to a group of genetic syndromes called ‘RASopathies’. These disorders are characterised by occurrence of similar phenotypic features. Among the most common are short stature, specific dysmorphic facial features and congenital heart anomalies. ‘RASopathies’ are caused by mutations in genes resulting in dysregulation of RAS-MAPK signaling pathway. Mutations in PTPN11 account for approximately 50% of all cases of Noonan syndrome.

Aims: The comparison of phenotype features in patients with PTPN11 mutation and without PTPN11 mutation.

Methods: Mutation analysis of PTPN11 gene was performed in 19 Slovak patients with phenotype of NS. It consisted of polymerase chain reaction and direct sequencing of 15 coding exons and exon/intron boundaries of the PTPN11 gene.

Results: We identified PTPN11 mutations in 47% of our patients (N=19) with clinical diagnosis of Noonan Syndrome. Comparing the prevalence of phenotypic features in patients with PTPN11 mutation with prevalence of the same features in patients without PTPN11 mutation, we found the most significant differences in Cryptorchidism (100% vs. 0%), pulmonary stenosis (78% vs. 10%), pterygium colli (0% vs. 70%) and birth length which was short only in 22% of patients with PTPN11 mutation in comparison to 80% in patients without PTPN11 mutation.

Conclusions: Our findings show that cryptorchidism, pulmonary stenosis, pterygium colli and short birth length are the most important features that distinguish patients with mutation in PTPN11 from patients without PTPN11 mutation.

P11.106-M
Noonan syndrome - the usefulness of whole exome sequencing in the identification of known and new causative genes and disease differential diagnosis
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Noonan syndrome (NS) is a rare disorder belonging to the group of RASopathies caused by the germline mutations in genes encoding proteins of RAS/MAPK signaling pathway. The majority (about 50%) of cases is caused by PTPN11 mutations. Also mutations in RAF1 and SOS1 are quite common (up to 17% and 13%, respectively). The aim of the study was the identification of molecular defect responsible for the NS phenotype using whole exome sequencing (WES). Forty two patients with primary clinical diagnosis of Noonan syndrome and excluded mutation in PTPN11, SOS1 and RAF1 genes were included in the study. The WES was performed using Illumina sequencing platform. The WES analysis allowed for the identification of mutation in RASopathies-related genes in 7/42 (16.7%) NS patients. The pSer296 mutation in SHOC2 was identified in 2 patients. The mutations in NF1 (p.Met1035Arg, p.Tyr2556*), BRAF (p.Gln257Arg, p.Thr241Pro) and KRAS (p.Asp153Val) genes were found in 2, 2 and 1 patients, respectively. In two patients, mutations (p.Rho291Val and p.Met90Ile) in the same NS-related gene RIT1 were found and subsequent analysis of follow-up cohort identified p.Gly95Ala mutation in further two patients. In several cases, the primary NS diagnosis was refined to Kabuki syndrome (2 patients, ML2 mutation), Andersen-Tawil syndrome (1 patient, KCN2 mutation) and Aarskog syndrome (1 patient, FG21 mutation). Our results support the usefulness of whole exome sequencing in the identification of known and new mutations related to specific disease as well as in the differential diagnosis. Supported by NCN research projects no. 2011/01/D/NZ5/01347 and 2013/09/B/NZ2/03164.

P11.107-S
Noonan syndrome-like disorder with loose anagen hair (mazzanti syndrome): a new case with neuroblastoma
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Mazzanti syndrome also known as Noonan-like syndrome with loose anagen hair (OMIM #607721) associates facial features resembling Noonan syndrome, cardiac defects, cognitive deficits, reduced growth generally associated with GH deficit, and hair anomalies. It is caused by an invariable mutation of the SHOC2 gene. We report on a subject with molecular confirmed diagnosis of Mazzanti syndrome with neuroblastoma, first suspected at the age of 3 months by abdominal ultrasound.

At first evaluation he showed a round face, blond, thin and sparse hair; slight non-salting palpebral fissures, slightly posterior rotated ears, evident palmar creases, mild generalized hypotonia and dystrophy, especially in his face and legs. Because of severe feeding difficulty and failure to thrive he underwent an abdominal ultrasound and subsequently abdominal MRI, which confirmed the presence of a circumscribed retroperitoneal nodular lesion between inferior cava vein and aortic artery at the level of the liver (2.7 x 1.5 x 2.2 cm), hyperintense in T2 and isointense in T1, with slight enhancement with contrast. The mass was completely removed and the histological analysis documented a neuroblastoma, poorly differentiated with intermediate MKI. Tumoral cells were negative for the MYCN amplification and bone marrow aspirate was N-Myc negative. The patient did not require any subsequent chemotherapy or radiotherapy.

The present finding emphasizes the importance of monitoring these patients to obtain a precarious diagnosis of possible malignancies. The growing evidence of a variably increased cancer risk in apparently all RASopathies demands further studies to substantiate and specify risk figures and tumor spectrum.

P11.108-M
A novo de macroduplication 3q29 in a patient with ocular uveal vertebreal spectrum
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Oculoauriculovertebral spectrum (OAVS; OMIM 164210) is a phenotypically and probably derived from an abnormally developmental of the first and second branchial arches. Main clinical characteristics include defects of the aural, oral, mandibular, and vertebral development. Anomalies of the cardiac, pulmonary, renal, skeletal, and central nervous systems have been described in OAVS. In a 3-year-old male with preauricular pits and tags, unilateral absence of the auditory meatus, dysgenesis of the inner ear and unilateral microphthalmia, clinical features fitting with the oculoauriculovertebral spectrum. Using SNP array analysis we identified a de novo macroduplication of 723Kb on chromosome 3q29, which was absent in 52 OAVS patients and in 80 ethically matched non-OAVS individuals. This de novo macroduplication was proximal to the 3q29 microdeletion syndrome region and reciprocal microduplication. The identified macroduplication encompassed 9 genes including ATP13A3 and XXYL1, which are involved in organogenesis and regulation of Notch pa-
thaw, respectively. The present observation is in accordance with the hypothesis that OAS is a genetically heterogeneous condition, underlying the importance of SNP array analysis in patients with OAS features.

P11.10-S Screening of CD96 and ASXL1 in twelve patients affected with Opitz C or Bohring-Opitz syndromes

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Opitz trigonocephaly (or C syndrome, OTCS) and Bohring-Opitz syndrome (or C-like syndrome, BOS) are two rare genetic disorders which show phenotypic overlap. The genetic bases of these diseases are not well understood. Two genes have previously been associated with OTCS or BOS with a dominant pattern of inheritance. Whereas the CD96 gene has been related to OTCS (one case out of 25) and to BOS (one case in 4 patients analyzed), ASXL1 (Additional Sex Combs-Like 1) has been related to BOS only (around half of the patients). In this study we analyzed CD96 and ASXL1 in a cohort of twelve individuals, including two sibs. Eight of them were diagnosed with OTCS, two displayed a BOS phenotype and another two could not be accurately diagnosed. Exome sequences were available for six patients with OTCS and three couples of parents, in which CD96 and ASXL1 were inspected using bioinformatic tools. For the remaining patients, Sanger sequencing of all exons in these genes was carried out. Detailed scrutiny of the sequences allowed identification of only one potentially pathogenic mutation in one of the patients. The remaining, affected with BOS, we identified a de novo mutation in ASXL1 (c.210dupT). By nature and location within the gene, this insertion resembles those previously described in other BOS patients and we conclude that it may be responsible for the disease. Our results indicate that for eleven out of the twelve patients, the disease (OTCS or BOS) is not caused by mutations in CD96 or ASXL1.

P11.110-M A case of Opitz GBBB syndrome: clinical presentation

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Opitz G/BBB Syndrome is a multiple congenital anomaly disorder affecting midline structures, characterized by variable expressivity of clinical signs. Primarily clinical features are facial anomalies (including ocular hypertelorism, broad nasal bridge and cleft lip and/or palate), laryngotracheoesophageal abnormalities and hypospadias. Imperforate anus and congenital heart defects are also present; patients may also show developmental delay and brain abnormalities. We report the case of a patient born prematurely with cleft lip and palate. Some dysmorphic features were evidenced: hypertelorism, flat nasal bridge and bifid nasal tip, macrostomia. Cystic murmur and mild hypospadias were noted on clinical examination. An echocardiogram defined congenital heart disease (patent ductus arteriosus together with persistent left superior vena cava and coronary sinus). During follow-up neurological examination and EEG were normal, but delay in speech and motor development emerged. MRI examination showed inferior vermis hypoplasia and small pituitary gland. Differential diagnosis among a number of clinical entities was considered, but the hypospadias and the marked hypertelorism oriented towards the diagnosis of Opitz GBBB syndrome. The analysis of the MID1 gene confirmed a partial deletion, spanning from exon 3 to the end of the gene. Opitz Syndrome is genetically heterogeneous presenting with X-linked and autosomal dominant form; the two forms cannot be distinguished on the basis of clinical manifestations. The autosomal dominant form is linked to a still unidentified gene located on a large region of chromosome 2q24.2. The X-linked one is associated with mutations in the MID1 gene located on the short arm of the X chromosome (Xp22.2).

P11.111-S Two cases of Opitz GBBB syndrome

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Opitz GBBB syndrome is a rare congenital syndrome characterized by hypertelorism, hypospadias, cleft lip/palate, laryngotracheoesophageal abnormalities, imperforate anus, developmental delay, and cardiac defects. Opitz GBBB syndrome is genetically heterogeneous, with both X-linked and autosomal dominant forms. X-linked form of Opitz GBBB syndrome is caused by mutations in the MID1 gene (OMIM #300552). We here report two male cases with Opitz GBBB syndrome. One of them was referred with preliminary diagnosis of FND due to hypertelorism at the age of 14 months. On physical examination he showed hypertelorism, notched nares, bifid incisory tooth, pectus excavatus, accessory right nipple, Simian line, bilateral fifth finger clinodactyly, glabrous hypospadias and hypoplastic scrotum. Clinical evaluation prompted the diagnosis of Opitz GBBB syndrome and MID1 sequencing revealed homozygous c.1798dupC [p.His600ArgfsX12]. Since the mother had one of the major signs of the syndrome, widows peak, X-linked inheritance was suspected. Second patient, at the age of three and a half, displayed hypertelorism and penoscrotal hypospadias on physical examination and displayed additional findings: such as sparse hair, telecanthus, depressed and broad nasal bridge, micrognathia, long philtrum, v shaped upper lips, diastatic recti and umbilical hernia. He had a striking resemblance to the first one and his mother also had widows peak. He clinically diagnosed as Opitz GBBB syndrome and MID1 analysis is still pending. We will discuss Opitz GBBB syndrome in view of the literature.

P11.112-M Mutations in a new gene cause a novel overgrowth syndrome with macrocephaly, hypoglycemia, enlarged ventricles, mild/moderate intellectual disability and recurrent inflammatory diseases

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Overgrowth syndromes (OGS) are a group of disorders in which all parameters of growth and physical development are above the mean for age and sex. Partial, localized, or regional OGS are those disorders in which excessive growth is confined to one or a few regions of the body. We carried out a series of 270 families from the Spanish Overgrowth Syndrome Registry with no known overgrowth syndrome. We identified a deletion and three missense mutations in a new gene in six patients from 4 families with overgrowth, macrocephaly, intellectual disability, mild hydrocephaly, hypoglycemia and inflammatory diseases resembling Sjögren syndrome. Our studies of this gene point to disruption of three important signaling pathways as the putative final effectors.

P11.113-S An apparently balanced translocation in a boy with multiple congenital anomalies of the eye

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A 2 year old boy product of the 1st pregnancy was referred for karyotype testing because of multiple congenital anomalies, including bilateral cataracts, diagnosed at birth, hypospadias and right cryptorchidia, hypotonia and failure to thrive. Glaucoma was diagnosed at 3 months of age. MRI showed generalized brain atrophy. At one year of age developmental delay was noted, microcephaly, weight, and head circumference had fallen below the 3rd percentile. There was no family history of similar findings and the parents are first cousins. Karyotype showed an apparently balanced translocation 46,XY,t(3;6)(p25;q15). The karyotype of both parents was normal. Further testing of the breakpoints of the translocation did not find mutations in the translocation site, including a whole genome sequencing. Whole exome sequencing ruled out mutations in known cataract genes, but identified a homozygous mutation in the PAH gene (NM_000277:c.1139C>T, p.(Thr380Met)). This mutation has been reported to cause Hyperphenylalaninemia (HPA) when combined with another deleterious mutation. Homozygosity for this mutation has not been reported previously, to our knowledge. We are confirming this result by Sanger sequencing and analysis of parental samples is ongoing. We are still investigating the link between HPA/PKU and congenital cataracts (and
reviewing variants in other genes in the WES data), but untreated PKU has been clearly linked to microcephaly and learning disabilities.

P11.114-M

New severe learning difficulty syndrome with skeletal features due to de novo germline mutation in the polycomb group ring finger protein 2 (PCGF2) gene

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Two UK patients, each known to a clinical genetics service (Northern Peninsula) were independently recruited to the Deciphering Developmental Disorders (DDD) study. Patient 1 presented with poor weight gain and hypotonia in infancy, and subsequently global developmental delay. He had relative macrocephaly, enlarged cerebral ventricles, and normal myelination on brain MRI at 17 months. At 2½ years a skeletal survey revealed delayed epiphysial ossification, particularly carpal bones, pseudo-epiphyseal dysplasia and mild metaphyseal deformity of the feet, and multiple pigmented naevi. Patient 2 presented at 6 months of age with poor weight gain. Subsequently his development was delayed with a moderate learning disability. He has relative microcephaly; an MRI scan performed at 7 years of age was normal. She developed constitution which was sufficiently severe to require an ACE procedure aged 12 years. She has fine hair; a long face with high arched palate and prognathism, long thin hands and fingers and mild generalised joint hypermobility. She does not have spine or foot abnormalities. Both patients were found to have a de novo p.P65L (c.194C>T) missense variant in exon 4 of the PCGF2 gene (17q12). Previously, PCGF2 had no associated human phenotype. This identical de novo finding in 2 subjects is strong evidence for a new syndrome associated with a novel morbid gene, despite some divergence of clinical features.

P11.116-M

A nonsense mutation in BMP2 causes a syndromic form of Pierre Robin sequence

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Pierre Robin sequence is a disorder of craniofacial development comprising mandibular hypoplasia, cleft palate and glossoptosis. It can be isolated or associated with other malformations in a syndromic form, with a high genetic heterogeneity. Here we report on a 7 years old boy presenting with Pierre Robin sequence, developmental delay, microcephaly, growth retardation, brachyactly, deep palmar flexion creases and supra-ventricular tachycardia. Exome sequencing revealed the heterozygous, nonsense mutation c.460C>T (p.R154X) in exon 2 of BMP2 (Bone morphogenetic protein 2). Microdeletions at 20p12 spanning exon 2 through DDD gene have been clearly linked to microcephaly and learning disabilities. This novel nonsense variant in BMP2 has been found in 2 other patients with similar phenotype including a boy aged 2 years. We suggest that BMP2 causes a new syndromic form of Pierre Robin sequence, associating intellectual disability, microcephaly, growth retardation, brachyactly and risk of heart rhythm disorder.

P11.117-S

Functional validation of a novel germline mutation in the PTK3CA gene in a child without the typical segmental overgrowth

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We report on a 6 year boy with body overgrowth at birth, macrocephaly and motor delay, who is a carrier of a de novo heterozygous mutation in PTK3CA (NM_002818.2:c.3357T>A, p.Ile1121Asn). The mutation appeared germline in peripheral blood, buccal swabs and skin fibroblasts (Sanger sequencing); it has not been observed in published PTK3CA-related segmental overgrowth patients. Phospho(yl)sinositol-3,4,5-triphosphate immune-staining of patient cells and Epidermal Growth Factor-mediated PI3K-AKT-mTOR pathway stimulation proved that p.Ile1121Asn leads to increased PI3K activity. The p.Ile1121Asn change lies adjacent to the p85 (PIK3CA) catalytic subunit of PI3K. Altered stoichiometry within the p85-p110 complex could underlie the hyperactive PI3K-AKT-mTOR signaling in this instance. Further experiments to investigate the function of the p.Ile1121Asn change are currently ongoing.

Interestingly, the phenotype of this child appears unique compared to published PTK3CA-related segmental overgrowth patients. The boy did not show somatic asymmetry, focal overgrowth, capillary malformations, epidermal nevi, or digital abnormalities. His brain MRI at 6 years showed a thick corpus callosum, large cerebellar vermis and possible restricted right posterior perisylvian polymicrogyria but his brain cortex otherwise looked largely normal.

Our observation adds an isolated megalencephaly with mild body overgrowth to the PTK3CA-associated clinical spectrum. The identified novel mutation is among the few known germline PTK3CA mutations. We demonstrate robust and rapid functional assays to enable interrogation of PI3K-activity in this context from patient-derived cells. The constitutional distribution of PTK3CA mutation might be of prognostic advantage regarding somatic overgrowth, cortical malformations and consecutively favorable development. However, this hypothesis needs further assessment.

P11.118-M

A clinical score system as useful tool in selecting subjects with clinical presentation in the spectrum of Pitt-Hopkins syndrome

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Pitt-Hopkins syndrome (PHS) is characterized by ID, typical facial gestalt, and additional features, including breathing abnormalities. The overlapping phenotype of severe ID, epilepsy, and constipation makes it difficult to differentiate the PHS phenotype from that of Angelman (AS), Rett (RTTS), and Mowat-Wilson (MWS) syndrome. However, comprehensive analysis of many patients led us to define a checklist of the most consistent phenotypic manifestations of this condition, and to suggest a clinical score ≥13 as tool for enrolling patients into TCF4 analysis first. We analyzed a total of 176 patients, of whom 22 had a proven mutation in TCF4 (and on average a clinical score of 14-15) and 154 did not. Of them, 53 were selected by means of the same score system, as having a clinical presentation within PHS. These 53 subjects were grouped in two clinical categories: 1) Group A (tot 11), clinical score > 13 and facial phenotype highly consis-
tent with PHTHS; 2) Group 2 (tot 42), clinical score of 10-11; 2A (tot 16): PHTHS-like phenotype that appears homogenous to large extend to other; 2B (tot 11): PHTHS-like phenotype that appear different from 2A, but homogenous as well; 2C (tot 15): with certain clinical heterogeneity. We performed semiquantitative analysis of TCF4 mRNA in 5 subjects in Group A, and found that it was significantly increased in two. Sequencing of the promoter region and UTRs regions is ongoing. Strategies and preliminary results of the diagnostic approach in the remaining patients, including saliva analysis and exome sequencing, are presented.
Array-CGH analysis disclosed a de novo 5.6 Mb microdeletion of Xq22.1-q22.3 chromosomal region. The pattern of X-inactivation was markedly skewed. The microdeleted region includes 53 genes of 3 of which are OMIM morbid: PLP1, RAB40AL and SERPINA7. There have been only two published reports of whole PLP1 gene deletions. PLP1 mutations have been associated with a continuum of neurologic phenotypes from severe forms of Pelizaeus-Merzbacher disease (PMD) to spastic paraparesis. Null alleles tend to associate with milder PMD features in hemizygous males but higher rate of neuronal manifestations in heterozygous females. PLP1 haploinsufficiency could explain at least part of the girl’s neurologic phenotype since it encodes for a primary constituent of myelin in the brain.

P11.120-M
Dissecting the genetic bases of Poland Syndrome by exome sequencing
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Poland syndrome (PS) is a congenital disorder presenting withagenesis/hypoplasia of the pectoralis major muscle variably associated with thoracic and/or upper limb anomalies. Incidence was reported to be 1/30000 births, with higher prevalence in males. Most cases are sporadic. Familial recurrence has been observed but PS genetic etiology remains to be clarified. Since PS involved structures may originate from the same embryonic tissues, genes controlling cell proliferation, migration, and differentiation of these tissues might be involved. Alternatively, one common assumption is that PS may origin from an embryonic vascular insult, indicating genes controlling vessel development as indirectly involved.

We recruited a large cohort of PS patients (more than 250, about 16% familial). Karyotype analysis in 128 patients did not show any relevant alterations. ArrayCGH revealed the presence of chromosome anomalies in 19 out of 119 analyzed patients (10 duplications and 9 deletions); bioinformatic data analysis indicates enrichment in different pathways including those involved in cell-cell adhesion and muscle structure development. We selected 3 sporadic and 6 apparently dominant familial cases for whole-exome sequencing. Patients’ parents, affected and non-affected siblings to a total of 31 subjects were sequenced. Preliminary data from one trio confirm involvement of genes regulating cell-cell and cell-matrix interaction and also indicate a possible contribution by genes implicated in vascular development.

Analysis of other sequenced families is now ongoing in order to check sharing and segregation of newly identified variants between different patients and to clarify their role in pathogenesis of PS.

P11.121-S
PTPN11 mutations in Noonan and LEOPARD syndromes: molecular spectrum, structural and functional insights on pathogenic mechanisms, and genotype-phenotype correlations
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Noonan syndrome (NS) is a genetically heterogeneous developmental disorder characterized by reduced growth, dysmorphic facial features, congenital heart defects, skeletal and hematological anomalies, and variable cognitive deficit. The majority of NS cases are due to aberrant signaling through the RAS-MAPK cascade, and we previously identified PTPN11 as the major gene underlying this condition. PTPN11 encodes SHP2, an SH2-domain-containing protein tyrosine phosphatase functioning as a signal transducer that positively modulates RAS function. Mutations in the same gene are implicated in LEOPARD syndrome (LS), a disease clinically related to NS, or contribute to leukemogenesis. Here, we explored further the molecular spectrum of germinal PTPN11 lesions and their associated phenotype. Mutation scanning of the entire PTPN11 coding sequence was performed in large NS/LS cohorts collected in the frame of the NEuroNet Consortium. Among the 452 mutation-positive subjects, 68 different variants deemed to be of pathologic significance, including 12 nonsense changes and 2 in-frame indels, were identified. Besides the previously characterized mutations destabilizing SHP2’s inactive state or increasing binding to phosphotyrosyl-containing partners, a novel mutation cluster was recognized. Specifically, eleven changes affecting Leu278, Leu283 and Arg288 were identified in ten unrelated subjects with clinical features fitting NS. Biochemical and structural characterization of these mutations provided novel insights on disease pathogenesis. Finally, these data and published records were used to define more accurately the mutational spectrum of germine and somatic PTPN11 mutations in human disease.

P11.122-M
A paternally inherited intestinal deletion of 15q11.2 causing clinical features of PWS: refinement of the PWS-IC
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Imprinting occurs on chromosome 15q11.2-12 by differential methylation of genes on the paternal and maternal chromosomes. Prader-Willi syndrome (PWS) is caused by the absence of paternally imprinted genes at chromosome 15q11.2, whether by deletion, uniparental disomy, or imprinting center (IC) defect. The PWS-IC is defined by the 4.1 kb shortest region of overlap (SRO) of reported deletions, which includes the promoter and exon 1 of the SNRPN gene. Mutation of the PWS-IC blocks the switch from maternal-to-paternal imprint within the male germ line.

We present an infant with a classical clinical presentation of PWS. As a neonate, he was hospitalized for severe hypotonia and required tube feeding. He has bitemporal narrowing, and typical facial features and hand morphology. Methylation-sensitive (MS) PCR across the differentially methylated CpG island of SNRPN showed amplification of only the maternal-specific product. High-resolution chromosome microarray analysis detected a copy loss at 15q11.2 which overlaps the 4.1 kb PWS-SRO; this copy loss is at least 1.6 kb in size and may be as large as 5.8 kb. MS-MELPA confirmed the deletion and showed a methylation pattern identical to that seen in other individuals affected with PWS due to paternal deletion. Familial studies showed that the father carried the mutation but the paternal grandparents did not, consistent with a de novo deletion occurring on the grand-maternal allele. To the best of our knowledge, this is the smallest copy loss causing PWS reported to date, and further refines the PWS-IC to a 1.8 kb region.

P11.123-S
Clinical phenotypes in patients with genomic anomaly detected by aCGH
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High resolution molecular karyotyping has been implemented in recent years in our group of 11 outpatients as a diagnostic test for nonsyndromic dysmorphism or developmental delay. Genomic imbalance in different chromosomal regions was detected in seven cases. Principally there were detected deletion/duplication in the range of 110 kb to 2.8 Mb. Clinical features and aCGH data will be summarised. In two patients was found a genomic rearrangement in more than one region. In one child patient with skeletal dysplasia like phenotype was identified a duplication at three different chromosomes. Genotype/Phenotype correlation in our patients with different kind of genomic anomaly as a deletion/duplication has a direct impact on a health care management including reproductive outcome in the family. An assessment of significance of genomic imbalance along with the incidence of benign CNV in the light of acquiring new knowledge is still a considerable assessment of significance of genomic imbalance along with the incidence of benign CNV in the light of acquiring new knowledge is still a considerable assessment of significance of genomic imbalance along with the incidence of benign CNV in the light of acquiring new knowledge is still a considerable assessment of significance of genomic imbalance along with the incidence of benign CNV in the light of acquiring new knowledge is still a considerable assessment of significance of genomic imbalance along with the incidence of benign CNV in the light of acquiring new knowledge is still a considerable assessment of significance of genomic imbalance along with the incidence of benign CNV in the light of acquiring new knowledge is still a considerable assessment of significance of genomic imbalance along with the incidence of benign CNV in the light of acquiring new knowledge is still a considerable assessment of significance of genomic imbalance along with the incidence of benign CNV in the light of acquiring new knowledge is still a considerable assessment of significance of genomic imbalance along with the incidence of benign CNV in the light of acquiring new knowledge is still a considerable assessment of significance of genomic imbalance along with the incidence of benign CNV in the light of acquiring new knowledge is still a considerable.
13 years old female was referred for genetic counseling because of intellectual disability (ID), short stature, microcephaly, dysmorphic features (low frontal hairline, left convergent strabismus, wide nose root, abnormal shape of the pinnae, flat philtrum, large mandible, hypodontia, malocclusion of teeth). Long bone length fell below third centile of 11-12 mos. A sister of proband’s mother presented with ID, delay of speech, club-foot and additional features not common to proband: normal stature, obesity, short philtrum, high narrow palate, crowded teeth, LDL toes overlapping thumbs. Mother’s second sister died in infancy because of multiple congenital anomalies. Array-CGH of proband’s DNA revealed trisomy of the distal bands of chromosome 10 short arm (1p13.3p12.2) and monosity of the distal bands of chromosome 10 long arm (10q26.21q26.2). Subtelomeric FISH analysis confirmed a rearranged chromosome 10 [rec dup (10q)] due to a large, maternal pericentric inversion. A reverse segmental imbalance [rec dup (10q)] was detected in proband’s aunt by subtelomeric FISH. The high frequency of recombiant in this family and data from literature review suggests a high recurrence risk in similar cases with large pericentric inversions comprising almost entire chromosomes. The research leading to these results was funded by the Lithuanian-Swiss cooperation programme to reduce economic and social disparities within the enlarged European Union under project agreement No. CH-3-5MM-01/04, UNIGENE project.

P1.112-5
Functional characterization of L440Xfs mutation in the thyroid hormone receptor beta (TRβ) in an individual with RTH syndrome

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Resistance to thyroid hormone is an autosomal dominant disorder that affects 1 in every 40,000 births. It is characterized by reduced soft tissue responsiveness to thyroid hormone, with increased levels of T4 and T3 and non-suppressed thyroid hormone (TSH), due to mutations present in the thyroid hormone receptor β gene (TRβ), particularly in its T3 binding domain. The clinical phenotype varies both between different families and between affected family members. Individuals with RTH can have variable resistance in different tissues, as a consequence of mixed features of hypothyroidism and hyperthyroidism. Our goal was to assess whether there was a functional alteration due to the mutation L440Xfs in TRβ gene that may generate RTH in our patient. In this study, we performed molecular and functional characterization of the L440Xfs mutation found in a male patient, who was diagnosed with RTH at 15 months of age. The patient harbors a novel mutation in exon 10 of the TRβ gene, which consists of a deletion of a cytosine at nucleotide 1609 in the position 440, leading to a stop codon. The mutation was found in neither his parents nor his two healthy sisters, indicating a de novo mutational event. Transfection studies showed that the mutant TRβ was unable to carry out the transcription of luciferase gene in the presence of T3. Therefore, it is likely that the impaired receptor generates the severe RTH phenotype in our proposition.

P1.112-6
Restrictive Demyelopathy: report of two new cases

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Restrictive demyelopathy (RD) is a very rare, lethal genetic condition with peculiar phenotypic, histologic and genetic characteristics. The first case we describe was clinically diagnosed after death and confirmed thanks to precise histological and molecular characterization. The fetus was stillborn at 31+1 weeks of gestation by cesarean section. He did not show any spontaneous respiratory activity at birth nor after cardiopulmonary resuscitation. Post mortem, clinical examination showed multiple joint contractures and peculiar facial features like thick hair, absent eyelashes and eyebrow, hypertelorism, small nose, small mouth in the “O” position with thin lips, micrognathia, low-set ears. The skin was thin, erythematous and translucent. The gynecomastia was particularly in the axillary and inguinal folds, nails were long. It was hypothesized the diagnosis of RD, confirmed by the microscopic examination of the skin. Molecular analysis performed on fetal DNA, showed a homozygous duplication in ZMPSTE24 gene, already described as causative for RD. The second patient is a SGA boy, born at 32 weeks of gestation by urgent cesarean section because of premature membrane rupture and pathologic CTG. Aggar was 3-0 and cardiodiopulmonary resuscitation was effective only after orotracheal intubation because of choanal stenosis, midface hypoplasia, micrognathia, microstomia and tight trismus. At this moment, spontaneous breathing was maintained only by respiratory support. The clinical diagnosis of RD is supported by the typical facial dysmorphism, multiple joint contractures, arthrogryposis, thin, tense and translucent skin with some erosions, long nails. Molecular characterization is still in progress.

P1.121-7
Rubinstein-Taybi syndrome lymphoblastoid cells show impaired DNA damage response to oxidative stress as a possible mechanism of mutation accumulation

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The Rubinstein-Taybi syndrome (RSTS) is a genetic disorder associated with growth defects, intellectual disability, and increased risk of tumors. About 60% of RSTS individuals carry heterozygous mutation/deletion of CREBBP gene, while ~3-8% of RSTS are caused by mutations in the EP300 gene. CREBBP and p300 proteins play a key role in many aspects of DNA metabolism, such as DNA repair. However, the efficiency of DNA damage response (DDR) in RSTS is not yet elucidated. Here, we have investigated DDR in lymphoblastoid cell lines from 3 RSTS patients carrying different types of mutations of CREBBP gene. RSTS cells showed signs of hyper-H2AX phosphorylation, indicating an endogenous DNA damage. In addition, all RSTS cell lines tested were more sensitive to treatment with the oxidative agent KBrO3. No significant differences were observed in protein expression levels of PCNA, and DNA repair proteins, such as XP proteins, PARP-1, XRC21 to DNA damage sites, suggests that in RSTS the DNA repair process is slowed or impaired. Preliminary results on the efficiency of DNA repair, as assessed by the Comet test, have suggested a delayed kinetics in the repair of oxidative lesions in RSTS cells, in particular at the level of DNA incision. These results suggest that RSTS cells show a reduced efficiency in DNA repair, explaining the greater sensitivity to oxidative stress, genomic damage, and accounting for the increased susceptibility to cancer of RSTS patients.

P1.128-M
Report of five novel Schinzel-Giedion patients with mutation in SETBP1. Further delineation of the neuroradiological phenotype

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Schinzel-Giedion syndrome was first described in 1979, and is characterized by typical facial gestalt, severe developmental delay, frequent epilepsy and various malformations (urinary tract, genitalia, heart and skeletal). The reported cerebral malformations are ventriculomegaly, thin or absent corpus callosum, cortical atrophy, gyration anomalies. Hypoplastic pons was reported cerebral malformations are ventriculomegaly, thin or absent corpus callosum, cortical atrophy, gyration anomalies. Hypoplastic pons was found in 2 patients. In 2010, SETBP1 gene was identified as responsible for Schinzel-Giedion syndrome, with identification, by exome, of de novo missense mutations, some recurrent, all localized in the SKI oncogene homologous domain. Some recurrent, all localized in the SKI oncogene homologous domain. Some recurrent, all localized in the SKI oncogene homologous domain. Some recurrent, all localized in the SKI oncogene homologous domain. Some recurrent, all localized in the SKI oncogene homologous domain. Some recurrent, all localized in the SKI oncogene homologous domain. Some recurrent, all localized in the SKI oncogene homologous domain. Some recurrent, all localized in the SKI oncogene homologous domain. Some recurrent, all localized in the SKI oncogene homologous domain. Some recurrent, all localized in the SKI oncogene homologous domain. Some recurrent, all localized in the SKI oncogene homologous domain.
These novel cases further delineate the cerebral spectrum of malformations in Schinzel-Giedion syndrome, in particular with similar posterior fossa anomalies. The 3 novel mutations, as all the previously described mutations, are localised in the known mutational hotspot.

P11.129-S
Towards a better understanding of de novo germline mutations in SETBP1 in Schinzel-Giedion syndrome
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We report on a case of a 3-year-old girl, with left hemiplegia, psychomotor delay and high pitched neonatal crying, epilepsy with MRI evidence of right open-lip schizencephaly; GGH array analysis discloses a paternal inherited 5p complex rearrangement consisting of a 7.6 Mb deletion (del5p15.33-31) and a 2.6 Mb duplication (dup5p15.31). Indeed, the Sp1.52 critical Cri du Chat (G6C) region is spared and accordingly our patient does not present the typical dysmorphic and severe developmental features of G6C syndrome. The proband’s father has a high pitched voice, a history of hyperactivity and poor school performances and normal EEG. Intrafamilial phenotype variability is not uncommon in contiguous gene syndromes and it can be explained by differences in modifying genes and in critical allele polymorphisms. Schizencephaly is a full-thickness cleft within the cerebral hemispheres, that can result from disruption or malformation. It has never been reported before in patients with 5p-syndrome, who typically show a variable degree of hypoplasia, especially of subcortical structures.

Based on the already published phenotypic data and CNV databases as well as on the increased knowledge on the function of the genes located in the deleted fragment, we discuss the genotype-phenotype correlation in our patient and the consequence of haploinsufficiency of NSUN2 in determining the phenotype. Sanger sequencing of the whole coding region of SKI (NM_003036.3) revealed heterozygous mutations in two patients. The mutation c.100G>T (p.Gly34Cys), but not the sequence alteration c.95T>A (p.Leu32Gln) has already been described as a pathogenic mutation for SGS. However, the description of two other pathogenic missense mutations in the same codon 32 within the SMAD binding domain as well as in silico predictions underline the role of the sequence alteration c.95T>A as a pathogenic mutation. Furthermore, we identified an unclassified heterozygous variant, c.185C>G (p.Ala62Gly) in the third patient (Table).
In conclusion, the molecular analysis of SKY should be considered to differentiate between marfanoid phenotypes such as MFS, LDS and SGS.

P11.134-M
Submicroscopic genomic alterations detected by array CGH analysis in a cohort of patients with Silver Russell Syndrome found negative to classical genetic and epigenetic tests

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Silver Russell syndrome is characterized by pre- and postnatal growth retardation, variable facial dysmorphism, clinodactyly of the fifth fingers, and sometimes asymmetry of face, trunk and extremities. Genetic and epigenetic aberrations on chromosomes 7 and 11 are commonly found in SRS, leaving out, however, a fraction of up to 50% of cases with unknown genetic etiology. A cohort of 32 clinically selected SRS patients, without detected genetic or epigenetic abnormality, was analyzed by array CGH analysis to identify possibly pathogenic CNVs. Twenty-eight patients (87.5%) were found to carry one or more rare CNVs, according to the Database of Genomic Variants and, overall, 55 rare CNVs, 25 gains (45.5%) and 30 losses (54.5%) were identified. Inheritance, established for 36 of the identified rare CNVs, showed that 7 occurred de novo (19.5%). Interrogation of public databases allowed us to pinpoint genomic regions containing genes, either imprint or not, according to their function, among plausible candidates for SRS. Interestingly 4 CNVs span genomic regions already associated with growth defects, 3 CNVs contain known genes found altered in previously described SRS patients and 3 CNVs include genes implicated in growth control pathways not yet associated with SRS. These results confirm the genetic heterogeneity of SRS and the high percentage of potentially causative imbalances, answering the wide clinical expression of patients with a phenotype strongly suggestive for this syndrome. Genome-wide scan is reconfirmed an appropriate and powerful tool to achieve a differential diagnosis between SRS and SRS-like patients.

P11.135-S
Mosaicism of the H19 hypomethylation in a patient with very low weight and severe insulin resistance

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A 6-year-old boy, born to healthy non-consanguineous parents, presented a history of severe stature-ponderal deficiency, bilateral cryptorchidism and hypogonitalism. The propositus was delivered at 38 weeks by cesarean section for intrauterine growth retardation (IUGR). Birth weight was 1400 g (-3SD), birth length 38 cm (-4SD) and OFC 52 cm (-2SD). He had moderately delayed developmental milestones, a poor appetite and feeding difficulties since the first year. Physical examination showed dysmorphic features suggestive of Silver-Russell Syndrome (SRS): triangular face, relative macrocephaly with frontal bossing, prominent forehead, mild low-set prominent ears, micrognathia, downturned mouth, thin lips, fifth finger brachydactyly/ clinodactyly, and delayed motor development. At 6 years he was 90 cm (-5.12 SD) tall and weighed 8000 kg (-9.22 SD). His basal levels of free thyroxine, TSH, cortisol and coeliac disease screening were normal. Plasma concentrations of IGFB-1 and IGFBP-3 were low for age and sex (-2.30 and -1.95 SD). Growth hormone stimulation tests revealed a classic growth hormone deficiency. He also had severe insulin deficiency and normal glucose response after OGTT, negative diabetes autoantibodies and normal glycated haemoglobin, features not reported in association with SRS.

Molecular studies revealed a hypomethylation of the paternal H19/IGF2 imprinting Control Region. Insulin like growth factor 2 (IGF2) is an imprinted gene, which has an important role in foetal growth. IGF2 is downregulated through hypomethylation of a differentially methylated region in SRS, characterized by growth restriction. Insulin metabolism abnormalities have never been described in SRS: could it be a specific manifestation of reduced IGF2 expression?

P11.136-M
Regulatory element deletion cause a down-regulation of ZDHHC15 gene in a proband with Smith Magenis syndrome phenotype

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Here, we describe a boy aged 3 years who showed mid facial minor anomalies such as brachycephaly, square face, thick eyebrows, broad palate, gene- ralized hypotonia, developmental delay, variable facial dysmorphisms, clinodactyly of the fifth fingers, and Seviroial problems (self injury), sleep disorders and congenital heart defect.

Based on suspected Smith Magenis syndrome (SMS), chromosomal analysis was performed and showed a 46,XY karyotype. FISH analysis of the SMS locus at 17p11.2, as well as MLPA, mutational analyses and quantitative expression of the RAI1 gene, gave normal results. High-resolution array-CGH analysis revealed two rare paternal deletions at 4q35.2 and Xq13.3, both yet unreported in healthy subjects according to the Database of Genetic Variants. Whereas it is not possible to assign a pathogenetic role to the 4q35.2 deletion, the Xq13.3 loss of 54 kb involves a predicted conserved insulator that maps 29 kb far from the 5’ end of the ZDHHC15 gene. ZDHHC15 acts as a palmitoyl-transferase in brain, and its null expression has been reported in a syndromic patient. We thus investigated in the patient’s blood the causative role of the identified CNV on ZDHHC15 by RT-qPCR. The ZDHHC15 expression level was significantly reduced in the male proband compared to controls, whereas the expression level in the mother was within the control range. Our results suggest the involvement of ZDHHC15 perturbation in the onset of the proband’s phenotype and point to this gene, sharing interactors with RAI1, as a novel candidate gene for SMS.

P11.137-S
A novel NSD1 mutation in Sotos syndrome with constriction of vena cava

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Background: Sotos syndrome, first described in 1964, is characterized by typical facial appearance, overgrowth (height and/or head circumference ≥2 SD above the mean), and sometimes other features such as learning disability, behavioral problems, congenital cardiac anomalies, neonatal jaundice, renal anomalies, scoliosis, and seizures.

The occurrence of Sotos syndrome is 1 in 10,000 to 1 in 14,000 newborns. However, many cases are assumed to be undiagnosed.

Methods: Genetic analyses including karyotype, array Comparative Genomic Hybridization and exome sequencing (HTS) was performed in a two year old girl with an unknown syndrome.

Results: HTS analysis revealed a novel de novo nonsense mutation in the NSD1 gene.

Her facial appearance was consistent with Sotos syndrome whereas her associated balloon dilated vena cava inferior constriction has not previously been associated to Sotos syndrome.

Conclusion: We describe a novel NSD1 nonsense mutation causing Sotos syndrome. To our knowledge, this is the first time a Sotos patient has presented with a vena cava constriction.

P11.139-S
Unbalanced translocation t(8;17)(q23;q24) in a patient with developmental delay and epilepsy

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We present a 2-year-old male patient with poor growth, developmental delay and epilepsy. He is the second-born child of healthy non-consanguineous parents, with an unremarkable family history. He was born at gestational week 40 after an uncomplicated pregnancy. Seizures appeared at 16 months and the EEG revealed focal abnormalities in the left frontal and parietal regions during sleep. The array-CGH analysis identified a de novo 2.32 Mb
deletion on chromosome 8q23.1q23.2 due to an unbalanced translocation t(8;17)(q23;q24). The analysis detected two additional anomalies which were both inherited from the healthy father: a 16.58 Kb duplication involving chromosome 7p15.2 and a 9.14.6 Kb duplication involving 22q13.2. In the deleted region of KCNV1, an additional candidate gene for the patient's neurological and electroclinical phenotype. In fact, SYBU encodes a protein which is a part of a kinase-motor-adaptor complex that is critical for the anterograde axonal transport and contributes to activity-dependent presynaptic assembly during neuronal development. KCNV1 encodes a neuronal modulatory subunit of a voltage-gated potassium channel and it is predominantly expressed in the brain.

**P11.140-M**

TBC1D7 mutations are associated with intellectual disability, megalencephaly, patellar dislocation and celiac disease

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Mutations in both TSC1 and TSC2 cause the tuberous sclerosis complex (TSC), a multisystemic disorder characterized by the development of hamartomas or benign tumors in various organs as well as epilepsy, intellectual disability (ID) and autism. Whereas the binding of TBC1D7, the third constitutive subunit of the TSC1-TSC2 complex, is required to maintain its integrity, sequencing of TSC patients with no TSC1-TSC2 mutations indicated that TBC1D7 is unlikely to represent a "TSC3" gene. Loss of function of TBC1D7 results in an increase in mTORC1 signaling, and consequently a delay in the induction of autophagy.

Mutations in TBC1D7 were recently reported in a family with ID and macrocrania. Using exome sequencing we identified two sisters homozygous for a novel TBC1D7 truncating mutation. In addition to the already described malformations and mild ID, they share osteo-articular defects, patella dislocation, behavioral abnormalities, psychosis, learning difficulties, celiac disease, proptosis, myopia and astigmatism. Consistent with a loss-of-function of TBC1D7 the proband's cell lines show an increase in the phosphorylation of e4BP, a direct downstream target of mTORC1 and a delay in the initiation of the autophagy process.

This second family allows enlarging the phenotypic spectrum associated with TBC1D7 mutations and defining a TBC1D7 syndrome. Our work reinforces the involvement of TBC1D7 in the regulation of mTORC1 pathways and suggests an altered control of autophagy as possible cause of this disease.

**P11.141-S**

Temple syndrome - introducing a new name for a characteristic chromosome 14 imprinting disorder

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Chromosome 14 harbours an imprinted locus at 14q32. Maternal uniparental disomy of chromosome 14, paternal deletions and loss of methylation at the intergenic differentially methylated region, result in a human phenotype of low birth weight, hypotonia, early puberty and short stature. This rarely diagnosed imprinting disorder has considerable overlap with other better known imprinting conditions such as Russell Silver and Prader Willi syndromes.

We have reviewed the world literature of 51 cases to identify the key diagnostic features to increase awareness, enhance diagnosis and improve treatment.

Key findings

1) Small for gestational age: The median birth weight standard deviation score (SDS) was -1.88 and none had a birth weight SDS > 0. The median birth length SDS was -1.64.
2) Hypotonia and motor delay (93% and 83%).
3) Mildly reduced intellectual ability: IQ 70-95.
4) Small hands and feet (87% and 96%).
5) Early puberty (86%).
6) Short stature in adulthood; the median final height SDS was -2.04
7) Metabolic syndrome; the median final adult weight SDS was -1.07 demonstrating a relatively greater weight for height in adults; median BMI of 2.7. Of 15 patients over the age of 11 years, three developed insulin dependent diabetes mellitus at the ages of 12 years, 19 years and 20 years.

The facial appearance distinguishes this condition from other imprinting disorders. The use of the name Temple syndrome is not universal, yet, as but rather than the somewhat cumbersome use of 'maternal uniparental disomy of chromosome 14 related conditions' we propose this name change.

**P11.142-M**

Testicular regression and cerebral abnormalities: a new syndrome

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We report three male siblings affected by a Multiple Congenital Abnormalities/Intellectual Disability (MCA/ID) syndrome which, to the best of our knowledge, has not been reported to date. Patient 1 presented with a clinical phenotype including microopenis, anorchia and a very low serum level of antimullerian hormone, consistent with progressive testicular regression; severe developmental delay; spasticity; epilepsy; dysmorphic features; deafness; retinopathy; cerebral atrophy, corpus callosum hypoplasia and peri-ventricular cysts. The two subsequent pregnancies were terminated at 34 weeks gestation because of recurrence of the phenotype. Genetic analysis revealed a 7p15.2 duplication in patient 2, therefore we propose this name change.

**P11.143-S**

Autosomal recessive POLR1D mutation with decreasing of TCOF1 mRNA is responsible for Treacher Collins syndrome

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We report on a family of Italian origin in whom we identified a novel POLR1D truncating mutation. The clinical presentation included severe developmental delay and hearing impairment. Anterograde axonal transport and contributes to activity-dependent presynaptic assembly during neuronal development. KCNV1 encodes a protein which is part of a kinesin motor-adaptor complex that is critical for the anterograde axonal transport and contributes to activity-dependent presynaptic assembly during neuronal development. KCNV1 encodes a neuronal modulatory subunit of a voltage-gated potassium channel and it is predominantly expressed in the brain.
P11.144-M
Molecular and cytogenetic characterization of an unusual case of partial trisomy 13q mosaicism
Trisomy 13 (Patau syndrome) is a rare multiple malformation syndrome and includes anomalies of the central nervous system, cardio-vascular and urogenital system. The probability of survival for live-born to one year is 3%. Partial or mosaic forms of trisomy 13 can occur and they cause a variable phenotype.

We report a case of a live-born baby with multiple congenital anomalies including severe failure tetralogy, bilateral postaxial polydactyly of fingers and toes, monolateral hydropneumosis, microcephaly with trigonocephaly and brain anomalies (hypoplastic corpus callosum and dysmorphic cerebellar vermis). The child also presents some facial anomalies (sloping forehead, hypotelorism, low-set ears and microretrognathia) and scalp defect (aplastic cutis of vertex).

Cytogenetics, fluorescence in situ hybridisation and array-\textit{CGH} analysis showed the presence of two cell lines in which a normal chromosome 13 was replaced in one by an isochromosome (13q) and in the other by a isochromosome 13 derivative, with a deletion spanning from 13q11 to 13q14, resulting in partial trisomy 13q. The two cell lines were present either in peripheral blood lymphocytes or skin fibroblasts.

Chromosomal mosaics made up of cell lines with distinct structural rearrangements of the same region on isochromosome 13 derivative. We suggest a common mechanism producing the coexistence of distinct structural rearrangements at common breakpoints on chromosome 13. Phenotype of the baby is discussed with respect to other cases showing partial trisomy mosaicism in Patau syndrome.

P11.145-S
Classical karyotyping vs molecular karyotyping (array\textit{CGH}) in a case of trisomy 9 mosaicism
Institut für Klinische Genetik, Stuttgart, Germany.
Trisomy 9 mosaicism is considered to be a rare chromosomal abnormality with limited survival and a characteristic pattern of multiple anomalies. The features commonly associated with trisomy 9 include growth retardation, facial dysmorphism, skeletal abnormalities, congenital heart disease and intellectual disability. More than 50 cases have been reported, most of which were diagnosed after birth.

We report a case of a one month old baby girl with craniofacial abnormalities, hydropneumosis and multiple contractures. Array\textit{CGH} analysis was performed and detected a trisomy 9 mosaicism in approximately 40% of the cells. Classical karyotyping of lymphocytes revealed trisomy in about 3% of the metaphases. Previous prenatal analysis of cell cultures from amniotic fluid had not shown the mosaic trisomy 9 constellation. Mosaicsms are expected to be exhibited at different levels in different tissues. In addition, cell culturing leads to a bias in terms of the ratio between trisomic and diploid clones and very likely underestimates the percentage of trisomic cells in conventional karyotyping. Our data show the importance of using uncultured samples (amniotic fluid and blood) and the value of microarray technology in the assessment of mosaicsms.

P11.146-M
Genetic-Environmental characterization of an idioc (Xq) in a female with Turner syndrome and her daughter
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The following is the case of a patient born 2 weeks premter weighing 2.800 kg. She had a multicystic kidney that was removed immediately after birth. At age 9, due to a significant growth delay, a cytogenetic analysis was made on peripheral blood revealing a homogenous 45,\textit{X} karyotype thus leading to Turner syndrome. Growth hormone treatment contributed to stature increase but with the development of secondary sex characterstics and the menstral cycle, it became necessary to repeat blood cytogenetic analysis that confirmed the 45,X karyotype. At 30 years, following absence of menstrual cycle, she realized she was pregnant. She had to undergo a caesarian delivery and was simultaneously operated for ovariectomy. Karyotype obtained from gonadic tissue was 45,\textit{X};46,\textit{X},der(X). The insufficient amount of cells however did not help to investigate on marker nature. As the daughter presented the same stature growth delay, at 5 years her constitutional karyotype was analyzed and was the same one found in her mother’s ova.

P11.147-S
SNP array revealed maternal UPD16 in a boy with short stature, craniosynostosis and psychomotor retardation
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We report on a 15 months old boy (geminii after IVF, healthy sister) with positive prenatal amnionesis (IUGR, oligohydramnion), craniosynostosis, short stature, hypotonia, hypoplasia, cryptorchidia, pulmonary vein stenosis and delayed psychomotor development. Chromosomal examination revealed normal male karyotype 46,XY. The whole genome genotyping was performed using SNP array (300K, Illumina) and no pathogenic CNV was found. However, three extensive blocks with loss of heterozygosity spanning almost one third of the whole chromosome 16 were found. Parental DNAs were analyzed and the maternal UPD16 was confirmed. Chromosome 16 showed the mixture of isodisomy and hetendisomy with homozgyous haployte within the centromere. This haployte arrangement implies the following origin of the UPD16: meiosis II nondisjunction, recombination and the loss of the paternal chromosome 16 (trisomy rescue). Data for UPD16 are inconsistent, prominent phenotype of maternal UPD16 is IUGR with or without catch-up growth. Other features may include heart defects, inguinal hernia, hypoplasia, pulmonary hypoplasia, although these features may be partly due to hidden mosaic trisomy 16. Mental development ranges from normal to severely delayed. We consider a potential impact of two imprinted genes and 52 predicted imprinted genes on chromosome 16 to the distinct phenotype of the UPD16.
**P11.149-S**

WHSC/NSD2 is the major candidate gene for growth delay and facial dysmorphisms in Wolf-Hirschhorn syndrome: expression analysis and functional studies on primary fibroblasts and immortalized peripheral lymphoblasts from three patients.

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1Institute of Medical Genetics, Catholic University, Rome, Italy; 2Institute of General Pathology, Catholic University, Rome, Italy.

Wolf-Hirschhorn Syndrome (WHHS, OMIM194190) is a contiguous gene syndrome caused by partial deletion of the short arm of one chromosome 4. The core of WHHS phenotype includes growth delay, intellectual disability, distinct facial appearance and seizures. It maps within the terminal 1.9 Mb region on 4p16.3, where the critical region, WHSCR-2, was described. With respect to pathogenic genes falling within WHSCR-2, WHSC1 is the major candidate gene for both facial characteristics and growth delay. WHSC1 is expressed mainly in embryonic tissues and presents homology with Drosophila dismorph gene. Its PWWP, HMG-box, PHD and SET structural domains suggest an histone methyltransferase activity and a role in the epigenetic regulation of morphogenetic transcriptional programmes. However, to which extent when 1 expression and activity are reduced in patients’ cells due to 4p16 deletion has not been thoroughly investigated. Additionally, while very little is known about when 1 regulation, it is intriguing that this gene is a potential target of hsa-miR948, that also maps to 4p16.3, and whose concomitant decreased intestinal biopsy on the back of TGF-β signaling (response, inflammatory signaling) reportedly related to WHSC1 biological activity at least in tumor cells.

**P11.150-M**

Coeliac disease in Williams syndrome

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Coeliac disease is a genetic, immune, gastrointestinal disorder characterized by intolerance to the dietary grain protein gluten. An increased prevalence of Coeliac disease has been reported in Down syndrome and Turner syndrome, but there has been only few previous reports in Williams syndrome. In this report we decided to evaluate at a mRNA and protein level the expression of WHSC1 and of hsa-miR948 in primary and immortalized cell lines from selected patients and healthy individuals as controls. Furthermore, the expression levels have been correlated with biochemical characteristics (H1K36 and H4K20 methylation) and cellular phenotypes (DNA damage response, inflammatory signaling) reported related to WHSC1 biological activity at least in tumor cells.

**P11.152-M**

Early diagnosis of Kallmann syndrome in a 4 year old boy with a translocation tXY translocation

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Kallmann Syndrome (KS) is a rare, X-linked syndrome characterized by hypogonadism and anosmia. This condition is caused by a de novo X;Y translocation (tXY) in about 40% of patients. The symptoms include hypogonadism and anosmia. Many of the patients with tXY inherited from a carrier mother. The phenotype was characterized by mesomelic short stature, mid-face retrusion, ichthyosis, ocular-albinism, mental retardation, and Kallmann syndrome (KS). Here we describe a boy with tXY inherited from a carrier mother. His phenotype was characterized by mesomorphic short stature, midface retrusion, ichthyosis, microcephaly,舗 and hypogonadism. The patient’s mother and sister showed mesomorphic short stature and Turner syndrome. The possible mechanism is postulated in this report.

**P11.153-S**

Chromosome Xq21 deletion syndrome - rare cause of deafness and mental retardation

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Our case is a family, where Chromosome Xq21 deletion syndrome was diagnosed. Genetic counseling was recommended because of mental retardation, bilateral hearing loss and paleo-ocular aspects. We found four cases with Xq21 deletions. The presence of a point mutation in exon 2 that disrupts magnesium RNA splicing by eliminating exon 2, causing a premature stop codon. After this first report of Jordanian patients, no further Xq21 deletions have been reported worldwide. In conclusion, we describe a Jordanian patient with a translocation tXY with a mild phenotype due to X inactivation and are fertile. Women with tXY show a range of phenotypes with variable degree of severity according to the size and position of the deletion of the X chromosome. The responsible gene for WFS2 is considered a phenotypic and genotypic variant of WFS, whose minimal criteria for diagnosis are diabetes mellitus and optic atrophy. The responsible gene for WFS2 is named CISD2, a highly conserved zinc-finger gene encoding for the Endoplasmic Reticulum Intermembrane Small (ERIS) protein, which plays a pivotal role in calcium homeostasis. It was identified for the first time in three consanguineous families of Jordanian descent who carried a point mutation in exon 2 that disrupts messenger RNA splicing by eliminating exon 2, causing a premature stop codon. After this first report of Jordanian patients, no further CISD2 mutations have been reported worldwide. In conclusion, we describe a Jordanian patient with a translocation tXY with a mild phenotype due to X inactivation and are fertile. Women with tXY show a range of phenotypes with variable degree of severity according to the size and position of the deletion of the X chromosome. The responsible gene for WFS2 is considered a phenotypic and genotypic variant of WFS, whose minimal criteria for diagnosis are diabetes mellitus and optic atrophy. The responsible gene for WFS2 is named CISD2, a highly conserved zinc-finger gene encoding for the Endoplasmic Reticulum Intermembrane Small (ERIS) protein, which plays a pivotal role in calcium homeostasis. It was identified for the first time in three consanguineous families of Jordanian descent who carried a point mutation in exon 2 that disrupts messenger RNA splicing by eliminating exon 2, causing a premature stop codon. After this first report of Jordanian patients, no further CISD2 mutations have been reported worldwide. In conclusion, we describe a Jordanian patient with a translocation tXY with a mild phenotype due to X inactivation and are fertile. Women with tXY show a range of phenotypes with variable degree of severity according to the size and position of the deletion of the X chromosome. The responsible gene for WFS2 is considered a phenotypic and genotypic variant of WFS, whose minimal criteria for diagnosis are diabetes mellitus and optic atrophy. The responsible gene for WFS2 is named CISD2, a highly conserved zinc-finger gene encoding for the Endoplasmic Reticulum Intermembrane Small (ERIS) protein, which plays a pivotal role in calcium homeostasis. It was identified for the first time in three consanguineous families of Jordanian descent who carried a point mutation in exon 2 that disrupts messenger RNA splicing by eliminating exon 2, causing a premature stop codon. After this first report of Jordanian patients, no further CISD2 mutations have been reported worldwide.

**P11.154-S**

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Next time the family was examined in the following pregnancy of the mother, but the case was complicated because of twin pregnancy. Prenatal diagnosis was recommended and chorionic villus sampling was performed with the result one male and one female foetus. Molecular genetic examination of the 17q21.31 microdeletion syndrome in the male foetus was performed and the same familiar deletion was identified. Mother underwent selective fetocide of the affected male foetus at 16 weeks’ gestation. Further course of gravidity is normal.

P11.154-M
17q21.31 microdeletion syndrome: neurocognitive functioning in two Italian Italian young patients
G. Vizzardi, M. Venemendia1, F. Ortolani1, M. Settembre1, A. Rosselli1, D. Cornacchia1, G. DiNelio1, A. Labbate1, R. Tripoli1, M. Riccardi1, M. Lucarano1, F. Nicastro1, M. Carella1, M. Gentile1, E. Piccinno1, F. Papadav, R. Fischer2; 1Servizio Psicologia Clinica-Direttore P.O.Giovanni XXIII AOU Policlinico, Bari, Italy, 2UOC Malattie Metaboliche Genetica Clinica e Diabetologia Ospedale Pediatrico Pediatri, Bari, Italy, 3UOC Malattie Metaboliche Genetica Clinica e Diabetologia, Ospedale Pediatrico, Giovanni XXIII, Bari, Italy, 4UOC Genetica Medica ASL BARI ROLDI Venere, Bari, Italy, 5UOC Malattie Metaboliche Genetica Clinica e Diabetologia Ospedale Pediatrico Giovanni XXIII, Bari, Italy.

The 17q21.31 microdeletion syndrome causes a well-known syndrome recently described in the scientific literature. Clinical features are: neonatal hypotonia, low birth weight, craniofacial dysmorphism, developmental delay, intellectual disability and amiable behaviour disposition. The present study reports on a psychological assessment in two young Italian patients with a genetically described 17q21.31 microdeletion. Case 1: 18-year-old male. Case 2: 13-year-old female. Neuropsychological Assessment Wechsler Adult and Children Intelligence Scales Revised, Italian Neuropsychological Battery, Peabody, Vineland Adapative Behavior Scales, Adaptive Behavior Inventory, Parent Stress Index, Brief Cope, Multidimensional Scale of Perceived Social Support, Child Behavior Checklist. Case 1: severe mental retardation (Intelligence Quotient, IQ = 32), immature representative capacity, impairment of receptive and expressive communication, anxiety and apprehension. The Adaptive functioning is impaired in all areas (communication, skills of daily living, socialization and motor skills). Mental Age 4y2m. Case 2: moderate to severe mental retardation, expressive and receptive language deficits, decline in all areas of adaptive functioning (M.A. 5y7m), socially indiscriminate attachment behavior, hyperactivity. Reading and writing disabilities, semantic memory deficits, verbal and visuospatial short-term memory deficits, ideational and motor dyspraxia, deficit in scheduling tasks and spatio-temporal organization were present in both patient. The emotional systems are characterized by trust in peers and adults, kindness, dependence and poor individual autonomy. The attachment is secure with important people. To our knowledge these observations can be added to preliminary evidences already present in literature: 17q21.31 microdeletion syndrome correlates to low intellectual capacities, learning disability, good interpersonal skills and an approaching behaviour.

P12.001-S
Impact of Aberrant Promoter Hypermethylation on down-regulation of MTS2 and MTS1 in Childhood ALL
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The tumor suppressor genes MTS1 and MTS2 are cyclin dependent kinases inhibitors inactivated in some human neoplasms via several mechanisms such as hypermethylation. We have investigated the methylation status of MTS1 and MTS2 in its effect on transcriptional down-regulation in 125 bone marrow aspirate (7 cases T-cell and 118 cases B-cell phenotypes) from childhood ALL patient and 100 healthy control in north Indian population by using MSP-PCR, bisulfite sequencing, SQRT-PCR and RT-PCR. There were significant differences in pattern of hypermethylation between patients and healthy controls of MTS2 (p=0.000) and MTS1 (p=0.001) and also when both genes methylated. Patients with hypermethylated of both genes showed an increasing risk for 2.33 fold (95%CI=2.33(1.97-2.77), p=0.03). Significant association of hypermethylation was observed only among the male patients (p=0.004) in contrast hypermethylation of MTS2 was significantly associated with increasing risk of ALL among both genders. Down-regulaition of mRNA expression was found in cases in which MTS1 and MTS2 were hypermethylated. In conclusion our data also indicate the impact of hypermethylation mediated inactivation of these genes which is associated with risk of childhood ALL. This abnormality particularly in promoter of MTS2 occurs in leukemogenesis and can be considered as an important factor in predicting the clinical outcome of ALL and it is able to suggest a diagnostic tool for some stage of ALL and even provide the better prospective for treatment strategy by applying some demethylating agents.

P12.002-M
The A785G CYP2B6 germiline polymorphism may affect the risk of de novo Acute Myeloid Leukemia
K. Kakosaiou1, A. Daraki2, S. Zachaki1, K. Stavropoulou2, K. Sambani1, V. Alepouri-Marinou1, P. Kollar1, K. Manola1; 1Pathology Department, Athens, Greece, 2National and Kapodistrian University of Athens, Athens, Greece.

The etiology of acute myeloid leukemia (AML) is currently unknown although genetic background and environmental exposure postulated to be one possible cause of AML development. The CYP2B6 enzyme plays a vital role in the degradation of many genotoxic compounds, protecting cells from oxidative damage. CYP2B6 gene is subjected to the A785G germline polymorphism which reduces enzyme activity. Thus, individuals homozygous or heterozygous for mutant allele (G/G or A/G) present decreased enzymatic activity. The purpose of this study was to investigate the role of the A785G polymorphism in the AML susceptibility. Possible associations with specific AML-chromosomal abnormalities were also investigated. CYP2B6 genotyping was performed in 220 de novo AML patients and 243 healthy donors by RCR-RFLP and Real-Time PCR assays. Cytogenetic analysis was successful in 192 of 220 patients (87.2%) . Significant higher incidence of 2.33 fold (95%CI=2.33(1.97-2.77), p=0.03). Significant higher incidence of the mutant genotypes (A/G and G/G) was observed in de novo AML patients compared to the controls (p<0.0001). The mutant allele frequency was similar between the different gender and age groups. Interestingly, a higher frequency of heterozygotes A/G was observed in normal karyotypes compared to abnormal (5.17% vs 3.00%, p=0.010). Furthermore, a significantly higher incidence of G/G genotype was observed in patients with t(9;21) and AML rearrangements compared to patients with normal karyotypes (28.5% and 20.0% vs 6.6%, respectively). Our study comprises the first investigation of the A785G CYP2B6 polymorphism in AML susceptibility. Our results reveal a possible implication of this genetic variant in AML development and its specific chromosomal aberrations.

P12.003-S
Higher incidence of co-existing Anaplastic Lymphoma Kinase (ALK) rearrangements and Epidermal Growth Factor Receptor (EGFR) mutations in Non-Small Cell Lung Cancer (NSCLC) in a South-East Asian population
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Both EGFR mutations and ALK rearrangements in NSCLC are associated with sensitivity to EGFR and ALK tyrosine kinase inhibitors, respectively. The incidence of ALK rearrangements is about 5% worldwide and EGFR mutations and ALK translocations are generally known to be mutually exclusive. The aim of this study is to determine the incidence of ALK rearrangements and the co-existence of co-existing EGFR and ALK rearrangements in South-East Asian population. FISH using an ALK break-apart probe was performed on formalin-fixed paraffin embedded (FFPE) tumor tissues from 1183 NSCLC cases from year 2011 till 2013. EGFR mutation test was performed using direct sequencing of EGFR exons 18-21. ALK FISH results were obtained from 1152 samples (97.4%). A total of 105 cases showed a rearranged ALK gene (9.1%) of which 76.2% showed the typical FISH pattern while 21.9% showed an atypical pattern with loss of the 5’ ALK gene segment and 1.9% showed loss of the 3’ALK gene. Among the ALK rearranged patients, 95 cases had concurrent EGFR mutation assays performed of which EGFR mutations were identified in 17 cases (17.9%). The incidence of coconstant ALK and EGFR alterations in the entire cohort was 1.49%. In our cohort study, the incidence of ALK gene rearrangement is relatively similar to the reporerted incidence. The co-existence of ALK rearrangements and EGFR mutations has been found to be rare in Western populations (0.33-6%). The higher incidence of coconstituent mutations in our South-East Asian population suggests differences in terms of genetic alterations.

P12.004-M
FISH analysis of four unbalanced translocations involving the 1q in hematologic neoplasms
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Unbalanced whole-arm translocations (WATs) of the long arm of chromosome 1q result in complete trisomy 1q. These are rare chromosomal abnorma-
Pathogenetic mechanisms of pancreatic acinar cell carcinomas (ACCs) are poorly characterized. There is no data about gene hypermethylation and chromosomal aberrations in ACCs. In a subset of ACCs we reported the impairment of APC/B-catenin pathway including mutations of APC gene. However, it is not known whether the loss of APC function can occur even through alternative genetic and epigenetic mechanisms. In this study, we investigated the methylation profile of 34 tumor suppressor genes, Copy Number Alterations (CNA) of 52 chromosomal regions, and APC alterations (mutation, methylation, and loss) together with the measurement of APC mRNA level in 45 ACCs and related available peritumoral pancreatic tissues using different methodologies: MS-MLPA, FISH, mutation analysis, and reverse transcription-droplet digital PCR. ACCs did not show an extensive global gene hypermethylation profile. RASSF1 and APC were the only two genes frequently methylated (60% and 56% of cases, respectively). APC mutations were found in 7% of cases, while APC loss and methylation were more frequently observed (48% and 56% of ACCs, respectively). They were more frequently observed (48% and 56% of ACCs, respectively). They were more frequently observed (48% and 56% of ACCs, respectively). They were more frequently observed (48% and 56% of ACCs, respectively). They were more frequently observed (48% and 56% of ACCs, respectively). They were more frequently observed (48% and 56% of ACCs, respectively). They were more frequently observed (48% and 56% of ACCs, respectively). They were more frequently observed (48% and 56% of ACCs, respectively). They were more frequently observed (48% and 56% of ACCs, respectively). They were more frequently observed (48% and 56% of ACCs, respectively). They were more frequently observed (48% and 56% of ACCs, respectively). They were more frequently observed (48% and 56% of ACCs, respectively). They were more frequently observed (48% and 56% of ACCs, respectively). They were more frequently observed (48% and 56% of ACCs, respectively).
ABSTRACTS POSTERS

P1.010-M SEP79/SYHR1, novel fusion gene identified in bladder cancer by RNA-seq.

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Bladder cancer is one of the most common genitourinary malignancies in the world. The urothelial carcinoma (UC) has multiple genetic alterations, but only a few low-frequency fusion genes have so far been reported for this disease. In this study, we present a thorough search for novel fusion transcripts in UC sample using high-throughput RNA sequencing. Sequencing was performed according to the paired-end RNA sequencing protocols from Illumina for Solexa sequencing on a Genome Analyzer II. We used the fusion discovery software tool ChimeraCan. From 51 million paired-end sequence reads, we identified 563 candidate fused transcripts. By stringent requirements, we nominated the one candidate fusion transcript for further experimental validation, which was positive by RT-PCR and Sanger sequencing. The transcript was intrachromosomal SEP79/ CYHR1 fusion gene. Septin 9 (17q25) is a member of the septin family of GTPases that have diverse cellular activity, including roles in cytokinesis, apoptosis, and vesicle trafficking. A chromosomal translocation involving this gene and MLL gene is described for acute myelomonocytic leukemia. CYHR1 (8q24) (cysteine and histidine-rich cytoplasmic protein) is involved in cell trafficking and support of gaitin 3. We found two transcript isoforms: SEP79 exon 2 was fused to CYHR1 exon 3 and SEP79 exon 1 was juxtaposed to CYHR1 exon 3. We also revealed SEP79/CYHR1 in 1/12 of UC FFPE samples. Further investigation of functional and clinical relevance of novel fusion gene remains to be elucidated to reveal the role of SEP79/CYHR1 in the carcinogenesis of bladder.

P1.012-M Phenotypic and clinical characteristics of 3300 Israeli BRCA1 and BRCA2 mutation carriers

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The most commonly mutated high penetrance genes in hereditary breast and ovarian cancer (HBOC) are BRCA1 and BRCA2. Germline loss-of-function mutations in these genes confer a high risk of breast cancer to the individual carrier. However, besides the clear loss of function mutations, there are still a large number of sequence variants of uncertain clinical significance (VUS) in both genes. These VUS generate a huge challenge both for genetic counseling and prophylactic surgery.

In the current study, the consequences of some BRCA1 and BRCA2 VUS identified in Norwegian cancer patients were evaluated. Initially, their possible influence on RNA splicing was investigated by targeted sequencing of cDNA. In addition, the complete BRCA1 and BRCA2 cDNA were sequenced in order to uncover eventually alternatively spliced transcripts. Some of the variants affecting the BRCT domains of BRCA1 were tested for their influence on BRCA1 trans-activation function. In total, 19 individuals with BRCA1 VUS and 18 individuals with BRCA2 VUS from families with HBOC were included in this study. Three of the variants identified, BRCA1 c.213-5T>A, BRCA1 c.5434C>G and BRCA2 c.687T>A, were shown to influence RNA splicing. Investigation of the full-length cDNA of BRCA1 and BRCA2 proved to be challenging due to the presence of several alternatively spliced transcripts which were also present in the controls. A functional assay, developed to assess the trans-activation ability of BRCA1, indicated that some of the variants may have a deleterious effect.

P1.015-S Two new cases of double heterozygosity for BRCA1 and BRCA2 gene mutations identified in a cohort of Italian breast and ovarian cancer families

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Double heterozygosity for BRCA1 and BRCA2 mutations is a very rare finding, particularly in non-Ashkenazi individuals, and only a few cases have been reported to date. Here we describe genetic and clinical data of two female double heterozygotes for both BRCA1 and BRCA2 mutations found in a cohort of 201 mutated Italian breast/ovarian cancer families out of 942 cases analyzed. The first one is a female patient affected by bilateral breast cancer at 47 and 49 years of age, carrying both a BRCA2 nonsense mutation (c.7408A>T - p.Arg2394X) and a BRCA1 proven splicing defect (IVS5-12A>G or c.331_332ins11 - p.Arg71SerfsX21). The second one is a female patient affected by ductal breast cancer at 42 years of age, carrying a BRCA1 nonsense mutation (c.3726C>T - p.Arg1203X) and a BRCA2 frameshift mutation (c.3036_3039delAACA - p.Ala938ProfsX21). Although this event is rare (2/201: 1%) in our clinical records, consistent with literature.
data) and the phenotype is not worse than carriers of a single mutation, it has to be considered in the assessment of the biological effect of variants of uncertain biological effect. Furthermore the presence of a second mutation has important consequences for genetic counselling of relatives. We suggest that mutation analysis of index cases should always be extended in order to avoid missing a second BRCA mutation.

P12.018-M
BRCA gene mutation carriers in an Irish Tertiary referral centre
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Aims: Germline mutations in BRCA1 and 2 confer a high risk of breast cancer. The aim of our study was to outline the disease phenotype and management of BRCA gene mutation carriers in the West of Ireland.

Methods: A longitudinal cohort study was undertaken. The study group included patients proven to carry a single gene mutation in BRCA1 or BRCA2 between 2000 and 2013. Clinicopathological information was obtained by chart review.

Results: Fourteen pathogenic mutations were identified in BRCA1 in 45 individuals from 24 families. The most common mutations were large genomic rearrangements, with deletion of exons 1-23 in 5 families, deletion exons 14-20 in 3 families and deletion exons 21-24 in 2 families. Fourteen pathogenic mutations were identified in BRCA2, in 32 individuals from 19 families. The most common was frameshift mutation 8525delC, in 5 families. Of forty-one patients affected with breast cancer, 17 carried mutations in BRCA1 and 24 mutations in BRCA2. Median age of onset of breast cancer in BRCA1 mutation carriers was 40 years (25-67), and 45 years (35-64) in BRCA2 mutation carriers (p=0.02, Mann-Whitney). Eight patients developed bilateral breast cancer, including 6 BRCA1 mutation carriers. Five of six patients with ovarian cancer carried BRCA1 mutations. Ten of 51 pre-symptomatic carriers underwent surgical prophylaxis, including 9 prophylactic mastectomies and 5 oophorectomies. Conclusions: BRCA gene mutations account for a small proportion of inherited predisposition to breast cancer. Carriers of these mutations require intensive surveillance or surgical prophylaxis. Counselling and testing of pre-symptomatic family members can facilitate intervention and modify disease phenotype.

P12.019-S
Characteristics of Greek patients with breast cancer rearrangements in BRCA1 gene
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In most countries large genomic rearrangements (LGRs) in BRCA1 and BRCA2 genes occur in a small percentage of patients tested for hereditary breast-ovarian cancer. Instead, in the Greek population, four specific LGRs have been identified in BRCA1 (deletions of exon 20 (4kb), exon 20 (3kb), exon 24 (4.5kb), and exons 23-24 (11kb)), the latter three of which have been characterized as founder mutations. Several factors may be associated with LGRs, such as younger age at breast cancer (BC) diagnosis, bilaterality and estrogen receptor-negative status. The study’s objective was to assess the possible establishment of criteria leading to targeted screening, as well as to increase our insight into the role and clinical significance of specific BRCA1 pathogenic findings.

In a cohort of 2,100 hereditary BC or OC patients, 74 (3.5%) were found to carry one of the four LGRs. We have investigated the possible association between BRCA1 LGRs and the aforementioned factors. The mean age at diagnosis was 40.6 years. Among the 74 patients, 43 (60%) developed BC, 15 (20%) both BC and OC, where 16 (20%) developed OC only 14 out of 74 developed bilateral (3/4), contralateral (9/14) and ipsilateral (2/14) BC. Histopathology data was available for 40 out of 58 BC patients, demonstrating that 29/40 (72%) were triple-negative, 6/40 (15%) were ER+/PR−/Her2−, 3 (7.5%) were ER+/PR+/Her2+, 1 (2.5%) was ER−/PR−/Her2− and 1 (2.5%) was ER+/PR+/Her2+. In conclusion, LGRs compared to other loss-of-function mutations of BRCA1 gene do not seem to be associated with specific clinical or histopathological features.
disease may contribute to the identification of new targets for future therapies. Tp53, Tp63 and Tp73 tumor suppressor family members encode for transcription factors which control genome integrity. They take part in cell response and in tumor suppression. Wild-type p53 protein is a growth modulator and its inactivation is a critical event in malignant transformation of breast cancer stem cells (SCs). Otx1 is a homeobox gene involved in central nervous system development, and when deregulated, plays a role in tumorigenesis. We showed that Otx1 is over-expressed in ductal and lobular invasive breast cancers and that is involved in adult mammary gland development. We demonstrated that p53 directly regulates Otx1 gene expression binding to the 3’p53 responsive element (RE) on its promoter, and that this pathway regulates the LA7 breast CSC differentiation. Here we will show that the Tp73a isoform of p73 is able to bind the S’p53 RE on the Otx1 promoter, leading to the LA7 cell differentiation and the asymmetric division of breast CSCs. Furthermore we will demonstrate that Otx1 and Tp73 are over-expressed in ductal and lobular invasive breast carcinoma. Finally we will show the functions of the Tp73a/Otx1 pathway in differentiation of mammospheres obtained from wild-type and c-ErbB2 transgenic mice, and the response to cisplatin treatment in MCF7 and MBA-MB-231 breast cancer cells.

P12.021-S
One in three Greek patients with early onset or familial breast cancer carries a loss of function mutation in a known or candidate breast cancer gene
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The antiquity of the Greeks as a population defined by language and culture, and the complexity of Greek historical demographics, present challenges to genetic testing for predisposition to cancer. The Greek population harbors ancient founder mutations in many genes, including five BRCA1 damaging alleles, as well as many disease alleles that are specific to one or a few families. The aim of this study was to identify loss-of-function mutations in 22 genes in 736 patients with breast cancer diagnosed at a young age (<35 years) or with a strong family history of breast, ovarian, and/or pancreatic cancer. Targeted capture and multiplexed sequencing was carried out using BROCAtm, which captures the entire loci of 22 genes. Of 736 patients with young onset or familial breast cancer, 252 (34%) carried loss-of-function mutations in one of 11 genes. Frequencies were: 103 BRCA1 founders, 61 other BRCA1, 45 BRCA2, 29 CHEK2, 7 PALB2, 7 ATM, 2 PTEN and 1 in each in RAD51C, FAM175A, BRIP1, PIK3CA and TP53. PALB2 p.R753X was observed in four Greek families and may be a founder allele. We conclude that among Greek patients with familial or early onset breast cancer more than a third carry loss-of-function mutations in a breast cancer-related gene. Founder mutations account for about 50% of the BRCA1 and BRCA2 mutational burden and about 40% of the mutational burden in all known and candidate breast cancer genes. Generations of these heterozygous patients benefit from an approach that detects all classes of mutations in known breast cancer genes.

P12.022-M
Mutations in CHEK2 and NBN genes among Macedonian breast cancer patients
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Heterozygotes for mutations in CHEK2 and NBN genes were found to be associated with increased risk of developing cancers, including breast cancer (BC). The aim of our study was to determine the frequency of three common mutations in CHEK2 gene (1100delC, 1157delT and ISV2*1G>A) and two mutations in NBN gene (R215W and 657del5) among Macedonian BC patients and controls from the general population. For this purpose we have designed a multiplex PCR followed by SNaPshot analysis. A total of 299 BC patients, of whom 112 with a cancer family history and 183 controls were included in the study. Mutations were more frequent among BC patients (n=13, 4.3%) than among controls (n=1, 0.3%), although without statistical significance. Twelve patients were heterozygous for one of the analyzed mutations, while one patient had two mutations (NBN R215W and CHEK2 I1157T). The most frequent mutation was CHEK2 I1157T, found in 10 BC patients and 4 controls. The frequency of this mutation was statistically higher among BC patients with a cancer family history when compared to the controls (p=0.028). NBN R215W was found in one BC patient and one control, while CHEK2 1100delC and NBN 657del5 were found each in one BC patient and one control. CHEK2 ISV2*1G>A has not been found in our study. In conclusion, our study suggested that mutations in CHEK2 and NBN genes might play a role in the development of breast cancer among Macedonian patients. A study including larger number of patients and controls are needed to confirm these results.

P12.023-S
BRCA1 and BRCA2 mutation detection by a Next Generation Sequencing approach: an epidemiological study conducted in Southern Italy
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Hereditary breast and ovarian cancer (HBOC) accounts for about 10% of all breast cancer cases and BRCA1/BRCA2 mutations are considered to be the most frequent pathogenic alteration. Several studies have investigated the role played by BRCA1/BRCA2 germline mutations in detecting the risk of developing HBOC by up to 20 fold. Testing for BRCA gene mutations is important to improve the clinical management of the high-risk patients and of their mutation carriers family members. A NGS screening for BRCA1/2 germline mutations of 300 patients, with early-onset breast cancer ("under forty") and/or with positive family history, is reported in order to identify mutation carriers. BRCA1/BRCA2 coding regions were amplified using the BRCA MASTR v2.1 Assay (Multiplicom). Sequencing reactions were performed with the 454 GS FLX System (Roche) and the downstream data analysis was carried out through the SeqNxt tool (JSI Medical Systems).

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genes are indeed intermediate-risk BC susceptibility genes (OR= 2.88, P = 0.0090). In addition we found that key domain missense substitutions were more frequent (24 vs 12 observations) than the truncating variants and conferred a slightly higher OR (3.07 vs 2.61) with a lower P-value (0.029 vs 0.014). Testing the sequence of pathogenic variants in MRN genes includes, as for ATM and CHEK2, a relatively high proportion of missense, and differs notably from the BRCA1/2 pattern where most susceptibility alleles are protein-truncating variants.

P12.025-S Search for recessive cancer predisposition genes: the advantage of multiple primary cancer cases vs. family history-positive patients

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Conclusion: Taken together, we demonstrate that NGS is a fast and cost efficient genetic screening tool to analyze for variants in genes associated with hereditary breast cancer.

We identified 15 mutations that are known to cause HBOC as well as mutations in the BRCA1 and BRCA2 genes. High depth (> 50x) was observed only for the pathogenic (c.1070delTAAG in BRCA1 and c.731delTC in BRCA2) and common (c.3051delTAAG in BRCA1 and c.1132delTC in BRCA2) variants. The rare variant (c.3051delTAAG in BRCA1) was deleted from the BRCA1/2 pattern where most susceptibility alleles are protein-truncating variants.

P12.026-M High-throughput genetic analysis in 100 hereditary breast cancer patients

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Objective: Here, we report our first results from our HBOC-NGS-panel that includes genes associated with breast and ovarian cancer. So far, 100 samples were analyzed in a diagnostic setting.

Methods: A custom NGS-panel (HaloPlex, Agilent) was used to target 56 genes. Depending on the patient’s family history, a set of “diagnostic” genes (mostly BRCA1/2, RAD51C/D) as well as “screening” genes were defined. DNA was isolated from all samples, enriched and sequenced on the Illumina MiSeq (2x 150 bp paired-end) following standard protocols. For diagnostic genes, regions with low depth (< 20x) were complemented by Sanger sequencing as well as MLPA. All mutations were confirmed by conventional Sanger sequencing.

Results: So far, we have sequenced 100 hereditary breast cancer patients. Overall 91-99.9 % of all targeted exons were represented with a “diagnostic” average depth of > 20x. Roughly 70 SNVs were identified per sample and stringent filtering resulted in less than seven variants for validation. We identified 15 mutations that are known to cause HBOC as well as mutations likely to cause HBOC. Further experiments and segregation analysis is required to determine the pathogenicity in the latter. In addition heterozygous mutations were found in genes relevant for different autosomal recessive cancer syndromes.

Conclusion: Taken together, we demonstrate that NGS is a fast and cost efficient genetic screening tool to analyze for variants in genes associated with the development of hereditary breast cancer. By applying this approach we were able to uncover both known and novel sequence variants.

P12.027-S BRCA1 and BRCA2 germline mutational spectrum among Macedonian women with breast cancer detected by next generation sequencing

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Virtually all known tumor predisposition genes have been identified via the analysis of familial cancer cases. Here we argue that this approach is likely to miss recessively acting cancer genes and suggest the analysis of family history-positive patients as a more cost-effective approach for mutational screening of genes, regions with low depth (< 20x) were complemented by Sanger sequencing as well as MLPA. All mutations were confirmed by conventional Sanger sequencing.

In conclusion, our work demonstrates that using TruSeq Custom Amplicon technology for mutational screening of cancer-predisposing genes offers notably from the BRCA1/2 pattern where most susceptibility alleles are protein-truncating variants.

P12.028-M Testing for genetic predisposition to breast cancer by NGS

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Testing for genetic predisposition to breast cancer by NGS seems a useful tool for the identification of genetic risk factors. In this study, DNA samples were obtained from 27 individuals with positive family history – affected or still not affected. Informed consent was obtained from all the subjects. Sequence analysis for BRCA1/BRCA2 or 94 cancer predisposing genes and 284 variants associated with cancer was performed on Illumina MiSeq system.

A wide range of variants were identified in the BRCA1 and BRCA2 genes. About 5-10% of breast cancer is due to inherited mutations. Genetic testing for predisposition in individuals with family history is recommended to determine their risk for developing this cancer type. Next generation sequencing enables us to perform analysis for large number of predisposing genes.

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Germline deleterious mutations of PALB2 are associated with breast cancer risk and have been reported in several populations. In our initial survey, truncating PALB2 mutations were detected in 12/575 (2.1%) familial breast cancer cases negative for BRCA gene mutations (BRCAx), recruited at two large cancer centres in Milan (Istituto Nazionale Tumori and Istituto Europeo di Oncologia). One mutation (c.1027T>C; p.Gln343X) reoccurred in both cases, of whom original family came from the province of Bergamo. The genotyping of the c.1027T>C in 113 BRCA cases ascertained at Azienda Ospedaliera HPG23 of Bergamo, detected a total of 6 carriers (5.3%), while only 2 carriers were observed among 477 female blood donors recruited in the same area (0.4%). The estimated age-adjusted odds ratio of these frequencies was 13.4 (95% confidence interval: 2.7–67.4). This value is similar to those previously observed for a few PALB2 mutations recurrent in other countries and suggests that the c.1027T>C-T is associated with a relatively high breast cancer risk. Of note, we also found that among breast/ovarian cancer families recruited in Bergamo the two analysed spectra or both BRCA1 and BRCA2 were much less heterogenous than those families ascertained in Milan. In particular, one BRCA1 founder mutation was observed in >8% of Bergamo families (Caleca et al, 2014). Further analyses are required to verify whether genotyping for specific recurrent mutations can be proposed as a cost-effective strategy for the rapid identification of individuals genetically predisposed to breast/ovarian cancer in the Bergamo area.

**P12.030-M**

RNA-Sequencing in MCF-7 cells: identification of a new transcript of SEMA3F and its expression in breast cancer

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Breast cancer is the most common tumor in women, and the second leading cause of death. Tumor cell invasiveness is mainly due to an alteration of cell-cell and cell-matrix connections. Thus, an altered expression of adhesion molecules and their receptors is a crucial event in this process. Among them, semaphorins, a large family of transmembrane or secreted molecules that regulate cell migration and adhesion, are of peculiar interest. A growing number of studies has recently been reported on the role of these molecules and their receptors in cancer progression, often with divergent functions.

In order to simultaneously investigate gene expression and alternative splicing for all the genes encoding adhesion molecules, and particularly for semaphorins, their receptors and co-receptors, we performed RNA-Sequencing experiment on MCF-7 cells, a well-established and widely used cellular model of breast cancer. Interesting preliminary results were obtained, particularly for SEMA3F gene. Indeed, we identified a novel transcript generated by alternative splicing predicted to encode for a truncated semaphorin. Moreover, through semi-quantitative PCR and quantitative Real-Time assay we measured SEMA3F expression on a panel of breast cancer tissues compared to those previously observed for a few PALB2 mutations recurrent in other countries and suggested that the c.1027T>C is associated with a relatively high breast cancer risk. Of note, we also found that among breast/ovarian cancer families recruited in Bergamo the two analysed spectra or both BRCA1 and BRCA2 were much less heterogenous than those families ascertained in Milan. In particular, one BRCA1 founder mutation was observed in >8% of Bergamo families (Caleca et al, 2014). Further analyses are required to verify whether genotyping for specific recurrent mutations can be proposed as a cost-effective strategy for the rapid identification of individuals genetically predisposed to breast/ovarian cancer in the Bergamo area.

**P12.031-S**

Investigating the importance of variants at 12p11, 12q24 and 21q21 in breast cancer in the west of Ireland


Introduction: Recent genome-wide association studies have identified new breast cancer susceptibility loci in women of European ancestry at 12q24 (rs10717399 and rs2823099), 12p11 (rs10717399) and 21q21 (rs2823099). The aim of our study was to investigate the prevalence of variants at these three loci in a specific Irish subpopulation, and to examine the association between these variants and breast cancer in this cohort.

Methods: DNA was extracted from the whole blood of patients with breast cancer and from healthy female controls using a salting out method. Genotyping of each sample for each of the three targets was carried out using the Taqman®-based platform. Statistical analysis was performed using SPSS software after testing for Hardy-Weinberg equilibrium.

Results: A total of 1639 samples were included in the study group, comprising 1191 cases and 448 controls. The minor allele at locus 12p11 was found to confer a significant protective effect, with a per allele odds ratio of 0.7 (0.5–0.9; p=0.002, X2). The minor allele at 12q24 had a slight protective effect (per allele OR =0.9 (0.8-1.1, p =0.29, X2)). The minor allele 21q21 did not have a protective effect, and was in disequilibrium as per test of Hardy-Weinberg.

Conclusion: All three genetic variants were detected in the population in the west of Ireland. The G allele at 12p11 was associated with reduced breast cancer risk. Population-specific genome wide association studies are required to identify susceptibility loci specific to the Irish subgroup.

**P12.032-M**

DNA-diagnostics for inherited breast and/or ovarian cancer: standard approaches and novel technologies

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Modern molecular techniques allow revealing the most characteristic genetic changes responsible for hereditary breast and/or ovarian cancer (bH/BC; OC), calculating the risk of neoplasia development, defining treatment and surveillance strategies. In routine laboratory practice, a cost-effective strategy for the rapid identification of individuals genetically predisposed to breast cancer is the DNA diagnostics for inherited breast and/or ovarian cancer.

Analyzing inherited breast and/or ovarian cancer syndromes, such as the Li-Fraumeni syndrome, the Cowden syndrome or the Bardenheuer syndrome, it has been demonstrated that many mutations lead to cancer risk and have been reported in several populations. In our initial survey, truncating PALB2 mutations were detected in 12/575 (2.1%) familial breast cancer cases (BRCAx). Recruited at two large cancer centres in Milan (Istituto Nazionale Tumori and Istituto Europeo di Oncologia). One mutation (c.1027T>C; p.Gln343X) reoccurred in both cases, of whom original family came from the province of Bergamo. The genotyping of the c.1027T>C in 113 BRCA cases ascertained at Azienda Ospedaliera HPG23 of Bergamo, detected a total of 6 carriers (5.3%), while only 2 carriers were observed among 477 female blood donors recruited in the same area (0.4%). The estimated age-adjusted odds ratio of these frequencies was 13.4 (95% confidence interval: 2.7–67.4). This value is similar to those previously observed for a few PALB2 mutations recurrent in other countries and suggests that the c.1027T>C-T is associated with a relatively high breast cancer risk. Of note, we also found that among breast/ovarian cancer families recruited in Bergamo the two analysed spectra or both BRCA1 and BRCA2 were much less heterogenous than those families ascertained in Milan. In particular, one BRCA1 founder mutation was observed in >8% of Bergamo families (Caleca et al, 2014). Further analyses are required to verify whether genotyping for specific recurrent mutations can be proposed as a cost-effective strategy for the rapid identification of individuals genetically predisposed to breast/ovarian cancer in the Bergamo area.

**P12.033-S**

The experiences and views of health care professionals and researchers regarding the feedback of results in the context of next-generation sequencing in oncology

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Next generation sequencing (NGS) allows the production of large volumes of sequence data (and potentially genetic results) and the ethical and practical issues regarding feedback of results become particularly pertinent to address. Should (any) results be given to research participants? If so, which results and who should provide them? Within two EU funded projects in oncology (CAGEKID and EUROTARGET), in order to gather researchers’ and health care professionals’ views and experiences on providing results we distributed a questionnaire to attendees of genetics meetings in Europe in 2011 and 2013. Of the 95 respondents, 88% work as researchers and/or clinicians in a field related to oncology and half (52%) use NGS in some aspect of their work; 56% of respondents state that they provide specific information about NGS to participants or patients before enrolling them in a study or using their samples for sequencing. The majority, 83% had never received requests for results of tests from participants or patients for access to NGS data to inform treatment decisions. Regarding feedback of results in a research setting, 54% or respondents think that results stemming from NGS studies should be provided to individual participants and 72% think that actionable incidental findings should be disclosed to participants. Finally, 53% of respondents think that specific measures and/or limitations should be implemented for the sharing of NGS data/results with colleagues in the scientific community. Such empirical data from stakeholders is a valuable contribution to the ongoing discussion of how to responsibly handle and feedback results to patients and research subjects.
whole-genome profiling of cervical carcinomas patients with CGH+SNP microarrays: correlations with clinical outcome

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Alterations in the genome that lead to changes in DNA sequence copy number are characteristic features of solid tumors. In this study, we used CGH+SNP microarray technique for detailed screening of copy number alterations (CNAs) in a cohort of 26 patients with uterine cervical carcinoma (UCC) and the findings were correlated with the incidence of lymph node metastasis and myometrial invasion. The whole-genome screening discovered CNAs in 73.1% of samples. Frequent areas of gains were observed in 3q (50.0%), 1q (42.4%), 19q (23.1%), while losses were commonly observed in 11q (30.8%), 4q (23.1%), 13q and 2q (both 19.2%). Regions of loss of heterozygosity were observed in 15.4% in 1q23, 14q21, 18q12.2 and 8q21. The incidence of gain 3q was associated with gain 1q (P=0.035), while loss of 4p was commonly observed with loss of 13q (P=0.010). Higher occurrence of CNAs was associated with patients under 45 years (P=0.016). Patients with adenocarcinoma have statistical trend to carry genomic profiles without CNAs (P=0.051). Incidence of lymph node metastases was associated higher number of CNAs (12 vs 9; P=0.045) and patients without LVS had trend to higher incidence of gains in chromosome 1q. Taking together array-CGH technique allowed us to precisely detect specific cytogenetic abnormalities in UCC, which can be used for prognosis of the disease as well as novel genomic markers associated with the development of invasive cervical cancer.

Supported by OPVK (Z.1.07/2.30/00183)

P12.035-M

Human bile contains microRNA-laden extracellular vesicles that can be used for cholangiocarcinoma diagnosis

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Cholangiocarcinoma (CCA) presents significant diagnostic challenges, resulting in late patient diagnosis and poor survival rates. Primary Sclerosing Cholangitis (PSC) patients pose a particularly difficult clinical dilemma, since they harbor chronic biliary strictures that are difficult to distinguish from CCA. MicroRNAs (miRs) have recently emerged as a valuable class of diagnostic markers; however, thus far, neither extracellular vesicles (EVs) nor miRs within EVs have been investigated in human bile. We aimed to comprehensively characterize human biliary EVs, including their miR content. Conclusion. We have established the presence of extracellular vesicles in human bile. In addition, we have demonstrated that human biliary EVs contain abundant miR species, which are stable and therefore amenable to the development of disease marker panels. Furthermore, we have characterized the protein content, size, numbers and size distribution of human bile EVs. Utilizing Multivariate Organism of Combinatorial Alterations (MOCA), we defined a novel biliary vesicle miR-based panel for CCA diagnosis which demonstrated a sensitivity of 67% and specificity of 96%. Importantly, our control group contained 13 PSC patients, 16 patients with biliary obstruction of varying etiologies (including benign biliary stricture, papillary stenosis, choledocholithiasis, extrinsic compression from pancreatic cysts, and cholangitis), and 3 patients with bile leak syndromes. Clinically, these types of patients present with a biliary obstructive clinical picture that could be confused with CCA. These findings establish the importance of using extracellular vesicles, rather than whole bile, for developing miR-based disease markers in bile.

P12.036-M

Functional studies on post-transcriptional regulation of FAS/FASL in chordoma

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Chordoma, originating from notochord remnants, is characterized by chemoresistance. Since the apoptotic Fas/FasL pathway is involved in notochordal cell death, we wanted to investigate its potential role in chordoma. In this work we detected the lack of FAS mRNA and the presence of both FAS anti- and pro-apoptotic isoforms and the inactive Caspases 8 and 3 forms in most of the tumors analyzed, suggesting the Fas/FasL pathway inactivity in chordoma. The enhancement of apoptosis in U-CH1 chordoma cells, expressing Fasl and Fas anti- and pro-apoptotic isoforms after Soluble Fasl treatment, indicates that this pathway can be re-activated. These findings lead us to hypothesize that Fas receptor binds Soluble Fasl that, undermining Fas anti-apoptotic isoform, activates apoptosis. Soluble Fasl isoform could have a key role as antiapoptotic factor as the alternative splicing underlying the expression regulation of the two Fasl isoforms. Knowing that HuR is one of the splicing factors leading to Fas transmembrane isoform, we are performing functional studies aimed at modulating the reciprocal amount of Fas isoforms by interfering with the expression of HuR. U-CH1 apoptosis, cellular vitality and migration will be evaluated after HuR (per)expression and silencing to assess the role of Fas antiapoptotic isoform in chordoma apoptosis regulation. We are also silencing miR-21 targeting FASL mRNA to verify whether the increase of endogenous Fas may activate apoptosis. This study, providing findings on the involvement of Fas/Fasl pathway in chordoma could help to elucidate the role of apoptosis in chordoma tumorigenesis, addressing the identification of new potential pharmacological targets.

P12.037-S

Transcriptome analysis of cancer cells in chronic myeloid leukemia

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Long term observation studies confirm high efficiency of targeted therapy of chronic myeloid leukemia (CML) by tyrosine kinases inhibitors (TKI). However part of CML patients demonstrate primary resistance to TKI. This resistance appears to be connected with activation of alternative BCR-ABL independent signaling pathways. Transcriptome analysis of cancer cell in CML is a perspective approach to elucidate molecular mechanisms of TKI resistance and to find new approaches to CML treatment. We aim to find and to study differences in expression levels of cancer cells in primary CML patients, who demonstrated sensitivity or resistance to TKI. Gene expression profiles were analyzed using Illumina HT-12 Expression Bead Chip. These chips quantitate expression levels of more than 47000 transcripts. According to European Leukemia Net (2013) criteria patients were divided into resistant to TKI therapy - molecular response <10% in 6 months of therapy and optimal responders with molecular response >1% in 6 months of therapy. Comparative transcriptome analysis revealed 2672 of differently expressed genes in responders and non-responders. Enrichment analysis of the differently expressed genes showed the following molecular networks involved (p < 0.05): HTLV-1 infection (FZD10, ADCY1, MYB); PPAR signalling pathway (SCD-1, OLR1); Transcriptional misregulation in cancer (MPO, CEBPE, ELANE); Melanogenesis (ADCY1, FZD10). Detailed analysis demonstrated that all of the selected genes are overexpressed in cancer cells of patients sensitive to TKI therapy. Differently expressed genes and expression particularities of the selected pathways may be useful molecular markers for prognosis of the TKI therapy efficacy and may be used to optimize treatment of CML.

P12.038-M

XRC4 gene (Intron 3 VNTR) polymorphism predisposition to chronic phase chronic myeloid leukemia (CML) and XRC1 gene (399) polymorphism in associated with event-free survival in CML treated with imatinib in Turkish population

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According to European Leukemia Net (2013) criteria patients were divided into resistant to TKI therapy - molecular response >10% in 6 months of therapy and to study differences in expression levels of cancer cells in primary CML patients, who demonstrated sensitivity or resistance to TKI. Gene expression profiles were analyzed using Illumina HT-12 Expression Bead Chip. These chips quantitate expression levels of more than 47000 transcripts. According to European Leukemia Net (2013) criteria patients were divided into resistant to TKI therapy - molecular response <10% in 6 months of therapy and optimal responders with molecular response >1% in 6 months of therapy. Comparative transcriptome analysis revealed 2672 of differently expressed genes in responders and non-responders. Enrichment analysis of the differently expressed genes showed the following molecular networks involved (p < 0.05): HTLV-1 infection (FZD10, ADCY1, MYB); PPAR signalling pathway (SCD-1, OLR1); Transcriptional misregulation in cancer (MPO, CEBPE, ELANE); Melanogenesis (ADCY1, FZD10). Detailed analysis demonstrated that all of the selected genes are overexpressed in cancer cells of patients sensitive to TKI therapy. Differently expressed genes and expression particularities of the selected pathways may be useful molecular markers for prognosis of the TKI therapy efficacy and may be used to optimize treatment of CML.
Whole-exome sequencing identifies rare coding variants in new predisposition genes for familial colorectal cancer
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Colorectal cancer (CRC) represents one of the most frequent neoplasms in Europe. Besides classical hereditary forms, around 30% of cases present familial aggregation mostly with an unknown inherited cause. Next generation sequencing permits to characterize genetic variation in one individual and to discover new disease predisposition genes. Material and methods: Patients were selected from high-risk clinics from Spanish hospitals as well as from the EPICOLON consortium. Forty-two individuals from 29 families with strong CRC aggregation compatible with an autosomal dominant pattern of inheritance and without alterations in the known CRC hereditary genes were selected. Exome sequencing was performed in germline DNA with subsequent quality control, removal of sequencing artifacts, data annotation and variant filtering. An automatic CRC-specific pipeline was used for prioritization.

Results: Sequencing mean coverage was >95x. Only very rare variants, producing a clear loss of function and located in genes with a function related to CRC or cancer were selected as final candidates. Afterwards, they were validated by Sanger sequencing and segregation was studied in additional affected family members when available. Loss of heterozygosity in tumor DNA was analyzed in variants with correct disease segregation. Best candidate variants included those located in interesting genes such as CDKN1B, XRCC4, EPX1, NRBP2, SMARCA4, BRCA2 and BAP1. Three variants are expected to abolish protein function and 4 missense changes had strongly deleterious in silico predictions.

Conclusions: We identified variants in DNA repair genes that represent good candidates to become new CRC predisposition genes, some of them previously involved in other neoplasms.

Whole Exome Sequencing of Hereditary Colorectal Cancer Families from Canada
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Familial Colorectal Cancer Type X (FCTX) is an inherited predisposition to colorectal cancer (CRC) in families fulfilling the Amsterdam-I criteria. A syndrome of unknown etiology, FCTX is genetically heterogeneous. FCTX families appear to have a moderate to high lifetime risk of developing CRC. A cohort of 91 FCTX patients (66 families) from the Canadian provinces of Ontario and Newfoundland & Labrador (NL) were selected for whole exome sequencing of genomic DNA. A pooled segregation analysis of all missense variants recurrent in patients within two or more families identified 93 candidate missense variants. We then prioritized missense variants with low minor allele frequency (<1%), deleterious prediction of protein consequence, and to discover new disease predisposition genes.

Colorectal cancer susceptibility genes are not limited to classical hereditary forms, but also include a large group of common genetic factors. The role of genetic factors in the etiology of CRC is a complex one, involving multiple genes and genetic variations. The identification of novel candidate genes for CRC susceptibility will provide conclusive evidence for the role of these genes in CRC development.
**Colorectal cancer (CRCs) with microsatellite instability (MSI)** represent 15% of all CRCs, and occur in patients with Lynch syndrome. MSI CRCs have a more dense cytotoxic T lymphocyte (CTL) infiltration and a better prognosis than microsatellite stable (MSS) CRCs. Stronger MSI and microsatellite instability (MSI) is commonly explained by the presence of frameshifting mutations in genes containing repeated coding sequences which could lead to the synthesis of neo-antigens recognized by specific CTLs. First, to explore the direct link between infiltrating CTL density and frameshifting mutations, we quantified within 121 MSI tumors from two independent series: CTL (CD8+) density, using tissue microarrays, and we searched for frameshifting mutations in repeated coding sequences of 19 genes. We found that infiltrating CTL density significantly increased with the number of frameshifting mutations, and was particularly correlated with frameshifting mutations in AST1E, HNF1A and TCCT7L2 genes. Second, we undertook to stimulate, in vitro, patients’ peripheral blood CTLs specific of their own tumor neo-antigens, using artificial antigen presenting cells (AACPs) developed in our laboratory and expressing the corresponding neo-peptides. We could indeed easily and efficiently activate such CTLs, in two tested Lynch syndrome patients. These results suggest that, in MSI CRC patients, and especially in Lynch syndrome patients, it should be possible to propose new personalized adoptive cell immunotherapy strategies based on the characterization of frameshifting mutations within their tumor and on the construction of AACPs expressing the corresponding neo-peptides.

**P12.04-M Diagnostic criteria for constitutional mismatch repair deficiency (CMMR-D) syndrome: suggestions of the European consortium “Care for CMMR-D” (C4CMMRD)**

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Constitutional mismatch repair deficiency (CMMR-D) syndrome is a distinct childhood cancer predisposition syndrome that results from biallelic germ-line mutations in one of the four MMR genes, MLH1, MSH2, MSH6, or PMS2. The tumour spectrum is very broad, including mainly haematological, brain and intestinal tract tumours. Patients show a variety of non-malignant features that are indicative of CMMR-D. However, currently no clinical criteria that should entail diagnostic evaluation of CMMR-D exist. The recently established European consortium care for CMMR-D (C4CMMRD) proposes a 3-step diagnostic strategy for patients presenting for the first time a potential diagnosis of a young adult cancer patient. Tumours highly specific for CMMR-D syndrome are assigned 3 points, malignancies overrepresented in CMMR-D 2 points and all other malignancies 1 point. According to their specificity for CMMR-D and their frequency in the general population additional features are weighted with 1 - 2 points. They include multiple hyper- and hypopigmented skin areas, brain malformations, pilomatrixomas, a second childhood malignancy, a LS-associated tumour in a relative and parental consanguinity. According to the scoring system CMMR-D should be suspected in any cancer patient who reaches a minimum of 3 points by adding the points of the malignancy and the additional features. We expect that application of the here suggested strategy for CMMR-D diagnosis will increase the number of patients being identified at the time when they develop their first tumour. This will allow adjustment of the treatment modalities, offering surveillance strategies for second malignancies and appropriate counselling of the entire family.

**P12.044-M Fourfold increased detection of Lynch syndrome by raising age limit for tumour genetic testing from 50 to 70 years is cost-effective**

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**Objective:** Recognizing colorectal cancer (CRC) patients with Lynch syndrome (LS) can increase life expectancy of these patients and their close relatives. To improve identification of this under-diagnosed disease, experts suggested raising the age limit for CRC tumour genetic testing from 50 to 70 years. The present study evaluates the efficacy and cost-effectiveness of this strategy.

**Design:** Probabilistic efficacy and cost-effectiveness analyses were performed comparing tumour genetic testing of CRC diagnosed at age 70 or below (experimental strategy) versus CRC diagnosed at age 50 or below (current practice). The proportions of LS patients identified and cost-effectiveness including cascade screening of relatives, were calculated by decision analytic models based on real life data.

**Results:** Using the experimental strategy, 4 times more LS patients can be identified among CRC patients as compared to current practice. Both the cost of genetic test one LS patient (£ 9437/carrier versus £ 4855/carrier) and the number needed to test for detecting one LS patient (42 versus 19) doubled. When including relatives the experimental strategy was found to be highly cost effective because CRC prevention in relatives neutralised the extra genetic testing costs, resulting in lower costs (£ 2180 per extra tested patient) and an average of 9 life years gained.

**Conclusion:** Testing CRC tumours diagnosed at age 70 or below for LS is cost-effective. Implementation is important as relatives from the large number of LS patients that are missed by current practice, can benefit from life-saving surveillance.

**Clinical relevance of 8q23.3, 15q13.3 and 18q21.1 SNP genotyping to evaluate colorectal cancer risk**

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The aim of this study was to determine if the at risk SNP alleles for colorectal cancer (CRC), previously identified by GWAS studies, could either alone or in combination contribute to clinical presentations suggestive of an increased genetic risk for CRC. We performed a prospective national case-control study based on highly selected patients (CRC in two first degree relatives, one being diagnosed before 61 years; or CRC before 51 years; or multiple primary CRCs, the first before 61 years; exclusion of Lynch syndrome, adenomatous and hamartomatous polyposes), and appropriate controls corresponding to healthy volunteers, between 45 and 60 years of age, without personal or familial history of CRC. We included 1029 patients and 350 controls. We confirmed the association of CRC risk with 5 SNPs, with odds ratio (OR) higher than previously reported: rs16929766 on 8q23.3 (OR: 1.99; p=0.0006); rs4779584 on 15q13.3 (OR: 1.45; p=0.0033), and rs4939827 and rs58920878 on 18q21.1 (OR: 1.49; p=0.0016 and OR: 1.48; p=0.039). We found a significant cumulative effect of the at risk alleles or genotypes with OR at 1.62, 2.11, 2.94 and 3.95 for 1, 2, 3 and at least 4 at risk alleles or OR at 1.71, 2.32 and 6.31 for 1, 2 and 3 at risk genotypes. This study suggests that genotyping of a limited number of SNPs, such as 8q23.3 rs16929766, 15q13.3 rs4779584 and 18q21.1 rs58920878, should allow to identify subjects at increased risk of CRC who might benefit from early CRC detection.

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**References:**

1. Sie AS, Mense AR, Madon AM, Ligtjen MJ, Hoogerbrugge N. Fourfold increased detection of Lynch syndrome by raising age limit for tumour genetic testing from 50 to 70 years is cost-effective. 
2. Sie AS, Mense AR, Madon AM, Ligtjen MJ, Hoogerbrugge N. Fourfold increased detection of Lynch syndrome by raising age limit for tumour genetic testing from 50 to 70 years is cost-effective.
P12.047-S

Constitutional mismatch repair deficiency due to a homozygous MSH6 mutation in a 14-year-old boy with colonic polyps, colorectal cancer and retinal lesions

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Constitutional Mismatch Repair Deficiency (CMMR-D) is a rare cancer predisposition syndrome in patients with biallelic mutations in one of MLH1, MSH2, MSH6 or PMS2. Heterozygous mutations cause the adult onset, dominant cancer syndrome Lynch syndrome. However, CMMR-D causes childhood onset cancers, particularly hematologic, brain and gastrointestinal malignancies. Children with CMMR-D also may have skin pigment findings suggestive of neurofibromatosis type 1. We report on a 14-year-old boy with a previous kaposiform hemangioendothelioma who was diagnosed with at least 100 colonic polyps and 6 invasive adenocarcinomas of the colon and rectum. He had café au lait macules, axillary and inguinal freckling and bilateral, multifocal congenital hypertrophy of the retinal pigment epithelium (CHRPE). Therefore he fit diagnostic criteria for both NF1 and Familial Adenomatous Polyposis. He also had a previous clinical diagnosis and family history of Cleidocranial Dysplasia. There was no family history of colon cancer and he had 2 siblings.

The colon cancers were MSI-H and by immunohistocmetry the tumour cells and adjacent normal mucosa were negative for MSH6 and positive for MLH1, MSH2 and PMS2. A homozygous pathogenic MSH6 mutation was identified (c.3202C>T) in the patient and both parents were found to be heterozygous. Genetic testing of the APC and MUTYH genes was normal.

To our knowledge, this is the first reported case of CMMR-D with CHRPE and demonstrates difficulties in making this diagnosis in a patient who fits the clinical criteria for both NF1 and FAP. It also illustrates ethical dilemmas around genetic testing of at risk siblings.

P12.048-M

Recurrent copy number alterations in prostate cancer: an in silico meta-analysis of publicly available genomic data

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We present a meta-analysis of somatic copy number alterations (CNAs) from eleven publications that examined 662 prostate cancer patient samples, derived from 546 primary and 116 advanced tumors. Normalization, segmentation and identification of corresponding CNAs for meta-analysis was achieved using established commercial software (Nexins). Unsupervised analysis identified five genomic subgroups in which ~80% of the samples had an abnormal profile characterized by qg gains. The most common loss was at 8p (NIX3.1). The CNA distribution in other genomic subgroups was characterized by losses at 2q, 3p, 5q, 6q, 13q, 16q, 17p, 18q and 1q (PTEN), and acquisition of 21q deletions associated with the TMPRSS2-ERG fusion rearrangement. Parallel analysis of advanced and primary tumors in the cohort indicated that genomic deletions of PTEN and the gene fusion were enriched in advanced disease. A supervised analysis of PTEN deletion and the fusion gene showed that when PTEN was deleted the overall percentage of the genome altered was significantly higher, suggesting that this important genomic subgroup was likely characterized by intrinsic chromosomal instability. Predicted alterations in expression levels of candidate genes in each of the recurrent CNA regions characteristic of each subgroup showed that signaling networks associated with cancer progression and genome stability were likely to be perturbed at the highest level in the PTEN deleted genomic subgroup.

P12.049-S

In-depth comparison of available targeted resequencing strategies for BRCA gene panels

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Next-Generation Sequencing is currently the technique of choice for genetic testing in genetic heterogeneous diseases. The broad spectrum of available target enrichment methods complicates the selection of the most suitable technique considering the size of the targeted region, the required depth of coverage to correctly identify variants, the number of samples to be sequenced, the turnaround time and the overall cost. Here we report a comprehensive comparison of four different commercial targeted enrichment kits (Haloplex, Nexten, Multiplicom and SureSelect) for the selection of the most convenient strategy to fully characterize the coding regions of BRCA genes in an Illumina benchtop sequencer. Most of samples were analysed with 1-3 different kits. Following clinical standards, we evaluated the analytical sensitivity, the analytical specificity, false positive and negative rates, the assay robustness and the precision for each of the considered capturing techniques. Our results demonstrate that technical and functional differences exist between capturing methods that could compromise clinical diagnosis if specific method-associated deviations are not considered.
Evx1 gene expression analysis in ESCC patients globally. To the best of our knowledge, this is the first report of VentX and roles in development of the disease through malfunction of BMP signaling progression of tumor stage and increased depth of tumor invasion. Our results - VentX was significantly underexpressed in 76% of ESCC tissues (P<0.05). Ewing sarcoma is a cancer that often presents in the second decade of life the great attention in studying the malignant progression of cells through ly defined. According to the importance of ESCC in this high risk area and - cancer, shows striking variations in geographic distribution reflecting expos -cancer (OC) predisposition. Given the fact that most risk families do not carry -cell-line, was conducted on an Ion Torrent Proton™ system to profile the -myeloablative chemotherapy. Whole-transcriptome and exome sequencing and is usually associated with a chromosomal translocation that results in a EWS/FL1 gene fusion. Four independent cell-lines have been established from a subject who succumbed to metastatic disease following relapse after myeloablative chemotherapy. Whole-transcriptome and exome sequencing of primary bone marrow-derived stromal cells and Ewing Sarcoma Tumor-Derived Virus (EBV) transformed normal lymphoblasts, a pre-therapy primary tu- mor-derived cell-line, and a post-chemotherapy metastatic tumor-derived cell-line, was conducted on an Ion Torrent Proton™ system to profile the differences in gene expression and in exon DNA sequence to characterize the molecular changes associated with primary tumorigenesis and disease persistence after treatment. The presence of the EWS/FL1 fusion gene in primary tumor cells was confirmed by the blocking of the breakpoint and observation of chimeric reads in the RNA-seq data and exome sequence analy- ses. Exome datasets indicate apparent loss of heterozygosity genome-wide in CHLA10 consistent with cytogenetic analysis that shows tetraploidy in this cell-line. Results from RNA-seq also indicate numerous instances, genome-wide, of differing transcript isoform expression and exon usage between normal, primary tumor, and metastatic tumor cells suggesting an increasing genomic mutational burden in the evolution of the disease, and pointing in particular toward aberrant regulation of RNA-splicing components. Taken together, the combination of RNA-seq and exome-sequencing on normal cells and primary vs. post-chemotherapy tumor is providing a broad and deep view of molecular signatures in tumor progression and indicating that a significant role is played by changes in non-coding RNA expression.

P12.055-S Beyond BRCA1 and BRCA2: results from screening 94 genes in 100 patients with familial breast and ovarian cancer using panel-sequencing and custom array-GCH


Breast and ovarian cancer predisposition has been associated with a num- ber of high-, moderate -, and low-penetration susceptibility genes. Here we report on the results of high-resolution custom array-GCH for deletion/dup- lication analysis and panel-based screening of 94 genes associated with hereditary cancer predisposition.

Selection criteria for the 100 patients were defined by the German Consorti- um for Breast and Ovarian Cancer: NGS was performed on an Illumina MiSeq and target enrichment was done with the Illumina TruSight cancer panel, whereas custom array CGH was performed using Agilent technology. In 28 % of the patients, BRCA1 or BRCA2 variations have been found. These were either clearly pathogenic protein truncating mutations (12 %) or very rare, unclassified missense variations with high probability of effect (16 %). In 39 % of the patients we found rare, unclassified missense variants in low penetrance susceptibility genes, especially NBN and ATM. In one case with early onset of breast cancer and familial history, a putative splice relevant penetration susceptibility genes, especially NBN and ATM. In one case with early onset of breast cancer and familial history, a putative splice relevant mutation in TP53 could be identified, which is currently being investigated on cDNA level. 33 % of the patients did not reveal any convincing sequence variation. In 10% of the patients we identified deletions in either BRCA1, CHEK2, or ATM. The extension of mutation screening beyond BRCA1 and BRCA2 reveals disease-causing mutations in high-penetration genes, like TP53, as well as mutations in low-penetration susceptibility genes. Here we report the results of high-resolution custom array-GCH for deletion/duplication analysis and panel-based screening of 94 genes associated with hereditary cancer predisposition.

First evidence for FANCJ as a novel breast cancer predisposing gene


Several members of the FANC (Fanconi anemia complementation group) gene family such as BRCA2 (FANCJ), RAD51C (FANCJ), PALB2 (FANCN) and BRI1 (FANCJ) have been associated with breast cancer (BC) and ovarian cancer (OC) predisposition. Given the fact that most risk families do not carry mutation in BRCA1 or BRCA2, these genes have been studied as BC and OC susceptibility genes. In this study we screened for mutations in FANCJ in a selection of high-risk breast and ovarian cancer families. Interpretation of findings was done considering the known role of FANCJ as a novel breast cancer predisposing gene.

P12.054-M Retrospective analysis of genomic and transcriptional changes in a case of Ewing sarcoma tumor progression determined by whole transcriptome and exome semiconductor-based sequencing

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Ewing sarcoma is a cancer that often presents in the second decade of life and is usually associated with a chromosomal translocation that results in a EWS/FL1 gene fusion. Four independent cell-lines have been established from a subject who succumbed to metastatic disease following relapse after myeloablative chemotherapy. Whole-transcriptome and exome sequencing of primary breast marrow-derived stromal cells and Ewing Sarcoma Tumor-Derived Virus (EBV) transformed normal lymphoblasts, a pre-therapy primary tu- mor-derived cell-line, and a post-chemotherapy metastatic tumor-derived cell-line, was conducted on an Ion Torrent Proton™ system to profile the differences in gene expression and in exon DNA sequence to characterize the molecular changes associated with primary tumorigenesis and disease persistence after treatment. The presence of the EWS/FL1 fusion gene in primary tumor cells was confirmed by the blocking of the breakpoint and observation of chimeric reads in the RNA-seq data and exome sequence analy- ses. Exome datasets indicate apparent loss of heterozygosity genome-wide in CHLA10 consistent with cytogenetic analysis that shows tetraploidy in this cell-line. Results from RNA-seq also indicate numerous instances, genome-wide, of differing transcript isoform expression and exon usage between normal, primary tumor, and metastatic tumor cells suggesting an increasing genomic mutational burden in the evolution of the disease, and pointing in particular toward aberrant regulation of RNA-splicing components. Taken together, the combination of RNA-seq and exome-sequencing on normal cells and primary vs. post-chemotherapy tumor is providing a broad and deep view of molecular signatures in tumor progression and indicating that a significant role is played by changes in non-coding RNA expression.
Clinical experience with screening formalin fixed and paraffin-embedded (FFPE) archival tissue for mutations in BRCA1 and BRCA2

Background: Women carrying a germline mutation in BRCA1 or BRCA2 (BRCA1/2) have an increased lifetime risk for breast- and ovarian cancer. Men carrying the same mutations are facing an increased risk of prostate- and breast cancer. Until now, screening for BRCA1/2 mutations required high quality DNA (from blood or other fresh specimens). This has ruled out families in which the relative(s) suffering from breast or ovarian cancer have already died. Several attempts to screen for mutations in archival formalin-fixed, paraffin-embedded (FFPE) tissue failed. Germline CDH1 mutation has been associated with limited success.

Aim: We present the first clinical data from our newly developed NGS based analysis, screening archival FFPE samples of non-tumor tissue for germline mutations in BRCA1/2.

Results: In 33 FFPE samples we found 3 pathogenic BRCA1 mutations, 3 pathogenic BRCA2 mutations and 2 variants of unknown significance in BRCA2. In 17 samples coverage was sufficient to disclose a negative result and in 8 samples the coverage was too low, and hence the result was designated inconclusive. The quality of the data varied between samples with a strong negative correlation between age of the tissue and sequence quality.

Conclusions: Mutations in BRCA1/2 can now be sought after in deceased relatives, in families with suspected germline mutations. For clinical use, mutations found in FFPE samples should always be confirmed in other samples from either the same individual, or from samples from close relatives.

P12.059-S
Comprehensive genetic characterization of pediatric patients with B-lineage ALL reveals novel mutations and gene fusions

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High-throughput sequencing technologies provide new opportunities for personalized treatment approaches as well as for the identification of novel genetic biomarkers in cancer. We analyzed a total of 9 tumor and reference samples of patients with primary and relapsed B-lineage ALL by whole-exome-/transcriptome sequencing and SNP-microarrays. The results were combined to obtain a comprehensive overview on the genomic landscape of B-lineage ALL. Only few genes were altered recurrently and our data confirms that hyperdiploidy is the predominant cytogenetic feature in B-ALL. In contrast, interstitial deletion variants are highly individualized therapeutic approaches. Besides common and well known genetic variations like mutations (e.g. in NRAS, JAK, IL17; 3' untranslated region 1630 C>T polymorphism and copy number variations (CNVs), e.g. a deletion of PAX5) and fusion genes (e.g. BCR/ABL) a novel gene, PYGO2, was identified to be altered in three patients. Besides a fusion gene (MEF2D:PYGO2) a mutation and a duplication of PYGO2 were found in different patients. Our data suggest that PYGO2 plays a role in leukemogenesis.

P12.060-M
Unraveling the genetic predisposition of early-onset and familial gastric cancer

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Gastric cancer is the second leading cause of cancer-related deaths worldwide. PHB has been reported as an oncogene and tumor suppressor in several neoplasias, including in gastric cancer. Here, we evaluated whether the PHB...
copy number and the rs6917 polymorphism affect its expression in gastric cancer. Forty-eight pairs of gastric cancers and corresponding non-neoplastic gastric samples were evaluated. PHB expression was analyzed by real-time quantitative PCR and by immunohistochemistry. Gene copy number was investigated by quantitative PCR. Allele-specific expression was determined by sequencing and by TaqMan assay. down-regulation and up-regulation of PHB was observed in the tumors (45.5% and 20.5%, respectively). Reduced PHB expression was associated with dedifferentiation (P = 0.029), lower intravision (P = 0.002), absence of lymph node metastasis (P = 0.040) and early gastric cancer (P = 0.002). In all cases, the PHB immunoreactivity was detected in neoplastic and non-neoplastic cells. PHB was mainly expressed in the cytoplasm and PHB expression was decreased in 34.2% tumors. PHB gain was associated with higher gene expression (P = 0.003) and late-onset gastric cancer (P = 0.022). In our sample, 22% patients were heterozygous and 4.17% were homozygous for the minor allele in the rs6917 polymorphism. The presence of the T allele in this polymorphism was associated with reduced expression in GC (P = 0.001) and in non-neoplastic samples (P = 0.001). Only one patient presented a higher C/T ratio in both tumor and non-neoplastic samples. In most cases, lower C/T ratio was detected. Thus, PHB copy number variation and differential expression of the rs6917 polymorphism may have a role in PHB transcriptional regulation.

**P12.063-S** Extrachromosomal driver mutations in glioblastoma and low grade gliomas


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Alteration of the number of copies of Double Minutes (DMs) with oncogenic EGFR mutations in response to tyrosine kinase inhibitors (TKis) is a novel adaptive mechanism of glioblastoma. In this study we provide evidence that such mutations in DMs, called here Amplification Linked Extrachromosomal Mutations (ALEMs), originate extrachromosomally and could therefore be completely eliminated from the cancer cells. By exome sequencing 7 glioblastoma patients we revealed ALEM in EGFR, PDGFRα and other genes. These mutations together with DMs were lost by cancer cells in derived gliospheres. We confirmed the extrachromosomal origin of such mutations by showing that wild type and mutated DMs could coexist in the same tumor. Analysis of 4198 tumors from TCGA collection suggested the presence of ALEM across different tumor types with the highest prevalence in glioblastomas and Low Grade Gliomas. In these tumors, driver oncogenic mutations in DMs were 13 - 25 fold more frequent as compared to the distribution of passenger mutations confirming the effective expansion of extrachromosomal drivers. The extrachromosomal nature of ALEM provides a powerful mechanism of rapid regulation of the copy-number of mutated oncogenes including their massive increase or complete loss in response to extracellular stimuli such as RTK inhibitors or growth factors.

**P12.064-M** New loci of loss of heterozygosity (LOH) in glioblastoma

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Despite recent advances in the diagnosis and treatment of glioblastoma, the prognosis for patients with this highly malignant tumor remains poor. Research of glioblastoma at the molecular genetic level can help reveal characteristic features of this tumor, identify potential markers of diagnosis of the disease and response to therapy. An efficient approach of identifying candidate genes, the disturbance of structure and functioning of which may be associated with the development of cancer, is the analysis of LOH. We have assessed 7 genomic loci for which LOH in glioblastoma have not been previously reported: 2q31.2, 3p25.1, 5q14.3, 7q21.2, 12q12.13, 18q11.2 and 21q21.1. None of these loci is associated with glioblastoma susceptibility according to OMIM. The selected loci were examined by microsatellite analysis in 86 glioblastoma samples. Four of the seven loci, 2q31.2, 12q12.13, 18q11.2 and 21q21.1 revealed no LOH cases. However, LOH was identified at 3p25.1, 5q14.3 and 7q21.2 with a frequency of 25.8% (8/31), 20.0% (3/15) and 30.3% (10/33), respectively. Identified areas of LOH can contain potential candidate genes that may be of interest in terms of the molecular pathology of glioblastoma. Some of these genes are SLCA6A6 (3p25.1), CCNH (5q14.3), AKA9 (7q21.2). Thus, we have identified new loci of LOH in glioblastoma, in which candidate genes that play a role in tumor development and represent potential molecular genetic markers may be located. We have also confirmed LOH at 8q21.3 and 14q13.3 with a frequency of 17.9% (5/28) and 60.0% (9/15), respectively, which other authors have reported.

**P12.065-S** Effects of NDRG2 downregulation on glioblastoma patient survival

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**Background.** Glioblastoma multiforme (GBM) is the most lethal malignant human brain tumor with the poor survival prognosis exceeding to 12-15 months after diagnosis. No reliable molecular marker has been identified yet, therefore, extensive research in molecular changes of GBM is essential to better understand this pathology. NDRG2 gene has been reported to be downregulated in GBM, whereas overexpression of this gene promotes glioblastoma cell proliferation in vitro. To further address the role of NDRG2 in gliomagenesis, we analyzed NDRG2 expression at mRNA and protein level in gliomas of different malignancy grade.

**Material and Methods.** The study included 78 different malignancy glioma tumors (9 astrocytomas grade-I, 29 grade-II, 14 grade-III and 26 glioblastomas). NDRG2 mRNA expression study was performed using quantitative real-time Reverse-Transcription PCR analysis, while protein expression was estimated by using Western blot technique. **Results.** We found a strong decrease of NDRG2 mRNA expression in glioblastoma to about 10-fold compared to I-III grade gliomas (Kruskal-Wallis, p<0.0001). In line with the mRNA data, NDRG2 protein level was markedly reduced in glioblastomas (to about 3-4-fold) as compared to grade I-III gliomas (p<0.0001). Spearman correlation analysis demonstrated significant correlation of NDRG2 transcripts and protein expression levels (r=0.662, p<0.0001). Survival analysis showed negative correlation between NDRG2 expression and tumor progression (Log-rank p=0.0001). Our results highlight the importance of NDRG2 gene tumor tumorigenesis as well as being an indicating factor for glioma malignancy and patient survival.

**P12.066-M** Genomic profiling reveals three molecular relapse patterns in IDH1/2 wild-type glioblastoma

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Molecular changes associated with the relapse of glioblastoma after standard radiochemotherapy remain poorly understood. Here we compared genomic profiles of 27 pairs of primary and recurrent IDH1/2 wild-type glioblastomas by genome-wide array-based comparative genomic hybridization. By bioinformatic analysis, primary and recurrent tumor profiles were normalized and segmented, chromosomal gains and losses called taking the tumor cell content into account, and difference profiles deduced. Seven of 27 (26%) pairs lacked DNA copy number differences between primary and recurrent tumors (Equal pairs). The recurrent tumors in 9/27 (33%) pairs contained all chromosomal imbalances of the primary tumors plus additional ones, suggesting that a major subclone successively accumulated aberrations (Sequential pairs). In 11/27 (41%) pairs, the profiles of primary and recurrent tumors were divergent, i.e. the recurrent tumors contained additional aberrations but had lost others, suggesting a polyclonal composition of the primary tumors and considerable clonal evolution (Dispersant pairs). Losses on 9p21.3 harboring the CDKN2A/B locus were significantly more common in primary tumors from non-Equal pairs. Non-Equal pairs showed ten regions of recurrent genomic differences between primary and recurrent tumors harboring 46 candidate genes associated with tumor recurrence. In particular, copy numbers of genes encoding apoptosis regulators were frequently changed upon recurrence. In summary, approximately 25% of IDH1/2 wild-type glioblastoma pairs have stable genomic profiles of 27 pairs of primary and recurrent IDH1/2 wild-type glioblastomas undergo further genomic aberrations and alter their clonal composition upon recurrence impacting their genomic profile, a process possibly facilitated by loss on 9p21.3 in the primary tumor.
P12.067-S
Targeted resequencing for analysis of gene mutations in pediatric Glioblastoma Multiforme

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ABSTRACTS POSTERS

Background: Among 103 patients with glioblastoma and II and III grade gliomas, to further understand: 1) correlation with grading, 2) prognostic value. Pyrosequencing analysis of MGMT promoter showed that methylation levels decrease from low to high grade tumors (p < 0.001), confirming that hypermethylation (methylation levels >20%) correlates with a better progression free survival (PFS). Interestingly, a mildly methylated subgroup (methylation 20%-40%) revealed a better PFS. Microsatellite and a-CGH analyses showed that 10q LOH and MGMT methylation in high grade tumors and associates with MGMT hypomethylation, while absence of LOH and hypermethylation correlate with low grade tumors. IDH1 mutation was investigated by pyrosequencing and detected in all of low grade gliomas (10 cases), in 7/11 II grade gliomas. We did not find mutations in IV grade gliomas, indicating a strong association between tumor grading and IDH1 mutation status (p<0.001). In addition, we found a correlation between IDH1 mutation and MGMT methylation level (p<0.001), both decreasing with increasing tumor grade. In conclusion, our data reinforced the role of MGMT methylation and IDH1 mutation as hallmarks of better prognosis, and conversely 10q LOH as a poorer one. Therefore, we evidenced the existence of a mildly methylated glioma subtype correlated with a better PFS.

P12.070-M
miRNA expression profile in tumor cell lines treated with the histone deacetylase inhibitor LBH589

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Introduction: The presence of HDAC proteins is necessary for the correct regulation of gene expression. Multiple studies have demonstrated that inhibition of these proteins can lead to deregulation of gene expression and abnormal cell proliferation. In our study we have analyzed the miRNA profile of three epithelial cell lines (HCT116, HT29 and HCC1937) and two mesenchymal cell lines (IP29 and MM1S) after treatment with a histone deacetylase inhibitor, currently in phase III clinical trial.

Methods and materials: We have analyzed miRNA expression comparing the profiles before and after treatment. RNA was isolated from cell lines and miRNA labeling was performed using a conventional assays. An Exiqon chip miRCURY LNA microRNA™ was used for analysis. Comparisons between the different study groups were performed using SAM to identify those miRNAs whose expression showed statistically significant differences.

Results: Comparing the expression profiles of the cell lines between 24h and 72h after treatment with the histone deacetylase inhibitor, we found that epithelial cells show a group of 18 up-regulated and 16 down-regulated miRNAs. Whereas mesenchymal cells show 20 up-regulated and 20 down-regulated miRNAs. Both types of cells share 3 up-regulated miRNAs at 24h, 36h and 72h after treatment. A differential expression pattern was used for identifying those miRNAs that show an influence on the cellular origin of tumor cells, which could explain different outcome of patients treated with this approach.

P12.072-M
Whole-genome sequencing of matched primary and metastatic hepatocellular carcinomas

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To gain biological insights on tumor metastasis, we used whole-genome sequencing at 3X-43X coverage to profile somatic mutations in primary HCC (HBV+1) and metastatic lung metastases (>2 years interval). In total, 5,027-13,961 and 5,275-12,624 somatic single-nucleotide variants (SNVs) were detected in primary HCC and lung metastases, respectively. Generally, 39.88-78.49% of SNVs detected in metastases were present in primary tumor mutations. We identified 65-221 structural variations (SVs) in primary tumors and 60-232 SVs in metastases. Comparison of these SVs shows very similar and largely overlapped mutated segments between primary and metastatic tumors. Copy number alterations between primary and metastatic pairs were also found to be closely related. Together, these observations in genomic profiles from liver primary tumors to metastatic lung metastases indicate that the genomic features during tumorigenesis may be retained during metastasis. Additionally, a few mutations were found specifically in lung metastases, which may explain the clinical observation that...
both primary and metastatic tumors are usually sensitive or resistant to the same systemic treatments.

P12.073-S Identification of a novel CDH1 gene mutation in a family with hereditary diffuse gastric cancer

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Introduction: Hereditary diffuse gastric cancer (HDGC) is an autosomal dominant genetic predisposition syndrome caused by CDH1 germline mutations. A 42-year-old woman was referred to our institution for cancer screening. CDH1 is relatively uncommon, representing about 2% of gastric cancer. The majority of inactivating mutations in HDGC families are of the truncating type (75%), whereas the remaining are missense type. The estimated lifetime risk for DGC is more than 80% in mutation carriers and progerplastic total gastrectomy is recommended.

Subjects and methods: A 47-year-old female patient diagnosed with DGC was screened for CDH1 gene mutations. DNA and RNA extraction was carried out from blood and tissue (gastric biopsy) and the entire coding sequence and flanking intronic portions of the CDH1 were sequenced. Other family relatives (three sisters, an uncle and a nephew) were also screened. Results: A deletion in exon 9 of CDH1 gene (c.1220delC, p.407*), not previously described, has been found. This mutation generates a stop codon that leads to a pathogenic variant. The presence of the mutation was corroborated both at DNA and RNA level in blood and tissue. The proband presented the deletion and had a previous history of malignant colorectal polyp at the age of 49. Her son (nephew of the proband) presented also the deletion. Neither of the other relatives harboured the mutation. Conclusion: HDGC has a poor prognosis, mainly because of its difficult early detection. The identification of CDH1 mutations offers the opportunity of carry out prophylactic strategies for unaffected at-risk individuals.

P12.074-M Novel mutations in Juvenile polyposis syndrome

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Juvenile polyposis syndrome (JPS) is an autosomal dominant disorder characterized by multiple juvenile polyps (JP) mainly in the colon with increased risk of colorectal cancer. Subsets of patients develop severe gastric polyposis with increased risk of gastric cancer. Of fifty patients evaluated for polyposis in 2013, two JPS patients with novel mutations were diagnosed.

1st: 27yrs after right colectomy for multiple hyperplastic, and JP resulting in increased risk of colorectal and gastric cancers. Sequencing and MLPA for SMAD4 were negative. BMPRIA sequencing yielded c.367G>C (p.123E*) , a novel nonsense mutation.

2nd: At 32 yrs total colectomy for multiple adenomatous polyps suspicious of Familial Adenomatous Polyposis. Gastroscopy detected multiple hyperplastic polyps. APC sequencing and two common MUTYH mutations were normal. Due to severe bulky gastric polyposis a revision of the pathology was performed and JPS was clinically diagnosed. A total gastrectomy was performed for persistent anemia and cancer risk. Epistaxis and telangiectases on his back and chest raised the possibility of hereditary hemorraghic telangiectasia (HHT). Sequencing of SMAD4 found c.406_407delGT and c.407delC(p.136E*), a de novo novel frameshift mutation. JPS comprises 10% of polyposis syndromes. About 20% of SMAD4 patients have JPS-HHT combined syndrome. 8 patients with SMAD4 mutations may have gastric polyposis with a significant risk of gastric cancer. These JPS patients illustrate the need for careful history collection and thorough pathological analysis, that should guide the genetic evaluation. Genetic workup of JPS patients is important for family counseling and to establish the need for HHT evaluation in patients with SMAD4 mutations.

P12.075-S Targeting the functional relevance of the novel tumor suppressor gene FOCA1 (KIAA1797) in glioma pathogenesis

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Malignant gliomas have a highly invasive phenotype, which is a main determinant for the poor prognosis of patients suffering from these tumors despite multimodal therapy. In previous studies, we used 24-color-FISH to characterize the chromosomal translocation events in glioblastoma cell lines, and detected a (7;9) disrupting the FOCA1 gene encoding a then uncharacterized protein. FOCA1 was deleted in around 50% of primary glioblastomas, and its gene product, focadin, has an impact on glioblastoma growth in vivo and cell motility in vitro. The aim of this project was to further characterize focadin by targeting its unknown binding partners. Therefore, we performed two independent yeast two-hybrid screens using a human brain cDNA library. In total, we isolated 206 clones, from which we eliminated 155 clones, because they could grow without auxotrophic marker expression. The DNA sequences of the remaining 51 prey inserts were identified. In 26 prey plasmids, the cDNAs were protein-coding alterations. Finally, HPLC analysis of potential binding partners were identified, which can be categorized into the functional groups of calcium signaling, protein folding, RNA processing and cell metabolism. To confirm these interactions, we performed retransformation experiments using fresh yeast. Three candidates were also analyzed by pull-down assays and co-localization studies using human cells. Two interactions have been confirmed so far. In the next step, the functional relevance of the verified interactions will be investigated in glioblastoma cells with stable focadin knockdown and cells with homoyzgyous deletion of the FOCA1 gene with or without FOCA1 rescue by viral transduction.

P12.076-M Targeted deep sequencing of fusion transcripts - developing clinical workflows for targeted cancer therapy

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One of the main focuses of the National Genomics Infrastructure-Sweden (NGI) is to bring high-throughput sequencing to clinical use. Here we describe recent work on PCR- and hybridization-based deep sequencing of specific fusion transcripts using Pacific Biosciences RSI (PacBio) and Ion Torrent PGM/Proton instruments, methods that can help to guide the treatment of leukemia patients.

By long-read PacBio sequencing we are developing clinical workflows for detection of BCR-ABL1 kinase inhibitor (TKI) resistance mutations in chronic myeloid leukemia (CML). Our assay enables rapid sequencing of a 17 587 bp BCR-ABL1 cDNA amplicon without the need of a nested PCR, Mutations down to a level of 1% are clearly detected using this approach. Moreover, the long PacBio reads makes it possible to resolve the mutational composition of all different clones present in CML patient samples. Since compound mutations might confer cross-resistance to multiple TKIs, the information provided by our assay can directly influence the choice of therapy.

In another project we study MLL-rearranged leukemias, characterized by chromosomal translocations involving the MLL gene at 11q23. MLL can form fusions transcripts together with different genes, some of which are still unknown. In some cases conventional assays like G-banding and FISH fail to detect these fusion partners. We are therefore evaluating a hybridization-based assay for targeted capture of MLL cDNA, and screening with Ion Torrent sequencing. Our preliminary results indicate that our method increases the sensitivity for detecting MLL fusion genes by several orders of magnitude compared to a regular RNA-sequencing approach.

P12.077-S Results of 20 years of Li-Fraumeni syndrome diagnosis in France

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The Li-Fraumeni syndrome (LFS), due to germline TP53 mutations, represents a remarkable cancer predisposition characterized by the extent of tumors spectrum. For 20 years, a network of over 1700 families allowed us to identify 214 French families with TP53 mutation, thanks to the Chompret’s criteria gradually elaborated by the French LFS working group. Updated analysis of over 1700 families allowed us to identify 214 French families with TP53 mutation, thanks to the Chompret’s criteria gradually elaborated by the French LFS working group. Updated analysis of over 1700 families allowed us to identify 214 French families with TP53 mutation, thanks to the Chompret’s criteria. Updated results from 1700 families showed that 214 French families had TP53 mutations. From data from 1700 families, we identified 214 French families with TP53 mutation, thanks to the Chompret’s criteria. Updated results from 1700 families showed that 214 French families had TP53 mutations.
Identification of a characteristic copy number alteration profile by high-resolution SNP arrays associated with metastatic sporadic colorectal cancer


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Background: Metastatic dissemination is the most frequent cause of death in sporadic colorectal cancer (sCRC). The metastatic process is considered, at least in part, to be related to a specific background of genetic alterations accumulated in cells from primary tumours, the identification of such genetic alterations being critical for the identification of sCRC patients at risk of developing metastases.

Methods: In this study we used high-resolution 500K SNP-arrays for the identification of copy number alteration profiles present at diagnosis in primary tumours from metastatic (n=43) versus non-metastatic (n=26) sCRC.

Results: Our results showed a characteristic pattern of copy number alterations among metastatic sCRC which involved losses of 23 regions at chromosomes 1p, 17p and 18q, together with gains of 35 regions at chromosomes 7 and 13q.

Conclusion: As could be expected, such copy number profile involved multiple genes previously associated with sCRC (ie. SMAD2) and/or the metastatic process (ie. PODXL) and it was further associated with a poorer outcome.

Oligogenic germline mutations predispose to early lung adenocarcinoma in non-smokers

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Despite the great bulk of research to identify genetic susceptibility genes in lung cancer by genome-wide association studies, only three loci have been identified and replicated consistently in subsequent studies. In addition to confer a very low risk, they have been associated with lung cancer in smokers, but not in non-smokers. The polygenic nature of common cancers has frequently been suggested, but its biological basis still remains elusive. We tested the hypothesis that genetic susceptibility may rely on germline mutations of a restricted number of genes. A combination between an advanced technical tool, ie the exome sequencing, and a new patient selection strategy was used. Among 964 lung adenocarcinoma patients we selected two patients with very early onset disease (mean age 43) in absence of cigarette smoking and with a normal health status. We identified 132 somatic copy number alterations (from 1k to 10k) and 40 genomic deletions in 8 and 5 different cancer predisposing genes in each affected subject, respectively, but not in the healthy sib (p=0.0026). Some of them are well known cancer players in lung tumors and others are genes previously identified in other cancer tissues. This study demonstrated for the first time that never-smoker patients with lung adenocarcinoma carry a specific and private oligogenic combination of mutations in cancer predisposing genes. Exome sequencing of another pair of sibs with slightly later age onset is ongoing. These findings, if replicated with further studies, support the hypothesis of an oligogenic nature of early onset common cancers.
Multiple primary tumors of the gastrointestinal tract, uterus, ovaries, central nervous system and other organs are hallmarks of Lynch syndrome, caused by heterozygous germline mutations in the mismatch repair (MRR) genes MLH1, MSH2, MSH6 and PMS2. Bi-allelic mutations of the same genes have been associated with a rare childhood cancer syndrome characterized by hematological malignancies, sarcomas, brain and gastrointestinal tumors together with cafe-au-lait spots resembling Neurofibromatosis 1. Here we report on a female patient who developed multiple primary tumors between 21 and 27 years of age including two metachronous colorectal cancers, a filloid tumour of the breast, a glomablastoma and a clear cell carcinoma of the ovaries. Genetic testing identified two germline heterozygous MSH6 mutations: a frameshift mutation (c.1610_1613delAGTA) inherited from the father and a suspected deleterious missense mutation (p.Arg706His) inherited from the mother. No TP53, MLH1 or MSH2 mutations were found. Microsatellite analysis revealed instability of BAT26 and BAT40 in both the colon and the ovarian carcinomas. The MLH1, MSH2 and PMS2 proteins showed nuclear staining in all specimens whereas MSH6 was completely absent in tumor and normal cells, including the filloid tumor of the breast and the normal mucosa of the colon. Germline mutations in the MMR genes, either mono-allelic or bi-allelic, can therefore underlie a wide spectrum of cancer syndromes characterized by variable age at onset and severity of the cancer risk. Development of multiple primary tumors in young adults appears to be suggestive for the presence of bi-allelic mutations of the MMR genes.

P12.083-S

Constitutional epimutation of MLH1 gene coexisting with a genomic deletion in Lynch Syndrome

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A few Lynch Syndrome patients display constitutional epimutations of the mismatch repair genes. Two models of MLH1 epimutations have been hypothesized: the "primary" type arises spontaneously and is reversible between generations; the "secondary" type is caused by an unknown cis-acting genetic-based alteration, with a classical Mendelian autosomal dominant inheritance pattern.

We report here a case with a heritable large genomic deletion of MLH1 associated with constitutional promoter methylation of the same gene. Both aberrations were found by MLPA analysis in a patient with colorectal and endometrial cancers (30 and 40 years), displaying MSI-H and loss of the MLH1 protein. They were also detected in three other affected relatives and in tumors.

We have explored the link between MLH1 deletion and methylation by different molecular approaches. The deletion of 997bp included the ATG codon, exon 1 and part of intron 1. Bisulfite sequencing demonstrated that CpG-deletion and methylation by different molecular approaches.

P12.084-M

Appearances deceive twice. Lynch-like syndrome ends up being MUTYH-associated polyposis. Apparent homzygous MUTYH mutation ends up being compound heterozygous with a large deletion

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Significant phenotypic similarities among Lynch syndrome (LS) and MUTYH-associated polyposis (MPP) have been reported. We describe a familiar suspicion of having LS. A 69 years old female diagnosed of an endometrial cancer (T1N0M0) and right CRC (T3N0M0) with multiple polyps at ages 60 and 65, respectively. Endometrial tumor had loss of MLH1 and PMS2 expression, MSI, no BRFV, V600E mutation, absence of MLH1 methylation, and KRAS_G12 mutation. No pathogenic variant was found in the MLH1 mutation screening and MPAP suspicion was considered.

MUTYH testing was approached by Sanger sequencing screening of recurrent variants at exons 7 and 13. Patient did show homzygous pattern for c.1187G>A (p.G396D) variant and was diagnosed as MAP syndrome. Predictive test for relatives unveiled an unexpected finding, we found one of her sons with an apparent wild type sequence. Further analysis evidenced a large deletion comprising exon 4-16 in both mother and son. Breakpoint characterization of this deletion allowed us to precisely define this alteration as c.348+33_641+164del428insTA with a precise size deletion of 4,285 kb. The same deletion has been published in other two unrelated families from French and Portuguese origin suggesting a founder effect. Large rearrangements at MUTYH locus are rare although probably under-diagnosed. Based on these findings we recommend first, to include MUTYH gene in the testing strategy for LS and second, to test for MUTYH large rearrangements in heterozygous cases after whole gene sequencing as well as in apparent homzygous cases.

P12.085-S

Male breast cancer in the Netherlands: uptake and outcome of BRCA testing. Results of study the Dutch Breast Cancer Research Group (BOOG 2009-04) in collaboration with the EORTC 10085 study.


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Background: In male breast cancer (MBC) the prevalence of BRCA1/2 mutations varies considerably between countries. In the Netherlands data were collected of all MBC patients diagnosed in the last 20 years. The nationally agreed criteria for DNA testing are rather broad, implying that many MBC cases with or without a family history for breast cancer have been tested. Aim of this study is to get more insight in the percentage of BRCA1/2 mutations among an unselected cohort of MBC patients. Methods: All diagnosed MBC patients between 1989-2009 (n=1487) were linked to databases of all clinical genetic centers. Data of BRCA testing, family history and tumor characteristics were collected. Results: 334 (22%) of MBC patients were tested for BRCA1/2. Ten (3%) BRCA1, 51 (15%) BRCA2 mutations were identified and also 7 (2%) variants of uncertain significance (VUS). At least one first or second degree relative with breast cancer <50 yr was seen in 80% of BRCA1, 20% of BRCA2 and 6% of non-BRCA1/2 MBC patients. Preliminary studies did show that the majority (66%) of BRCA associated MBC were ductal carcinomas of no special type. Conclusion: Roughly one fifth of unselected MBC patients had undergone DNA testing, in 10% of these a BRCA mutation was found. Most striking family histories were seen in BRCA1 families, whereas the majority of BRCA2 MBC patients had no strong family history. Based on our results and previous studies genetic testing for BRCA1/2 should be recommended for any MBC case, regardless of family history for breast cancer.

P12.086-M

Role of MAP/Microtubule Affinity Regulating Kinase 4 in the regulation of cell cycle progression and cytoskeleton dynamics

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MAP/microtubule affinity regulating kinase 4 (MARK4) is a serine-threonine kinase that phosphorylates and regulates MAP proteins. MARK4 differs from the other members of the MARK protein family, for encoding two isoforms (MARK4L and MARK4S) differentially expressed in the nervous system, and for being crucial for mitotic spindle assembly and normal mitosis. In order to better define the role of MARK4 in cell cycle and cytoskeleton dynamics, we performed cytofluorimetric analysis of fibroblasts and glioma cells, showing that MARK4 is expressed throughout the cell cycle and is preferentially activated during mitosis and cytokinesis. Using the same cell system we demonstrated the role of MARK4 in cell cycle progression and cytoskeleton regulation by knockdown and overexpression experiments. Knockdown of MARK4L silencing and glioma cells, showing that MARK4L is matched with a reduction in proliferation rate and mitotic fraction. In addition, silenced cells show duplicated centromeres positioned apically to the nucleus, a feature typical of the G1/S phase transition. Overexpression of MARK4L or MARK4S reduced the density of the microtubule network, confirming microtubules as the main target of MARK4. Furthermore in fibroblasts MARK4L was found to colocalise with vimentin and to reorganise...
intermediate filaments. Overexpression of kinase-dead mutants indicated that the effects on cytokinase compartments are due to MARK4 kinase activity. The overall data highlight MARK4 as a key component in the regulation of MT dynamics, demonstrate its role in cell cycle progression, particularly at the G1/S transition, and point to vimentin as a new plausible MARK4 interactor.

P12.087-S Multiple primary melanoma (MPM) as a valid criteria for genetic assessment: an Italian IMI multi-center study

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The prevalence of mutations in the CDKN2A melanoma candidate gene correlates with number of affected family members and number of MPM/neoplastic events. International referral guidelines for genetic counselling and testing (GenoMEL) indicate that in low melanoma incidence populations individuals developing two melanomas may be considered for candidate-ration for genetic testing, even in the absence of family history (FH). Aims of this multicenter case-control study performed within the Italian Melanoma Intergroup were to verify the likelihood to identify mutations carriers in MPM vs single primary melanoma (SPM) patients recruited, to update the current Italian shared protocol for hereditary melanoma (SIGU-ONC recommendations; Biachetti et al., 2004) and to include the presence of MPM, in the absence of family history, as a criterion. Despite regional differences (i.e. founder mutations), 118/587 (20.1%) of the recruited MPM patients (including those with melanoma FH) and 52/443 (11.7%) of sporadic MPM harbored CDKN2A mutations, suggesting that the development of MPM (2 or more events) even in the absence of FH can be considered a criterion for genetic testing on national basis. The presence of MPM cases in a family was confirmed as a strong mutation predictive parameter, while CDKN2A mutations in sporadic SPM was under 5%. The search for the MITF E318K mutation, recently identified as a novel intermediate risk allele, showed that the mutation was associated with the risk of sporadic MPM (3.7% of sporadic MPM compared to 0.8% of SPM), underscoring the importance of the MITF mutation to the burden of MPM susceptibility.

P12.088-M Genetic variants in the interleukin locus at 1q32.1 as markers of melanoma survival


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This study strongly suggests that germline variants in the interleukin locus at 1q32.1 should be considered as novel prognostic markers with potential clinical utility.
tumor were measured by real-time PCR with subsequent quantification using a 2-ddCT method. Obtained results were then analyzed for association with clinical-morphological parameters: age, cancer stage, and tumor cells differentiation. The expression of let-7a was significantly decreased in tumors compared to adjacent tissue at both 2 and 5 cm. Let-7a and miR-155 levels in tumor were substantially lower than in adjacent tissues in patients under 63 years. The expression of let-7a and miR-155 in tumor was also suppressed in patients with III-IV stages of NSCLC. Besides that patients with poorly differentiated NSCLC had significantly lower let-7a level in tumor compared to adjacent tissue. The levels of let-7a, miR-155, miR-205 were different even in adjacent tissue, in patients with differentiated tumors it was observed that tumors with poorly differentiated tumors. The study showed that let-7a expression is suppressed in tumors compared to adjacent normal tissue at 2 and 5 cm from tumor. The decrease of let-7a and miR-155 is distinctive for younger patients, III-IV stages of NSCLC, and poor tumor cells differentiation. Altogether these findings consider miRNAs let-7a and miR-155 as markers of unfavorable prognosis for NSCLC patients.

P12.093-S A germline mismatch repair mutation possibly leading to a de novo NF1 germline mutation C. Bracco1, L. Borelli2, M. Micheleletti1, D. Martorana1, E. Grossi1, B. Pasi1.
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We report on a female patient diagnosed with breast, colon, and endometrial cancer at the age of 38, 40, and 41 respectively, presenting with café au lait macules, multiple neurofibromas and axillary freckling. With respect to the family history, her paternal grandmother died at the age of 63 for a colon cancer diagnosed when she was 35, whereas her father died at the age of 42 because of an accident. As the proband met the criteria for Neurofibromatosis 1 (NF1), analysis of the NF1 gene was performed. A frameshift mutation (c.7096_7101delAACTTT) was identified confirming the clinical diagnosis. The parents showed no clinical signs of NF1 nor did the two proband’s siblings, which tested negative. Because of the co-occurrence of endometrial and colon cancer in the paternal grandparent, mother and the presence of an early-onset colon cancer in the paternal grandchild, expression of mismatch repair protein and Microsatellite Instability (MSI) were analyzed on endometrial and colon cancers. Both tissue showed loss of staining for the MSH2-MSH6 heterodimer and high MSI, therefore analysis of MSH2 was performed, leading to the identification of a nonsense mutation of exon 2 (c.289C>T, p.Gln97*), consistent with the presence of the Lynch syndrome. As women with NF1 have a fivefold risk of developing premenopausal breast cancer, no further analysis was performed. Although it was not possible to analyze any member of the paternal family, an intriguing possibility is that the inherited germinal mutation affecting the mismatch repair system led to a de novo NF1 germline mutation in our patient.

P12.094-M Whole genome sequencing of mitochondrial DNA in low and high-risk neuroblastoma patients
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Mitochondrial DNA (mtDNA) mutations may contribute to tumor initiation and progression. mtDNA mutations have never been considered as causative and secondary events for neuroblastoma progression. We sequenced 3.6 full-length mitochondrial genomes belonging to 16 low-risk and 20 high-risk (HR) Italian neuroblastomas by Sanger method.

Mutations were selected considering variability ~0.01 (SiteVar algorithm) with respect to 14,144 mitochondrial genomes from healthy individuals. To determine whether the selected mutations were somatically acquired, we sequenced the matched germ-line DNAs.

We found 3 somatic missense mutations in CO1, CO2, CYTB genes in HR patients and 1 in (mt)-tRNA gene in LR patients. Moreover, we identified 47 germ-line mutations: 5 were novel (2 missense and 2 tRNA mutations in HR and 1 insertion in LR patients) and 10 (8.33%) rare missense mutations occurred in HR and 2 (16.7%) in LR patients. Genes with higher germ-line mutation frequency in HR patients than in LR ones included CYTB (20.0% vs 2.6%), ND1 (11.0% vs 0.0%), and (mt)-tRNA (25.0% vs 12.5%). All CYTB and ND1 mutations in HR patients were missense. The highest rate of rare mutations was found in mt-tRNA gene (19.4% of cases). In HR patients, two mutations [one novel] in tRNA-thr altered (mt)-tRNA secondary structure and one novel mutation occurred in the anticodon loop of tRNA-gly. In agreement with recent high-throughput screenings of nuclear DNAs, the rate of somatic mitochondrial mutations in neuroblastoma is low. Rare and novel germ-line variants in CYTB, ND1 and (mt)-tRNA loci might be important contributors to HR neuroblastoma development.

P12.095-S A heterozygous deletion in BUB1B predisposing to pediatric cancer, outside the context of classic Mosaic Variegated Aneuploidy syndrome
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Biallelic mutations in BUB1B cause Mosaic Variegated Aneuploidy (MVA) syndrome, characterized by microcephaly, growth retardation, intellectual disability and cancer predisposition. BUB1B encodes a kinase involved in spindle assembly checkpoint (SAC) function. We describe a boy with PDDNOS who developed acute lymphoblastic leukemia at age 9 years and primary diffuse leptomeningeal gliomatosis at age 17 years. His father, aged 54 years, had a head circumference ~2 SD and developed a brain tumor at the age of 19 years. They both were found to carry a monoallelic deletion of exons 9–23 of BUB1B. Karyotyping of EBV-transformed lymphoblastoid cell lines (LCL) from both father and son, revealed an enrichment of aneuploid cells (10% in both) and a significant increase in premature sister chromatid exchanges when compared to controls. We sequenced the BUB1B gene and documented a monoallelic deletion in each of these two patients presented with features of the MVA syndrome, but did not present the full-blown phenotype, we hypothesized that this could be explained by BUB1B protein expression levels below 50%. To study this hypothesis, BUB1B mRNA and BUB1B protein expression levels were measured in EBV-transformed LCLs of the two cases. Expression levels were ~50% and, therefore, in concordance with a deletion of one BUB1B allele. Our findings suggest a role for heterozygous germline mutations in BUB1B in cancer predisposition, outside the context of classic MVA syndrome. An explanation might be found in digenic inheritance. To study this hypothesis, we will perform exome sequencing on germline DNA of the son and initially we will search for aberrations in SAC genes in particular.

P12.096-M Somatic mosaicism and transmission of an MSH2 pathogenic variant in a parent of a Lynch Syndrome proband
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We describe the finding of somatic mosaicism for a pathogenic variant of the MSH2 gene in the parent of the proband with Lynch Syndrome. The proband was diagnosed with a colorectal tumor at age 50 that was immunodeficient for MSH2 and MSH6. Sequencing and MLPA analysis of MSH2, MSH6 and MLH1 in a blood sample revealed the presence of a heterozygous pathogenic variant in the MSH2 gene, c.920-923delTACG (p.Val307Glufs*23). The father was found to have a low level of mosaicism, <10%, for this variant in DNA from blood. This finding was confirmed on a buccal sample from him which showed a greater proportion of the pathogenic variant, ~20%. The father had been diagnosed with prostate cancer at age 70 and his family history is not suggestive of Lynch Syndrome. The mother’s sample was negative and parentage was confirmed using microsatellite loci BRC1/2 analysis was negative in the proband’s sister who was diagnosed with uterine and ovarian cancer at age 37. She was found to be a carrier of this MSH2 pathogenic variant.

This patient provides evidence that mosaicism can be transmitted from a parent to a child. A case for applying VUS classification based on family history should be considered.

P12.097-S A study to investigate the genetic basis of multiple primary tumours
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Multiple primary malignant tumours (MPMT) are frequently taken as an indicator of potential inherited cancer susceptibility and occur at appreciable frequency both among unselected cancer patients and referrals to cancer genetics services. Analysis of a referred based series of 212 MPMT cases sho-
P2.100-M
Next-generation panel based characterisation of breast/ovarian cancer genetic predisposition
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BACKGROUND. Genetic predisposition to breast and/or ovarian cancer is largely confined to mutations in BRCA1/2 genes, although rarer mutations in other known genes (e.g. TP53, PTEN, CDH1, PALB2, BRI1, CHEK2 etc.) are also important. Massively parallel (or next-generation, NGS) resequencing technology is attractive for identifying cancer predisposing mutations in known genes (panels) and discover new associations.

METHODS. We aimed to better characterise cancer predisposing landscape in clinically selected 96 breast and 96 ovarian cancer cases (with strong family history or early age at diagnosis and negative for previously tested BRCA1/2 genes mutations) by performing NGS based analysis of 94 genes previously associated with both common (e.g. breast, colorectal) and rare cancers (TruSight Cancer Nextera Custom hybridization-based target enrichment) on MiSeq (Illumina). VariantStudio software was used for annotation and filtering of genetic variants.

RESULTS. Of 192 tested subjects, 8% carried germline loss-of-function mutations (confirmed by Sanger sequencing) in 10 cancer predisposing genes, 3 of them were not previously implicated in hereditary breast/ovarian cancer predisposition.

CONCLUSION. NGS panel based resequencing is effective way for better characterising of cancer predisposition landscape.

P2.102-M
Role of inflammatory gene variants in oral cancer in an Indian population
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Oral squamous cell carcinoma (OSCC) is the eighth most common cancer worldwide. Alcohol, tobacco and smoking are well known risk factors for OSCC. During oral cancer development, inflammation, angiogenesis and cell proliferation are involved which correlate with immune cells involved in the production of cytokines, growth factors and adhesion molecules. The study was to evaluate association of cytokine gene variants viz. IL-1RN Variable Number of Tandem Repeats (VNTR in intron 2), IL-1p-511C/T [rs16944], IL-6-597G/A [rs1800797] and TNFα-308G/A [rs1800629] with oral cancer in a north Indian population. Clinical and addiction details of healthy age/sex matched controls (n=140) and OSCC patients (n=77) were recorded after ethical clearance and consent. DNA was extracted and SNPs genotyped by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP). Minor allele frequencies; genotype and allele frequencies were calculated by chi-square (χ2) analysis (SPSS v.15.0). Gene-gene interaction, pairwise linkage disequilibrium (LD) based on D’ statistics and correlation coefficient (r2) of frequencies were analyzed using SHEsis (online version). Genotypic frequencies of IL-1RN, IL-1β and IL-6 while allelic frequency of IL-6 showed significant association with OSCC (P<0.001). TNF-α increases risk of OSCC up to 1.68 times in alcoholic subjects. GATI* and GGTII* haplotypes increased the risk up to 2.863 and 38.285 times respectively. This is the first report from India showing the effect of cytokine gene polymorphisms in OSCC to predict individuals at risk of oral cancer. The knowledge of risk alleles will enable individuals to take precautionary measures before hand and prevent or delay the onset of disease.
Aims and methods: BRCAl/2 mutations occur in 10-15% of ovarian cancer (OC) patients, regardless of family history. In November 2012 a pilot protocol was developed by the Unit of Hereditary Cancer (UHC) and the Oncology Service (OS) caring most OC patients in our Institute, to assess the feasibility of offering genetic counseling (GC) to all OC women. Oncologists agreed to propose GC during their clinics and to refer all interested OC patients by directly arrange a contact with UHC. After the first year we evaluated oncologists’ adherence to protocol, patients’ compliance with GC and testing, and prevalence of BRCAl/2 mutations.

Results: 104 OC women underwent an oncology visit from November 2012 to December 2013. Ten patients were excluded because they had GC in the past. Only 29 patients (29/94; 31%) were referred to GC; 22/29 attended GC (76%) and 21/22 had genetic testing (95%). Three pathogenic BRCa mutations were detected (3/21; 14%); among healthy female close relatives, seven were tested and three were mutation-positive. Referral was much higher for patients attending the first visit (14/26; 54%); than for follow up patients (15/66; 22%). The main differences in these two settings are the time schedule (one vs half an hour) and the checklist in the clinical record (including or not family history).

Conclusions: Patients’ compliance to GC/testing was high, and BRCa mutation prevalence was as expected. However, oncologists’ compliance was low, mainly because of practical barriers. Efforts are needed to integrate GC-focused tools and procedures in oncology practice.

P12.104-M
Targeted resequencing approach to investigate the mutational landscape associated to platinum resistance in EOC

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INTRODUCTION: Despite initial response to first line platinum-based chemotherapy, more than 80% of high grade serous ovarian cancer patients relapse and develop resistance. The molecular and genetic features involved in drug resistance are still unknown. By gene expression profile in a cohort of patients from which matched biopsies were taken at primary surgery (PS-0) when tumor was sensitive to chemotherapy and at time of relapse (SCR) when the tumor was resistant, we identified EMT pathway as a key player in tumor relapse (Marchini et al., 2013). Here we investigate the genomic alterations driving drug resistance by performing targeted DNA resequencing on our cohort of SCR and PS-0 samples.

METHODS: DNA libraries enriched in a selected panel of 30 genes encompassing key players of signal transduction, cell cycle and DNA repair were generated using TruSeq Custom Amplicon kit and sequenced on MiSeq (Illumina). Data were analyzed using a high performance cluster computing platform (Cloud4CARE project).

RESULTS: Analysis identified a total of 166 mutations (152 SNPs and 14 InDels), of which 51 affecting PS-0 and 115 SCR. With a 500X coverage, we observed BRCa1, BRCa2 and TP53 mutated in the majority of cases. In addition, the PI3K pathway was found mutated in SCR samples only.

CONCLUSIONS: Our preliminary results suggest two main conclusions: 1- genomic alterations (SNPs or InDels) were more frequent in SCR compared to PS-0; 2- most of the mutations affected genes belonging to DNA and PI3K pathways.

P12.105-S
Transcriptome and pathway analysis identifies IRF1 as a predictor of progression free and overall survival in ovarian carcinoma

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Epithelial Ovarian Cancer (EOC) is the most lethal female reproductive tract malignancy. Most patients present with advanced stage disease and the cornerstone of treatment is surgical debulking followed by platinum-based chemotherapy. The major contributor to the high fatality-to-case ratio is chemoresistant disease. We sought to identify candidate biomarkers/pathways which could distinguish between platinum sensitive and platinum resistant and test their prognostic ability. Ovarian tumor samples from patients with primary high-grade serous ovarian cancer were divided into two groups based on response to platinum status. Transcriptome analysis was performed using RNA-Seq and Ingenuity Pathway Analysis (IPA) was used to explore differences between these two sets of samples. Findings were validated using qRT-PCR. Survival analysis was performed in two independent sample sets: gene expression data (GE); relapse-free/overall survival in formation (EGA, TCGA). IPA highlighted that Interferon regulatory factor 1 (IRF1) was differentially expressed between the two clinical groups and was upregulated in the platinum-sensitive group. Validation studies performed on 31 patient tumor samples demonstrated a significant difference in PFS between the low and high IRF1 groups (P = 0.027) as well as a distinct difference in the probability of recurrence. In conclusion, we have shown that high levels of IRF1 strongly correlated with increased overall survival in late-stage disease regardless of debulking status and grade in those patients who received platinum therapy.

P12.106-M
Role of p53 gene in the pathogenesis of Acinar Cell Carcinomas of the pancreas


INTRODUCTION: The role of p53 (17p13.1) in the pathogenesis of acinar cell carcinomas (ACCs) has not been fully clarified yet. Few studies, mostly on immunohistochemistry, have investigated the role of p53 in ACCs. The aim of our study is to evaluate the expression of p53 and the point mutations in the p53 gene in a series of primary ACCs and to correlate them with clinical and pathological features.

METHODS: We retrospectively analyzed 22 cases of primary ACCs (sex 11 M and 11 F; median age 65 years, range 21-84), histologically confirmed. Following the guidelines of the Working Group on Acinar Cell Carcinoma of the European Society for Medical Oncology: 2013 consensus conference, we included tumors with acinar pattern, at least 40% of the tumor volume, without evidence of ductal differentiation. The expression level of p53 protein was evaluated by immunohistochemistry (IHC) with the anti-p53 antibody (DO-7, 1:100 dilution). Mutations in the p53 gene were investigated using Sanger sequencing.

RESULTS: p53 mutations were found in 8/22 (36.4%) cases, correlated with higher stage tumor (5 cases were at stage IV). In one case, p53 mutation was observed only in the metastasis of a primary p53 wild-type ACC. Methylation of p53 was observed in only one case. Loss of p53 gene, carried the 617delT mutation in BRCA1/2 gene.

P12.107-S
Do pancreatic cancer patients diagnosed with BRCA1/2 Ashkenazi mutations have less critical risk factors?

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Recent studies have reported a higher occurrence of cancer cases in Ashkenazi Jewish families. Many of these cases are due to a BRCA1/2 mutation. BRCA1/2 genotyping in patients with pancreatic cancer has been recommended by multiple international guidelines. However, the proportion of patients with BRCA1/2 mutations found in patients with pancreatic cancer is still debated.

METHODS: Pancreatic cancer patients who underwent genetic testing for BRCA1/2 mutations were retrospectively reviewed from 1996 to 2013. Demographic, clinical and pathological characteristics were collected for each patient and stratified based on the presence or absence of BRCA1/2 mutations.

RESULTS: Fifty-eight (21.3%) patients were enrolled in the study. Patients with BRCA1/2 mutations were similar to their counterparts without mutations regarding age, smoking status and family history. However, patients with BRCA1/2 mutations had a significantly higher proportion of the following risk factors: a history of breast cancer (20% vs 10%, p=0.03), a history of ovarian cancer (20% vs 1%, p=0.001), a family history of pancreatic cancer (20% vs 1%, p=0.03), and a personal history of pancreatic cancer (20% vs 1%, p=0.03). On the other hand, patients with BRCA1/2 mutations had a significantly lower proportion of diabetes (20% vs 40%, p=0.001), hypertension (20% vs 40%, p=0.001), and hyperlipidemia (0% vs 30%, p=0.001).

CONCLUSIONS: Our study suggests that patients with BRCA1/2 mutations presenting with pancreatic cancer may have a lower proportion of critical risk factors compared to pancreatic cancer patients without BRCA1/2 mutations.
Germline mutations in these genes in patients with apparently sporadic PHEO/PGL were screened. Germline mutations were investigated by using direct sequencing for point mutations in RET, VHL, SDHx, and TMEM, and multiplex ligation-dependent probe amplification for gross deletions in VHL gene.

Results: In 30/200 (15%) PHEO/PGL tumors, germline variants were identified: 12 heterogeneous germline mutations (7 novel nonsense: W218X, frameshifting: c.661delG, p. Asp221ThrfsX27, splicing: c.424-12deltCTCT; missense: R116M; frameshift: c.663_665insA, p. Met213AsnfsX8 and missense: H132R; splicing: c.424-1insG -7 delA) of the SDHB gene, 10 in VHL51 exon, V84M, in 3 families, 6 variants in SDHx, 1 in RET and 1 in TMEM gene. Family members were also tested. Most of the patients with mutations in SDHB gene found to have malignant PHEO/PGL. Patients with mutations in VHL, RET and TMEM genes developed PHEO.

Conclusion: The most commonly mutated gene was SDHB, which carries the highest risk of malignancy. Our patients with extra-adrenal disease needs careful follow-up, since they are in higher risk for the development of metastases or novel adrenal/extra-adrenal PHEO. The patients with VHL mutation (V84M) are apparently classified as 2C. These patients may develop some other tumors than PHEO.

P12.111-S

Post-transcriptional regulation of PHOX2B gene expression

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Neuroblastoma (NB) is one of the most frequent and severe solid tumors in childhood. Mutations in the genes coding for the transcription factor PHOX2B and for its transcriptional target ALK have been detected in sporadic and familial cases of NB and over-expression of the two genes have been identified in NB samples and cell lines.

In this work, in order to investigate the mechanisms underlying PHOX2B overexpression in NB, we report in silico and in vitro characterization of the PHOX2B 3' untranslated region (3'UTR). The first, in silico search for elements known to regulate the mRNA stability allowed us to identify three AU-Rich elements (AREs) in the 3'UTR of the 3'UTR, in addition to several putative miRNAs binding sites.

Regions of the distal PHOX2B portion likely responsible for mRNA regulation were eventually defined by combining the above predictions with results from the phylogenetic conservation of the PHOX2B 3'UTR.

In vitro experiments in IMR32 NB cells have shown that PHOX2B mRNA is stable, thus suggesting it as target of post-transcriptional regulation mechanisms. The successive cloning of the 3'UTR PHOX2B downstream the Luciferase gene in the pmiRGlo vector allowed us to confirm such a hypothesis and, following the generation of constructs containing progressively shorter deletions of the 3'UTR, to map position and extent of the regions responsible for the PHOX2B mRNA stability.

As a consequence of all the above described observations, we suggest that regulation and modulation of PHOX2B post-transcriptional stability may be considered a pharmaceutical target in NB.

P12.112-M

In vitro drug screening approaches targeting PHOX2B over-expression in neuroblastoma

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PHOX2B is a transcription factor involved in the regulation of neurogenesis and in the correct development of autonomic nervous system. Several evidences report a pathogenic role of PHOX2B in neuroblastoma (NB): (i) somatic and germline gain of function mutations in familial, sporadic and syndromic cases of NB; (ii) the finding of ALK, a transcriptional target of PHOX2B, as major familial NB predisposition gene; and (iii) the observation of ALK and PHOX2B over-expression in tumor samples and NB cell lines. Starting from these observations, we have performed an in vitro drug screening targeting PHOX2B over-expression as a potential pharmacological means in NB. First, we have evaluated the effects of (a) a small subset of molecules and an (ii) epigenetic library in a IMR-32 cell line stably expressing Luciferase gene under the control of PHOX2B promoter to identify molecules able in down-regulating PHOX2B expression. Curcumin, SAHA and trichostatin A showed a down-regulation of PHOX2B promoter activity and a decrease of both protein and mRNA expression. To deepen into curcumin mechanisms of action, we have investigated the role of transcription factors (TF) predicted by in silico analysis to bind the PHOX2B promoter.

Supporting: several susceptibility genes have been found to be associated with development of phaeochromocytoma (SFHE2) or paraganglioma (PGL) harbouring an underlying germline mutation in a known PCC/PGL gene. Mutations in SDHx genes (SDHB, SDHD, SDHD and SDHA) encoding a component of the TCA cycle, succinate dehydrogenase (SDH), are a major cause of inherited PCC/PGL. SDHB mutations are also associated with inherited renal cell carcinoma (RCC). Inactivation of SDH in tumour cells results in abnormalities of cellular metabolism associated with activation of hypoxia gene response pathways and epigenetic alterations (e.g. DNA methylation). Similar findings have recently been reported in cases with mutations in the FH gene, which encodes the TCA cycle component directly downstream of SDH, fumarate hydratase.

However, the clinical phenotype of germline mutations in SDHx genes and FH is usually distinct with FH mutations classically associated with hereditary cutaneous and uterine leiomyomatosis and renal cell carcinoma. In order to identify potential novel PCC/PGL predisposition genes we undertook an exome sequencing study in a case of childhood PCC. After identifying a candidate FH missense mutation (p.Glu53Lys) we sequenced FH in a further 71 patients with PCC, PGL or head and neck paraganglioma (HNPG) and identified a further candidate missense mutation (p.Glu353Lys). We then performed in vitro analyses and demonstrated that both missense mutations were associated with elevated intracellular fumarate levels compared to a wild-type rescue construct. These findings (a) confirm that germline FH mutations may present, albeit rarely, with PCC or PGL and (b) extend the clinical phenotype associated with FH mutations to paediatric PCC.

P12.109-S

Molecular analysis of somatic mutations in Phaeochromocytoma and Paraganglioma

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At least a third of patients with phaeochromocytoma (PCC) or paraganglioma (PGL) harbour an underlying germline mutation in a known PCC/PGL gene. Identification of germline mutations and key somatic mutations is important for the application of personalised medicine. To investigate the complete spectrum of mutational hotspots in 50 oncogenes and tumour suppressor genes and HRAS and inherited PCC/PGL gene mutations might be mutually exclusive.

hras and JAK3. Activating mutations in HRAS (p.Gln61Arg and p.Gly13Arg) and deletions of the 3'UTR, to map position and extent of the regions responsible for mRNA regulation and modulation of PHOX2B gene expression in neuroblastoma (NB), we report in silico and in vitro characterization of the PHOX2B 3' untranslated region (3'UTR).

Deletions of the 3'UTR, to map position and extent of the regions responsible for mRNA regulation and modulation of PHOX2B gene expression. In silico analysis to bind the PHOX2B promoter.
Among these TF, we have demonstrated that treatments with 8 or 20 μM curcumin led to a decrease of PBX1/MEIS1 expression and modulated the activity of NFκB and AP-1. Moreover, combined drug treatments showed successful effects of down-regulation of the expression of both PHOX2B and its target ALK, thus supporting the notion of the effectiveness of molecule combination in tumor therapy.

P12.13-S PIK3CA mutations in non-small cell lung cancer
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Background: Most of the proteins that are encoded by oncogenes which play a role in molecular pathogenesis of cancer, function as protein kinases. Acquired constitutional activity of these proteins leads to the activation of signalling pathways which are involved in cell proliferation, apoptosis, protein synthesis, cell migration and many other cellular processes. One of the most important signaling pathways that plays role in the pathogenesis of cancer is phosphatidylinositol 3 kinase (PI3K) signaling pathway. Especially class IA PI3Ks are known to play a role in the pathogenesis of cancer. Basing on this fact, PIK3CA and PIK3CB mutations have been previously reported in many cancers.

Methods: All exons of the PIK3CA gene were sequenced in 40 NSCLC tumor samples. All individuals provided informed consent, and the study was performed in accordance with ethical guidelines.

Results: The 1634A>C mutation which has already been identified in many cancers and NSCLC was determined in 7,5% of the tumor tissue samples. This mutation rate is higher than reported in the literature. Interestingly a second mutation (1658_1659delGTinsC) was identified in these patients. The concurrence of these two mutations has been reported as Cowden syndrome in the literature which is known to be a cancer predisposition syndrome. Conclusion: This finding is quite important since it can be an indication of underlaying cancer predisposition syndrome in NSCLC patients. Besides previously reported PIK3CA mutations some novel mutations have been defined.

P12.14-S Development of Acquired Resistance to Anti-EGFR Therapy in Colorectal Cancer Identified by Whole-Genome Plasma DNA Sequencing
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Introduction: EGFR-targeting monoclonal antibodies, cetuximab and panitumumab, are important therapeutic options in KRAS wild-type metastatic colorectal cancer (mCRC) patients. However, secondary resistance inevitably ensues in all patients within 3-12 months from the start of therapy. The mechanism and timing of emergence of resistance which limit the efficacy of these drugs, is highly relevant for designing therapeutic strategies.

Methods: We examined the plasma DNA of 10 KRAS wild-type mCRC patients who received anti-EGFR therapy, using a high-throughput whole genome sequencing (Plasma-Seq) and ultra-deep sequencing of genes associated with resistance to anti-EGFR therapy such as KRAS, BRAF, PIK3CA and EGFR with Illumina’s MiSeq.

Results: Genome-wide characterisation of the plasma DNA and corresponding primary tumor revealed several tumor specific aberrations such as over-representation of chromosomes 8g, 13 and 20g, and losses of 8p, 4 and 18. The development of resistance to anti-EGFR therapy was observed to be associated with novel foci amplification of KRAS (n=3), MET (n=2) and ERRB2 (n=1) or high level polyclony of 12p which includes KRAS (n=1). Overexpression of EGFR gene was associated with initial good response to therapy. However, ultra-deep sequencing of KRAS, BRAF, PIK3CA and EGFR did not reveal any novel mutations. Conclusion: Overall, predictive biomarkers associated with the anti-EGFR therapy efficacy, correlating well with treatment response was identified in 70% of our patients. The tumor genome is prone to continuous changes and plasma-seq, a fast and affordable tool enables identification of novel druggable sites and guides real-time modification of treatment regimen to delay or prevent disease progression.

P12.15-S PMS2 mutation detection and PMS2 mutation spectrum in the Netherlands
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Background: Most of the proteins that are encoded by oncogenes which play a role in molecular pathogenesis of cancer, function as protein kinases. Acquired constitutional activity of these proteins leads to the activation of signalling pathways which are involved in cell proliferation, apoptosis, protein synthesis, cell migration and many other cellular processes. One of the most important signaling pathways that plays role in the pathogenesis of cancer is phosphatidylinositol 3 kinase (PI3K) signaling pathway. Especially class IA PI3Ks are known to play a role in the pathogenesis of cancer. Basing on this fact, PIK3CA and PIK3CB mutations have been previously reported in many cancers.

Methods: All exons of the PIK3CA gene were sequenced in 40 NSCLC tumor samples. All individuals provided informed consent, and the study was performed in accordance with ethical guidelines.

Results: The 1634A>C mutation which has already been identified in many cancers and NSCLC was determined in 7,5% of the tumor tissue samples. This mutation rate is higher than reported in the literature. Interestingly a second mutation (1658_1659delGTinsC) was identified in these patients. The concurrence of these two mutations has been reported as Cowden syndrome in the literature which is known to be a cancer predisposition syndrome. Conclusion: This finding is quite important since it can be an indication of underlaying cancer predisposition syndrome in NSCLC patients. Besides previously reported PIK3CA mutations some novel mutations have been defined.

P12.16-M A new POLD1 germline mutation as cause of Familial Colorectal Cancer Type X
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The genetic basis for Familial Colorectal Cancer Type-X (fCRC-X) is unknown. Mutations at the proof-reading domains of DNA polymerase ε (POLE) and δ (POLD1) have been recently identified in families with multiple colorectal adenomas and CRC. We aimed to assess the prevalence of POLE and POLD1 mutations in fCRC-X. A total of 63 index cases fulfilling Amsterdam II criteria with normal expression of mismatch repair proteins and microsatellite stable tumors were included. Sanger sequencing of POLE-exon13 and POLD1-exon11 was performed.

None of the cases carried mutations in POLE. We found one case with a new POLD1 variant in heterozygosis: c.1427T>C (p.Leu474Pro). The patient was a woman who underwent a synchronous CRC and large bowel gastrointestinal stromal tumor at age 36. Her maternal aunt had metastatic CRC (dx33y) and endometrial cancer (dx56y) and was a carrier of this variant. The patient was the index case's mother, who underwent endometrial cancer (dx52y), was an obligate variant carrier. It has been described that the homologous residue of POLD1 p.Leu479Pro mutation in S. cerevisiae causes a mutator phenotype. Furthermore, this is the paralogous residue of the hot spot at POLE (p.Leu472). In silico prediction analysis strongly suggests a pathogenic nature for this variant. Integration of all these evidences drove us to classify this new variant as probably damaging.

POLD1 mutations might explain about 1.6% of fCRC-X. POLD1 testing should be included in the diagnostic strategy for unexplained familial CRC.
P12.117-S Polymerase Proofreading - Associated Polyposis (PPAP): an unusual family with the highly penetrant POLE p.Leu424Val mutation with colorectal cancer, polyposis, duodenal cancer and diabetes
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Mutations in the exonuclease domain of the polymerase E (POLE) gene have recently been identified as causing a rare but highly penetrant predisposition to colorectal cancer and colorectal polyps. Pulle et al employed whole-genome sequencing and detected the p.Leu424Val (L424V) mutation in 12 families, with no evidence of a shared common ancestor. The phenotypes of these families were consistent with an autosomal dominant predisposition to colorectal adenomas and carcinomas, with some individuals having multiple tumours. Tumours from these subjects were all microsatellite stable (MSI-S) and did not show any preponderance of site within the colon or particular morphology. There were no extra-colonic tumours described in any affected individuals in these families. Following the identification POLE L424V in our family through the CORGI study, subsequent testing identified the familial mutation in an individual with an MSI unstable (MSI-H) tumour and synchronous duodenal adenocarcinoma, rectal carcinoma and adenomas. It was also noted that all the individuals affected with colorectal cancer in this family also have diabetes, consistent across 3 generations. The co-segregation of colorectal cancer and duodenal cancer requires formal confirmation in some relatives. This association warrants further study. An increased risk of CRC in patients with diabetes mellitus has been recognised for some years. Emerging evidence that polymerase D (POLD1) gene has an important function in adipose tissue homeostasis lends weight to the association. This family provides evidence of a broader phenotype of malignancy in POLE and an opportunity for new investigations into the possible mechanism linking diabetes and colorectal cancer.

P12.118-M Association of variant -765G>C in the PTGS2 gene promoter with melanoma in Italian patients and its relation to gene expression in dermal fibroblasts
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Aim: The production of prostaglandins, especially prostaglandin E synthetase (PGES) is hypothesized to influence carcinogenesis by promoting cell proliferation, inhibiting apoptosis, stimulating angiogenesis, and mediating immune suppression. Cyclooxygenase-2 (COX-2), the inducible isosform of cyclooxygenase coded by the PTGS2 gene, is the key enzyme in the production of prostaglandins involved in inflammatory processes including cancer. In melanoma skin cancer, COX-2 is overexpressed in primary malignant melanoma and in their corresponding metastases. Aim of this study was to investigate if polymorphisms -765G>C (rs20417) and -1195A>G (rs689466) in the PTGS2 gene impact on its expression in dermal fibroblasts and are associated with individual susceptibility to malignant cutaneous melanoma.

Methods: Two hundred forty patients presenting melanoma and 342 control individuals were genotyped for polymorphisms -765G>C (rs20417) and -1195A>G (rs689466) by restriction fragment length polymorphism (PCR-RFLP) analysis. PTGS2 gene expression was performed by Real Time PCR using Sybr Green.

Results: The allele -765C was associated with an increased prevalence of melanoma. No association of -1195A>G polymorphism was observed. Haplotype analysis of both variations showed that the haplotypes carrying the minor alleles were associated to a higher risk of melanoma (p<0.02). Expression analysis indicated that allele -765C is associated to a higher gene expression and thus could represent a risk allele by affecting the functionality of the promoter.

Conclusion: In conclusion, variant -765G>C may be associated to malignant cutaneous melanomas with a low penetrance effect and this effect could be a consequence of altered gene expression.

P12.119-S DNA methylation profiles of PDGFB and FGF2 are potential biomarkers of disease progression in primary myelofibrosis
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The primary myelofibrosis (PMF) is characterized by clonal proliferation of the hematopoietic precursors, fibrosis, osteosclerosis and angiogenesis. Recent evidence revealed that fibrosis is a major cause of mortality, with accelerated fibrosis proliferation – in response to cytokines, such as platelet derived growth factor (PDGF) and the basic fibroblastic growth factor (FGF2), produced by malignant megakaryocytes or monocytes. No specific prognostic markers are today available to refine the clinical classification and the risk to develop fibrosis. The purpose of this study is to investigate DNA methylation of PDGFB and FGF2, to verify possible associations among the epigenetic profile fibrosis progression and prognosis of PMF. The methylation is evaluated by pyrosequencing in a cohort of 58 PMF cases and 20 controls. The methylation percentages of PDGFB and FGF2 in PMF ranged from a complete demethylation to hypermethylation (PDGFB range: 3-95, mean value: 39; FGF2 range: 1-96%, mean value: 40), in controls the methylation values of both genes are clustered in more restricted intervals (PDGFB: 28-46% mean value: 35; FGF2: 16-43% mean value: 28). The methylation values of PDGFB and FGF2 are significantly increased in the prefibrotic cases compared to controls (PDGFB: 71 vs. 33, p<0.0005 and FGF2: 56 vs. 27.5, p<0.0005). Interestingly the hypomethylated PDGFB was an indicator of better prognosis for fibrosis, International Prognostic Scoring System (IPPS) and Dynamic International Prognostic Scoring System (DIPPS) progression (p=0.03, p=0.02 and p=0.01 respectively).

P12.120-M Synergistic effect and VEGF/HSP70-hom haplotype analysis: relationship to prostate cancer risk and clinical outcome
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Prostate cancer is a complex disorder resulting from the combined effects of multiple environmental and genetic factors. Our previous single locus analysis showed that VEGF and HSP70-hom polymorphisms were significantly associated with prostate cancer susceptibility and prognosis. Both genes encoding these proteins were located on chromosome 6p21.2 and combining the neighboring SNPs into haplotypes may increase the association with the disease. Three tagging polymorphs, the HSP70-hom 2437 T/C, the VEGF-1154 G/A and the VEGF-634 G/C SNPs were genotyped in 101 cases and 80 controls. For the combined analysis of VEGF and HSP70-hom we found a positive gradient in the ORs related to the number of high risk genotypes with a 3.53-fold increase of prostate cancer risk (OR=11.9, P=0.05). Furthermore, The TAG and CAG haplotypes at positions HSP70-hom, VEGF-1154 and VEGF-634 exhibited a twofold (OR= 0.46; P= 0.014) and a sevenfold (ORs = 0.14; P= 0.0005) reduction in prostate cancer risk, respectively. Regarding prostate cancer prognosis, the TAG haplotype had a negative association with the aggressive phenotype as defined by the histopathological grade (OR= 0.28; P= 0.006). Our findings confirm the role of at-risk haplotype across the HSP70-hom/VEGF gene cluster in determining susceptibility to prostate cancer.

P12.121-S Frequency and clinical implication of rare BRAF variants other than BRAF V600E mutation in a large cohort of consecutive thyroid fine needle aspiration cytology samples
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Background: BRAF mutation analysis is a useful adjunctive tool in diagnosing thyroid nodules. The BRAF V600E (c.1797T>A) mutation comprises over 95% of all BRAF gene mutations in papillary thyroid carcinoma (PTC). The clinicopathological association of rare BRAF variants other than V600E
mutation is still obscure.

Methods: We evaluated a total of 1067 consecutive patients with malignant or indeterminate thyroid nodules by ultrasonography. All fine needle aspiration cytology (FNAC) samples were tested for BRAF mutation using mutan t variants analytical oligonucleotide microarray (MOMA) sequencing with real-time PCR concurrently. Rare BRAF variants were evaluated with regard to cytology and/or histology results.

Results: BRAF mutations were detected in 37.9% (404/1067) of all samples. The V600E mutation was detected in 98.3% (397/404), and six rare variants were detected by MEMO sequencing. Three types of variants were identified: c.139A>G, c.1606del (p.Val600Leu); c.634del (p.Thr212Pro); c.1794T>1794insGTT (p.Val598_Thr599insGTT). The former two are known mutations to be associated with PTC and three patients with these mutations were diagnosed as PTC histologically. The third variant was novel and presented with c.1801A>T was diagnosed as a benign follicular nodule.

Conclusions: Out of 1067 thyroid nodule cytology samples, six (0.56%) had rare BRAF variants. A novel variant was identified in a cytologically benign thyroid nodule. Targeted testing for detecting only the V600E mutation may be good enough to increase the diagnostic value of FNAC, however, identification of other variants in BRAF gene may provide additional valuable information on the nature of thyroid nodules in the future.

P12.122-M Association between the cytogenetic profile of tumor cells and response to neoadjuvant radiochemotherapy in locally advanced rectal cancer


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Neoadjuvant radiochemotherapy to locally advanced rectal cancer patients has proven efficient in a high percentage of cases. Despite this, some patients show non-response or even disease progression. Recent studies suggest that different genetic alterations may be associated with sensitivity vs. resistance of rectal cancer tumors to neoadjuvant therapy. We investigated the relationship between intratumoral pathways of clonal evolution as assessed by iFISH (5 Idifferent probes) and response to neoadjuvant radiochemotherapy, evaluated by Dworak criteria in 45 rectal cancer tumors before (n=45) and after (n=31) treatment. Losses of chromosomes 1p (4%), 1q (5%), 17p (4%), 18q (38%) and gains of 1q (49%), 13q (75%) as well as amplification of 8q (38%) and 20q (47%) chromosomal regions were those specific alterations found at higher frequencies. Significant association (p<0.05) was found between alteration of 1p, 1q, 1p, 12p and 17p chromosomal regions and degree of response to neoadjuvant therapy. A clear association was observed between specific cytogenetic profile of the ancestral tumor cell clone and response to radiochemotherapy; cases presenting with del(17p) showed a poor response to neoadjuvant treatment (p=0.03), while presence of del(1p) was more frequently observed in responder patients (p=0.002). Moreover, a significantly higher number of copies of chromosomal regions 8q (p=0.004), 13q (p=0.003) and 20q (p=0.002) were found after therapy vs. paired pre-treatment rectal cancer samples. Our results point out the existence of an association between tumor cytogenetics and response to neoadjuvant therapy in locally advanced rectal cancer. Further studies in larger series of patients are necessary to confirm our results.

P12.123-S Epigenetic Profile of Early Relapsed Childhood ALL

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Relapsed acute lymphoblastic leukemia (ALL) is one of the leading causes of death among children with cancer. The prognosis for early relapse children remains poor. To discover the underlying epigenetic pathways that may play a role in drug resistance and relapse, we performed Infinium HumanMethylation450K BeadChip arrays and to characterize the molecular evolution of relapsed childhood ALL, we used Ilumina HumanCytoSNP-12 arrays to identify somatic copy number alterations (CNAs) in 16 diagnosis/relapse pairs. For sorted tumor cell population subclonations and CD4+CD8+ T-cell purified from thymus were used as controls. Analysis variant CpG sites in an unsupervised manner, we identified three distinct DNA methylation profiles in samples according to their structural variations. ALL cases did not show significant differences between paired diagnosis/ relapse samples though they had hyper DNA methylation than control samples. Aversion DNA methylation had been detected in negative regulatory region and c1410 and DNA damage repair genes in ALL. Copy number analysis of patients revealed varying numbers of genetic lesions ranging from 0 to 45 CNAs per sample. The vast majority of CNAs observed were shared between diagnosis and relapse in the same patients. One of the most frequent CNAs involved deletions of CDKN2A/B, occurring in 8 patients, 2 of 8 patients lost the CDKN2A/B at relapse time. Integration of methylation and genotyping data revealed high concordance. Early relapse samples were more likely to be similar to their respective diagnostic sample that suggests early-reapse results from the emergence of a related clone.

P12.124-M Genome-wide methylation analysis of tubulocystic and papillary renal cell carcinoma

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This study was undertaken to characterize and compare molecular signatures associated with tubulocystic renal cell carcinoma (TRCC) and papillary renal cell carcinoma (PRCC). We performed methylated DNA immunoprecipitation (MeDIP) coupled with genome wide microarray analysis (Roche NimbleGen) in 6 PRCC and 2 TRCC together with analysis of control tissues of normal histological appearance adjacent to each examined tumor sample. All examined tumors and their control tissues of histological normal appearance share alteration in regulation of specific pathways. In TRCC we found higher number (48) of methylated tumor suppressor genes (TSG) than in PRCC (35). Only TSG DPH1 and VHL were found to be distinctive methylated in all PRCC. The methylation of gene sequences SPIB, PLAT, PCDHB1 and FAF2 was found exclusively in TRCC tumor samples, the methylation in genes KIF3C, MDH2, PAG1, BC1D1, PLG and CTRL was limited to all PRCC tumor samples only. Using bioinformatics tools (DAVID and GSEA), we found the pathways and genes which are altered by differential methylation and which may be responsible for developmental divergence of both tumors from precancerous renal tissue. The genes involved in transmembrane transport of small molecules, lipoprotein metabolism and transmission across chemical synapsis were found to be differentially overexpressed in both tumors compared to normal renal tissue. The genes involved in transmembrane transport of small molecules, lipoprotein metabolism and transmission across chemical synapsis were found to be differentially overexpressed in both tumors compared to normal renal tissue. Using bioinformatics tools (DAVID and GSEA), we found the pathways and genes which are altered by differential methylation and which may be responsible for developmental divergence of both tumors from precancerous renal tissue. The genes involved in transmembrane transport of small molecules, lipoprotein metabolism and transmission across chemical synapsis were found to be differentially overexpressed in both tumors compared to normal renal tissue. Using bioinformatics tools (DAVID and GSEA), we found the pathways and genes which are altered by differential methylation and which may be responsible for developmental divergence of both tumors from precancerous renal tissue. The genes involved in transmembrane transport of small molecules, lipoprotein metabolism and transmission across chemical synapsis were found to be differentially overexpressed in both tumors compared to normal renal tissue. Using bioinformatics tools (DAVID and GSEA), we found the pathways and genes which are altered by differential methylation and which may be responsible for developmental divergence of both tumors from precancerous renal tissue.

P12.125-S Identification of mosaicism in patients with sporadic retinoblastoma using nGS

G. Gómez-Mariano1, C. Rodriguez-Martín1, L. de la Vega1, A. Zaballori1, C. Sahade1, T. Vendrell1, J. C. Ferreter2, N. Martín1, P. García-Miguel1, A. Sastre2, J. Abellardia, A. Alonso3;1Instituto de Investigación de Termedmoléculas Bases, Majadahonda-Spain, Centro Nacional de Microbiologia, Majadahonda, Spain, 'Hospital Vall d'Hebron, Barcelona, Spain, 5Hospital Infantil La Paz, Madrid, Spain.

Objectives: Retinoblastoma is the paradigm of hereditary cancer. Approximately, 40% of patients carry germline mutations in the retinoblastoma gene RB1 that predispose to develop retinoblastoma. These mutations can be detectable in blood DNA using standard techniques. However, these techniques are not sensitive enough to detect mosaicism in sporadic cases of the disease (without family history). This study was thus designed to determine the frequency of mosaicism in patients with sporadic retinoblastoma by using next generation sequencing (NGS) technologies.

Methods: We selected for this study 22 patients with sporadic retinoblastoma in which we had previously identified two inactivating mutations in the RB1 gene in tumor DNA, but in which none of those mutations were detected in blood DNA using Sanger sequencing. Blood DNA of these patients was analyzed by nGS (Roche/454) in order to identify in low proportion
P12.126-M

BRC1 and TP53 germline mutations are associated with gynecologic sarcomas

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Introduction: gynecologic sarcomas comprise less than 1% of all gynecologic malignancies and represent very heterogeneous group. Very little is known about etiology of these malignancies, the only documented etiologic factor in less than 25% of these tumors is previous pelvic irradiation and some have been linked to tamoxifen treatment. We report that BRC1 & TP53 germline mutations are associated with gynecologic sarcomas.

Methods:

During the last ten years 16 patients diagnosed with gynecologic sarcoma were seen at Genetic Counseling Unit at Cancer Center & Institute of Oncology in Warsaw. All the patients were offered genetic testing, after genetic counseling and after obtaining the written informed consent. The consent protocol was accepted by the local ethical committee. BRC1 analysis was performed on DNA from the peripheral blood leukocytes. The mutations in BRC1 were detected using DHPLC and sequencing of exons 2, 5, 11 and 20, where the Polish founder mutations are found most frequently. TP53 gene was sequenced from exon 2 to 10.

Results:

Among 16 patients with gynecologic sarcomas, we found 6 (37%) BRC1 germline mutation carriers. We have found only one TP53 mutation carrier, who had been diagnosed with vulvar angiosarcoma at exceptionally young age.

Conclusions:

In women diagnosed with gynecologic sarcoma screening for the germline mutations in the BRC1 gene is important strategy. In pediatric patients gynecologic sarcoma should prompt Li-Fraumeni syndrome diagnosis.

Table 1

<table>
<thead>
<tr>
<th>Patient ID number</th>
<th>Diagnosis</th>
<th>Age at diagnosis (years)</th>
<th>Mutation in BRC1</th>
<th>Mutation in TP53</th>
</tr>
</thead>
<tbody>
<tr>
<td>DN 121</td>
<td>Oviductal carcinosarcoma and serous G3 ovarian adenocarcinoma</td>
<td>67</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>DN 110</td>
<td>Carcinosarcoma uterine serous</td>
<td>54</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>DN 1430</td>
<td>Carcinosarcoma uterine adenocarcinoma</td>
<td>52</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>DN 1659</td>
<td>Vulvar sarcoma fungocellulare</td>
<td>75</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>AN 245</td>
<td>Oviductal carcinosarcoma and uterine adenocarcinoma G3 and uterine stromal sarcoma</td>
<td>60</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>AN 1263</td>
<td>Aterine leiomysarcoma</td>
<td>45</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>DN 5098</td>
<td>Vulgar angiogamesoma</td>
<td>0</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

P12.127-S

Glutathione-S-transferase P1 gene in secondary Acute Myeloid Leukemia


Interactions between genetic, epigenetic and genotoxic factors play a pivotal role in secondary acute myeloid leukemia (s-AML) development. The GSTP1 is a well-known housekeeping gene engaged in the detoxification of a variety of carcinogens. We hypothesized that genetic and epigenetic mechanisms resulting in reduction or inactivation of GSTP1 expression may be implicated in s-AML pathogenesis. Thus, we investigated the possible implication of the A31G germline polymorphism in s-AML development and/or its specific chromosomal abnormalities. Moreover, we studied the possible contribution of GSTP1 promoter hypermethylation in s-AML development and the methylation status in respect to patients' genotype. Concerning GSTP1 genotyping, a case-control study in 75 s-AML patients and 185 controls was performed by Real-Time PCR. The GSTP1 hypermethylation was studied by methylation-specific PCR in 40 of the above cases and 15 controls. The genotypic distribution between cases and controls revealed a statistically higher frequency of the variant genotypes (A/G, G/G) in s-AML compared to the controls (p=0.001). Allele frequency distribution analysis showed that s-AML patients exhibited an almost 2-fold increased risk of carrying at least one variant G allele compared to the controls. Stratification of patients according to the karyotype revealed a significantly increased frequency of A/G heterozygotes in patients carrying -7/ del(7q) (89.5%). The GSTP1 promoter was hypermethylated in a large proportion of s-AML patients (32.5%), but in none of the controls. No statistically significant associations were found between the methylation status and GSTP1 genotype. Our findings provide evidence for an important role of the GSTP1 gene in AML pathogenesis.

P12.128-M

TP53 gene mutation analysis in breast cancer: Our experience of six years

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Hereditary breast cancers account for 5-10% cases and are predominantly due to BRC1/2 genes and less commonly due to other high penetrant (TP53, STK11, Pten) and less penetrant (CHEK2, ATM, PALB2, BRI1) genes. Breast cancer is an important component of Li-Fraumeni Syndrome (LFS) cancer spectrum related to TP53 gene mutation. Genetic testing for BRC1/2 is generally not offered in young breast cancer women (<30 years) unless they meet the testing criterion (Manchester score etc). However TP53 testing is suggested for young breast cancer alone cases without family history of LFS tumours

We analysed our data of past 6 years (2008-2013) of TP53 gene testing in women with breast cancer. Information was obtained regarding family history of cancers, age of onset, receptor pathology and BRC1/2 testing. No TP53 mutation was found in our breast cancer cohort. The solitary TP53 mutation was detected in a woman with family history suggestive of LFS. The TP53 gene mutation detection rate in our cohort was only 4.5% (1/22).

Although our sample size is small TP53 gene mutation rate is likely to be better in breast cancer cases with LFS family history and may be influenced by receptor pathology as published previously. Genetic centres with limited resources need to consider these factors before requesting expensive genetic tests. Availability of affordable NGS panel testing for breast cancer genes may address this issue in future and would also help in understanding the contribution of the above mentioned genes to hereditary breast cancer.

P12.129-S

Expression analysis of TNF related apoptosis inducing ligand and its receptors in gastrointestinal tumors

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TRAIL (TNF related apoptosis inducing ligand) is a member of the tumor necrosis factor superfamily. Due to its ability to selectively induce apoptotic death in transformed cells, TRAIL pathway has been considered as a promising drug target for cancer therapy. Our purpose was to examine TRAIL pathway components expression in gastrointestinal tumors.

mRNA relative expression levels in 45 colon, 11 esophageal, and 11 gastric cancers (37 RCL2-fixed, 30 fresh frozen,4 male and 24 female, median age 67 years) along with matching normal tissues were analysed using RT-PCR for TRAIL pathway genes, namely TRAIL, DR4, DR5, DcR1, DcR2, OPG. Colon cancer samples displayed elevated mRNA levels in 20% (9 of 45), 42% (19 of 45), 47% (21 of 45), 64% (29 of 45), 44% (20 of 45), 31% (14 of 45) of the cases for TRAIL, DR4, DR5, DcR1, DcR2, OPG genes respectively. Furthermore, TRAIL receptors were found simultaneously overexpressed in a subset of colon tumours. TRAIL overexpression was correlated with low stage tumours (p=0.03) in our cohort. Esophageal cancers overexpressed TRAIL and its receptors DR4, DR5, DcR1, DcR2 at 18%, 54.5%, 27%, 45.5% and 45.5% of the cases, respectively. Finally, in gastric cancer samples overexpression was found in 45% of the cases for TRAIL, DR4, DcR1, DcR2 and TRAIL. TRAIL receptor mRNA levels were found elevated in a significant percentage of gastrointestinal tumours. In addition, TRAIL expression emerged as an early event, which may delay disease progression.
P12.130-M
Discordant molecular breakpoints in an apparent recurrent translocation (3;12)(q13;p13)

D. Costa\(^1\), C. Muñoz\(^2\), A. Carrión\(^2\), A. Arias\(^3\), C. Gómez\(^4\), F. Solé\(^5\), B. Espanet\(^6\), G. Anascea\(^7\), M. Calafat\(^8\), E. Campo\(^9\), B. Matute\(^10\).

\(^1\)Unitat d’Hematopatologia. Hospital Clinic, Barcelona, Spain, \(^2\)Institut de Recerca contra la Leucèmia Josep Carreras, Badalona, Spain, \(^3\)Llaboratori de Citogenètica Molecular Hospital Mar, Barcelona, Spain, \(^4\)Hospital Clínic Universitario de Zaragoza, Barcelona, Spain, \(^5\)Universidad de Navarra, Barcelona, Spain, \(^6\)Departament d’Hematologia. Hospital Clinic, Barcelona, Spain.

Background: The identification of recurrent translocations in neoplasia has been used to identify the genes involved in the neoplastic process in order to develop new therapies.

The objective of our study was to determine if the breakpoints determined by conventional cytogenetics in two apparently recurrent translocations (3;12)(q13;p13) were the same at the molecular level. Material and Methods: Two patients with myelodysplastic syndrome had a complex karyotype including a translocation (3;12)(q13;p13). In addition to the translocation, both karyotypes shared a deletion of chromosome 5 and a trisomy of the chromosome 8. A set of 35 Bacterial Artificial Chromosomes (BACs) were used to cover the 3p11.1-q13.32 region and a set of 12 BACs were used to cover the 12p12.1-p13.31 region. Results: The breakpoints were precisely established only for the translocation (3;12)(q13;p13) in patient 1, within the BAC RP1-491D12 at the 3p12.2 region and within the BAC RP1-705C15 at the 12p13.31 region. A region of 22.458 kbp was deleted including the 12p13.2 - 12p11.21 region. In patient 2 the breakpoints on chromosomes 3 and 12 were not found, but based on the BACs study they should be more centromeric than the breakpoints determined in patient 1. A deleted region of 23.163 kbp including the 12p13.2 - 12p11.21 region was found in patient 2. Conclusion: BACs studies revealed that the breakpoints in two apparently recurrent translocations were different at the molecular level. Both translocations showed a deletion of 22.458 kbp - 23.163 kbp near the breakpoint, including the 12p13.2 - 12p11.21 region.

P12.131-M
Prevalence of BRCA1 and BRCA2 germline mutations in triple-negative breast cancer

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University of Newcastle, Hunter Medical Research Institute, New Lambton Heights, Australia.

Triple-negative breast cancer (TNBC) is a subtype of breast cancer that lacks ER, PR and HER2 expression. TNBCs have a gene expression profile similar in Australia.

Material and Methods: Two patients with myelodysplastic syndrome had a complex karyotype including a translocation (3;12)(q13;p13). In addition to the translocation, both karyotypes shared a deletion of chromosome 5 and a trisomy of the chromosome 8. A set of 35 Bacterial Artificial Chromosomes (BACs) were used to cover the 3p11.1-q13.32 region and a set of 12 BACs were used to cover the 12p12.1-p13.31 region. Results: The breakpoints were precisely established only for the translocation (3;12)(q13;p13) in patient 1, within the BAC RP1-491D12 at the 3p12.2 region and within the BAC RP1-705C15 at the 12p13.31 region. A region of 22.458 kbp was deleted including the 12p13.2 - 12p11.21 region. In patient 2 the breakpoints on chromosomes 3 and 12 were not found, but based on the BACs study they should be more centromeric than the breakpoints determined in patient 1. A deleted region of 23.163 kbp including the 12p13.2 - 12p11.21 region was found in patient 2. Conclusion: BACs studies revealed that the breakpoints in two apparently recurrent translocations were different at the molecular level. Both translocations showed a deletion of 22.458 kbp - 23.163 kbp near the breakpoint, including the 12p13.2 - 12p11.21 region.

P12.132-M
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\(^1\)Unitat d’Hematopatologia. Hospital Clinic, Barcelona, Spain, \(^2\)Institut de Recerca contra la Leucèmia Josep Carreras, Badalona, Spain, \(^3\)Llaboratori de Citogenètica Molecular Hospital Mar, Barcelona, Spain, \(^4\)Hospital Clínic Universitario de Zaragoza, Barcelona, Spain, \(^5\)Universidad de Navarra, Barcelona, Spain, \(^6\)Departament d’Hematologia. Hospital Clinic, Barcelona, Spain.

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P12.133-S
Comparison between splicing reporter minigene assays and patient blood RNA analyses used for the assessment of splicing defects caused by variants in the DNA mismatch repair genes


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A fraction of sequence variants found in disease-causing genes induce aberrant splicing. At the moment, reliable splice-prediction tools are only available for variants in the consensus splice site regions, and even these cannot predict the exact molecular nature of the aberrant transcription. Wet-lab splicing assays, either RTPCR analyses of patient RNA or functional splicing reporter minigene tests, have to be performed to confirm an aberrant splicing. Here, we present results of splicing reporter minigene assays performed for 37 disease-gene variants of unknown significance (VIS), mainly found in the four DNA mismatch repair genes MLH1, MSH2, MSH6 and PMS2 that are associated with Lynch syndrome. Twenty variants are located in the consensus splice site regions, 13 are exonic and 4 are deep-intronic variants. We used a previously described minigene vector, and transfected HEK293 and/or HeLa cells with wildtype and variant constructs. For 32 variants also results from patient RNA analyses were available, either performed by our laboratory or presented in literature. For comparison with minigene assay splicing data, we especially included variants that showed multiple aberrant transcripts in patient RNA analysis, or another splice effect than the prevalent exon skip. We found 100% concordance between patient RNA analyses and minigene assays in terms of showing an effect on splicing or not. However, for 6 variants discrepancies in the molecular nature of aberrant transcription were observed. Possible explanations for these discrepancies, and implications for the assessment of pathogenicity of the variant are discussed.

P12.134-M
Genetic risk factors in a vulvar cancer cluster among young Indigenous women in Arnhem Land, Australia

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Vulvar cancer is usually rare, and occurs most often in postmenopausal women. Among young (<50 years) Indigenous women living in remote Aboriginal communities in Arnhem Land, however, the incidence of this malignancy is more than 70 times the national Australian rate for the same age group. Previously, we found that neither excess human papillomavirus (HPV) incidence nor a particularly virulent strain of HPV could explain the very high incidence of vulvar cancer in this population. Reports from the Gynaecology Outreach Service that cases appeared to cluster in family groups suggested that a genetic susceptibility, either to the effects of HPV or another cause of vulvar cancer, may be involved in this cluster. To investigate the role of genetic risk factors, 30 cases and 61 controls, matched on age and community of residence, were recruited to the study. DNA was extracted from saliva samples, and genotyped to provide information on approximately 2.5 million variants. These data were analysed using both genome-wide association and identity-by-descent techniques. We found clear evidence for the involvement of a genetic risk factor predisposing this population to vulvar cancer, and identified three genomic regions of interest. Bioinformatics analysis prioritised biologically plausible candidate genes within these regions and functional studies to further elucidate the role of genetic variants in the aetiology of vulvar cancer are currently underway. This is the first genetic
study of this population, and these findings continue to inform health care delivery in Arnhem Land, especially vaccination policy and screening strategies.

P12.135-S

Whole exome sequencing identifies novel mutations from DNA repair pathways in familial esophageal squamous cell carcinoma

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Introduction: Esophageal cancer (EC) is the fifth leading cause of cancer death in the world, and squamous cell carcinoma (SCC) is a major subtype. This aggressive subtype comprises 95% of all Iranian EC cases. High existence of familial aggregation among ESCC cases necessitates the identification of new germline mutations for the purpose of surveillance and personalized medicine.

Methods: We analyzed the mutation spectra from 100x whole exome sequencing (WES) of peripheral blood mononuclear cell’s DNA extracted from 10 affected probands of familial ESCC using SureSelect target enrichment system capture process (Agilent, USA). All family members directly sequenced for confirmation of targeted mutations in patients.

Result: We identified 9 mutated genes from DNA repair pathways including ATM, BRCA1, AXIN2, FANC, FANCB, FAHACE, RAD51AP1, RAD51AP2, RECQL5 with the allele frequencies below 0.01 according to 1000 Genome project (October 2011). Novel non-synonymous substitution found in FANCE causing glutamate to aspartate amino acid change. All mutations identified as damaging variants using SIFT and PolyPhen softwares. Conclusion: The use of WES identified that familial cases of ESCC harbor mutations from DNA repair, especially homologous recombination and Fanconi anemia pathways. These findings have potential implications on the surveillance and treatment of ESCC patients. Particularly, these patients may benefit from treatment with DNA cross-linking chemotherapeutic drugs, such as cis-platinum and mitomycin C, or from a PARP [poly (ADP-ribose) polymerase] inhibitor. Additionally, genetic counseling and mutational analysis of the proband’s relatives will considerably benefit these individuals for their surveillance and early diagnosis leading to an improved disease management.

P12.136-M

Whole transcriptome analysis of testicular germ cell tumors

L. Degoricja1, K. Y. Lee2, S. Patel3, A. J. M. Gillis4, B. M. Biljarens1, L. C. J. Dorssers1, L. Lee1,2,3,4,5; 1Thermo Fisher Scientific, South San Francisco, CA, United States, 2Erasmus MC University Medical Center, Rotterdam, Netherlands, 3Medical University, Fairfax, VA, United States, 4INT National Cancer Institute, Aviano, Italy, 5Treviso General Hospital, Treviso, Italy.

Next generation sequencing of the whole transcriptome enables high resolution measurement of gene expression activity in different tissue and cell types. This methodology provides an in depth study of known transcripts and depending on the data analysis, allows identification of additional transcript types such as transcript variants, fusion transcripts, and small and long ncRNAs. In this study we performed RNA-Seq using the Ion Torrent Proton platform to compare the expression profile of testicular germ cell tumors (Sertoli cell type, n=3) and normal testis (n=3). Using Partek Flow and Star or TopHat aligners, we aligned the reads to the human genome and mapped sequences to the RefSeq database. We identified a large number of genes that were up and down regulated with high degree of significance (p<0.01, >2X FC). These included genes related to testicular tissue type, stem cell pluripotency (NANOG, POU5F1) and proliferation (KRAS, CCND2). In addition, many non-coding and recently discovered ncRNAs were identified (SNORD12B, XIST). The method was validated on a small set of genes (>20) using qPCR (TaqMan Assays). We used the Open Array platform to quantitatively screen a larger number of differentially expressed genes (~24) across a number of different testicular germ cell tumor types (non-seminoma).

P12.137-S

Upregulation of key WNT signaling molecules in human astrocytic brain tumors

N. Pecina-Slaus1, A. Kafka2, B. Tomas3, B. Kruslin2; 1Medical School University of Zagreb, Zagreb, Croatia.

The knowledge on molecular profiles of astrocytic brain tumors still needs elucidation. In the present study key players of Wnt signaling, beta-catenin (CTNNB1), TCF1 and LEF1, adenomatous polyposis coli (APC) and axin (AXIN1) were investigated in the set of human astrocytic brain tumors. The investigation of beta-catenin demonstrated 10% of samples with potential activating mutations. The results on protein levels demonstrated that 50% of glioblastomas (WHO grade IV) and 56% of astrocytomas (WHO grades II and III) showed upregulation of beta-catenin and nuclear localization was found in 5.2% of glioblastomas. Transcription factors of the wnt pathway were also upregulated. Strong TCF1 and LEF1 expression was observed in 51.6% and 7.1% of glioblastomas. Analysis of variances performed on the total sample indicated significant differences in the values of TCF1 weak expression (F=2.804; p=0.045) and LEF1 weak expression (F=5.498; p=0.002) with regard to malignancy grade. The F-ratios for two variables (LEF1 strong and LEF1 weak) indicated that differences between astrocytomas (II, III) and glioblastomas were statistically significantly higher (p=0.02).

Allelic losses of APC gene were frequent with glioblastomas showing 60% and diffuse astrocytomas (grade II) 20%. Allelic losses of AXIN1 were found in 10% of glioblastomas. In 31% of glioblastomas and 22% of astrocytomas downregulation of axin proteins was detected. In 31% of glioblastomas axin was localized in the nucleus. Our findings contribute to understanding of human astrocytic brain tumor genetic profile and suggest that molecular changes of wnt signaling play important roles in astrocytic tumor etiology.
significantly decreased cell proliferation and cell cycle perturbation asso-
ciated with upregulation of p21 and p27 cell-cycle inhibitors, reduced cell
migration (p=0.048) and anchorage-independent growth (p=0.02). In CaKi
cell line, YAPI silencing induced significantly increased sensitivity and cell-
death response to cisplatin treatment (p=0.001) as well as reduction of in-
vivo tumorigenic potential (p=0.027).
Overall, these results establish that YAPI is a direct oncogenic target of the
11q22 amplicon in previously unreported cancer types and support the re-
llevance of such genetic aberration in carcinogenesis in a fraction of multiple
tumor types.

P12.140-M
No change in the rate of bilateral mammographies after BRCAl/2
testing among true non-carriers
G. Larouche1, J. Chiquette2, J. Simard3, M. Dorval3,4
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The majority of women who are true non-carriers of the BRCAl/2 familial
mutation may be reassured that they are no longer considered at high risk
for breast and ovarian cancer. For this reason, most should be encouraged
to adopt the same cancer screening practices as those recommended to
women of the same age in the general population. The aim of this study is to
compare the rate of bilateral mammographies after BRCAl/2 testing that
took place among true non-carriers of BRCAl/2 mutation. Information from
the Quebec Health Insurance Board was used to identify all registered bilateral
mammographies done between May 1, 1998 and March 31, 2012 among a
cohort of 143 French Canadian unaffected true non-carriers. The Cox pro-
portional hazards model for repeated events, with women's age as the time
was used, to obtain hazard ratios of bilateral mammographies. The rate of
mammographies did not change after BRCAl/2 testing, neither glob-
ally (HR=0.93, p=0.22), nor by age (<50 years HR=0.81, p=0.13; ≥50 years
HR=1.01, p=0.84). Although women <50 years had a lower rate of mam-
mographies than women ≥50 years (HR=0.55; 95% CI =0.43-0.70) after
genetic testing, 74% still continued to be screened, which is not generally
recommended to women of the same age group in the general population.
In conclusion, genetic testing information did not have a significant effect
on mammography screening in our cohort of true non-carriers of BRCAl/2 mutation.
Clear-cut recommendations for the follow-up of true non-carriers
of BRCAl/2 mutation are needed.

P12.141-S
EGFR and ALK gene mutations screening in Non-small-cell lung
cancer (NSCLC) specimens
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BACKGROUND: Testing for genetic abnormalities in epidermal growth factor receptor (EGFR) and anaplastic lymphoma receptor tyrosine kinase (ALK) is a critical tool in the care of advanced NSCLC. We investigated the incidence of epidermal growth factor receptor (EGFR) mutations and anaplastic lymphoma kinase (ALK) rearrangements in Lithuanian patients with non-small cell lung cancer (NSCLC). MATERIALS AND METHODS: 326 NSCLC paraffin-embedded, formalin-fixed (FFPE) specimens were collected, DNA extracted, and using real time quantitative analysis in EGFR gene and translocation in AKL gene by IHC following confirmation positive test by FISH analysis. RESULTS: We screened 326 consecutive patients with NSCLC for the presence of concomitant EGFR mutations and ALK rearrangements Mutations in EGFR gene appeared mutated in 17%; ALK translocation was found in 4% of NSCLC cases.

P12.142-M
miR-106b-5p may act as tumor suppressor by down-regulation
C1orf24 expression in human thyroid carcinoma.
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We previously have shown that the C1orf24 gene is highly expressed in
follicular thyroid carcinomas while is not expressed in benign thyroid le-
sions. However, little is known about the molecular mechanism involved
in C1orf24 expression. It is widely demonstrated that microRNAs (miRs) are
potent regulators of gene expression. The miRs expression varies in accor-
dance with tissue, development stage, and tumor types. We therefore, investi-
gated whether miRs could modulate C1orf24 expression in thyroid cancer.
In this study, we show that the miR-106b expression is lower in thyroid
carcinomas, than in benign thyroid lesions (p<0.01). Functional analysis
was performed in follicular thyroid carcinoma cell line (WRO), which highly
express C1orf24 gene. The ectopic expression of miR106b into WRO resul-
ted in down-regulation of C1orf24 at both mRNA and protein levels, when
compared to negative control. Mutations made at miR-106-5p binding sites
in the C1orf24 mRNA 3’ UTR showed that miR-106b directly interacts with
C1orf24. Additionally, miR-10b overexpression significantly decreased the
mRNA levels and transcriptional capabilities in LiPa whilst increased an
apoptosis rate. Our findings indicate that miR-106-5p may play a role in thyroid carcinogenesis by nega-
atively regulating C1orf24 expression and, therefore, by acting as tumor
suppressor. However, further analyses are needed to fully demonstrate the
role of miR-106b-5p in thyroid carcinogenesis.
Financial Support: FAPESP (2012/02902-9) and CNpq (740041/2013-5)

P12.143-S
SOX2 gene expression and copy number variation in squamous cell
lung cancer
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Transcription factor SOX2 might have an important oncogenic role in
development and progression of lung cancer. The aim of our study was to estima-
tate copy number variation (CNV) of SOX2 in primary tumours and to analyse
SOX2 expression in primary tumour tissue and in blood samples in relati-
on to CNV status in squamous cell lung cancer patients. 28 patients with
metastatic squamous cell lung cancer were prospectively included between
years 2010 and 2013. Total RNA was isolated from whole blood collected in
PAKgene Blood RNA Tube before any systemic treatment. For 10 patients
primary tumour tissue was extracted from the same tissue samples; for those
patients RNA and DNA were extracted from FFPE tumours. SOX2 expression
levels and CNV status were determined by quantitative RT-PCR. Copy num-
er analysis revealed high copy number of SOX2 in 3/10 (30%) tumours and
gain of function in another 3/10 (30%) tumours. Median SOX2 expression in
FFPE tumour tissue was 3.9 (0.1-29.1) and in blood samples 5.4 (0.4-17.9).
Elevated copy number of SOX2 correlated with higher SOX2 expression in
tumours (p=0.92). Moreover, correlation between SOX2 expression in blood
samples and FFPE tumour tissue was observed (p=0.60); patients with high
SOX2 expression in blood tended to have high SOX2 expression in tumour
tissue. According to our observation genetic alterations in SOX2 seem to be
common event in squamous cell lung cancer. Furthermore, good correlation
between SOX2 expression in blood and tumour samples supports the idea of
liquid biopsy in lung cancer.

P13.01-S
A French series of 731 patients with 22q11.2 microdeletion
Variable phenotype in adults: some had intellectual disability with typical gestalt, some had psychiatric disorders, and a minority was almost asymptomatic parents when the diagnosis was first made in their children.

Molecular data: Thirteen cases were diagnosed with array-CGH. The majority of these patients were referred for intellectual disability (n=10/13) and only 3 had heart defect. The size of the deletion was variable: 745 - 2904 kb and surprisingly only 46.2% had deletion between LCR22-A and LCR22-D. The 22q11 deletion was inheritable in 22.8% of cases and mainly maternally (86.7%).

Conclusion: We report the largest series of 22q11DS postnatal diagnosis. We plan to study more patients using array-CGH to investigate whether the size of the deletion or the presence of other CNV may explain the phenotype variability.

P13.02-M Transgenerational inheritance of 22q11.2 atypical deletion: instability of LCRs in a family with discordant clinical phenotype

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Genomic rearrangements in 22q11.2 region are caused mainly by LCRs elements or repetitive sequences, leading to different clinical phenotypes including velocardiofacial or DiGeorge syndrome. Most of the deletions encompass the ~3 (LCR A and D) or ~1.5 Mb (LCR A and B), however atypical deletions have been described in a few cases. Patients with genomic alterations in 22q11.2 region present a spectrum of phenotypic manifestations and this condition can be more severe compared with the relatives. We report on a family, including the maternal grandfather, the mother and daughter, with discordant clinical phenotype and 22q11.2 atypical deletion using SNP-array (Illumina 850K) in order to better delineate the size of deletion. In three generations we found a difference in the deletion size in the 22q11.2 region, range of approximately 0.1 Kb to 0.5 Kb, encompassing the A and B LCRs for mother and daughter, and also the A and C LCRs in the grandfather. Although there are rare, atypical variant deletion endpoints could provide important insights related to the role of genomic architecture in chromosomal rearrangements, chromosome evolution, and in human disease.

P13.03-S A catalog of hemizygous variation in 127 22q11.2 deletion patients

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The 22q11.2 deletion syndrome (22q11.2DS) is the most common chromosomal deletion syndrome in humans with an incidence of 1 in 2-4000 live births. The clinical presentation of 22q11.2DS is extremely variable, but the underlying reason for this variation remains unknown. Individuals with 22q11.2DS most often have a clinically associated 3Mb or 1.5Mb deletion. However, nested deletions as well as atypical deletions also occur that can contribute to the broad spectrum of phenotypic abnormalities. It is also hypothesized that variations in the remaining allele could underly the phenotypic variability.

To investigate variation within the non-deleted allele we performed targeted resequencing of the 22q11.2 region for 127 patients, identifying multiple structural variations in two atypical deletions. We cataloged over 18 thousand hemizygous variant positions, of which sixty percent were previously annotated. As expected, in this gene dense region more variants were intronic (52%) than intergenic (36%). Within the coding regions we identified 213 non-synonymous variants, 6 stop gains, and 5 frameshift insertions. In addition, the observed number of variants per gene was higher or lower than expected for some genes in both our data and 1000 genomes data, indicating some genes may tolerate variation more or less than others. This extensive catalog of hemizygous variants will serve as a blueprint for future experiments to correlate 22q11DS variation with phenotype and serve as an analysis model as we extend to whole genome sequencing of 22q11DS patients.

P13.04-M New splicing mutation in SERPINA1 gene causing severe alpha-1 antitrypsin deficiency

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Alpha-1 antitrypsin (AAT) deficiency is a common hereditary disorder associated with reduced AAT serum level, predisposing to pulmonary emphysema or liver disease. It is caused by inheritance of mutations in the AAT gene (SERPINA1). Most of the deficiency alleles occur in the coding sequence of the gene due to amino acid substitution or deletion resulting in reduced protein level or altered functionality. Rarely mutations affecting RNA splicing in the AAT gene have been described so far.

We have identified a new null allele, QOmadrid, in two siblings with significantly reduced serum levels of AAT. QOmadrid allele results from a duplication of a timine in the position +2 of the donor splice site of exon 1C (+2dupT). In these patients QOmadrid occurred in combination with another previously described null variant, QOporto. These two variants correspond to splicing mutations in a regulatory region of the gene, both causing disruption of the normal splicing of intron 1C. Analysis of transcripts in patients samples and in vitro assays using minigenes revealed abnormal splicing leading to absence of transcription from exon 1C, where the hepatocytes transcription start site is located. Thus, in these patients no normally spliced RNA products are expected to be produced in the liver, causing the AAT deficiency. This mutation constitutes a new null allele, QOmadrid, contributing to explain the disease.

P13.05-S Upstream open reading frames regulate cannabinoid receptor 1 expression under baseline conditions and during cellular stress

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The endocannabinoid system (ECS) plays a crucial role in the regulation of a variety of physiological functions, such as learning and memory processing, vegetative control, energy homeostasis, immunity and stress response. It acts through different endocannabinoid receptors, which are able to bind to the cannabinoid receptor subtypes 1 and 2 (CB1R and CB2R). The CB1R is not only associated with phenotypes such as cognitive performance, addiction and anxiety, but is also known to be crucially involved in cellular responses to acute and chronic stress conditions. The molecular mechanisms leading to altered CB1R expression under acute or chronic stress are not completely understood so far. It is known that the 5'- and 3' untranslated regions (UTRs) of genes can harbor regulatory elements, such as upstream open reading frames (uORFs) that are capable of influencing the expression pattern of the main protein coding region.

In our study, we investigated the influence of putatively functional uORFs present in the five known mRNA variants of the human CB1R gene on transcriptional and translation under baseline conditions and various stress conditions in vitro. The functional analysis performed with reporter gene assay and quantitative real-time PCR revealed that two of these variants contain upstream open reading frames that modulate gene expression both under baseline condition and conditions of cellular stress. Thus our findings suggest that the functionally relevant uORFs found in the 5'UTR variants of the gene are part of the cellular stress response mechanisms.

P13.06-M Clinical, cytogenetic and molecular analyses in seven patients with a constitutive autosomal ring chromosome.

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Autosomal ring chromosomes have a frequency of 1/30,000 to 1/60,000 in the general population. Furthermore, they are part of the cellular stress response mechanisms. Thus, our findings suggest that the functionally relevant uORFs found in the 5'UTR variants of the gene are part of the cellular stress response mechanisms.
births. We report 7 patients identified from the cytogenetic registers of the HIMFG, who attended during the last 8 years. They corresponded to r(4) [p16q35], r(5)[p15.3q55.3], r(9)[q24.3q34.3], r(14)[p11q32.33], r(19) [p13.3q3.4], r(21)[p11q23.23] and idic (r)(21)[q22.2.q23] cases. We suspect a genomic rearrangement generating an inv dup del mechanism as there are a deletion and a duplication of 14q. This large dentricif r(21) may have been formed by breakage and reunion of long arms of an isochromosome. The phenotypes described included short height, microcephaly, variable facial dysmorphism and psychomotor developmental delay, corresponding to autosomal ring syndrome. Some patients showed hirsutism, renal abnormalities and hypoaesthesia, data corresponding to monosomy of the specific chromosomal region involved. Cases 1 and 7 presented characteristics of Wolf-Hirschhorn and Down syndromes, respectively. Patients 2 and 4 had different control seizures. We consider that the variable clinical data reported in these patients are due to the specific chromosomal region involved and to the ring chromosome instability.

P13.07-S A new translocation involving 4 and 11 chromosomes in a adult man S. Barati, A. Giaufreda, E. Ferro, M. Santangiu, C. D. Solpierio, R. Civa; Genetic and Immunology Pediatric Department, AOI Policlinico G.Martino, Messina, Italy. Chromosomal translocations occurring between the chromosome 11 (region q12) and other chromosomes are the most recurrent chromosomal alterations observed in some subtypes of leukemia, lymphoma and sarcomas. In many cases, identification of these chromosome abnormalities is crucial to select appropriate treatment protocols.

To our knowledge we are the first group to describe a new translocation, involving 11q12 and 4q21 regions, in a 35 years old man. The proband came to our observation to perform cytogenetic analysis because of a suspected infertility and we found 46,XY t (4;11) (q21;q12) karyotype. The man had a cortical dysplasia and seizures from birth. We extended the cytogenetic analysis to the parents and sister of the proband and the same translocation was found only in the father. It is known that some types of dysplasia are linked to the presence of genetic alteration but so far it has not been described any association between dysplasia and chromosomal alteration. The region 11q12 is involved in many chromosomal translocations such as t(2;11) (q31; q12), t(11; 17) (q22; p13), t(11; 17) (q22; p13), and t(6; 11) (p21; q12) which are all related to tumor development. The common breakpoint region (11q12) has been delineated by FISH and contains the FOSL1 gene which has been associated with fibroblast growth or cancer development, such as colorectal, gastric and melanoma. Patients 2 and 4 had different control seizures. We consider that the variable clinical data reported in these patients are due to the specific chromosomal region involved and to the ring chromosome instability.


Chromothripsis (CTH) is a newly described phenomenon of chromosome shattering where multiple localized breakpoints result in catastrophic genomic rearrangements. CTH is found both as a somatic (cancer) and germline rearrangement. The limited G-CTH cases (n=16) described so far are all balanced rearrangements due to GCTH. Our study is the first report suggesting that G-CTH may not always be associated with an abnormal phenotype but may also have a neutral effect. It is therefore possible that G-CTH may be found not only among individuals with apparently balanced rearrangements but also karyotypically normal asymptomatic individuals. We describe CTH on the cytogenetic level based on recommendations of ISCN-2013 (International System for Human Cytogenetic Nomenclature) as: 46,XX [t(3;5)(q22.2.q23.1)arr

G-CTH involving six breakpoints and a ~108kb deletion within a ~6,4Mb region on 3q22.23-q23. Although six protein-coding genes were affected by the breakpoints the 10 carriers of the G-CTH do apparently not have common associated disorders. However, there are several spontaneous miscarriages for the family, suggesting that there may be DNA damage resulting from unbalanced rearrangements due to CTH.

Conclusion: Our study is the first report suggesting that G-CTH may not always be associated with an abnormal phenotype but may also have a neutral effect. It is therefore possible that G-CTH may be found not only among individuals with apparently balanced rearrangements but also karyotypically normal asymptomatic individuals. We describe CTH on the cytogenetic level based on recommendations of ISCN-2013 (International System for Human Cytogenetic Nomenclature) as: 46,XX [t(3;5)(q22.2.q23.1)arr

P13.10-M A family-based genome-wide scan shows 10q25.2, LRFN2, TGFB2 and CRISPLD2 loci associated with cleft lip with or without cleft palate in a high-prevalence cluster in South America F. M. de Carvalho1, F. A. Poletta1, R. F. Fonseca2, D. Montaner1, J. Mereb1, M. M. A. Moreira1, H. N. Souza2, A. R. Vieira1, E. E. Castilho1, L. M. Orioli1; 1, B. Bertelsen*1, W. Sun*2, M. Bak3, G. Xie2, W. Chen2, L. E. Hjermind4,5, J. T. d.

Background: In South America four cleft lip with or without cleft palate (CL±P) high prevalence regions were detected, one of them being the Pata
gonía (Argentina). We aim the identification of independent autosomal seg-
ments containing polymorphic markers that may contribute to CL±P with a family-based design for genome-wide association scan.

Methods: The study sample included 26 families with isolated CL±P (27 affected and 99 total individuals), they were genotyped on the Affymetrix Genome-Wide 6.0 array. Only “independent” SNPs were included in the associ-
ation analysis. We calculated linkage disequilibrium (LD) between each pair of SNPs into a window of 50 SNPs, shifting the window 5 SNPs forward and repeating the procedure to scan all autosomes. Then we pruned the data removing one SNP of each pair that was in strong LD (r >0.8). We perform the transmission disequilibrium test (TDT). We identified segments of a maximum length of 250kb with more than one SNP significantly associated with CL±P.

Results: A total of 88 genomic segments with two or more independent SNPs significantly associated with CL±P were identified. An intergenic region of 33kb on 10q25.2 showed the most significant association with CL±P (p=0.00007). Furthermore, we found the other significant association with 6p2.1 including the marker rs4153154 (p<0.00009). Besides, others genomic seg-
ments including the genes TGFB2 (1.4Kb on 1q41) and CRISPDL2 (61.3Kb on 16q24.1) deserve our attention because they have previously been asso-
ciated disorders. However, there are several spontaneous miscarriages for the family, suggesting that there may be DNA damage resulting from unbalanced rearrangements due to CTH.

Conclusion: Our results suggest 10q25.2 and some genes spread across associ-
tomes as candidate loci to CL±P.

P13.11-S The FRA14B common fragile site maps to a region of frequent somatic and germ-line rearrangements within the GPHN gene D. Zheglov1, L. Brandt2, L. Savery1; 1, B. Bertelsen*1, W. Sun*2, M. Bak3, G. Xie2, W. Chen2, L. E. Hjermind4,5, J. T. d.

Common fragile sites (CFS) are heritable chromosomal loci that exhibit non-random breaks on metaphase chromosome in response to replication stress. Chromosome breakage at CFSs contributes to cancer genome evolu-
tion, de novo pathogenesis and presumably resulting in genomic alterations. Approximately 90 CFSs have been identified at cytogenetic band resolution, but just few of them have been molecularly characterized. Precise mapping of CFSs can reveal new rearrangement-prone candidate genes critical for the development of cancer and hereditary conditions.

We performed FRA14B mapping in cultured lymphocytes treated with a replication inhibitor, aphidicolin, using six-color FISH with contiguous BAC probes on metaphase chromosomes. FRA14B was restricted to a 765 kb region within 14q23.3, overlapping with a major part of the GPHN gene.
GPHN encodes a protein involved in molybdenum cofactor biosynthesis and clustering of postsynaptic neurotransmitter receptors. Computational analysis of the FRA14B sequence revealed a large hairpin-prone motif. Targeted oligonucleotide array CGH in 160 cancer cell lines and primary tumors detected hundreds of structural alterations at FRA14B. Sequence analysis of cancer breakpoint junctions indicated involvement of microhomology-mediated repair. In 2 cell lines, exonic deletions resulted in the expression of aberrant GPHN transcripts. A survey of publicly available copy number profiles revealed that FRA14B is a hotspot of focal losses in cancer cell lines, loss-in-size rare CNVs, and rare and de novo deletions in patients with neurodevelopmental diseases.

In summary, intrinsic chromosome instability at FRA14B may account for pathogenic germ-line and somatic GPHN alterations, providing new insight into the role of CNVs in cancer and neurological disorders.

### P13.12-M

**Signature of germline chromothripsis in a 14-break complex rearrangement associated with deletions at 7q33-q35;11p13 and CNTNAP2 gene disruption**

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We report an apparently balanced complex chromosome rearrangement (CCR) t(7;10)(q35;p12.1)ins(7;11)(q35;p14.1p2) associated with intellectual disability, developmental delay, absent speech and anhidria. The breakpoints were mapped by FISH. The CNTNAP2 gene was truncated by the breakpoint at 7q35 (chr7:146,808,760-146,922,794; hg 19). The breakpoint interrupted the normal reading frame (chr10:27,897,056-28,081,590) contains the MOKX gene. The distal deletion breakpoint interval at 11p14.1 includes the BDNF gene (chr11:27,180,086-27,297,856), whilst the proximal breakpoint was mapped to a segment devoid of genes at 11p12 (chr11:37,068,735-38,957,367). Array-CGH (180K; Agilent) detected four deletions of 2.3Mb, 1.6Mb, 724Kb (chr11:27,718,086-27,929,856), whilst the proximal breakpoint was mapped to this CCR formation. Twelve germline chromothripsis were previously reported, most of them balanced (Kloosterman et al. Curr Opin Cell Biol 2010; 23:102-110). Here we identify this CCR formation. In vivo and in vivo analyses of the breakpoint region revealed novel genetic mechanisms, and define genomic regions which can be mutated without any direct phenotypic consequence.

### P13.14-M

**Insight into the mutational mechanism of the recurrent CREBBP c.3852G>A (p.Glu1278Lys) mutation in patients with Rubinstein-Taybi syndrome**

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Rubinstein-Taybi syndrome (RTS) is a rare disorder affecting approximately 1/100,000 newborns. The syndrome is characterized by mental and growth retardation and a particular dysmorphology mainly concerning the face, hands and feet. The most frequent cause of RTS are de novo mutations in the CREBBP gene, leading to somatic pathway activation and consequent growth overactivity. RTS patients show a broad spectrum of phenotypes, varying from patients with very mild to patients with profound clinical symptoms. The molecular mechanism underlying most Guanine to Adenine substitutions in the CREBBP gene is not completely elucidated. We report a case of a patient with a c.3852G>A (p.Glu1278Lys) mutation in exon 21 of the CREBBP gene. The mutation was identified in six independent patients out of a group of 101 RTS patients with a CREBBP mutation. Five of these patients were from the Netherlands, and another was from Spain. The molecular mechanism underlying more general disease causing mutations might be homologous recombination and DNA replication failure involving loop formation. One might get insight in more general disease causing mutation mechanisms and find an explanation for recurrent mutations. This may have implications for counseling when hotspots are predictable based on knowledge about mechanisms leading to disease causing mutations.

### P13.15-S

**In vivo and in silico analyses of impact of the p.Val322Ala mutation on CFTR protein in a Britany family**

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Cystic Fibrosis (CF) is an autosomal recessive severe genetic disease. Mutations on CFTR (Cystic Fibrosis Transmembrane conductance Regulator) gene could induce CF or CFTR-RD (related disorder). A missense mutation detected in a Britany family p.V322A (c.965T>C) was studied since this mutation is present in a family owning a risk to have a child carrying this mutation and another severe mutation in trans (p.Phe508del). To provide a wise genetic counseling to this family, in silico and biological studies of the impact of the p.Val322Ala on CFTR protein were realized. Aim: This mutation was studied to elucidate its impact on CFTR process (maturation and localization), and in silico (protein structure) as well as in silico (protein structure) as well as in vivo (protein expression and function).

**Background:**

Cystic Fibrosis (CF) is an autosomal recessive severe genetic disease. Mutations on CFTR (Cystic Fibrosis Transmembrane conductance Regulator) gene could induce CF or CFTR-RD (related disorder). A missense mutation detected in a Britany family p.V322A was studied since this mutation is present in a family owning a risk to have a child carrying this mutation and another severe mutation in trans (p.Phe508del). To provide a wise genetic counseling to this family, in silico and biological studies of the impact of the p.Val322Ala on CFTR protein were realized. Aim: This mutation was studied to elucidate its impact on CFTR process (maturation and localization), and in silico (protein structure) as well as in vivo (protein expression and function).

**Methods:**

- pCF plasmids containing CFTR-WT or CFTR-p.Val508del, pCF or pVal322Ala are transfected in eukaryotic cells for western blot analysis and in silico (protein structure) as well as in vivo (protein expression and function).

**Results:**

The p.Val322Ala leads to a normal processing and correct membrane localization. Both mutated protein and the WT-CFTR structures were analyzed, suggesting that this mutation is a milder mutation. Conclusion:

These results emphasize the importance of molecular studies in elucidating the impact of mutations on clinical phenotypes and therapeutic strategies.
Combination of molecular techniques in evaluation of de novo structural chromosomal abnormalities may be a reason of high infertility rates and recurrent pregnancy losses (RPLs) in humans. Karyotype and karyogram profiles of patients with RPLs are presented in the current study. A total of 722 patients (16.4% infertile and 200 (55.5%) RPL couples were included in the study.

Karyotype and structural chromosome analyses of both patient groups in Canakkale population were made between May 2011-December 2013, using peripheral lymphocyte cell culture and GTG banding technique. High frequency of chromosomal abnormalities (%7.45) were detected in 24 patients of the infertility group (n=322). 10 patients (42%) of this group (n=24) had numerical and 14 patients (58%) had balanced structural chromosomal abnormalities. A novel chromosomal insertion was found in one infertile male, one of the 22th chromosome was totally inserted in 9th chromosome [t(9;22)(qter-q12.22;q11.1-13.35;9q12-2q9)], This is the first report of germ line total inserion of a chromosome. Interestingly, this insertion was inherited from father. Balanced structural chromosomal abnormalities was also detected in 17 patients (4.25%) of RPL group without any numerical abnormalities.

Current results constitute the first report on the high incidence of structural chromosome aberrations in RPLs and infertile couples in Canakkale district.

**P13.17-S**

Combination of molecular techniques in evaluation of de novo chromosome rearrangements involving terminal regions

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Structural aberrations make a significant contribution to genetic disease. Balanced or unbalanced structural abnormalities may be inherited from a carrier parent or may occur as de novo rearrangements. When the abnormality occurs as a de novo event, the risk for genetic disease or phenotypic effects is increased, even when the rearrangement appears balanced. This may result from either submicroscopic deletions or duplications at the breakpoints.

We report three cases with structural chromosomal abnormalities that were detected using a combination of different techniques. Conventional karyotype revealed the presence of de novo chromosomal rearrangements in all three cases. The investigation continued with targeted array CGH (Comparative Genomic Hybridization) technique, using RAC (Bacterial Artificial Chromosome) clones for the identification of chromosome imbalances. Since the results obtained using cytogenetic analysis and targeted array CGH revealed the implication of terminal regions of abnormal chromosomes, we also applied MLPA (Multiplex ligation-dependent probe amplification) for subtelomeric regions, as an additional detection technique. In two cases additional information regarding the chromosome constitution was provided by MLPA.

Knowing the advantages and limits of each technique, complementary investigations were necessary to detect the architecture of chromosomal imbalances.

Further investigations for a specific case, although it means additional costs, are required to check the genotype-phenotype correlation. Any discrepancy between the clinical picture and the results of genetic testing imposes supplementary tests for an accurate diagnosis and a proper genetic counseling.

**P13.18-M**

Somatic mosaicism for deletion at 8p21.3p23.1: some new twists on generation of terminal 8p rearrangements

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Somatic mosaicism for terminal 8p deletions is rare. Here, we report on a case of a deletion at 8p21.3p23.1 affecting about 50% of cells addressed by SNP/poligonucleotide CGH. An 8 year old girl presented with intellectual disability, autistic features, microcephaly, large upper incisors, small lower jaw, hypertrichosis, and pectus excavatum. Cytogenetic analysis has indicated the presence of a deletion at the short arm of chromosome 8 in about 60% of cells. SNP/poligonucleotide CGH showed the presence of mosaic terminal deletion at 8p21.3p23.1 spanning about 11.152 Mb affecting 178 genes.

Molecular cytogenetic analysis showed that 48% of cells are affected by this deletion. Clinically, the index case resembles non-mosaic deletions of 8p23. Nevertheless, milder manifestations of intellectual disability, microcephaly and facial dysmorphisms were noticed, whereas autistic features were found to be more prominent. Interestingly, genomic loci flanking the breakpoint of mosaic deletion were regularly deleted. The region deletion spanned three oligaryctor (OR) genes (OR7E158P, OR7E161P, OR7E160P) and three beta-defensin (DEFB) genes (DEFB137, DEFB136, DEFB134). Since OR gene clusters are known to be involved in generation of 8p chromosome rearrangements, we have hypothesized that at least in the present case DEFB gene cluster is also involved in chromosomal abnormality formation. Finally, to our knowledge this is the first case of a mosaic 8p deletion addressed by SNP/poligonucleotide CGH and mediated by OR gene recombination. Supported by the Russian Federation President Grant (MD-440.120.13.7).

**P13.19-S**

Differential allelic expression of the SOS1 c.755C>T activating variant in a Noonan syndrome family

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Noonan Syndrome (NS) is a genetic condition characterized by congenital heart defects, short stature and characteristic facial features. We analyzed a girl with moderate learning disabilities, delayed language development, craniofacial features and skin anomalies reminiscent of NS. After a mutation screening of the known NS genes PTPN11, SOS1, RAF1, KRAS, GRB2, BRAF and SHOC2 we found the heterozygous c.755C>T variation in SOS1 causing the I252T substitution, which was considered possibly pathogenetic by bioinformatic predictions. The same mutation was present in the proband’s mother and maternal grandfather, both displaying some NS features, but also by a healthy subject on 1000 genomes analyzed. The functional analysis revealed that the SOS1 c.755C>T activated the Ras effector Erk1, confirming the predicted pathogenetic substitution. To explain the incomplete penetrance of the reported mutation we hypothesized that SOS1 may be subjected to a differential allelic expression (DAE). Interestingly, after sequencing the cDNA from peripheral blood compared to genomic DNA, we showed a DAE of some known SOS1 SNSs in healthy individuals and observed the mutated allele C 50% more expressed than the normal allele T in all our NS familial carriers. The similar level of SOS1 mRNA between mutated and control individuals, suggests that the mutation here described does not affect SOS1 expression. We are now evaluating the SOS1 promoter polymorphisms. This study, providing the first evidence of allelic imbalance of SOS1, pinpoint DAE as a possible mechanism underlying a different penetrance of some SOS1 mutated alleles in unrelated carriers.

**P13.20-M**

Association of genetic factors involved in folate metabolism and the occurrence of congenital heart defects in individuals with Down syndrome


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Down syndrome (DS) individuals with polyhydramnios in infants have increased risk for developing congenital heart defects, which are the leading cause of death in the first years of life. Moreover, these polyhydramnios in mothers may also be associated with the risk for congenital heart disease in DS offspring. This study investigated whether the presence of the MTHFR C677T, MTHFR A1298C, MTHFR T317C, MTR A2756G, RFC1 A80G, MTRR A66G, TC2 C776G, TC2 A67G, CBS 844ins68, CBS T833C, BHMT G742A, BHMTD1 G1958A, DFRD del 19 bp and SHMT CI420T polymorphisms in DS individuals and their mothers is associated with congenital heart defects. We evaluated 86 individuals with free trisomy 21 and their mothers, attended by Genetics Service at Faculdade de Medicina de São José do Rio Preto at the period 2005-2008. The investigation of polymorphisms was performed by Polymerase Chain Reaction (PCR), Real time PCR and PCR followed by digestion. The RFC1 80G polymorphic allele increased the risk for intestinal communication (IAC) (OR=7.92, CI=1.21-51.84, P=0.03) when in DS individuals. The BHMT 742A maternal polymorphic allele increased the risk of atrioventricular septal defect (AVSD) (OR=10.50, CI=3.16-81.06, P=0.02) and interventricular communication.
P13.21-S
Protective action of NADPH oxidase inhibitors and the role of NADPH oxidase in the pathogenesis of colon inflammation in mice
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AIM: To investigate the role of NADPH oxidase in colon epithelial cells in pathogenesis of acute and chronic colon inflammation using mice dextran sulphate sodium (DSS) colitis model.
METHODS: BALB/c mice were divided into three groups: 8 mice with acute DSS colitis, 8 mice with chronic DSS colitis and 12 mice without DSS supplementation as control group. The primary colonic epithelial cells were isolated using chelation method. The cells were cultured in the presence of mediators (lipopolysaccharide (LPS), apocynin or diphenyleneiodonium). Viability of cells was assessed by fluorescent microscopy. Production of reactive oxygen species (ROS) by the cells was measured fluorimetrically using Amplex Red. Production of tumour necrosis factor-alpha (TNF-α) by the colonic epithelial cells was analysed by ELISA. Nox 1 gene expression was assessed by real-time (RT) PCR.
RESULTS: Our study showed that TNF-α level was increased in unstimulated primary colonic cells both in the acute and chronic DSS colitis groups, whereas decreased viability, increased ROS production, and expression of Nox 1 was characteristic only for chronic DSS colitis mice when compared to the controls. The stimulation by LPS increased ROS generation via NADPH oxidase and decreased cell viability in mice with acute DSS colitis. Treatment with NADPH oxidase inhibitors increased cell viability decreased the levels of ROS and TNF-α in the LPS-treated cells isolated from mice of both acute and chronic DSS colitis groups.
CONCLUSION: Our study revealed the importance of NADPH oxidase in pathogenesis of both acute and chronic inflammation of the colon.

P13.22-M
Centromere of chromosome 9 presents unusual behavior in rearrangements leading to complete 9p duplication
Trisomy 9p is one of the most common partial trisomies found in newborns, possibly because this region is relatively poor in genes resulting in a high survival rate. We report four different chromosome rearrangements resulting in complete 9p duplication, three of them involving 9p centromere alterations. The rearrangements in the patients were characterized by G-banding, SNP-array and FISH (fluorescent in situ hybridization) with different probes. One presents 9p duplication concomitant to 18p deletion due to an inherited der(18)[9pter;18](p11.2;p11.31)mat. Two patients present de novo dicentric chromosomes: der(9;15)[9pter;15](p11.2;p13) and der(9;21)[9pter;21](p13.13;13.1), respectively. Another patient presents two rearranged chromosomes: a der(12)[9pter;12q21.13;p13.33] and concomitant i(9q)10(p) which showed a FISH centromeric signal smaller than its homologue. Besides the duplication 9p24.3p13.1, array revealed deletion in 9q13q21.13 (7.3 Mb). This rearrangement may have been originated by an in delivision centric fission resulting in a smaller centromere in (9p) and part of the 9q long arm being translocated to the distal 12p. The deletion 9q may have been caused during the rearrangement with the chromosome 12. The chromosome 9 is rich in segmental duplications, especially in pericentromeric region, with high degree of sequence identity to sequences in 15p, 16p and 21p. Chromosome 9 is involved in our rearrangements. In two patients the dicentric chromosomes formed may have been converted into stable functional monocentric chromosomes by epigenetic centromere inactivation followed by heterochromatization. Thus, we suggest that chromosome 9 is prone to illegal recombination, either intra or interchromosomal, that predisposes it to rearrangements, frequently involving pericentromeric regions (Financial support FAPESP, Brazil).

P13.24-M
Multiprobe FISH method for enhanced detection of chromosome 9 heterochromatin rearrangements
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Heterochromatin rearrangements are believed to be clinically insignificant variants of the human karyotype. However, several authors have studied the possible association of heterochromatin variants with certain clinical diagnoses, especially with reproduction failure. Variants of heterochromatin area of chromosome 9 are the most common. They involve enlargement (q+) or shortening (q-) of the heterochromatin block as well as the pericentromic inversion - inv(9)(p12q13). More complex variants of this area may include duplication and/or combination of above mentioned rearrangements. Distinguishing between benign and pathological rearrangement in this area can be challenging. The classical G and/or C-banding are not very specific and array methods like SNP-array/array-CGH are usually not able to analyse precisely this pericentromeric region, which is composed mainly of satellite DNA.
For enhanced analysis of heterochromatin area of chromosome 9 we implemented a special molecular cytogenetic method using three different FISH probes - centromeric alpha-satellite, centromeric II-DNA satellite and a specific BAC probe (hybridizing on 9p12 and 9q13 homologous sequences). The outcomes of this examination in 20 patients with different clinical indications are demonstrated.
Although believed to be benign, the heterochromatin variants of chromosone 9 have been repeatedly mentioned as potentially associated with reproductive failure. Since the majority of these variants are undoubtedly truly benign, presented molecular cytogenetic examination is able to analyse this region more precisely than standard banding methods and distinguish among different (sub)variants of chromosome 9 much better than karyotyping and point out these potentially harmful.
This study was supported by the GAUK 523456 grant project.

P13.25-S
De novo case of a mosaic small supernumerary marker chromosome leading to proximal partial trisomy 5p
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Small supernumerary marker chromosomes (sSMC) are structurally abnormal parts of the karyotype with unknown origin that may arise de novo or be inherited from parents. ~70% of people with sSMC grow and develop normally, while 30% show different clinical signs and symptoms. Here we present the clinical and cytogenetic findings in a 1-year-old female referred for genetic evaluation because of dysmorphic features, including hypertonia, umbilical hernia, hypertelorism, broad nasal bridge, microretrognathia, low set ears, and wide spaced nipples. Cytogenetic examination of GTG banded metaphases showed a female karyotype with mosaicism of an sSMC. Additional molecular cytogenetic analysis (cenM-FISH and subcenM-FISH) characterized the sSMC to be derived from chromosome 5 including heterochromatic and euchromatic material. The shape of sSMC was not clearly defined; either it is a ring or centric minute. The karyotype can be reported as: mos 47,XX,1qh+pat,+der(5)?(5)(::p1?4→q11.1::)[3]/der(5)?min(5)qh+) or shortening (qh-) of the heterochromatin block as well as the pericentromic inversion. The patients are affected with different diagnoses, especially with reproduction failure. Variants of heterochromatin area of chromosome 9 are the most common. They involve enlargement (q+) or shortening (q-) of the heterochromatin block as well as the pericentromic inversion - inv(9)(p12q13). More complex variants of this area may include duplication and/or combination of above mentioned rearrangements. Distinguishing between benign and pathological rearrangement in this area can be challenging. The classical G and/or C-banding are not very specific and array methods like SNP-array/array-CGH are usually not able to analyse precisely this pericentromeric region, which is composed mainly of satellite DNA.
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Although believed to be benign, the heterochromatin variants of chromosone 9 have been repeatedly mentioned as potentially associated with reproductive failure. Since the majority of these variants are undoubtedly truly benign, presented molecular cytogenetic examination is able to analyse this region more precisely than standard banding methods and distinguish among different (sub)variants of chromosome 9 much better than karyotyping and point out these potentially harmful.
This study was supported by the GAUK 523456 grant project.

P13.26-M
Defining the role of CGGBP1 protein in FMR1 gene expression
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Heterochromatin rearrangements are believed to be clinically insignificant variants of the human karyotype. However, several authors have studied the possible association of heterochromatin variants with certain clinical diagnoses, especially with reproduction failure. Variants of heterochromatin area of chromosome 9 are the most common. They involve enlargement (q+) or shortening (q-) of the heterochromatin block as well as the pericentromic inversion - inv(9)(p12q13). More complex variants of this area may include duplication and/or combination of above mentioned rearrangements. Distinguishing between benign and pathological rearrangement in this area can be challenging. The classical G and/or C-banding are not very specific and array methods like SNP-array/array-CGH are usually not able to analyse precisely this pericentromeric region, which is composed mainly of satellite DNA.
For enhanced analysis of heterochromatin area of chromosome 9 we implemented a special molecular cytogenetic method using three different FISH probes - centromeric alpha-satellite, centromeric II-DNA satellite and a specific BAC probe (hybridizing on 9p12 and 9q13 homologous sequences). The outcomes of this examination in 20 patients with different clinical indications are demonstrated.
Although believed to be benign, the heterochromatin variants of chromosone 9 have been repeatedly mentioned as potentially associated with reproductive failure. Since the majority of these variants are undoubtedly truly benign, presented molecular cytogenetic examination is able to analyse this region more precisely than standard banding methods and distinguish among different (sub)variants of chromosome 9 much better than karyotyping and point out these potentially harmful.
This study was supported by the GAUK 523456 grant project.
G6PD-Meyer: a new mutation causing compensated chronic haemolytic anemia

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An updated database reports 186 different G6PD mutations. Many of these are polymorphic in various populations; many are spondic, having been discovered because they cause chronic non-spherocytic haemolytic anaemia (CNSHA) associated with severe enzyme deficiency (WHO class I).

A little boy with a history of neonatal jaundice treated with phototherapy, and of anaemia (Hb 80-90 G/L) during his first semester, was seen at age 3.4 years. He is clinically well, with no significant physical findings; Hb 110 G/L, MCV 95, MCH 31, MCHC 33, reticulocytes 271x10⁹/L, bilirubin 22 µmol/L: indicating a well compensated haemolytic condition. G6PD activity was 0.89 IU/Gb (ref values 7-10 IU/Gbh). Sequencing the G6PD gene revealed an A->G change in exon 7 at nucleotide 655, predicting an Arg->Gly change at codon 219. The mother was shown to be heterozygous for the same mutation: her G6PD assay was normal. This mutation is not present in the database and therefore it is a new sporadic class I mutation. We interpret the CNSHA phenotype as related to the fact that Arg->Gly is a rather drastic amino acid change, since it entails a charge change as well as a steric change: such changes are likely to cause decreased stability of the G6PD protein and enzyme deficiency. It is interesting that Arg219 is not a conserved residue: we have previously reported that in terms of clinical phenotype the consequences in such cases would not be so drastic, and this may explain why this child does have haemolytic disease but only mild anaemia.

P13.28-M

Large duplication implicating only exon 1 of F8 gene in mild hemophilia A phenotype

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In 98% of mild hemophilia A (HA) patients, a missense mutation spread throughout the Factor B (F8) gene’s 26 exons can be identified using complete gene sequencing. In this study, 12 French-speaking Belgian with mild hemophilia A were screened for full mutations F8 and unmethylated full mutation FUM (UMF), demonstrating no differences between them. ChIP assays showed that GGBP1 binds unmethylated CGG triplets of the FMR1 gene proportionally to the length of the repeats. We also observed that GGBP1 binding to the FMR1 locus was restored after pharmacological demethylation with 5-aza-dc of F8 alleles, suggesting a possible role for GGBP1 in FMR1 expression. GGBP1 silencing with siRNA (reaching ~ 85% of GGBP1-mRNA depletion) did not affect the expression of F8 in WT and UFM fibroblasts. Although the GGBP1 silencing was specific and limited to the FMR1 gene, the results presented in this study allow us to suggest that GGBP1 is not a direct regulator of FMR1 transcription.

Supported by Telethon Orlus, FRAXA Foundation and Italian Association for fragile X syndrome.

P13.29-S

Subtelomeric chromosomal breakages characterization in patients with intellectual Disabilities/Congenital Anomalies and mechanisms for formation


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Abnormal CNVs are frequently found in subtelomeres of patients with intellectual disabilities (ID) and congenital anomalies (CA). The subtelomeric rearrangements are usually not present in recurrent breakpoints (BPs) and involve several different chromosomes ends. Although these regions encompass approximately 30% of pathogenic CNVs, the causes of subtelomeric breakages have not yet been explored. We have previously studied 105 unrelated patients with ID/CA using MLPA, FISH and arrays (BeadChip Illumina/GH-Array-Agilent) in order to characterize the subtelomeric BP. Within the set of subtelomeric rearrangement studied, the deleted regions ranged from 137 kb to 29 Mb, and the duplicated regions from 155 kb to 32 Mb. We identified 38 BPs, from 19 different regions. Our analysis showed repetitive elements in 31 BPs encompassing SINES (Alu; MIR). LINES (L1; L2), and LTRs. We also found six BPs presenting simple tandem repeats and two BPs with interstitial telomeric sequences (ITS). Rearrangements with exclusively deletions are suggested to be caused by non-homologous end joining (NHEJ) or microhomology-mediated end joining (MMEJ) mechanisms. Complex rearrangements are likely caused by fork stalling and template switching (FoSTeS) or microhomology-mediated break-induced replication (MBMR). Misalignments during replication due the repetitive sequences in these loci can lead to MMBIR and generate the complex dup/del rearrangements observed in 7 of our patients. Furthermore, genetic architectural features, like sequence motifs, non-B DNA conformations, and repetitive elements may increase the susceptibility for DNA breakage or promote FoSTeS in these regions. Mapping subtelomeric BPs should help to clarify the mechanisms involved in these genomic rearrangements.

P13.30-M

Two cryptic duplications detected in an apparently balanced inversion posing a diagnostic challenge

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Balanced rearrangements in patients with abnormal phenotype are often associated with cryptic copy number changes (CNCs). These CNCs might not always be the cause of the phenotype and one must be careful to interpret especially in aberrations involving the X chromosome. A 22-year-old female patient was referred for cytogenetic analysis because of certain neurological symptoms such as learning difficulties, developmental delay, and language delay. The patient’s mother and grandmother have no remarkable clinical features with the exception of hypothyroidism and bilateral hearing loss. Chromosomal analysis revealed a submicroscopic inversion on chromosome X with breakpoints at Xq21.11 and Xq23 delineated by FISH using several BACs. Surprisingly, array CGH analysis with a high resolution exome specific X-chromosome array (OGT) revealed two cryptic duplications, 116 kb and 184 kb in size, on each breakpoint. Cryptic duplications associated with apparently balanced inversions are very rare events. The duplications include coding genes such as POIF1, NZF711, SAT1, APOOL, and LHPL1. Therefore the duplication was suspected to be causative for the phenotype as some of these genes included in the duplicated regions might partially explain the phenotype of the patient, e.g. LHPL1 which was previously found to be associated with hearing loss. Family studies revealed the same duplication in the father and grandmother of the patient. The father and grandmother have no remarkable clinical features with the exception of hypothyroidism in the father, rendering the duplication as coincidental rather than pathogenic. This case highlights the need for extended family studies and careful detailed clinical evaluation of other family members carrying the same aberration prior to interpretation and genetic counselling.
is triggered by decreased expression of MARK4, the canonical isoform, associated with overexpression of MARK4, the alternative isoform with skipping of exon 16.

Having ruled out mutations, CNVs and transcript detection as causes of deregulated MARK4 expression, we searched alterations in alternative splicing at the origin of the observed isoforms imbalance.

Bioinformatic analysis of MARK4 genomic sequence revealed three binding sites for the splicing factor PTB in introns flanking MARK4 alternative exon 16. A functional role for these sites is suggested by the conservation in mouse and the surrounding polyypyrimidine rich context. Since in glioma PTB overexpression drives an oncogenic splicing switch favoring exon skipping isoforms, we performed Western blot on glioma and glioblastoma-derived cancer stem cell samples and found a significant overexpression of PTB, correlating with MARK4 expression. Splicing assay of the designed and differently deleted minigenes revealed that IVS15 contains a functional intronic splicing silencer (ISS). However, mutagenesis of the PTB binding site in this region does not affect minigene splicing, suggesting PTB binds to a non-canonical ISS. Electrophoretic mobility shift coupled to mass spectrometry confirmed the PTB binding to MARK4 IVS15 and its involvement in MARK4 alternative splicing.

Alternative splicing thus emerges as an oncogenic mechanism that through the PTB-mediated regulation of MARK4 isoforms expression fosters proliferation and de-differentiation in glioma.

P13.32-M
Normal and oncogenic proliferation under control of microRNAs: a functional high content screening
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Cancer cells share several characteristics with normal stem cells especially those concerning cell cycle regulation. Therefore it is believed that cancer cells might arise from stem cells possessing self-renewal capabilities. MicroRNAs (miRs) are small RNA molecules that act regulating gene expression post-transcriptionally by targeting hundreds of mRNAs simultaneously. With this in mind, we hypothesized that significant changes in the cell cycle could be a good indicator for miRs capable of regulating normal and oncogenic proliferation. We performed a focused screen in glioblastoma cell lines transfected with 50nM of 28 microRNAs mimics (pre-miR) and inhibitors (anti-miR) in 96-well microplates. After 5 days, proliferation was measured by XTT assay. Cell cycle classification (EdU assay) and viability (Sytox Green staining) following miR transfection were performed in a High-Content Screening platform. Nineteen treatments significantly altered the cell proliferation. Interestingly, while transfection of pre-miR of miR-20b, miR-101 and miR-181d decreased the proliferation, the corresponding anti-miRs had the opposite effect. Pre-miR-181d, pre-miR-20b and pre-miR-101 induced a significant most cytotoxic effect. Pre-miR-181d and pre-miR-101 significantly reduced the reduction in the percentage of cells in 5 phase. Cyclin D1 expression was elevated by anti-miR-101 and by pre-miR-24, indicating that this cell cycle regulator might be the mediator of these miR’s effects. These results indicate that the normal and cancer cell proliferation can be altered by miRNAs interfering with the cell cycle, such as speeding it up or slowing it down as desired. Also miR-101 acts as tumor suppressor in colorectal cancer and this role is under evaluation in our lab.

P13.33-S
The evaluation of apoptotic gene expressions, telomerase activity and the effects of insulin like growth factor-1 and erythropoietin on apoptosis in an experimental necrotizing enterocolitis mice model
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Necrotizing enterocolitis (NEC) is a major cause of mortality among preterm infants. Apoptosis, which occurs after hypoxia/reoxygenation (H/R), has an important role in the pathogenesis of NEC. Telomerase activity may also be important in the recovery process. The aim of this study is to evaluate the role of apoptotic gene expressions and telomerase activity in the H/R-induced intestinal mucosa and to investigate whether pre-treatment with insulin-like growth factor-1 (IGF-1) and erythropoietin (EPO) could protect the intestinal cells from apoptosis or intestinal injury. The study set was divided into 4 groups, each containing 10 young Balb/c mice. Group 1 mice were exposed to H/R. Group 2 and group 3 mice were pre-treated with IGF-1 and EPO for 7 days respectively before H/R. Group 4 served as a control group. Intestinal injury was evaluated by histological scoring. TUNEL test and caspase-3 activity was performed to assess apoptotic, Pro-apoptotic (p53, Casp3, Tnf, Bax, Fas, Bad) and anti-apoptotic (Bcl2, Bcl-W, Bcl-XL, NF-kB) gene expressions and telomerase activity were studied by Real-Time RT PCR.

IGF-1 and EPO treated animals showed decreased histological damage and decreased apoptosis which was confirmed by TUNEL test and caspase-3 activity. Telomerase activity increased in these groups in addition to increased expression of anti-apoptotic genes (p< 0.01). However pro-apoptotic gene expressions were not statistically different (p>0.05). In conclusion, the protective effects of IGF-1 and EPO in H/R damage may be due to increased expressions of anti-apoptotic genes.

P13.34-M
Phenotype variability in Slovak NF1 patients related to some mutation characteristics
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One of the most frequent autosomal dominant disorders is neurofibromatosis type 1 (NF1). In NF1 patients are usually found at least two of seven clinical features: cafe au latte macules, freckling, Lisch nodules, bone dysplasia, glioma of optical pathway, different types of neurofibromas and the first degree relative with confirmed NF1. Our cohort included 108 unrelated Slovak NF1 patients and we identified in 39% (42/108) patients framshtet, in 13% (14/108) misense, in 18.5% (20/108) splicing, in 17% (18/108) nonsense mutations, moreover in 4.5% (5/108) deletion of entire genes and in 6% (7/108) specific microdeletions and in 2% (2/108) small in frame deletions. 23% (25/108) of mutations were located in Ras-GRD (Ras GAP related) domain and 21% (23/108) in CSDL (cystein serine rich) domain. “Café au latte” macules were present in all patients, freckling in 87%, Lish nodules in 28%, glioma of optical pathway in 31%, neurofibromas in 56%, and bone dysplasia in 6% of patients. Patients with nonsense mutations show the lowest occurrence of Lish nodules (11%) and the highest incidence of neurofibromas (72%). These findings show no significant differences between clinical features of patient with mutations in Ras-GRD and CSDL domains. Complete mutation analysis in the first-degree relatives was performed in 51 families. Interestingly, we showed that 63.9% (32) of the patients from these families carry no NF1 mutation. Age of the patients was from 2 to 69 years, 41% patients were younger than 18 years.

P13.35-S
In vitro analysis of alternative splicing of OLR1, a gene involved in atherogenesis and tumorigenesis
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Lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) encoded by OLR1 gene, is the major endothelial ox-LDL receptor and plays a role in the pathogenesis of atherosclerosis and tumorigenesis. OLR1 is subjected to a physiological alternative splicing. Two different isoforms are known: Loxin [NM_00172632.1] lacks exon-4 and encodes for a putative truncated receptor [LoxN] with impaired binding activity; OLR1D4 [NM_00172632.1] lacks exon-4 and the putative protein has a different C-terminus domain. Functional role of OLR1D4 is unknown, while LOX-N is a natural inhibitor of LOX-1-mediated signalling and its up-regulation may have a potential therapeutic effect.

We transfected HeLa cells with minigenes (HIGH/LOW-risk) carrying two different C-terminus domain. Functional role of OLR1D4 is unknown, while LOX-N is a natural inhibitor of LOX-1-mediated signalling and its up-regulation may have a potential therapeutic effect. We transfected HeLa cells with minigenes (HIGH/Low-risk) carrying two different haplotypes of the six SNPs regulating OLR1/Loxin splicing. A dose-curve PMA treatment shows that 10nM PMA at 3h up-regulates Loxin expression (FC10nM=+1.6, p<0.05) in HeLa transfected with H-risk haplotype (HeLa-H). Loxin increase was not followed by OLR1 up-regulation and the OLR1/Loxin ratio after 10 nM of PMA was lowered to 28.1% than HeLa-H. These results suggest a role of PMA in modulating OLR1 splicing in vitro, leading to an increase of Loxin isoform. Moreover, we performed a computational analysis from different databases (TargetScan, miRanda, Pita) that predicted two microRNAs that bind the 3’UTR of OLR1 gene in nucleotide region in which are located SNPs. So these miRNAs may act as regulators of OLR1 expression and alternative splicing.

These are the first evidences of a regulation of OLR1 splicing, and provide new data addressing a future more selective and personalized therapy for diseases caused by OLR1 over-expression.
Exploring the role of a ceRNA-based regulatory network, involving GBA and GBAP1, in the pathogenesis of familial Parkinson disease V. Rimoldi1, N. Tonoli2, L. Stranieri2, M. Auroli3, S. Goldward1, S. Duga1, G. Soldi1, R. Asselta1
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Parkinson disease (PD) is a complex neuronodegenerative disorder characterized by the loss of dopaminergic neurons of the substantia nigra, which causes motor impairment and resting tremor. To date, mutations in the glucosecerebrosidase (GBA) gene represent the most frequent cause of genetic PD. A widespread deficiency of GBA activity was recently demonstrated in the brains of PD patients carrying GBA mutations. Most interestingly, also PD patients without GBA mutations had lower levels of GBA activity in the brains than in the peripheral blood leukocytes. This could imply that the GBA deficiency observed in PD brains may be the result of a single genome-shattering event resulting in multiple chromosome breaks that have involved GBA and several other genes. A GBA deficiency could thus contribute to PD susceptibility. Possible mechanisms modulating GBA expression include post-transcriptional networks involving microRNA (miRNAs) and competing-endogenous RNAs (ceRNAs). ceRNAs are a class of regulators that titrate away miRNAs from their targets, thus influencing miRNA expression. The highly-homologous and expressed GBA pseudogene (GBAP1), located 16 kb downstream of GBA, is particularly suited to act as a GBA ceRNA. To verify this hypothesis, we first bioinformatically selected five miRNAs potentially targeting both transcripts, and demonstrated that one of them is significantly overexpressed in whole blood from PD cases vs. controls (2-fold increase, P=0.0028). Then, the miRNA precursor was overexpressed in Hela/Hek293/HepG2 cells; in all cases, both GBA and GBAP1 endogenous miRNA levels were significant decreased (up to 70%). The specific interaction between the miRNA and its targets was demonstrated by luciferase-based reporter assays. Finally, preliminary overexpression experiments showed that the GBAP1 3′-untranslated region is able to act as a molecular “sponge” of the identified miRNA, thus suggesting the actual existence of a ceRNA-based regulatory network modulating GBA expression.

Non-coding PAH gene alterations act as new transcription regulators M. Stojiljkovic1, K. Khassanov2, B. Zukić2, M. Ugrin2, V. Spasović2, N. Nović3, N. Kataršić4, S. Srećan5, G. Nišević2, S. Pavlovic6
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Phenylketonuria (PKU) is a rare metabolic disease caused by mutations in phenylalanine hydroxylase gene (PAH). Mutations that abolish structure and function of PAH are main determinant of PKU phenotype. However, phenotype couldn’t be always predicted precisely. Previously, we found a transcription enhancer in PAH intron 8 that could affect genotype-phenotype correlation. In this study, we functionally analyzed additional non-coding PAH gene alterations to propose new transcription regulatory elements. In silico prediction for transcription factor binding sites pointed to a population-specific promoter alteration (PAH e-170delC) and VNTR alterations in 3′ region, that have never been analyzed before. We transiently transfected HepG2 cell line with various CAT reporter constructs to determine the effect of a PAH non-coding sequences on transcription. We found that a construct with additional binding site in promoter and constructs with VNTR3, VNTR7 and VNTR8 alterations had a 50-60% reduction of CAT activity in comparison to pBluCAt5. EMSA supershift showed binding of KLF1 transcription factor to the analyzed promoter sequence, while the full structure of VNTR3 was needed to obtain binding of C/EBPα/α.

Our study pointed to two new elements in promoter and 3′ region of PAH gene that could act as transcription silencers and thus influence genotype-based prediction of PKU severity. Given that these non-coding alterations are population specific, further validation of their relevance should be studied in different populations. New transcription regulators in non-coding regions of the gene could contribute to better understanding of PKU.

Apparent chromothripsis in a child with a complex chromosome rearrangement resulting in an 11p11.2 microdeletion within the Potocki-Shaffer syndrome region B. Argiroppolous1, O. Caluseriu1, K. Bowser2, J. E. Chernos1,2
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Chromothripsis is a phenomenon of genomic rearrangement arising during a single genome-shattering event resulting in multiple chromosome breaks involving one or more chromosomes. We report a case of a 21 month old female presenting with global development delay, hypotonia, minor dysmorphic features, nystagmus and multiple cutaneous lesions. Array-CGH analysis identified a 1.15 Mb interstitial deletion within chromosome 11p11.2 that partially overlaps the proximal interval of the Potocki-Shaffer syndrome (PSS) critical region. This deletion does not overlap the EXT2 and AXL4 genes which are associated with the multiple exostoses and parietal foramina phenotypes, respectively, frequently observed in individuals with this syndrome. This deletion does, however, overlap a 137 kb region previously identified as the critical region sufficient to cause hypotonia in PSS. Furthermore, haploinsufficiency of PHF21A, which is deleted in this patient, has been implicated in the intellectual disability and craniofacial anomalies associated with PSS. Chromosome and FISH analyses revealed an uniparental de novo complex rearrangement involving chromosomes 9, 10 and 11 with at least 8 chromosome breaks: 46,XX,der(9)[10pter->10q24.3::11p11.2::11p31.1::11p31.1::11q13.5::11qter]der(10) (10pter->10q24.3::11p11.2::11p13::11p13::11p13::11q13.5::11qter)der(11) (11pter->11p24::11q13.5::11p11.22::11q13.5::11qter)der(11) der(11) (11pter->11p24::11q13.5::11p11.22::11q13.5::11qter)der(11) der(11) der(11) der(11) der(11) der(11) der(11) der(11). Although the deletion within chromosome 11p11.2 was the only clinically significant copy number imbalance, it is unclear if the other chromosome breaks and possible gene disruptions contribute to the patient’s phenotype. Similar to other reported instances of apparent germline chromothripsis, this case showed minimal DNA loss presumably reflecting a selection against massive copy number imbalances. This case highlights the importance of performing both array-CGH and conventional cytogenetic methods as either investigation alone would have provided partial information.
P13.41-S
Mosaic ring chromosome 17: a new case
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Ring 17 syndrome is a rare disorder and only 19 cases have been reported so far. Severity of the associated phenotype is influenced by the presence or deletion of the Miller-Dieker critical region (MDCR) with presence of the MDCR being associated with milder phenotypes, including growth delay, intellectual disability, epilepsy, café au lait skin spots and minor facial dysmorphism. We report a patient born at 41 gestation weeks after an uneventful pregnancy, with normal birth parameters - weight: 3kg350, length: 49 cm. The psychomotor development was normal. When he was 3 years old the patient raised the developed frontoparietal epilepsy. Cytogenetic investigations from peripheral blood cells showed a mosaic ring chromosome 17 karyotype mos 46,XY,r(17)(p13q21) [42]/45,XY,-17 [5]/46,XY [3]. At 6 years of age, he had generalised epilepsy, developmental delay, school difficulties, and attention deficit disorder. Clinical examination showed no facial dysmorphism and no growth delay. Skin changes showed sparse café au lait spots but no axillary or inguinal freckling, and 2 small achromic spots. Cerebral MRI, cardiac ultrasound and ophthalmological fundus were normal. Molecular cytogenetic investigations confirmed that no terminal deletion had occurred, and an array-CGH (Agilent 44K) did not detect any submicroscopic deletion or duplication. Chromosome analysis were also obtained on café au lait skin spot and normal skin, and are currently being studied. Data from the patient will be compared to the literature, including the recent reports which raised the hypothesis of telomere shortening and telomere position effect in mild ring 17 syndrome.

P13.42-M
Somatic structural genome variations (chromosome rearrangements) are common in children with intellectual disability, congenital malformations and autism
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Classically, common pathogenic somatic genome variations are attributed to mosaic aneuploidy or polyplody. However, recent reports on somatic mosaicism have demonstrated that mosaic structural genomic rearrangements (chromosome abnormalities) are likely to underlie intellectual disability, congenital malformations and autism as low-level mosaic aneuploidy does. To get further insight into possible effect of somatic structural genome variations in disease, we have analyzed 202 patients with intellectual disability, congenital malformations and autism using SNP oligonucleotide array CGH (two array CGH platforms with a resolution of 1 and 15 kb). Low-level mosaic aneuploidy (additional chromosome Y in about 5% of cells) was found in 2 cases (1%). Mosaic structural genomic rearrangements associated with a phenotypic outcome (according to an original bioinformatic technology) were detected in 14 cases (6.9%). These were mosaic duplications at 1q21.1q21.2 (1.9Mb), 11p11.5p5.3 (1.3Mb), 11p14.3 (3.8Mb) and mosaic deletions at 1p33.3p35.3 (7.7Mb), 2q22.1q23.3 (10.2Mb), 2q23.3q24.2 (10.7Mb), 5q14.3q15 (6.3Mb), 7q35q36.3 (14.6Mb), 8p23.3p23.1 (11.1Mb), 11g23.3 (11.3Mb), 11q13.1q11.2 (11.4Mb), Xp22 (100Mb), Xp22.3q13.3 (100Mb). Moreover, two cases have demonstrated low-level mosaicisms for isochromosome 12p and small supernumerary chromosome, which represented min(17)(p11.2-q11.1) (8Mb). These rearrangements were further confirmed by FISH and/or multicolor banding. Additionally, two benign mosaic subchromosomal abnormalities (deletion at 14q11.1 in 2 cases; duplication at Xq28 in 2 cases) were observed. To our knowledge, such somatic genome variations (recurrent and benign) were not reported. Our data evidence that somatic genome variations manifesting as structural rearrangements are relatively common among children with intellectual disability, congenital malformations and autism. Supported by the Russian Federation President Grant (MD-440.2013.7).

P13.43-S
Assessing the impact of exonic variants on splicing regulation by functional analysis and bioinformatics predictions: BRCA2 exon 18 as a model system
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Sequence changes in exons can alter pre-mRNA splicing either by changing splice sites or by modifying exonic splicing regulatory elements (ESR). The effects on ESR are especially difficult to predict by using currently available bioinformatics tools. Here, we used as a model system the exon 18 of BRCA2 gene involved in breast-ovarian cancer predisposition, and tested the impact on splicing of 36 variants identified in patients or reported in public databases. Using a splicing minigena assay, we found that 13 (7 missense, 4 synonymous, 1 nonsense and 1 in frame deletion variants) out of these 36 variations induce an increase in exon 18 skipping by potentially modifying ESR. When patient blood RNA was available (n=4), the effects of the variants were confirmed. Recently, we demonstrated the predictive value of ESR hexamers’ scores established by Ke et al. (2011) in identifying splicing regulatory mutations within BRCA2 exon 17 (Di Giacomo et al. 2013). Here, we show that this approach is also able to predict the effect on splicing regulation of BRCA2 exon 18 variants. Together with segregation data, these results should contribute to the classification of variants of unknown significance in BRCA2 exon 18. In addition, this study extends the validation of a new in silico tool for predicting the effect of exonic variants on ESR. This tool may have important applications in the filtering strategy allowing the discovery of pathogenic mutations among the large fraction of variants detected by high throughput sequencing.

P13.44-M
‘Stress Entropic Load’ as a Transgenerational Epigenetic Response Trigger
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Epigenetic changes are generally based on the switching of alternative functional or structural states and result in the adaptation of cellular expression patterns during proliferation, differentiation or plastic changes in the adult organism, whereas some epigenetic information can be passed on to other generations while other is not. Hence, the principal question is: why is some information reset or resolved during the meiotic process and whether there is one mechanism that triggers the modification of epigenetic expression patterns during proliferation, differentiation or plastic changes in the adult organism? Hereto, we propose a theory which states that stress, or, more specifically, the energy cost of an individual’s adaptation to stress, represents a viable measure of energy released by a unit’s organ mass where k = heat) and the energy cost of an individual’s adaptation to stress, represents a viable measure of energy released by a unit’s organ mass where k = heat) and the irreversibility within the organism, resulting in faster organ degradation and consequent health problems for the entire biological system.

We therefore suggest a new variable: ‘stress entropic load’ actually reflecting the energetic cost of an individual’s adaptation to stress, represents a viable measure of energy released by a unit’s organ mass where k = heat) and the irreversibility within the organism, resulting in faster organ degradation and consequent health problems for the entire biological system.

P13.45-S
Prenatal diagnosis of partial trisomy 14 and partial trisomy 18 due to a 3:1 segregation of maternal reciprocal translocation t(14;18)

A 30-year-old woman was a known carrier of a balanced translocation 4401.2013.7).
mother was confirmed aminoacids because of advanced maternal age. Karyotyping was confirmed by FISH for five years ago. The woman with a known balanced translocation between chromosome bands 1q43 and 1q12 was referred for prenatal diagnosis by amniocentesis at 17 weeks of gestation. Cyto genetic lymphocytes showed a normal male karyotype 46,XY. Their first baby had normal karyotype 46,XX, also prenatally observed. We present the case of prenatally diagnosed tertiary trisomy with supernumerary derivative chromosome 14, due to a 3:1 segregation of maternal reciprocal translocation. The fetal karyotype was 47XX,+der(14) [t(14;19)(q13.1;12)mat, resulting in partial trisomy 14pter-q13] and partial trisomy 18 (q12.2-qter). There was no evidence of fetal malformations by ultrasound examination. After the amniocentesis procedure, amniotic fluid has leaked and the mother miscarried. In reciprocal translocation carriers these chromosomes can arise in different segregation modes, such as alternate, adjacent-1, adjacent-2, 3:1 or 4:0, resulting in the formation of 32 possible zygotes with different chromosome complements. This case supports the thesis that the incidence of 3:1 segregation is higher in females than in males. So far, most 3:1 segregation cases have been postulated as the underlying cause of developmental delay and/or tissue heterogeneity for the observed UPD containing cell lineages. This may be caused translocation between chromosome bands 14q13 and 18q12.2 was concluded to be balanced translocation. In reciprocal translocation carriers these chromosomes can arise in different segregation modes, such as alternate, adjacent-1, adjacent-2, 3:1 or 4:0, resulting in the formation of 32 possible zygotes with different chromosome complements. This case supports the thesis that the incidence of 3:1 segregation is higher in females than in males. So far, most 3:1 segregation cases have been postulated as the underlying cause of developmental delay and/or tissue heterogeneity for the observed UPD containing cell lineages. This may be caused.

P13.46-M

Mosaic Uniparental Isodisomy (UPD) of 11p, presenting as a regular beta-thalassemia carrier.


Recently we discovered three independent cases of “severe late onset” beta-thalassemia, all presenting with the mild phenotype of beta thalassaemia minor up to adult age and developing a severe transfusion dependent phenotype in the third and fourth decade of life when a presumed homozygosity for the beta-thalassaemia mutation is observed. Affymetrix and/or Illumina SNP-array analysis revealed incomplete homozygosity for beta’s along at least the entire short arm of chromosome 11 containing the beta-globin gene, indicating mosaicism for a partial uniparental isodisomy of chromosome 11p. Three patients were born asymptomatic as beta-thal carriers and developed a severe blood transfusion dependent beta-thalassemia major at different ages and with different percentages of mosaicism. Recently we discovered another case showing a similar mosaic UPD of 11p, presenting as a regular beta-thalassemia carrier. The fourth patient however did not develop the clinical severity despite of an almost 50% mosaicism determined from the DNA isolated from leucocytes. The most probable mechanism seems to be clonal selection for hematopoietic stem cells containing the uniparental isodisomy for the mutant beta-globin gene during life, this may account for the progressive development of the disease. However, there seems to be no correlation between the percentage of mosaicism measured in the DNA isolated from the white cells and the severity of the clinical phenotype related to the expression in red cells, which strongly suggests hematopoietic tissue heterogeneity for the observed UPD containing cell lineages. This may have serious consequences for disease prediction and counselling, as this is largely dependent upon DNA isolated from leucocytes.

P13.47-S

Cryptic genomic imbalances and developmental delay and/or congenital defects in apparently balanced rearrangements carriers

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Apparently balanced chromosomal rearrangements (ABCRs) are associated with an abnormal phenotype in 6% of cases. It has been described that in over 40% of cases this phenotype may be caused by cryptic genomic imbalances, both at the breakpoints (25%) and elsewhere in the genome (15%). However, cryptic genomic imbalances detectable by array-CGH have also been postulated as the underlying cause of developmental delay and/or congenital abnormalities (DD/MCA) in 10-15% of patients with normal karyotype. The aim of this study is to determine if cryptic copy number variants are in fact a major genetic defect associated with DD/MCA in ABCR carriers. We performed CGH-array studies in three groups of patients: G1.1: 21 ABCR carriers with DD/MCA; G1.2: 22 ABCR carriers with normal phenotype and G2: 45 cases with normal karyotype and DD/MCA. Similar number of pathogenic imbalances were detected in both groups of patients with DD/MCA, independently of their karyotype (5/21 of cases in G1.1 (2.4%) and 9/45 in G2 (20%). Only one of the ABCR carriers (5% of cases) had an imbalance at the breakpoints. Conclusion: Simple ABCR do not seem to confer an independent and significantly higher risk for DD/MCA associated with genomic imbalances.

P13.48-M

Patterns of X inactivation in abnormal X chromosome

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Background: X inactivation is a dose compensation mechanism which results in silencing majority of genes on one of the two X chromosomes in every somatic cell of human females. Early in embryonic development, cells inactivate all their X chromosomes except one. Once an X is chosen, it is stably inherited through subsequent somatic mitotic divisions. The process of X inactivation is under the control of X inactivation center. Purpose: Study of X inactivation patterns in cases with abnormal X chromosome and its correlation with patients phenotype. Methods: 15 selected patients having abnormalities of the X chromosome were subjected to Clinical examination, GTG banding, FISH technique to detect origin of some structural X abnormalities and Detection of X chromosome replication pattern (Late Replicating Chromatin) technique. Results: Cases were classified according to their karyotypes into three groups: Cases with numerical X abnormalities, Cases with iso X chromosome and Cases with other structural X abnormalities. In most of X aneuploides, each cell has only one active early replicating X, while other extra or abnormal X chromosomes are inactivated late replicating. Regarding the balanced X-autosome translocation, there was a mosaic pattern; in majority of cells translocated X was late replicating inactive, while in few cells translocated X was early replicating active X chromosome. Conclusion: It has been found that at least 30 X-linked genes are expressed on the inactivated X chromosome. The varying degree of phenotypes within each syndrome occurs because the genes that escape X inactivation are expressed at varying degrees.

P13.49-S

Uncovering the oligomeric structure of the y+LAT1/4F2hc amino acid transporter

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y+LAT1 and 4F2hc are protein subunits that form a transporter complex for cationic amino acids in the basolateral membrane of epithelial cells, mainly in the small intestine and proximal kidney tubules. Mutations of y+LAT1 and 4F2hc were found in patients with inborn error of amino acid transport (OMIM #222700), a rare metabolic disorder characterised by diminished intestinal absorption of the cationic amino acids lysine, arginine and ornithine, and by severe loss of these amino acids into the urine. The more detailed structure of this transport complex has so far been unclear - it has remained elucidated whether the complex is formed as a dimer or a tetramer of the subunits. What has been known, however, is that the y+LAT1 subunits cannot reach the plasma membrane without forming a complex with 4F2hc. We previously established fluorescence resonance energy transfer (FRET) microscopy and FRET-FACS as tools in studying the interactions of y+LAT1 and 4F2hc. We have now applied these techniques together with immunohistochemistry to the exploration of the heteromerisation status of the y+LAT1/4F2hc transporter complex. We discovered that when fused into fluorescent vectors and transfected into the HEK293 cells, the y+LAT1 subunits interact in the presence as well as absence of 4F2hc. Our initial results therefore suggest that the holotransporter is a multimer of the subunits. What has been known, however, is that the y+LAT1 subunits cannot reach the plasma membrane without forming a complex with 4F2hc. We previously established fluorescence resonance energy transfer (FRET) microscopy and FRET-FACS as tools in studying the interactions of y+LAT1 and 4F2hc. We have now applied these techniques together with immunohistochemistry to the exploration of the heteromerisation status of the y+LAT1/4F2hc transporter complex. We discovered that when fused into fluorescent vectors and transfected into the HEK293 cells, the y+LAT1 subunits interact in the presence as well as absence of 4F2hc. Our initial results therefore suggest that the holotransporter is a multimer of y+LAT1 and 4F2hc subunits.

P14.01-S

Technical aspects of ALK FISH test to improve therapy selection of lung cancer patients

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Introduction. ALK gene (2p23) rearrangement characterizes a subgroup of patients affected by lung adenocarcinoma who may benefit from the ALK-targeted therapy. ALK translocation is principally related to a small pathogenic inversion. FISH with break-apart strategy is considered the gold standard to investigate ALK. Evidence based studies settled the presence of ≥15% cells with rearrangements as cut-off to classify patients as positive (ALK_FISH+). Recently, same Authors identified a subset of borderline patients that might benefit from therapy, wondering whether this cut-off could reflect a real biologic distinction between ALK-positive and ALK-negative tumors.

Materials & Methods. We investigated ALK gene status by FISH, using ALK LSI Dual-Color Break-Apart (Abbott) and ALK Split-Signal (DAKO) probes, in
243 lung adenocarcinoma as collected in three Institutions. A series of stan- 
dardized ALK-negative lung cancer cell lines (Abbott) were used as negative 
controls. A specific scoring system considering not only the splitting of the 
signals but also their distance was developed. Results: We identified 12% ALK_FISH patients, with similar rate among the 
three Institution (13%, 12%, 9%). ALK_FISH+ cases showed: 53% invi-
version/translocation, 28% deletion and 18% both patterns. Concordance was 
observed between the different operators and between the two probes, 
both on patients and on the panel of negative controls. In this last, the cut-off 
was observed by calculating M+3SD was 14.9%. 

Conclusions: Difficulties in interpretation of ALK FISH signals pattern might 
be bypassed using our detailed scoring system, that is reproducible among 
operators, probes, and provide experimental evidence that 15% is a reasonable cut-off for patients selection.

P14.02-M

Allelic drop-out in large Iranian Brugada syndrome family revealed by new generation sequencing
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Background: Sanger sequencing is a gold standard of DNA diagnostic cur-
rently used for NGS validation. Nevertheless, it has its limitations produ-
cing false results. The well-known mechanism of allelic dropout is the 
presence of SNPs in 3’-region of PCR primers. The early recognition of allelic 
dropout prevents diagnostic errors.

Materials and Methods: The DNA samples from Brugada syndrome Irani-
an family were extracted from peripheral blood. Sanger sequencing of SC-
N5A gene coding areas was performed for proband and relatives. Control re-
sequencing via semiconductor PGM IonTorrent platform using Ampliseq 
primer pool encompassing coding area of 10 genes including SCN5A was 
performed for family members. 
The control group comprised 100 ethnically matched healthy donors.

Results: Sanger sequencing has revealed a novel heterozygous genetic va-
riant p.P1506S in exon 26 of SCN5A gene in proband. Surprisingly, his son 
carried p.P1506S variant in homozygous state. Carriage of p. P1506S va-
riant in mother had been excluded by Sanger sequencing and PCR-RFLP 
analysis. We performed control re-sequencing via semiconductor PGM 
IonTorrent platform for proband and his son. NGS results showed a discre-
pancy with primary Sanger sequencing results, proving heterozygosity of 
p.P1506S variant in proband’s son. Sanger re-sequencing using alternative 
goldenucleotide primers for exon 26 confirmed NGS outcome. Thus, SNP 
rS41315501 was identified in 3’-region of previously used forward primer. 
Presence of SNP rS41315501 was assessed by PCR-RFLP in 200 chromosomes 
cohort with MAF (minor allele frequency) accounted 9.4%. SNP was detected 
in proband’s wife and son in homozygous state.

Conclusion: Currently Sanger sequencing is used for validation of NGS tech-
niques. But vice versa, NGS technology can be applied for capillary sequen-
cing quality control.

P14.03-S

Scanning Alpha Haemoglobin-Stabilizing Protein (AHS) gene by High Resolution Melting Analysis
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Alpha-Haemoglobin Stabilizing Protein (AHS) is involved in the stability, 
folding and binding of alpha globin to the hemoglobin complex, regulates 
the free alpha globin pool and has been associated with heterogeneity of 
thalassaemia phenotypes. AHS is encoded by a relatively small gene and is 
a good candidate for High Resolution Melting Analysis (HRMA). The aim of this study was to develop a rapid, specific and sensitive HRMA approach for 
scanning AHS gene. Alpha haemoglobin heterozygotes with the same 
underlying mutation (αPA/sαt) but heterogeneity in haematological indi-
ces MCV and MCH and no iron deficiency were selected and divided in 
three subgroups. Ten normal samples were used as controls. AHS gene was 
divided in small sized overlapping amplicons and PCR conditions were op-
timized prior to HRMA. Samples presenting distinct derivative plots were 
Sanger sequenced. Two variations were detected. The variation 12895 G>T 
(exon III) was detected in samples regardless group and may represent a 
neutral polymorphism as reported previously. The second variation detec-
ted p.P1506S in proband’s son. Sanger re-sequencing using alternative 
methods for scanning AHS gene. This approach can be incor-
porated in order to analyze higher number of samples and strengthen the 
characterisation of these changes as neutral or not polymorphisms in the 
Hellenic population. Moreover, detection of other variations may associate 
them with heterogeneity of alpha / beta thalassaemia phenotypes.

P14.04-M

NGS network for diagnostic of autoinflammatory diseases (AID) 
rearrangements
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Hereditary autoinflammatory diseases (AID) are characterized by recur-
rent bouts of systemic inflammation caused by dysregulation of proteins of 
the innate immunity system. Identification of about 25 genes over the last 
17 years rendered possible genetic diagnosis, a major tool to discriminate 
patients with close phenotype. This genetic heterogeneity and the rarity of 
these conditions motivated an International concerted action for the use of 
next generation sequencing for diagnosis of AID.

A survey among AID experts was conducted in 2013. Twenty laboratories 
declared willing to participate in this network and filled out a questionnaire. 
Results were as follows:

- Sequencing equipments varied across the laboratories. Illumina MiSeq was 
the major device (29%), followed by Lifetech PGM (25%), Illumina HiSeq 
(18%), Roche Junior (14%), other (14%).

- Sequencing results were analyzed with either a software from the plat-
form or in house pipeline, a collaboration (21%) or a combination 
(37%).

- The number of AID genes included in the panels varied from the few most 
common to all published genes.

- Clinical expectations included ethics: develop new informed consent 
(53%) and guidelines (47%), and collaboration: sharing experience for va-
riant interpretation (31%), access to patients and database (28%), harmo-
nization of technology and reports (17%).

Through a close collaboration with clinicians, we could connect this project 
with two existing dedicated patient (Eurofever) and mutation (Infevers) 
registries. Integration of clinical and genetic data will help 1) elaborate a 
consensus for classification criteria and 2) identify new AID genes in orphan 
patients.

P14.05-S

Next Generation Sequencing approach to molecular diagnosis of auto-
inflammatory diseases: from gene panel design to variant call
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Auto-Inflammatory Disorders (AIDs) are monogenic diseases caused by 
primary dysfunctions of the innate immune system. However, molecular 
diagnosis performed by Sanger sequencing of known genes fails to detect 
mutations in around 80% of patients referred to our Unit. Clinical misdi-
agnosis, mutations in untested gene regions, genetic heterogeneity and/or a 
complex mode of inheritance are all possible explanations.

The Next Generation Sequencing approach has been undertaken to improve 
mutations detection in AIDs. By using the Ion Ampliseq™ Designer (Life-
Tech) online tool, we designed a panel of 203 amplicons including the 121 
exons of 11 genes already known to be involved in AID, for a total of 22Kbs. 
Eight samples can be sequenced in one Ion PGM™ 34-chip. The mean co-
verage has turned out to be 22X, with 93.4% of the target region covered 
>20X and 76.5% >100X. The analysis from FastQ to VCFs was carried out 
using three different workflows: i) Ion Torrent Alignment and Ion Repor-
ter™ 4.0, specific for data generated by PGM ii) in-house pipeline based on 
free-tools like BWA and GATK, iii) CLC Bio software.

Focusing on three representative DNA samples already genotyped for the 
respectively causative genes, Ion Reporter and CLC allowed to detect all the 
three expected mutations, while GATK did miss one of these. In order to ma-
ximize sensitivity and specificity for routinely use of the procedure in AIDs 
diagnosis, we will present data from 50 additional DNA samples carrying 
mutations at least at one of the 11 genes in the present panel.

P14.06-M

A robust approach for blind detection of balanced chromosomal 
rearrangements with whole-genome low-coverage sequencing
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Balanced chromosomal rearrangement (or balanced chromosome abnor-

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mality, BCA) is a common chromosomal structural variation. Next-genera-
tion sequencing has been reported to detect BCA-associated breakpoints
with the aid of karyotyping. However, the complications associated with this
approach and the requirement for cytogenetics information has limited its
application. We describe a whole-genome low-coverage next-generation ap-
proach to detect BCA events independent of knowing the affected regions
and with low false positives. First, six samples containing BCAs were used

to establish a detection protocol and assess the efficacy of different library
construction approaches. By clustering anomalous read pairs and filtering out
the false-positive results with a control cohort and the concomitant mapping
information, we could directly detect BCA events for each sample. Through
optimising the read depth, BCAs in all samples could be blindly detected
with only 120 million read pairs per sample for data from a small-insert
library and 30 million per sample for data from non-size-selected mate-pair
library. This approach was further validated using another 13 samples that
contained BCAs. Our approach advances the application of high-throughput
whole-genome low-coverage analysis for robust BCA detection, especially
for clinical samples, without the need for karyotyping.

P14.07-S
Using biotin-streptavidin interaction for binding plasmid to a cell-
penetrating peptide
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Gene therapy strategies based on plasmid DNA are more preferable than
viral-based gene delivery. Plasmids are more stable in vivo and known to
be less immunogenic. Retroviral-based vectors integrate in actively tran-
scribed genes that can lead to disruption of tumor suppressor genes. Gene
delivery methods based on plasmid DNA do not cause mutagenesis and they
do not integrate in chromosomes providing increased safety. Despite the
potential advantages of using plasmid DNA, delivery issues with plasmid
vectors exist. One of the ways of plasmid delivery into a cell can be biotin-
streptavidin interaction of the plasmid to a cell-penetrating peptide (CPP).
Streptavidin binds the small molecule biotin with femtomolar affinity. We
have created a biotinylated pEGFP-N3 circular plasmid DNA to further
its binding to the streptavidin-CPP fusion protein. Few thymines were replaced
by biotinylated uracils in the plasmid. The presence of biotinylated uracils
in purified plasmid was verified in restriction analysis followed by dot blot
procedure. Streptavidin-conjugated alkaline phosphatase was used to detect
the biotin-uracils. The ability to express green fluorescent protein was
tested in Hela cell culture. We have created a genetic construct for synthesis
of monovalent tetrameric streptavidin, which consists of one WT streptavi-
din and three inactive streptavidins. Each inactive streptavidin is fused to
CPP. The streptavidin-conjugated alkaline phosphatase was used to detect
the biotin-uracils. The expression of green fluorescent protein was
performed in Hela cell culture. We have created a genetic construct for synthesis
of monovalent tetrameric streptavidin, which consists of one WT streptavi-
din and three inactive streptavidins. Each inactive streptavidin is fused to
CPP.

P14.08-M
Methyl-DNA detection: a performance comparison of four commercial
kits
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Epigenetic alterations, including DNA methylation, play an important role in
the regulation of gene expression. Several methods exist for evaluating DNA
methylation, but bisulfite sequencing remains the gold standard by which
base-pair resolution of CpG methylation is achieved. The main limitation of
this method is its harshness to DNA and several commercial kits try to
find a balance between reagent harshness and conversion efficiency. Our
study compares four popular kits (Diagenode, Promega, Epigentek, Qiagen)
regarding their conversion efficiency and degradation effect. We used in-
vitro methylated and unmethylated forms of two A-phage PCR products to create
various DNA mixtures (Spikes), including ratios 1.0, 1.1, 1.3 and 0.7 methylated
methylated to unmethylated DNA. These were bisulfite converted with all 4
kits and then PCR amplified producing templates for Sanger sequencing and
NGS. Our Sanger sequencing results showed 100% conversion efficiency of
cytosines across all kits and there was a correct trend of methylation status
at CpG sites which reflected the expected ratios of the spikes. However, the
methylation was limited in displaying accurately the extent of methylation in
each case. DNA degradation was compared between kits based on the effi-
cency of PCR amplification and it was shown that the Diagenode and Prome-
ga kits had the least degrading effect.

Our NGS results offered higher resolving power as expected, allowing accu-
rate assessment of methylation ratios lower than 1:3. Our NGS results indicate
bisulfite-NGS applications are applicable for detecting small variations in
methylation while our comparison shows which kit is the least harsh and
the most efficient.

P14.09-S
Molecular testing of BRCA1&2 genes with NGS technology: a three-
step approach
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The aim of our study was to enhance the throughput of mutation detect-

ation analysis in order to supply high sensitivity, fast and accurate data on
BRCA1/2 genes’ mutational status of selected breast and/or ovarian cancer
patients. Starting from June 2012 a panel of 8 selected BRCA1/2 pathogenic
mutations in 8 breast and/or ovarian cancer cases were proposed on our NGS 454
Junior platform (Ro-
che) and 6 out of 8 pathogenic variants were successfully identified. One of
the two unidentified variants was a single nucleotide insertion just adjacent
to a homopolymer stretch of 8 Adenines in the coding region of BRCA2. The
second unidentified variant was a deletion of the whole exon 14 of BRCA1
gene. The latter two unidentified pathogenic variants were revealed subse-
quently using the BRCA Homopolymer (BRCA HP) kit from MULTIPLEXIC
and MLPA analysis. We then decided to adopt a Three-step mutational scree-
ing approach. As a first step BRCA1 & 2 amplicon libraries were generated with
BRCA MASTTM- assay from Multiplexic, and sequenced on our GS 454
junior platform. Sequencing data are then analysed with Amplicon Variant
Analyzer (AVA) software (Roche). The samples with no pathogenic variants
identified are then involved in the second step which is the processing with
BRCA HP kit in order to uncover insertions or deletions in homopolymeric
coding regions of both genes. The third step is MLPA analysis to uncover
large genomic rearrangements. So far 181 samples were analysed in our
laboratory and 29 pathogenic variants were identified with our three step
testing approach.

P14.10-M
Efficient sharing of BRCA1 and BRCA2 variant and phenotype data
during diagnostic labs
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The Dutch and Belgian working group for Breast Cancer DNA Diagnostics
(LOB) has decided to share over 7500 variants detected in the BRCA1 and
BRCA2 genes in breast cancer families since 1997. For this, the data, almost
evenly split among both genes, have been submitted to the LOVD 3 shared
gene variant database installation (1,2). Previously, variants identified in
Dutch and Flemish (Belgian) DNA diagnostic labs were collected yearly and
stored in an Excel data file. Advantages of the LOVD system include: simple
submission of new data in a standardized way, instant updates after curati-
on, easy maintenance and automatic backups. Although most data are pub-
licly accessible online, some data are shared by members only. Others can see
whether such information is available (password protected file links),
giving them the option to contact the submitter for further details. Mem-
bers can contribute their opinions about variant classification, increasing
its consistency, but being aware of potential misinterpretation they have
reservations sharing this information. Data are stored variant-by-variant and
coupled to each individual patient and submitting diagnostic lab. Using
existing LOVD functionality, users can perform queries per gene or individu-
al, use other linked resources of interest, get genome browser views of the
data and use web services to access variants stored in other gene variant
databases. In addition, LOVD has a new access level, designated “collabo-
rate”, allowing submitters to share otherwise non-public data with other
submitters, e.g., to share detailed phenotype information with other diagno-
labs only.

1) http://databases.lovd.nl/shared/genes/BRCA1
2) http://databases.lovd.nl/shared/genes/BRCA2

P14.11-S
Validation of a Next Generation Sequencing assay for BRCA1 and


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Next Generation sequencing (NGS) is rapidly finding a place in routine dia-
gnostics although, extensive validation of such platforms and the associated
bioinformatics is urgently required before they reach equivalent confidence
as Sanger sequencing. Routine testing of BRCA1 and BRCA2 for Breast
Cancer predisposition has resulted in numerous positive and negative controls
as Sanger sequencing. Routine testing of BRCA1 and BRCA2 for Breast Can-

ceister is significant. A further consideration when imple-
menting a new technology for routine testing is it’s suitability to the diagno-
tic labs only.
P14.12-M
Initial evaluation of the cardiomyopathy-specific content of Illumina’s TruSight ONE next generation sequencing assay

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Introduction: The recently launched TruSight ONE panel comprises 4812 genes and is meant to enable a one-for-all mendelian diseases test. Objective: To determine whether the cardiomyopathy-specific genes are covered sufficiently good enough to justify the use of TruSight ONE in clinical genetic testing. Subjects, Materials and Methods: In order to initially analyze control samples, DNA was obtained from three individuals presenting with conditions other than cardiomyopathy (CM). TruSight ONE DNA enrichment and next generation sequencing (NGS) was conducted according to the manufacturer’s instructions using a MiSeq instrument. A proprietary NGS data processing and filtering pipeline was used. Results: A) Performance characteristics for 46 core CM genes: The fraction of bases covered with less than 20 reads varied between 2.3 and 5.4% (mean: 3.7%). The average read depth varied in the range of 68- to 147-fold (mean: 120-fold). A mean of 4 relevant variants (i.e. missense, nonsense or splicing) was observed (range: 2-8). B) Performance characteristics for a set of 281 genes annotated for cardiomyopathy by NCOB’s Entrez Gene (including the 46 core CM genes): A mean fraction of low coverage bases of 4.7% was observed (range: 3.7-6.6%). The mean average read depth was 118-fold (range: 81-148-fold) and 15-30 (mean: 22) relevant variants were detected. Conclusions: The cardiomyopathy-specific genes could efficiently be analyzed using TruSight ONE and a MiSeq instrument. The sensitivity and specificity of the test will be determined and compared to that of Illumina’s separate 46 gene cardiomyopathy assay.

P14.13-S
Computer-aided facial recognition of Cornelia de Lange Syndrome (CdLS): a follow-up study

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CdLS is a genetically heterogeneous disorder, exhibiting a wide phenotypic spectrum. Approximately 70% of the clinically diagnosed CdLS patients are confirmed for a cohesin-related gene mutation. In “Computer-aided facial recognition of Cornelia de Lange syndrome: a comparison to the recognition by human experts” presented at the 2012 DSW workshop, the FDNA® technology successfully recognized facial dysmorphology associated with CdLS from 2D photos, producing results comparable with those of human experts. In this study, we collected and tested blindly 18 photos of probands with a molecularly-confirmed or clinical diagnosis of CdLS and 10 with confirmed diagnosis of various non-CdLS syndromes (e.g., kabuki, Aarskog, dubowitz, etc). For each photo, the system produced a score above (possible) or below (negative) a threshold, determined from the results of the original study. 4/5 probands with the NIPBL mutation (including 1 mosaic mutation), 3/4 with the SMCG1L1 mutation and 5/9 clinically diagnosed by geneticists as CdLS-like, but carrying various genomic imbalances, received positive scores. 10/10 non-CdLS probands received a negative score. The 6 false-negatives were reported to have a mild phenotype or not to meet the clinical diagnostic criteria, of which 3 received a score well below the threshold and 3 a score immediately below the threshold, suggesting that the threshold may be skewed upwards and requires further adjustment to increase system’s sensitivity from 67% to 83%, while maintaining specificity of 100%. Results show consistency with experts’ evaluation of the “classic” CdLS facial morphology, validating the system’s ability to discern between CdLS’s and other syndromes’ phenotype.

P14.14-M
Profiling circulating miRNAs in plasma samples of celiac disease patients

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Celiac Disease (CeD; “gluten intolerance”) is diagnosed by symptoms, detecting CeD-specific antibodies, and biopsy results. The only available therapy is a life-long gluten-free diet. Despite the availability of diagnostic tools, as many as 7 out of 8 adult CeD patients are either not or incorrectly diagnosed, highlighting the need for novel biomarkers. Circulating miRNA profiles have been shown to be disease-specific, even disease-stage-specific, in patients with cancer or gastrointestinal disease. We examined whether circulating miRNAs in plasma samples of CeD patients can be used as CeD biomarkers. By next generation sequencing we profiled miRNAs in 95 plasma samples from 12 CeD patients. 5 patients positive for CeD-associated antibodies and 5 control subjects from the Prevent CD cohort. In this cohort, newborns at risk for CeD were challenged with low levels gluten between 4-6 months of age. Plasma and urine samples were taken every 3 months until diagnosis. Comparing data from samples taken at diagnosis to samples taken at 3 months of age (first available sample), we found 62 miRNAs significantly differently expressed (FDR<0.05). Of those, 4 miRNAs were previously reported to be associated with CeD, 12 were implicated in other autoimmune diseases and 7 miRNAs have been implicated in immune signaling. One of these immune related miRNAs is miR-23b, which appears to be downregulated 2-fold in CeD patients at time of diagnosis. This miRNA is known to limit tissue inflammation. A downregulation of this miRNA would therefore contribute to the pro-inflammatory state in active CeD.

P14.15-S
Northern Lights Assay of cdDNA damage in body fluids

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Structural damage in cdDNA molecules in body fluids has been little studied. Such damage may reflect normal and abnormal cell turnover, genome instability or exposure to genotoxic agents. We analyzed cdDNA damage in plasma, urine and saliva. Standard methods of isolation of cdDNA in plasma and urine are based on inducing ssDNA with a chaotropic agent and selective coordination binding of lone pair electrons on guanine to silica. These methods were not usable. In contrast, we found that selective ion exchange chromatography allowed gentle isolation of DNA without inducing damage. Damage in isolated DNA was assessed with the Northern Lights Assay. This assay is based on Two-Dimensional Strandness-Dependent Electrophoresis (2D-SDE) in premade microgels. Each sample was run in duplicate i.e. uncut and cut with Mbo I, an enzyme which cuts both single- and double-stranded DNA, single-stranded breaks, single-nicks and gaps were detected as horizontal stearaks from uncut DNA molecules. Double-stranded breaks generated an arc in the gel. DNA molecules with interstrand crosslinks (ICL) migrated as an arc behind normal dsDNA molecules. DNA with intrastrand crosslinks and bulky adducts were bent and migrated in front of that arc. Single-stranded DNA molecules, too damaged for complementary strand binding, formed a diagonal line. Patterns of cdDNA in plasma of normal non-celiac tolerant, as well as celiac disease subjects showed an apoptosis pattern with single- and double-stranded breaks of nucleosomal fragments. cdDNA in urine showed composite patterns of apoptosis and non-specific degradation. The most extensive damage and variable patterns were seen in saliva including prominent single-stranded breaks.
Charcot-Marie-Tooth disease (CMT) is a clinically and genetically heterogeneous disorder of the peripheral nervous system. CMT1A is the most frequent autosomal dominantly inherited form caused by a 1.4-Mb tandem duplication in chromosome 17p12. In 1997 the first annual German external quality assessment (EQA) was performed for CMT1A. At this time the molecular genetic analysis was mainly based on 5 methods: RFLP Southern blotting, STR markers, PCR for junction fragments within the CMT1A-REP elements, PFGE and FISH. 10 laboratories in Germany participated. In the year 2000 the scheme was offered to a wider audience with 22 participating laboratories. Only one genotyping error occurred. Since 2000 the European Molecular Genetics Quality Network (EMQN) has organised the EQA scheme. In 2012 the number of participating laboratories increased to 66 from 22 countries (198 reports) representing countries from around the globe.

Methods like FISH, PFGE and Southern blotting have disappeared; PCR, qPCR and MLPA are now used. Next generation sequencing (NGS) allowing simultaneous sequence analysis as well as gene dosage determination is a future perspective. Currently at least 60 genes are associated with disorders of the peripheral nervous system. Consequently the scheme scope has been extended to sequence analysis of GB1 (CX32) (CMTX1). In 2012 again only 1 genotyping error occurred. The efforts that have been made for an EQA in molecular genetic diagnosis for Charcot-Marie-Tooth disease demonstrate very good laboratory analytical performance. Nevertheless national laws affecting human genetics are different, thus harmonization in political terms (e.g. predictive testing) remains as important task.

P14.17-S

Comparative-High Resolution Melting - a novel method of simultaneous screening for small mutations and copy number variations

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Efficient and cost-effective screening for DNA sequence changes, both small mutations and copy number variations (CNVs), is a crucial aspect for routine genetic diagnostics as well as for basic research. In this study we present a development and evaluation of comparative high resolution melting (C-HRM), a new approach for the simultaneous screening of small DNA changes and CNVs. In contrast to other methods, relative quantification in C-HRM is based on the results obtained during the melting process and calculation of the melting peak height ratio in the multiplex reaction. Validation of the method was conducted on DNA samples from 50 individuals from Duchenne muscular dystrophy (DMD) families, 50 probands diagnosed with familial adenomatous polyposis and a control group of 36 women and 36 men. The results of analyses conducted on fragments of the DMD and APC genes correspond completely (100%) with the results of previous studies. C-HRM sensitivity in CNV detection was assessed through the analysis of mixed DNA samples with different proportions of a deletion carrier and wild type control. The results are presented as a linear regression with R2 of 0.9974 and imply the capability of the method to detect mosaics. C-HRM is an attractive and powerful alternative to other methods of point mutations and CNV detection with 100% accuracy in our studied group.

P14.18-M

New molecular technique to monitor minimal residual disease in CML

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Chronic myeloid leukemia (CML) is a clonal myeloproliferative disorder characterized by the Philadelphia chromosome (Ph), resulting from the t(9:22) (q34;q11) balanced reciprocal translocation that generates the BCR-ABL1 fusion protein. The first line therapy of CML is imatinib Mesylate, which targets BCR-ABL1 protein, inhibiting proliferation pathway. Residual leukemia is assessed by a sensitive molecular quantitative manner evaluating levels of BCR-ABL1 transcripts by real-time reverse transcriptase PCR (qR-PCR). Undergone stem cell transplantation, however, can reflect either an effective elimination of leukemia cells, or the presence of a quiescent leukemia stem cells transcriptionally silent. We developed a novel highly sensitive method to identify quiescent leukemic cells and possible candidates for discontinuation of therapy through quantitative real-time PCR (Q-PCR) based on the DNA. Detection of BCR-ABL1 fusion gene is critical for the diagnosis of chronic myeloid leukemia and to follow the disease progress in patients under therapy. We applied targeted next-generation sequencing (NGS) to resolve sequence breakpoints in BCR-ABL1 fusion gene in CML patients and K562 cell line. In conclusion, DNA genomic q-PCR is a very sensitive and direct technique to identify quiescent leukemic cells and patients that could be possible candidates to stop imatinib therapy. We identified junction sequences in all samples using Agilent SureSelect enrichment and Illumina paired-end sequencing.

P14.19-S

Rapid Serial PCR Instrument with High Speed Melting (HSM) Analysis

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We have developed a prototype instrument capable of rapid serial PCR/HSM, enabling multiple targets to be tested sequentially. The current system processes eight samples simultaneously and has a liquid handling system that delivers PCR reagents to a cartridge with microfluidic channels used for rapid PCR and HSM. A unique feature is that once PCR/HSM is completed (<12 min per test), reagents for the next assay are then delivered, tested and analyzed. We tested 100 blinded clinical blood samples (obtained from ARUP Laboratories) for F2 (c.970G>A), F5 (c.1601G>A), and MTHFR (c.665C>T and 1286A>C) genotyping using this instrument. The samples were also tested on a LightScanner®32 (Biofire™) using high resolution melting analysis (HRMA) assays designed by the University of Utah. Data from both instruments was interpreted using custom Melting Wizard software. Results show that all four tests with all 100 samples were genotyped accurately. Follow-up testing was required on 1.4% of the assays for a definitive genotype. The prototype instrument described allows for extremely rapid PCR (each thermal cycle <15 seconds) and HSM (melting rate up to 2°C/s) due to unique design of the microfluidic chip. The on-board liquid handling system allows multiple tests to be performed sequentially, and adding tests during the run. This method facilitates reflex testing, such as exon scanning follow-up, by a reflexive genotyping assay or a second reflexive genotyping assay. These features are better aligned with a workflow that requires multiple tests run on a single sample adding flexibility compared to current batch PCR workflows.

P14.20-M

Challenges in the interpretation of variants identified in autosomal dominant familial nonsyndromic congenital heart defects by targeted next-generation sequencing

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High throughput sequencing technologies enable efficient large-scale genetic analysis, dramatically accelerating the genetic research and diagnostics. Our study aims to determine the proportion of familial nonsyndromic congenital heart defects (CHD) that can be explained by pathogenic mutations in currently known CHD associated genes, by implementing next-generati- on sequencing technologies in genetic diagnosis for familial nonsyndromic CHD. Targeted resequencing of known cardiac genes was performed in 36 patients from 13 nonsyndromic CHD families, using either array-based or solution-based method to capture the coding regions of 57 genes associated with CHD. Following variant analysis and Sanger validation, we identified 6 functional deleterious mutations in 3 genes, explaining the defects in 6 families. The genetic heterogeneity of CHD and remarkable variability of expression make it challenging to interpret the large number of identified variants in patients. Thus, setting up a well-defined analysis pipeline is necessary to identify causative mutations. In conclusion, by targeted resequencing of known CHD associated genes in well selected nonsyndromic CHD families and cautious variant interpretation, likely causative mutations were identified in 6/13 (46%) families. Reduced penetrance and possibili- ty of phenocopies complicate the interpretation. Targeted next-generation sequencing is a powerful tool in genetic testing of familial nonsyndromic CHD, however, variant interpretation remains a major challenge for the diagnostic application.
Cystic Fibrosis (CF) is a genetic disorder that affects about 1 in 2,500 live births. It is caused by mutations in the CFTR (Cystic Fibrosis Transmembrane Conductance Regulator) gene, which is responsible for the transport of chloride and sodium ions across cell membranes.

Sanger sequencing is a method used to determine the DNA sequence of a specific region of DNA. It is a simple method, but it is only effective for small regions of DNA and is time-consuming. PCR (Polymerase Chain Reaction) is a method used to amplify DNA. It is a faster method than Sanger sequencing, but it can also amplify unwanted DNA sequences.

In recent years, next-generation sequencing (NGS) has become a more popular method for genetic analysis. NGS is a high-throughput sequencing technology that can sequence large amounts of DNA at once. It is faster and more cost-effective than Sanger sequencing and PCR, but it can also produce a large amount of data that needs to be analyzed.

In this study, we analyzed 24 selected CF patients by full CFTR gene resequencing (8 with known CFTR genotype, 11 with only one mutation identified, and 5 with no mutations after conventional screening). A custom capture SeqCap EZ Library (Roche) and an HiSeq 2000 platform (illumina) were used. Multiplexing 6 samples in the capture phase and 12 in the sequencing step, we obtained a mean depth >1,000X, with a 98% coverage. An in-house developed pipeline was used for variant detection and annotation, which allowed the identification of all 14 previously known mutations. Moreover, 4 genetic lesions undetected by genetic screenings were found, including a large heterozygous deletion. Additionally, we found new intronic variants, whose possible role on RNA splicing was excluded by a combination of in-vivo and in-vitro analyses. In conclusion, NGS increased the percentage of genetically diagnosed patients, being able to disclose mutations escaped to “standard” mutational screening. Moreover, read alignment also allowed an immediate definition of large deletion breakpoints at the nucleotide level. Finally, even after this extensive sequencing strategy, 5 probands did not show any mutation in CFTR (nor in the SCNN1A, SCNN1B, and SCNN1G coding regions), suggesting the intriguing possibility of an additional locus for CF. These subjects are currently being processed for whole-exome sequencing analysis.
relevant. Therapy was initiated before release of results in 27%, after cytogenetics in 9%, after FISH in 3%, after array in 1% and supportive care in 60%. The preliminary data suggests a tier testing approach does not compromise patient care if tier testing is completed within 3-4 weeks of initial diagnosis. In presence of unfavorable prognostic abnormalities by cytogenetics, array testing may not be warranted. Based on this data, we would like to propose the optimal genetic testing algorithm.

P14.26-M

Real-time and Droplet PCR quantification for non-invasive determination of RHD incompatibility between mother and foetus

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The aim of this study was to compare two strategies of DNA quantification: RealTime PCR (qPCR) and Droplet PCR (dPCR). The benefit of dPCR against qPCR is represented by absolute quantification of target nucleic acid molecules without the requirement of calibration curves. The methods were compared by quantification of standard nuclear DNA of known concentration. DNA was eight-fold diluted (2-0.015 ng/ul) and twelve replicates were realized for each dilution. Concentrations were then measured as levels of amplicons of two genes, housekeeping gene GAPDH and human RHD gene (exon 10). The same genes were analyzed in plasma cell-free DNA and also in cell-free fetal DNA isolated from maternal plasma. Evaluation of these two methods’ performance was based on several parameters, such as degree of linearity (R²), accuracy, detection limit, etc. In case of standard nuclear DNA, both methods showed high accuracy and low detection limit. However, in plasma DNA, results showed that in both methods linear regression was obtained only in levels of 10 ng/ul or more. In case of data analysis were performed followed by a blind protocol. DNA from all the patients was analyzed with the Haloplex technology and only 24 of them were also analyzed with the SeqCap technology (they were sorted among the 44 patients). Data analysis was performed with the NextGene Software (Softgenetics) and also with specific BWA-GATK pipeline based on the recommendations of BroadInstitute. Our analysis will include coverage, sensitivity and false positive rate as well as a cost evaluation.

P14.28-M

Scaling up whole-exome sequencing on Ion Proton using AmpliSeq


The Ion Proton® and Ion PGM® (Life Technologies) platforms are used in the National Genomics Infrastructure - Sweden (NGI) to handle the different requests and needs for rapid massively parallel sequencing. (MPS). Ion Proton® enables a rapid workflow for human whole exome sequencing. The PCR based whole exome capture (Ion Ampliseq Exome Kit) provides consistent high coverage across annotated exonic regions, with the PP chip yieleding 50-60.000 SNPs per sample, with ~98% of these overlapping variants reported in dbSNP. Sample preparation and sequencing is performed in less than two days. We are exploring the possibility to scale-up the throughput by loading two PI chips on one initialization and to combine 3 exomes per PI chip. With this set up it will be possible sequence up to 30 exomes per week on one Ion Proton®.

The bioinformatics analysis has been streamlined using an in-house database system, based on R and MySQL, where all detected variants from all in house exome-sequencing runs are stored. This system allows for very efficient and fast filtering of SNPs or indels between any groups of samples and we currently have a success rate of almost 80% of finding disease-causing variants in small families or trios with rare Mendelian disorders. Ion Proton® is also being used for clinical applications such as identification of fusion transcripts from cancer samples, mutation screening using panels of candidate genes. The sample preparation will be simplified and faster when the automated system, Ion Chef® is introduced and established in the workflow.

P14.29-S

Evaluation of three sequence capture platforms for whole exome sequencing

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Whole exome sequencing (WES) can be effective for identifying sequence variants. Here, we present a comprehensive comparison of the most recent next generation sequencing (NGS) exome enrichment methods of Agilent (SureSelect V5 + UTR), NimbleGen (SeqCap V3 + UTR), and Illumina (Nextera Expanded Exome). Exomes of six human DNAs were captured by these methods and sequenced at 100x depth of coverage on an Illumina HiSeq 2000 platform by four vendors. Read depth, % of coverage, GC bias, and number of detected single nucleotide variations (SNVs) and small indels were compared. To examine the methods’ ability to identify SNVs and small indels, we analyzed heterozygous positions and small indels previously detected by Sanger sequencing (SS) for two DNAs. WES data were also compared to whole-genome-sequencing WGS of SureSelect and NimbleGen demonstrated highest average read depth in target region. Considering ≥20x for read depth, SureSelect covered the largest proportion of its targeted bases. SureSelect and NimbleGen showed the highest average read depth as well as detected the highest number of SNVs and small indels in RefSeq. However, all SNVs identified by SS were accurately called by all three methods. Less consistent was the detection of small indels, where a substantial difference among the platforms and vendors was observed. In addition, all three methods showed high GC bias which was not seen in WGS. Our analysis indicated a considerable variability among exome enrichment methods, DNA sequencing laboratories, and even among DNA samples. Our data revealed that both SureSelect and NimbleGen performed better than Nextera.

P14.30-S

The importance of root cause analysis following an external quality assessment (EQA) to improve the quality and accuracy of a diagnostic service

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EQA is essential to verify the quality and accuracy of the diagnostic service. When new technologies emerge, EQAs need to be developed to verify the laboratory diagnostic validation process and enable benchmarking of their performance. When laboratories introduce new technologies, efficiently identifying the most critical errors in EQAs are analytical. As laboratories gain more experience in the new technologies, the critical errors usually become limited to the interpretation of results.
Identification of point mutations and deletions in Fanconi anemia using a next generation sequencing approach


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Fanconi Anemia (FA) is a bone marrow failure disorder characterized by high clinical and genetic heterogeneity (at least 16 genes), which makes diagnosis complex and time-consuming. Next-generation sequencing technologies, such as the Ion PGM System (IPGM; Life Technologies), could improve the molecular genetic testing in FA. To test IPGM, we sequenced 30 DNA samples: 2 from controls and 28 from FA patients, whose mutations were previously identified by Sanger sequencing in 18 of them. According to the Ampliseq Designer software, the molecular target has a size of 74.2 kb covering 96% of the FA coding exons and their flanking regions. After exclusion of one sample for low coverage, we found that the coverage was higher than expected (>100X in 97%) of reads per run except for one sample that was excluded from the analysis. Then, comparing the IPGM and Sanger sequencing data, we assess sensitivity (100%), specificity (99%) and accuracy (100%). In addition to confirming all the mutations (31 alleles) identified by Sanger sequencing, this study allowed us to characterize another 18 out of the 54 FA alleles in our cohort. The remaining 5 are likely to be localized in genomic region not covered by the sequencing analyses. Moreover, quantitative analysis aimed at detecting copy number variations (CNVs) detected complete (n=1) and partial (n=7) deletions of FANCA, or entire removal of (n=3) of FANCC. All the CNVs were confirmed by SNP array or MLPA analysis. Our data suggest that IPGM is suitable for detection of point mutations and large deletion in FA.
P14.35-S Development of a next generation sequencing panel to assess hereditary cancer risk that includes clinical diagnostic analysis of the BRCA1 and BRCA2 genes
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Assessment of hereditary breast and ovarian cancer risk should include germline sequencing of BRCA1 and BRCA2 as well as additional genes with known associations in breast/ovarian cancer patients. Sanger DNA sequencing has been the gold standard for molecular genetic analysis but next generation sequencing (NGS) platforms could provide another sequencing alternative. However, optimized assay design and validation are critical to maximize the analytical sensitivity and specificity of NGS assays and to ensure high quality interpretation for clinical decision making. We developed a 25-gene NGS hereditary cancer panel that uses RainDance PCR technology for high-throughput sample preparation, Illumina HiSeq and MiSeq NGS technologies, and commercially available and lab-developed informatic tools. Initial assessment of analytical sensitivity and specificity was performed by comparing BRCA1 and BRCA2. NGS was performed on 186 anonymized patient samples, which had previously undergone BRCA1 and BRCA2 Sanger sequencing. Sanger sequencing identified 15,877 variants, of which 681 were unique and 482 were classified as disease-associated mutations. We identified 15,877 variants at an initial sensitivity of >99.99% for BRCA1 and BRCA2. One polymorphic variant was missed due to a variant under the primer. Sensitivity was subsequently optimized through process improvements. No additional variants were found by NGS, yielding a specificity of 100%. This preliminary analysis facilitated assay optimization and validation of all 25 genes in the NGS panel. This analysis indicates that a NGS gene panel designed to meet rigorous quality standards can be used to provide clinical sequencing results equivalent to those obtained from Sanger DNA sequencing analysis.

P14.36-M In silico analysis of the effect on splicing of single nucleotide changes at exon-intron junctions is predictive of pathogenicity. A study on MYBPC3 mutations.
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Hypertrophic cardiomyopathy (HCM) is one of the most common inherited cardiovascular disorders with an estimated prevalence of 1/500. About half of these cases are familial and mutations in MYBPC3 are among the most frequent cause of HCM. Here we report on 14 patients with HCM in which single nucleotide substitutions potentially affect splicing of the MYBPC3 gene. Twelve mutations are located either on the last nucleotide of an exon or within the consensus 3'- or 5'-splice site sequences, while two are situated outside these regions. Mutations at the exon-intron junctions can potentially affect splicing. However, only changes affecting the invariant AG-GT nucleotides are considered to be certainly pathogenic, while the rest require functional studies. Mutations were first analysed in silico, using a combination of 5 splice site prediction algorithms, integrated in the Alamut 2.0 Splicing prediction module. For 12 mutations splicing prediction analysis indicated an effect on splicing and for 2 there were no alterations. Subsequently, all mutations were analysed by cDNA sequence analysis and these results were compared to the prediction models. In all patients the results of sequence analysis were consistent with the predicted effect, namely exon skipping, intron inclusion, partial exon deletion, and retention of intronic sequences or no altered splicing at all. In conclusion, this study indicates that in silico splicing analysis can accurately and reliably predict the effect on splicing of mutations occurring at exon/intron junctions representing a helpful tool in DNA diagnostics for the assessment of the pathogenicity of a sequence variant.

P14.37-S Genetic diagnosis of familial colorectal cancer: Detection of large rearrangements in the MMR genes by molecular combing
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Hereditary Non Polyposis Hereditary Colorectal Cancer (HNPCC) or Lynch syndrome is an autosomal dominantly inherited cancer susceptibility syndrome that is caused by germline mutations in one of the DNA mismatch repair (MMR) genes (MSH2, MLH1, PMS2 and MSH6). Although most of the mutations reported in these genes are point mutations, large genomic rearrangements in one of the MMR genes occur with a frequency varying from 5 to 20% depending on the population. Effective methods to identify these types of mutation should be integrated in current diagnostic procedure to obtain a more comprehensive genetic screening strategy. Molecular Combing is a powerful FISH-based technique for the direct visualization of single DNA molecules which allows exploration of the genome at high resolution in a single analysis. We are developing a novel HNPCC genetic test based on molecular combing, for which specific "genomic Morse codes" (GMC) have been designed for each MMR gene. These GMC have been validated on comb-bed genomic DNA extracted from the cancer-derived cell lines LoVo and Sk-OV-3, which harbor MSH2 and MLH1 large deletion respectively, and from HNPCC patients. From these patients, large rearrangements corresponding to either deletions of one to several exons in one of the MMR genes and with sizes ranging from 4 kb to 53 kb or the paracentric inversion in the MSH2 gene have been detected. Importantly, when the sequencing and molecular combing confirm the results previously obtained by Southern Blot analysis on the same patients, with a resolution in the 1-2 kb range.

P14.38-M When an allele drops out of amplification: whole exome sequencing unmasks a case of false-negative genetic diagnosis
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Introduction: Sudden cardiac death without an attributable cause after clinical diagnostic work-up, is defined as idiopathic ventricular fibrillation (IVF). Mutations in genes primarily involved in inherited arrhythmia syndromes underlie some cases of IVF.
Methods: An IVF patient underwent routine mutational analysis of 6 arrhythmia-susceptibility genes ( KCNQ1, KCNH2, SCN5A, KCNE1, KCNE2 and RYR2) through direct sequencing. Afterwards, exome sequencing (Agilent SureSelect) was performed on the proband and his non-affected parents on an Illumina HiSeq2000 (minimum coverage 20x for 90% of bases, genotype quality score>40). Identified variants were subjected to a 3-step filtering strategy (de novo, non-synonymous, absent from dbSNP-1000Genomes).
Results: Traditional mutation scanning failed to identify a pathogenic variant. When exome sequencing was performed, a RYR2 candidate variant was identified (c.12006G>T, p.M4002I) that however could not be validated by Sanger sequencing. This prompted further investigation and all factors contributing to either a stochastic sampling or a systematic error were evaluated. A SNP near the primer’s 3’ site (rs709889, MAF=0.426) was identified and hypothesized to cause the mutant allele to drop out. Targeted screening with redesigned primers confirmed the presence of the variant.
Conclusion: In routine diagnostics often only screening procedures reported through literature dated at a time when genetic variation information was not widely available. Allelic dropout or, more precisely, the occurrence of a null/stop-coding variant, due to primer-target mismatch, constitutes an avoidable laboratory error. Our report highlights the need to periodically reevaluate even previously well established screening conditions.

P14.39-S Pyrosequencing Assay Panel For Imatinib Resistance Mutations in ABL kinase domain
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Background: Chronic myeloid leukemia (CML) is characterized by the presence of the Philadelphia (Ph) chromosome. Ph chromosome results from the translocation of ABL protooncogene from chromosome 9 and BCR gene on chromosome 22, which leads to the formation of BCR-ABL chimeric gene with constitutive tyrosine kinase activity. Imatinib (Gleevec) is the first BCR-ABL tyrosine kinase inhibitor used in the treatment of CML. Second generation drugs such as nilotinib and dasatinib, are now considered as alternative. However, optimized assay design and validation are critical to maximize the analytical sensitivity and specificity of NGS assays and to ensure high quality interpretation for clinical decision making. We developed a novel NHPCC genetic test based on molecular combing, for which specific "genomic Morse codes" (GMC) have been designed for each MMR gene. These GMC have been validated on comb-bed genomic DNA extracted from the cancer-derived cell lines LoVo and Sk-OV-3, which harbor MSH2 and MLH1 large deletion respectively, and from HNPCC patients. From these patients, large rearrangements corresponding to either deletions of one to several exons in one of the MMR genes and with sizes ranging from 4 kb to 53 kb or the paracentric inversion in the MSH2 gene have been detected. Importantly, when the sequencing and molecular combing confirm the results previously obtained by Southern Blot analysis on the same patients, with a resolution in the 1-2 kb range.
ANTS were used to study assay performance and precision. Totally 25 RNA samples of BCR-ABL positive patients were analyzed by pyrosequencing and mutation samples, which had mutation were also analyzed with sanger sequencing. 

**Results:** Our assays allows flexible, fast and reliable detection of mutations in CML patients from both blood and bone marrow with high concordance to Sanger sequencing. **Conclusion:** Our pyrosequencing assay for imatinib-resistant mutations provides a reliable method for the detection of the mutant BCR-ABL transcripts and also provides a significant value for the follow up of CML patients on imatinib.

**P14.40-M**

Next generation tissue profiling

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Cancer develops as a consequence of genetic mutations, which results in deregulation of gene expression and/or proteins with aberrant functions. These changes often interfere with cellular processes such as survival and proliferation. The cancer cells evolve and are subjected to natural selection through interplay with its microenvironment. As is the case with other ecosystems, the tissue microenvironment changes and responds to the populations in its niche and to external interference. Communication with other cells in the microenvironment will provide input signals that are interpreted by the malignant cells, and responded to, based on their altered genetic programs. Hence, the consequence of a mutation has to be viewed in the environmental context of each individual cell. The activity status of a protein or signaling pathway can be visualized with in situ proximity ligation assays (in situ PLA) using a pair of antibodies equipped with DNA oligonucleotides (proximity probes) to target interacting proteins. Proximal binding of such probes template the creation of a circular DNA molecule, which is a surrogate marker for the interaction. We recently developed a multiplexed version of in situ PLA by introducing unique tags as identifiers in each different proximity probe. The combinatorial events generating an in situ PLA signal will harbor a set of identifier tags that will be unique for each protein interaction. By combining in situ PLA with padlock probes, analysis of signaling activity can be achieved together with genotyping expressed mRNA in fixed tissue sections, retaining the architectural information while providing single-molecule resolution.

**P14.41-S**

Quantification of donor/recipient chimerism in leukemia samples by digital PCR

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During leukemia treatment mixed chimerism occurs in which both recipient and donor cells are present in the bone marrow or peripheral blood after transplantation. Chimerism analysis is performed to monitor peripheral blood or bone marrow in the recipient after allogeneic stem cell transplantation to monitor for leukemic relapse. Observation of increasing mixed chimerism after transplantation is associated with a higher risk of relapse in acute leukemia. Previously, a quantitative PCR (qPCR) technique, using insertion/deletion polymorphisms, was found to predict relapse in 88.2% vs. 44.4% of individuals analyzed by VNTR markers with a median anticipation period of 58 days and a sensitivity of 0.01% vs. 3%. Here we present results from research experiments performed to determine if a digital PCR (dPCR) method is able to predict relapse earlier and with greater accuracy than the qPCR method using retrospective leukemia samples. Research results showed that dPCR using the QuantiStudio™ 3D Digital PCR System and the qPCR method yielded similar percent recipient chimerism values when recipient DNA was present above the 1% level. Furthermore, dPCR using the system was found to be more sensitive than the qPCR method based on the ability to detect the recipient DNA in a relapsed individual about 2 months earlier where the percent recipient chimerism was 0.2% or less. The false positive rate was close to the complete chimerism value of 0.01% for peripheral blood samples.

**P14.42-M**

Hotspot mutation and fusion transcript detection from the same non-small lung adenocarcinoma sample


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The presence of certain chromosomal rearrangements and the subsequent fusion gene derived from translocations has been implicated in a number of cancers. Hundreds of translocations have been described in the literature recently but the need to efficiently detect and further characterize these chromosomal translocations is growing exponentially. The two main methods to identify and monitor translocations, fluorescent in situ hybridization (FISH) and comparative genomic hybridization (CGH) are challenging labor intensive, the information obtained is limited, and sensitivity is rather low due to the necessity to analyze RNA samples from tissue or cell lines, which are very limited in material making the downstream measurement of more than one analyte rather difficult; obtaining another biopsy, using a different section or splitting the sample can raise issues of tumor heterogeneity. The ability to study mutation status (DNA) as well as measuring fusion transcript expression (RNA) from the same sample is powerful because you’re maximizing the information obtained from a single precious sample and minimizing any sample to sample variation. We isolated DNA and RNA from the same non-small lung adenocarcinoma sample without splitting or dividing the sample, and both mutation analysis, as well as fusion transcript detection was performed using the Ion Torrent PGM™ platform on the same Ion 318™ chip. Using 10ng of DNA and 10ng of RNA input, we applied the Ion AmpliSeq™ Colon and Lung Cancer panel to detect 40 COSMIC mutations and the Ion AmpliSeq™ RNA Fusion Panel to detect 40 different fusion transcripts.

**P14.43-S**

Development and verification of an Ion AmpliSeq RNA gene fusion panel for lung cancer: an OncoNetwork collaborative research study


Chromosomal translocations and corresponding gene fusions play an important role in carcinogenesis. The recent establishment of ALK, ROS1, RET and NTRK1 fusion transcripts as predictive biomarkers for lung cancer therapy has increased the need for a technology that could detect these biomarkers starting from very limited amounts of material. Here we describe the development and verification of an Ion AmpliSeq RNA fusion panel that can simultaneously detect ALK, RET, ROS1 and NTRK1 fusion transcripts in a single reaction. For the development of the panel we used previously characterized samples comprising 6 cancer cell lines and 62 FFPE lung tumor samples. Upon RNA isolation and amplification using the panel, samples were sequenced on the Ion PGM system. Initially, using serial dilutions of RNA from cell lines in normal RNA a limit of detection of 1% was established. Next, using the PGM, 10ng of RNA from fusion positive cell samples were detected in 21 of the 24 known positive samples. Two of the non-considerant results were due to lack of representative tumor material. For the third, although no specific fusion transcript was identified, the internal control for ALK expression revealed the presence of an unknown ALK fusion transcript. Additionally, two new RET fusions were detected in samples not previously tested for RET translocations. These preliminary results are highly encouraging regarding the possibility of a reliable experimental protocol for the detection of fusion transcripts in the biological material routinely obtained for the diagnosis of lung cancer. An extensive verification is under way within the OncoNetwork.

**P14.46-M**

MicroRNA detection by real-time TaqMan® assays for translational research


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MicroRNAs (miRNAs) are small non-coding RNAs involved in the multilevel regulation of gene expression targeting a battery of mRNA genes. Researchers have discovered that miRNAs are efficacious biomarkers for the classification of tumors and provide important outcomes to support their role in the cancer progression, their evolutionary conservation, unique expression signatures, relative stability, and abundance. There has been an increased interest in recent years for the identification of circulating miRNAs in serum, plasma, and other body fluids because it holds great promise for a non-invasive approach to molecular diagnostic and therapeutics. However, detection of miRNAs in these clinically relevant samples has been difficult, often requiring greater sensitivity. We have developed a new method for the detection and quantification of miRNAs that is highly specific and sensitive. The highly efficient
upstream chemistry allows synthesis of miRNA template library that is used in the downstream real-time TaqMan qPCR for miRNA-specific detection. The universality of template synthesis simplifies the workflow and provides the flexibility for scalable content (miRNA coverage). Whether applied to basic or translational research, profiling, screening, or validation, this new and robust method allows detection and quantification of miRNAs to address the unmet needs in workflow and sensitivity that exists today with next generation sequencing and other qPCR technologies, especially with clinical samples. Results show miRNA-specific amplification with a 7-log linear dynamic range and sensitivity of 60 copies input to meet the needs of translational research and clinical/diagnostic application.

P14.47-S
A new strategy for genetic testing of neurodegeneration with brain iron accumulation (NBIA) using amplicon multiplexing and next generation sequencing
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In the expanding field of neurodegeneration with brain iron accumulation (NBIA), the development of sensitive brain imaging played a key role to characterize iron-related damages in patients. In parallel, new genes have been identified allowing genotype-phenotype correlations. However, overlapping phenotypes and non-informative pedigrees are still sources of delayed diagnosis. For these reasons, it would be more rapid and cost-effective to propose a simultaneous analysis of the nine genes identified so far.

Here, we propose a new Generation Sequencing solution, tested in our series. NBIA genes (ATP1A2, CPN10/11, DCAF17; FAH, PTL, PHA2G, PLATG and WDR45) were included in a custom panel of 35 genes dedicated to our routine molecular diagnosis platform. Two libraries of amplicons were designed and built with the AmpliSeqTM technology. Sequencing was performed according to the semi-conductor technology on a PGM. The Torrent Suite (Life Technologies) and AlamutTM interface (Interactive BioSoft®) were used for the mapping of reads, variant calling and the interpretation. Workflow analysis was validated on known PTL and PANK2 mutations previously identified using Sanger sequencing. DNA samples irrelevant for NBIA were used as control to avoid considering recurrent artifacts. In silico AmpliSeq design covered 99% of NBIA genes targeted regions, of which 98% were sequenced at least 40X. Preliminary results obtained from 3 patients showed a perfect match between NGS and Sanger sequencing results. Thirty patients with compatible symptoms and MRI signs of NBIA who were referred to our laboratory for genetic testing are currently screened.

P14.48-M
New generation sequencing dedicated to CFTR genotyping: comparison of two kits for multiplexed amplicons resequencing
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Cystic Fibrosis (CF) is the most frequent autosomal recessive disease among Caucasians (prevalence 1:2500). It depends on mutations in the Cystic Fibrosis Transmembrane Regulator (CFTR) gene that encodes a chloride exchanger. More than 1500 causative CFTR mutations have been identified, including missense, frameshift, splice site, nonsense and deletion mutations. Their accurate detection is important to improve the clinical management of CF patients and to correctly identify all CFTR carriers. Here, we report the use of a next generation sequencing screening for the identification of CFTR germline mutations. The study was performed on 60 patients, including CF carriers, patients, CFTR carriers and controls, previously analyzed by traditional methods (reverse dot blot and Sanger sequencing). CFTR coding regions (including their flanking sites) were amplified using the CFTR MASTR Assay kit (Multiplicom) to obtain a library/sample. The subsequent sequencing reactions were performed with both the GS Junior System (Roche) and the MiSeq System (Illumina) allowing the simultaneous analysis respectively of 10 and 60 patients/run. Finally, the downstream data analysis was carried out through the SeqNext tool (JSI Medical Systems). All the features and performances of the Next Generation Sequencing technologies are discussed in the light of results obtained with the two used systems. Comparative sequence analysis highlighted that the proposed method is reliable, since all mutations previously identified were confirmed and the time of analysis was remarkably reduced. Our results assess the feasibility of a next generation sequencing approach for CFTR mutation detection to be included in a routine diagnostic workflow.

P14.50-M
Validation of a next generation sequencing approach for rapid and accurate CFTR mutations screening
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Validation of a next generation sequencing approach for rapid and accurate CFTR mutations screening A. Teles1, V. Zitgenhofer1, I. Postiglione1, P. Naridello1, G. Castaldo1, E. Salvatore1,2

Cystic Fibrosis (CF) is one of the most frequent autosomal recessive disease among Caucasians (prevalence 1:2500). It depends on mutations in the Cystic Fibrosis Transmembrane Regulator (CFTR) gene that encodes a chloride exchanger. More than 1500 causative CFTR mutations have been identified, including missense, frameshift, splice site, nonsense and deletion mutations. Their accurate detection is important to improve the clinical management of CF patients and to correctly identify all CFTR carriers. Here, we report the use of a next generation sequencing screening for the identification of CFTR germline mutations. The study was performed on 60 patients, including CF patients, CFTR carriers and controls, previously analyzed by traditional methods (reverse dot blot and Sanger sequencing). CFTR coding regions (including their flanking sites) were amplified using the CFTR MASTR Assay kit (Multiplicom) to obtain a library/sample. The subsequent sequencing reactions were performed with both the GS Junior System (Roche) and the MiSeq System (Illumina) allowing the simultaneous analysis respectively of 10 and 60 patients/run. Finally, the downstream data analysis was carried out through the SeqNext tool (JSI Medical Systems). All the features and performances of the Next Generation Sequencing technologies are discussed in the light of results obtained with the two used systems. Comparative sequence analysis highlighted that the proposed method is reliable, since all mutations previously identified were confirmed and the time of analysis was remarkably reduced. Our results assess the feasibility of a next generation sequencing approach for CFTR mutation detection to be included in a routine diagnostic workflow.

P14.51-S
Molecular analysis of mutations in the CFTR gene by Next Generation Sequencing
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Cystic fibrosis (CF) is the most common autosomal recessive disease in Caucasians, affecting 1:2500 live births. CF is caused by mutations in the CFTR gene that encodes a chloride exchanger. More than 1500 causative CFTR mutations have been identified, including missense, frameshift, splice site, nonsense and deletion mutations. Their accurate detection is important to improve the clinical management of CF patients and to correctly identify all CFTR carriers. Here, we report the use of a next generation sequencing screening for the identification of CFTR germline mutations. The study was performed on 60 patients, including CF patients, CFTR carriers and controls, previously analyzed by traditional methods (reverse dot blot and Sanger sequencing). CFTR coding regions (including their flanking sites) were amplified using the CFTR MASTR Assay kit (Multiplicom) to obtain a library/sample. The subsequent sequencing reactions were performed with both the GS Junior System (Roche) and the MiSeq System (Illumina) allowing the simultaneous analysis respectively of 10 and 60 patients/run. Finally, the downstream data analysis was carried out through the SeqNext tool (JSI Medical Systems). All the features and performances of the Next Generation Sequencing technologies are discussed in the light of results obtained with the two used systems. Comparative sequence analysis highlighted that the proposed method is reliable, since all mutations previously identified were confirmed and the time of analysis was remarkably reduced. Our results assess the feasibility of a next generation sequencing approach for CFTR mutation detection to be included in a routine diagnostic workflow.
casians, with an Cystic fibrosis (CF) is the most common autosomal recessive disease in Caucasians, with an incidence of approximately 1 in 2500 and a carrier frequency of 1 in 25. The gene responsible for CF, named the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR), encodes the cyclic adenosine monophosphate (cAMP)-dependent chloride channel found in the apical membrane of secretory epithelial cells. The known mutations of the CFTR gene are 1965 (Cystic Fibrosis Mutation Database). The massive sequencing for mutation analysis and copy number variations (CNVs) by Next Generation Sequencing (NGS) allows investigating at the second and third level with the scanning of all exons, the flanking regions and the search for deletions and/or insertions in a single diagnosis. To check the reliability of massive sequencing by NGS as many as 100 control cases provided by an European network, were analyzed. All of the mutations previously obtained by Sanger Sequencing were confirmed by NGS that was shown to be highly sensitivity and specific. The massive sequencing offers the possibility to carry out a reliable and comprehensive analysis for all genetic variants in reduced time (16h) testing up to 20 cases-samples in a single run. The costs of genetic analysis are substantially decreased.

P14.54-M
Enabling high-throughput discovery of the RNA transcription landscape using a directional RNA workflow and a combinatorial multiplexing approach
Massively parallel next generation cDNA sequencing (RNA-Seq), has allowed many advances in the characterization and quantification of transcriptomes, including the detection of non-canonical transcription start sites and termination sites, and identification of alternative splice isoforms, transcript mutations and edits. Additionally, the ability to obtain information on the originating strand is useful for reasons including for example: identification of antisense transcripts, determination of the transcribed strand of noncoding RNAs, and determination of expression levels of coding or noncoding overlapping transcripts. However, standard methods for sequencing RNA do not provide information on the DNA strand from which the RNA strand was transcribed, and methods for strand-specific library preparation can be inefficient and time-consuming. To address this challenge we developed a streamlined, low input method for Directional RNA-sequencing that highly retains strand orientation information while maintaining even coverage of transcript expression. This method is based on second strand labeling and excision after adaptor ligation; allowing differential tagging of the first strand cDNA ends. We have also extended the utility of this method by developing additional adaptor and primers, including a dual barcoding approach for multiplexing up to 96 samples. As a result, we have enabled highly multiplexed, strand-specific mRNA sequencing, as well as whole transcriptome sequencing (Total RNA-seq) from ribosomal-depleted samples, enabling the discovery of a much broader picture of expression dynamics including discovery of antisense transcripts. This work presents a streamlined, fast solution for complete RNA sequencing, with high quality data that illustrates the complexity and density of the RNA transcription landscape.

P14.53-S
Highly efficient diagnostic testing in patients with hereditary hearing loss using Panel-based Next Generation Sequencing
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Genetic heterogeneity complicates the molecular diagnosis of hereditary hearing loss (HHL). Although a multitude of genes are attributed to HHL, patients are routinely tested only for mutations in GJB2, GJB3 and GJB6 (~5-10% of HHL). Thus, screening of all known hearing loss genes in parallel by high-throughput sequencing methods (next generation sequencing technology) is the most promising tool for a comprehensive detection of causative mutations, especially after negative testing for GJB2. Here we present the methodology of the ‘hearing loss’ diagnostic panel comprising 110 genes associated with non-syndromic and syndromic HL (Target enrichment, NGS library preparation and sequencing on the Illumina HiSeq2500 platform (2x100bp) and bioinformatic analysis and medical evaluation). A group of 2010 patients with profound hearing loss were analyzed in a clinical setting. Multiplexed samples were sequenced with high coverage per base and combined with bioinformatic analyses, single base substitutions, small deletions, and insertions in known genes of genetic hearing loss can reliably be detected. In conclusion, we have established a panel-based NGS pipeline which is a highly sensitive, fast and cost efficient tool for the genetic diagnostics of HHL. NGS-based mutation analysis allows us to detect causative mutations in >55% of HHL patients. These results imply consequences for counseling of patients and families and can also be the foundation for novel gene- or even mutation-specific treatment options in hearing loss.

P14.54-N
teaching challenges towards extended blood group genotyping by next-generation sequencing (NGS)
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Generating libraries by using the Ampliseq™ strategy (Ion Torrent) for next-generation sequencing (NGS) is very convenient in terms of technical handling and time. By these means we designed primers and generated libraries to investigate exons, flanking introns and UTRs of 18 genes involved in 15 human blood systems, for a total of ~57 kilobases. After normalization by two different approaches, libraries were sequenced by the Ion PGM™ Sequencer (Ion Torrent). While all coding DNA sequences, except in ABO, exhibited a significant ~90% coverage, data analysis of genotypes in homologous genes (i.e., RHD and RHCE, GYP, GYPB, and GYPE) resulted in discrepancy with what expected due to misassignment of sequencing data to target genes by analysis softwares. That prompted us to specifically PCR- amplify the exons of our genes of interest with gene-specific primers. PCR products were subsequently fragmented and sequences from homologous genes were labeled with different barcodes. All products were mixed with the Ampliseq™-generated products before sequencing. Sequencing data were finally found to be in accordance with known genotypes. This work actually made the proof of principle that 1 libraries generated by two different methods can be combined and sequenced together, and 2/ gene-specific primers are required to accurately investigate genotypes in homologous pairs of genes. Meanwhile we also define conditions to reduce both intra- and intersample variability. Overall this study illustrates that NGS is applicable for blood group genotyping, and may be used in a near future for molecular diagnosis at the laboratory level.

P14.55-S
Cardiovascular NGS-Panel testing: design and first experiences
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Syndromes resulting in primary structural heart disease or arrhythmia are characterized by a large clinical variability and a significant genetic heterogeneity. In fact, in a diagnostic setting it is not uncommon to have more than 100 genes as potential causes for an individual patient. For potential patients this situation is often not satisfactory because a precise diagnosis of the underlying cause of the disease directly affects patient treatment and allows counseling for relatives. To address these issues we designed an enrichment panel covering 79 genes, including all genes currently associated with LQT, SQT, Brs, IVFA, ARVC, HCM, DCM, RCM and SUDS/SIDS. After targeted enrichment, the DNA is sequenced on an Illumina MiSeq and subjected to analysis employing our analysis pipeline. Our strategy includes an indication specific analysis with an in depth evaluation for highly penetrant genes with a prevalence of >5% where we complement regions with a coverage below 50x by Sanger sequencing. For the remaining genes we generate a screening report and usually achieve coverage above 50x for more than 95% of bases. Up to date we applied our cardiovascular NGS panel to more than 25 patients, which allowed us to establish a definite diagnosis in many of these cases. For example, we detected mutations in MYBPC3 in 5 out of our 8 patients with suspected HCM. We will present a summary of interesting cases and our experiences with this interesting diagnostic tool.

P14.56-M
Is exome sequencing of single patients with intellectual disability an effective diagnostic strategy?
Trio-sequencing can be used in all disorders, and has particularly proven its value in finding causes of intellectual disability (ID) or multiple congenital anomalies. In contrast, we investigated whether sequencing only the affected patient without parents is sufficient to find the causative mutation, leading to a considerable reduce in costs. In this study, we enrolled 36 patients with unexplained ID, and sequenced the exome. The exome sequences were analysed with a stringent post-sequencing annotation pipeline including an
ID gene panel of ~500 genes for filtering of the data. All remaining variants with a potential clinical consequence were validated by Sanger sequencing and tested in the parents for inheritance. After variant filtering we noticed an average of 13 variants per patient (range 2-27) requiring further clinical interpretation. The majority of these variants were inherited from one of the parents. Hitherto, we identified 5 de novo mutations in 36 patients (14%). Without exome sequencing the parents, a relatively high amount of potentially pathogenic variants remain. All these variants require clinical interpretation which is very time-consuming, while most of these variants were likely benign because they are inherited from one of the parents. With trio-analysis, inherited variants can be filtered out suggesting that this strategy, at this moment, is more efficient in identifying the causative variant. In the future when databases are filled with more and more exome data and consequently with more rare benign variants, exome sequencing single patients will become a more realistic diagnostic approach.

P14.57-S Detection of large rearrangements in the CFTR gene: Comparison between custom CGH array and NGS
24 years after the discovery of the CFTR gene, more than 1900 anomalies are described, mainly single base-pair substitutions or micro-insertions/deletions, but many large rearrangements are also described. Identification of these rearrangements has important implications for genetic counseling, prenatal diagnosis, cascade screening in families, and for understanding the genotype-phenotype relationship.
Classical approaches for gene analysis aim either to look for point mutations or to identify large deletions or duplications, but no strategy allows, to date, to identify both types of mutations. Since a few years, NGS technologies enable us to overcome the classical approaches of whole coding sequence sequencing at single nucleotide resolution. The aim of this study is to validate a NGS data analysis to detect large rearrangements in the CFTR gene. 23 DNA carrying known large deletions (n=20) or duplications (n=3) previously identified by CGH-array or QPM-PCR and two control samples, one complete deletion, and the other corresponding to the wild-type sequence of the gene were included in this study. Analysis was conducted by SeqNext and Nextgene software and by a simple spreadsheet by comparing the average depths obtained for each amplicon. This method allowed us to confirm all the large deletions/duplications affecting more than one exon. Only one short deletion of 1 kb affecting exon 17b alone was not detected by both methods. In conclusion, NGS technologies is the first tool that allows in a single step both the identification of point mutations and large rearrangements in a gene.

P14.58-M The impact of NGS on genetic services: prioritization criteria and accountability
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Clinical use of Next Generation Sequences (NGS) technologies is appealing, but requires high accuracy, simple assays, small inexpensive instruments, flexible throughput, short run times and most importantly, robust data analysis as well as tools for biological interpretation of results. We have launched a pilot project to assess the clinical value of NGS technology according to the following criteria: 1) phenotypes characterized by genetic heterogeneity; 2) accurate selection of patients in collaboration with a clinical team; 3) the informed consent of the patient to perform the test. We performed targeted NGS analysis on 30 selected patients with Hypertrophic cardiomyopathy (HCM) and Epileptic Encephalopathies (EE) using custom panels of candidate genes on PGM (Life Technologies).
Our study highlight important issues which have to be considered for the accountability of management and transmission of the results: 1) increased identification of new variants with uncertain pathogenic value affecting the expectation of patients, especially in predictive or presymptomatic testing; 2) identification of deleterious variants in target regions leading to missing clinically relevant mutations. Accordingly, Sanger sequencing seems to be required to analyse specific genomic segment. 3) Our experience indicates that most of identified non-synonymous missense coding variants are validated by standard sequencing techniques. On the other hand small indels are more frequently false positives or mis-called.

The study confirms the potential of NGS techniques, indicates caution in interpretation and validation of data and suggests a soft transition between classical methods and NGS approach in clinical application.

P14.59-S Application of NextGeneDx, a validated NGS based procedure, in the clinical practice
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c We have developed a NGS based procedure, called NextGeneDx, with the main objective that it can be used for genetic diagnosis, by using specific designs targeted at specific clinical problems. High coverage and representativity (100%) guarantees a sensitivity and specificity comparable to Sanger sequencing. Validation has been performed by an extensive comparison between the results obtained with NextGeneDx and Sanger sequencing. Versatility and robustness of NextGeneDx allows to increasing gradually the number of genes and diseases analysed. Nine months after the validation of the whole procedure we have developed more than 46 NextGeneDx services for multigene or genetically heterogeneous diseases including more than 180 genes. The application of this technology in the clinical practice shows a significant reduction of the global costs of sequencing, making accessible the diagnosis of both single-gene and heterogeneous diseases, bringing to an increase in the detection of disease-causing mutations and, definitely, a higher number of patients with a genetic diagnosis. Cost effectiveness analyses, from DNA extraction to obtain the final report, bear an important reduction of the costs, especially that related with hands-on and turnaround time, without compromising the diagnostic accuracy of the analysis. In conclusion, NextGeneDx provides an analytical accuracy comparable to Sanger sequencing and permits the analysis of all of the coding region and adjacent intronic sequences including those refractory to other types of sequencing. Therefore, NextGeneDx is a cost effective approach for the genetic diagnosis of multigene and genetically heterogeneous Illnesses, with a negligible number of incidental findings or unsolicited genomic information.

P14.60-M Noninvasive prenatal testing for fetal trisomies: a validation study using the SOLiD Wildfire platform
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Next-generation sequencing of cell-free DNA, isolated from plasma of pregnant women, is widely used for noninvasive prenatal testing (NIPT) for fetal trisomies. Recently, a new upgrade for the SOLID platform was released: the SOLID Wildfire. The Wildfire has a simplified sample preparation protocol removing the laborious and expensive emulsion PCR step, an increased sequencing throughput, and the capability to use individual lanes and reuse unused flowchip lanes. Consequently, run time and costs are reduced significantly whilst obtaining equal numbers of mapped reads for statistical analysis. In this study, we aimed to validate the use of the SOLID Wildfire for NIPT. In total, 154 samples were tested (between 11-20th weeks of gestation) including sixteen T21, ten T19 and four T13 (validated by karyotyping or QF-PCR in chorion villus or amniocytes). Cell-free DNA was extracted from 1 ml plasma and processed using a NIPT optimized library preparation and multiplexed (up to 16-plex). Libraries were sequenced on the Wildfire (35pb) targeting > 10 million uniquely mapped reads without mismatches per sample. The in-house developed pipeline CHROMATTE (CHROMosomal Aneuploidy TEster) was used for GC-correction, read filtering, and statistical analysis. To calculate Z-scores from Illumina data, the Z-score from the wildtype and the NIPT read coverage was used to reliably detect fetal trisomies and that results are robust between runs and under suboptimal conditions. Low costs, ease of use, decreased run time, and robustness underlining the suitability of the Wildfire for cost-effective and rapid NIPT in clinical practice.

P14.61-S Epigenetic strategies for non-invasive prenatal diagnosis: The power of the fetal methylome
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Epigenetic modifications have proven to play a significant role in cancer development as well as fetal development. Taking advantage of the knowledge acquired during the last decade, great interest has been shown worldwide in deciphering the fetal epigenome towards the development of methylation...
based non-invasive prenatal diagnostic (NIPD) assays. We hereby highlight the different approaches implemented such as sodium bisulfite conversion, restriction enzyme digestion and methylated DNA immunoprecipitation, for the identification of differentially methylated regions (DMRs) between free fetal DNA and maternal blood DNA from maternal blood cells. Furthermore, we evaluate the use of selected DMRs identified towards the development of NIPD for fetal chromosomal aneuploidies. In addition, we perform a comparison analysis; we evaluate the performance of each assay and provide a comprehensive discussion on the potential use of different methylation-based technologies in retrieving the fetal methylome, with the aim to further expand the development of NIPD assays.

P14.62-M
Next-generation variant effect predictions and data integration
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The interpretation of variants flowing out of next-generation sequencing experiments is one of the largest bottlenecks for DNA diagnostics. Frequently used tools like PolyPhen and SIFT are not up to this task, and consequently there is an enormous need within the community for novel tools to reliably interpret the effects of polymorphisms. In response to the growing demand for high-quality variant effect predictions we present 3DM; a data integration & mutation prediction platform for protein families.

3DM relieves many of the burdens that researchers face in dealing with the growing amounts and complexity of biomedical data. For each protein family, a database generation that is extracted from protein structures, alignments and scientific literature, among others, is available. All this information is integrated and validated, and can be analysed via a number of different methods and tools. By intelligently combining all this heterogeneous information 3DM is able to provide state-of-the-art predictions about the effects of genetic variants. Collaborative work with a number of the largest hospitals in the Netherlands has shown that our solutions represent a major step forward in helping researchers and clinicians making accurate assessments of the pathogenicity of their variants, both by providing predictions, as well as enabling them to quickly navigate to literature and other relevant data.

P14.63-S
The Israeli experience of the first 300 Panorama™ tests that use 19,488 single nucleotide polymorphisms (SNPs) followed by high-throughput sequencing for common trisomies risk assessment
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Background Cell free DNA (cfDNA) has emerged over the last year as an alternative for amniocentesis for diagnosis of the common aneuploidies looking at trisomy 21, 13, 18, sex chromosomes and triploidy. Methods We present our experience with the first 300 Panorama™ tests sent from Israel. This method is based on massively multiplexed PCR amplification of cfDNA isolated from maternal plasma, targeting 19,488 SNPs, followed by high-throughput sequencing. The fetal fraction is determined. The SNP pattern of maternal DNA (from buffy coat) is compared to the SNP pattern of free DNA from maternal plasma, which contains maternal and fetal DNA. Paternal genomic samples, when available, were included in the analysis; in the absence of a paternal sample, the algorithm considers population allele frequencies. Combining the maximum likelihood ratio with a priori risk generates a risk score. Results The results of the first 300 sequential tests performed in Israel were analyzed. Fifteen samples necessitated redraw; two samples failed analysis. Four samples yielded high risk scores: two cases for trisomy 21, one for Kleifelter syndrome (KS) (47,XXY) and one for trisomy 18. Confirmation of both trisomy 21 and one K5 were done by CVS and amniocentesis. The suspected trisomy 18 is in the process of confirmation. There are no confirmed false positive results.

Discussion Panorama™ test is a reliable tool for identification of pregnancies at high risk for fetuses with the common aneuploidies with a high success rate. We recommend confirmation of the diagnosis for high risk scores pregnancies using invasive tests.

P14.64-M
Quality control procedure for assessing good manufacturing of a molecular diagnostics test in freeze-dried format
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Quality Control (QC) procedures are crucial in manufacturing of molecular diagnostics tests. The aim of this work is to describe QC procedures used to check properties of a freeze-dried PCR master mix for molecular diagnostics. A liquid bulk was prepared as follows: reaction buffer, dNTPs, MgCl2, DNA polymerase, primers and probe for detection of human beta-globin gene, preservatives and stabilizers. Prior to freeze-dry, a PCR was performed with an aliquot of this mix to assure the functionality (Pre-Lyophilization control, Pre-Lyo). The mixed bulk was then dispersed in aliquots of 25µL using a Freedom EVO 100 liquid handler (Tecan) and freeze-dried using an Epsilon 2-12D freeze-dryer (Martin Christ). A PCR was performed with lyophilized mix to check the functionality (Post-Lyophilization control, Post-Lyo). PCR performances were evaluated on a 7500 Real-time PCR System (Applied Biosystem). Moreover, to inspect the residual moisture in the lyophilized mix, an analysis using a G20 Compact Karl Fischer Coulometer (Mettler Toledo) was performed. Test performances were checked using as template different concentrations of human genomic DNA (hu gDNA; Roche). Similar threshold cycles in Pre- and Post-Lyo controls were obtained with a concentration ranging from 0.1 ng to 10 ng of hu gDNA. Moreover, Karl Fischer analysis after freeze-drying showed residual moisture content less than 5%.

The described QC procedure assures to control in an effective way the manufacturing of a freeze-dried molecular biology test in terms of performance evaluation and residual moisture content.

P14.65-S
Phenotype ontology driven gene panel construction in diagnostic next generation sequencing
A. Mauer, B. Peterlin; Clinical institute of Medical Genetics, Ljubljana, Slovenia.

Technological advances in determination of human genetic sequence have significantly facilitated genetic diagnostics in human disorders at progressively diminishing costs. Due to interpretative and ethical challenges faced in medical reporting of findings in whole exome sequencing (VES), sequencing of arbitrarily defined gene panels is commonly endorsed in clinical practice. Such selection of clinical sequencing target, however, narrows the diagnostic survey to genes directly associated with proposed diagnosis, is not resistant to ambiguity in determination of patients’ diagnosis and suffers from large disparity in definitions of gene panels between diagnostic centres.

The systematization of human phenotype annotation, notably with the Human Phenotype Ontology (HPO) project now offers a possibility for formalized dissection of human genetic features and offers a possibility for straightforward, systematic and individualised definition of clinical sequencing target based on distinct patients’ phenotypic features. We therefore propose a novel approach to outlining the clinical sequencing target for diagnostic next generation sequencing, where patient’s phenotype is first defined according to the HPO nomenclature and according gene panel is then dynamically constructed based on known phenotype-gene associations. Proposed algorithm automatically scores genes based on their phenotypic compatibility with disease under evaluation and defines a tailored sequencing target based on specific phenotypic characterization of disease.

We demonstrate that such an approach allows for judicious extension of currently established sequencing panels while assuring comparable power to establish genetic etiology and controlling for issues in either WES or targeted panel sequencing approaches.

P14.66-M
A new paradigm for prenatal chromosome microarray testing: increased resolution without equivocal findings
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A novel prenatal chromosome microarray testing strategy has been developed, that moves away from size-based detection thresholds, towards a more clinically relevant analysis, providing higher resolution than G-banded chromosomes but avoiding the detection of imbalances of unclear prognosis that cause parental anxiety. All prenatal samples fulfilling our criteria for karyotype analysis (n=353) were tested by chromosome microarray; only copy number variants of established deletion/duplication syndrome regions and any imbalance >3Mb were detected and reported. A retrospective full-resolution analysis of 249 of these samples was carried out to ascertain the performance of this testing strategy. Using our prenatal analysis, 28/353 (7.9%) samples were found to be abnormal. Of the remaining samples, 240 were anonymized and reanalyzed at full postnatal resolution; a further 46 regions of imbalance were detected in 44 of these traces (17.7%). None of these additional imbalances was of clear clinical significance. This prenatal chromosome microarray strategy therefore detected all CNVs of clear prognostic value. This strategy avoids the problems associated with interpre-
P14.67-S
Determining the sensitivity of diagnostic methods to maternal cell contamination (MCC) in prenatal diagnosis (PND)
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Background: MCC is a potential risk factor for misdiagnosis in PND. The purpose of this study was to quantify the percentage of MCC that can be present in a fetal sample without compromising the fetal genotyping result in each of the different assays used for PND in this laboratory.

Methods: Maternal DNA carrying X-linked and autosomal recessive disease was mixed with unaffected fetal DNA (prepared from dissected chorionic villi) in known proportions. Diagnostic methods examined included primer extension assays with detection by MALDI-TOF mass spectrometry, gap-PCR, MLPA, long range PCR and Sanger sequencing, STR analysis (AmpFISTR Identifier kit, Life Technologies) is routinely used in our laboratory to assess MCC and was run in parallel with each assay to assess the theoretical versus the detectable MCC.

Results and discussion: An unacceptable level of MCC was defined as the percentage of MCC that results in an equivocal or incorrect genotype in the fetus. This percentage of detectable MCC was assay dependent, ranging from 1.0% with the gap-PCR, 10-15% for long range PCR, 15-20% for Sanger Sequencing and 50% for MLPA. A correct genotyping result was obtained with the primer extension assay up to 20% MCC. STR analysis had a limit of detection of 1.0%, providing a useful tool for quantifying MCC. Results of this study have provided a basis for interpreting a PND result when MCC is present and advising requesting clinicians when repeat invasive procedure is warranted.

P14.68-M
Mutation screening in patients with PCD by a multi-gene panel and next generation sequencing technology
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Primary ciliary dyskinesia (PCD) is a rare, autosomal recessive genetic disorder characterized by defect in the action of the cilia lining the respiratory tract, fallopian tube and also of the flagella of sperm in males. The impaired ciliary function results in neonatal respiratory distress, chronic oto-sino-pulmonary disease, infertility, and organ laterality defects in approximately 50% of cases. Currently, the diagnosis of PCD involves the study of cilia morphology, motility and ultrastructure, nasal nitric oxide measurement and, more rarely, genetic analysis. This is mainly due to the high genetic heterogeneity of the disease which has been associated to mutations in 26 different genes.

This study aims to improve the genetic diagnosis of PCD that it is routinely limited to a subset of the most frequently mutated genes. This approach is expensive, time consuming and ineffective leaving many patients without a molecular diagnosis. Using the AmpliSeq technology we developed a panel which allows comprehensive, rapid and cheap analysis of the 26 PCD-causative genes. A total of 160kb genomic sequence including all the protein coding sequences, splice sites and 5'-UTRs will be amplified in 1151 amplicons using two primer pools and sequenced in the Ion Torrent platform. Twenty PCD patients diagnosed according to the protocol of the European Respiratory Society Consensus Statement have been selected for a test study.

Technical details, results and cost-effectiveness analysis will be presented.

P14.69-S
Public Health Genomic and genetic tests. Cost evaluation analysis and quality standards as relevant factors in health care planning
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Genomic era has improved a lot molecular diagnosis, but in the last five years the new revolution of next generation sequencing has open a new window not only on research but also on diagnostic testing.

The scientific framework and the laboratory activities are completely changed: the number of known disease genes has increased exponentially, automation is now applied, certification and accreditation procedures are in place in a growing number of laboratories.

Both public National Health systems and private laboratories or companies are facing with an increasing demand of genomic tests for the most common diseases. Correct information and awareness among public and all stakeholders are crucial.

Since it has not been clearly defined how many genomic tests have enough clinical utility, the investigation of their costs could be a way to establish a correct public health policy.

Activity-Based Costing is the methodology used to assigns the cost of each activity.

The systematic analysis of activities needed to perform genetics tests has identified a number of indicators to assess the workload for every professional who participates in the process of diagnosis and the proportion of material used for each activity. All these parameters have been incorporated into a software able to split all the lab costs (personnel, material and general costs) for each test provided in order to compare costs among different laboratories, to compare the performance of the same laboratory in subsequent years and to make a priority list of genetic/genomic tests to provide, taking into account costs/benefits data.

P14.70-M
Testing homopolymers in pyrosequencing-based next generation sequencing chemistry
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In the human exome, approximately 1.43 million homopolymers exist with the size of 4-mer and up. Majority (97%) of them are in the range of 4-mer to 40-mer. To detect indel mutations in homopolymers is of great importance in order to implement the corresponding sequencing system into the routine clinical practice. To test the analytical performance of the pyrosequencing-based GS Junior system in assessing homopolymer sequences, a series of plasmid vectors were generated using pcDNA3.1 as template. Altogether 12 clones were produced, 4-mer, 5-mer, and 6-mer homopolymers with all four nucleotides using site-directed mutagenesis. Mutations were confirmed using Sanger sequencing. For the test system, each homopolymer tract was tested using three pairs of PCR/sequencing primers to test the hypothesis whether the beginning of the sequencing reaction might provide the necessary signal-to-noise ratio to accurately test the size of the homopolymers. In the first, the forward, in the second, the reverse primer was located (i.e. the primer’s 3’ end) just next to the homopolymer to be analyzed, respectively. In the third, the homopolymer was in the middle of the sequenced product.

Average of the correct genotypings were 95.8% in 4-mers, 87.4% in 5-mers, and 70.0% in 6-mers, respectively. Contrary to the low genotyping accuracy in 5-mers and 6-mers, acceptable settings could be found in both with a minimum of 97% in 5-mer and 91% in 6-mer tracts. In conclusion, we have developed a test system that can be used for the assessment of genotyping accuracy of next generation sequencing systems.
suitable for analyzing HaloPlex data in clinical diagnostics. Data from scree-
nening of additional patients will be presented.

P14.72-M Next Generation Sequencing: New approach for diagnosis of autosomal dominant Retinitis Pigmentosa patients
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Introduction: Retinitis Pigmentosa (RP) is an inherited disease clinically and genetically heterogeneous causing degeneration of photoreceptors. Autosomal dominant RP is the most common inherited retinal dystrophy; it presents for 15% of all RP patients, and mutations in 24 known genes are responsible for 60% of them. The 40% of our cohort of 200 adRP Spanish patients has been characterized using classical tools: different methods of screening (Single Strand Conformation Polymorphism (SSCP), CG-clamped Denaturing Gradient Gel Electrophoresis (DGGE) and ADRP genotyping microarray (Asper Biotech)) followed by Sanger sequencing gene by gene. Patients and methods: 66 previously studied and not characterized adRP families, were analysed by Next Generation Sequencing (NGS) panel of 73 genes associate to RP and other Retinal dystrophies (RD). Sequence variants were detected using the sequence DNA capture Haloplex (Agilent) and sequencing with a MiSeq platform (Illumina). Bioinformatic analysis was performed with a specific DNA Nexus pipeline. Results: potentially pathogenic variants in known adRP genes were found in 42.4 % of families studied: SNP200P (7.7%), PRPF8 (6%), BESTI (5%), PRPF33 (3%), PRPH (3%), RPL13 (3%), CRX (1.5%), FSCN2 (1.5%), GUCA1B (1.5%), IMPG1 (1.5%), KCN13 (1.5%), PROM1 (1.5%), PRPF3 (1.5%), RGR (1.5%), RhO (1.5%), RP2 (1.5%), and TDP1 (1.5%). Additionally 7.6% of cases displayed mutations in ABCA4, RPGR and USH2A allowing us to identify a clinical or genetic reclassification. Conclusion: this new approach seems to be a fast and reliable tool to detect the disease-causing mutation for adRP patients.

P14.73-S Visualization of XIST expression in a female with structural X chromosome abnormality: Single-cell analysis by three-color interphase RNA-FISH
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An X-inactivation (XIA) analysis in females is commonly performed by metaphase R-banding or methylation-specific PCR (M-PCR) technique. The late-replicating X-chromosome can be recognized under a microscope at the single cell level by R-banding using BrdU incorporation, but there are limitations: 1) Not so many metaphases can be observed, and 2) Only an abnormal X chromosome can be detected. M-PCR can detect random or non-random XIA pattern in cell population, but the parental samples are needed to identify which is normal or abnormal chromo-

somes. A subject was a female with an X-autosome balanced translocati-

on; 46,XX,19(19p21.1q12), and having clinical manifestations of Duchenne muscular dystrophy. The breakpoint of the derivative X was on the BMD gene. We selected three kinds of BAC clones and used simultaneously for our protocol using the Agilent 2200 TapeStation system with the respective assays run on the 2100 Bioanalyzer system. The results from the QCs of the starting total RNA and final sequencing libraries generated from Agilent’s SureSelect strand-specific RNA library preparation kit reveal that the RNA and D1000 ScreenTape assays are a match to the visual and quantitative results obtained with the RNA 6000 Nano and DNA 1000 Kits.

P14.74-M Quality control for SureSelect Strand-Specific RNA library preparation protocol using the Agilent 2200 TapeStation system
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Transcriptomics reveal global changes in gene expression that may con-

tribute to the pathogenesis of a particular disease or help drive a fundamental biological process. RNA sequencing (RNA-Seq) has emerged as a promi-

nent and rapidly growing method for studying gene expression and characterizing cellular transcripts at single base pair resolution. Among different approaches for generating libraries for transcriptomics study, strand-specific library pre-

paration method has added advantage over other methods. The Agilent Su-

reSelect strand-specific RNA library preparation kit generates libraries with specific adaptors ligated to each strand enabling the identity of the DNA template strand of origin to be retained in downstream sequencing and data analysis. The high throughput deep-sequencing based RNA-seq studies gen-

erate more data compared to RT-qPCR and array based methods yet they are expensive and time consuming study. Proper quality control (QC) steps within the library preparation process are therefore crucial to ensure successful sequencing. The Agilent 2200 TapeStation system offers an easy to use automated electrophoresis system with rapid analysis time as well as flexible sample throughput capabilities. Here, we compare the performance and capabilities of the RNA and D1000 ScreenTape assays for use on the Agilent 2200 TapeStation system with the respective assays run on the 2100 Bioanalyzer system. The results from the QCs of the starting total RNA and final sequencing libraries generated from Agilent’s SureSelect strand-specific RNA library preparation kit reveal that the RNA and D1000 ScreenTape assays are a match to the visual and quantitative results obtained with the RNA 6000 Nano and DNA 1000 Kits.

P14.75-S Expanded carrier screening tests currently on the commercial market
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There has been a recent rise in the advertising of preconception genetic car-

rier screening tests directly to consumers via internet. We analyzed the of-

fers with particular focus on comprehensiveness, clinical validity and utility of the genetic tests proposed.

We gathered data from the websites of Counsyl, InheriGen, Pathway geno-

mics and Recombine in December 2013. Of about 1300 recessively inherited diseases listed in OMIM (causative gene known), current offer includes a range of 73 to 206 for a total of 286 diseases of which 30 are included in the panels of all providers and 92 in at least three providers. According to the ORPHANET catalogue, 31% of screened diseases are more frequent than 1/100,000 in the general population. On the other hand, there are relatively frequent recessively inherited diseases (e.g. Duchenne muscular dystrophy, Friedreich ataxia) not covered by any of the screening tests. 52% of screened diseases in an infant neonatal, 26% during childhood and 3% in adult life (e.g. Hemochromatosis). While the onset of the others is variable. 34% can be treated if diagnosed neonatally. On average, companies test for 4% (2.2% to 7%) of all known mutations on a particular gene (HGM). Currently, commercial companies offer screening for around 20% of known recessively inherited diseases. The great diversity of genetic diseases screened suggests a lack of clear inclusion criteria. Although advertised as comprehensive, all tests include population specific diseases which are extremely rare in the general population.

Next Generation Sequencing: New approach for diagnosis of frequent recessively inherited diseases (e.g. Duchenne muscular dystrophy, Friedreich ataxia) not covered by any of the screening tests. 52% of screened diseases in an infant neonatal, 26% during childhood and 3% in adult life (e.g. Hemochromatosis). While the onset of the others is variable. 34% can be treated if diagnosed neonatally. On average, companies test for 4% (2.2% to 7%) of all known mutations on a particular gene (HGM). Currently, commercial companies offer screening for around 20% of known recessively inherited diseases. The great diversity of genetic diseases screened suggests a lack of clear inclusion criteria. Although advertised as comprehensive, all tests include population specific diseases which are extremely rare in the general population. The lack of clear inclusion criteria has been criticized as being a result of a lack of clinical utility. However, there is evidence that screening for select genetic diseases can be beneficial. For example, screening for cystic fibrosis carrier status has been shown to be effective in identifying individuals who are at risk for having affected offspring. This information can be used by couples to make informed decisions about their reproductive choices.

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the population by PCR-RFLP, allele specific PCR or direct sequencing in each individual. Allelic frequency estimated by the NGS analysis using both pooled DNA as most correlated the real frequency calculated by the individual analysis. For sensitivity to detection of allelic frequency, less than 0.5% of allele in the population could be detected.

P14.77-S Increasing the detection rate of genome-wide high resolution SNP array analysis by using the right follow-up test procedures in constitutional genome diagnostics


We routinely perform genome-wide high resolution SNP array analysis as the first-line diagnostic test for patients with intellectual disability and/or congenital anomalies and prenatally in case of structural ultrasound anoma-

lies or intra uterine foetal death and a normal QF-QPCR test result. So far, a total of 9,471 patient and 3,348 parental samples have been tested by SNP array in our diagnostic laboratory. A (potentially) causative copy number variant (CNV) was detected in almost 27% of the patients and analysis of SNP genotypes revealed one or more significant stretches of homozygosity in an additional 8% of patients. Follow-up testing by either gene mutation analysis or patient-parent trio information analysis subsequently led to the respective identification of pathogenic mutations in recessive disease genes or uniparental disomies (UPD), thereby increasing the diagnostic yield with at least 1%. Using the SNP genotype information also improved the detection of mosaic imbalances and enabled us to detect clinically relevant, mo-

saic, copy neutral changes of homozygosity in four patients. A mosaic finding (CNV, aneuploidy or allelic imbalance) was detected in a further 26 patient samples and 10 parental samples, resulting in a dramatically increased re-

course risk for these parents. The percentage of mosaicism often differed between tissues samples of mesodermal, ectodermal or endodermal origin from each of these individuals. Genome-wide high resolution SNP array analy-

sis is a suitable and particularly effective technique in genome diagnostics to reliably detect in a single test CNVs, UPDs and mosaic imbalances as well as for homozygosity pre-screening.

P14.78-M Classifying variations as de novo without available paternal DNA using SNP-array J. Graaakjaer, A. Bogesen; Vejle Hospital, Vejle, Denmark.

Inheritance of a variation is often used as a guide for diagnostic classification in microarray analysis. If a variation is inherited from a normal parent, morbidity is less likely, compared to a variation that has appeared de novo. Unfortunately paternal DNA is frequently not available for this analysis. Therefore many variations are never properly classified and the morbid-

ity of the variation remains unknown. To overcome this problem we have incorporated a workflow that determines whether a variation is located on the maternal or the paternal allele in the patient. The workflow requires SNP-array analysis to be done on maternal and paternal DNA samples. If a variation is located on the maternal allele in the patient and the variation is not found in the maternal DNA sample, then the variation can be classified as de novo. Utilizing this workflow enhances the diagnostic value of vari-

ations found by SNP-array, when paternal DNA is not available.

P14.79-S EGFR and KRAS mutational profiling in fresh non-small cell lung cancer (NSCLC) cells

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Knowledge of tumor mutational status has become a priority for effective NSCLC-tailored treatment. NSCLC diagnosis is more often reached through biopsy; thus, there is a clear need to implement for routine tumor mole-

cular profiling on small cytological samples. This work aims to screen and compare the EGFR and KRAS mutational prevalence in fresh tumor cells and in corresponding routinely processed samples derived from trans-thoracic fine-needle aspiration. The latter currently represents the most appropriate diagnostic procedure in case of peripheral lesions, such as adenocarcino-

mas, which account for almost 40% of all NSCLCs and for the highest EGFR mutational rates.

Two hundred and forty-four patients carrying peripheral lung masses under-

went CT-guided aspiration. The obtained material was split, and a part was addressed to conventional histopathological analysis while the remain-

ning one was stored at −20 °C. In case of confirmation of adenocarcinoma, tum or genomic DNA was extracted from both fresh and fixed material, and EGFR and KRAS sequencing was performed.

We identified 136 adenocarcinomas; from 134, we could recover enough material for the study. A full match was demonstrated in 102 cases. KRAS mutational prevalences through the two approaches tested. We found EGFR mutations in 13 patients (9.7%); 7 were females and 11 never or former smokers. KRAS mutations occurred in 20 (14.9%) patients. EGFR and KRAS mutations were mutually exclusive. Mutualational screening on fresh cancer cells is an achievable, safe and cost-effective procedure which might allow routinely tumor molecular profiling as powerful integration of conventional histopathological analysis.

P14.80-M Identification of structural variation in whole exome sequencing and whole genome data

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Whole exome sequencing (WES) allows the detection of a wide range of vari-

ations. With one single test it is possible to detect small variants (SNVs), and structural variation. Whilst the role of copy number variants (CNVs) in intellectual disability (ID) is well known, the role of CNVs in other diseases is less well studied, although it has been suggested that CNVs can be found in up to 10% of deafness patients. We have performed read depth WES/CNV analysis on 600 patients across 5 heterogeneous disorders including ID, deafness, blindness, metabolic disorders, movement disorders. This analy-

sis revealed several clinically relevant CNVs exerting a dominant effect, as well as CNVs unmasking a recessive mutation that lead to pathogenic comp-

ound heterozygous events. The cohort of 310 ID patients had previously screened negative for CNV microarray analysis as well as WES SNV analysis. Nonetheless clinically relevant CNVs were identified in 2% of patients. Sys-

tematic screening of the remaining patient groups identified CNVs in ~4% of individuals, including in COLA1, EYS and deletions in USH2A. To further examine pathogenic structural variation, we performed whole genome se-

quencing on 50 patient-parent trios. In 7 patients we identified pathogenic large variants, including two single exon deletions, a tandem duplication, an inter-chromosomal duplication and one complex inversion/duplication/dele-

tion event. Discontant reads provided positional information for duplica-

tion/deletion events identifying a in-frame gene fusion. These results show that structural variation is not only an important cause of neurodevelopmental diseases but also a much broader range of genetic diseases.

P14.81-S Targeted exome sequencing as a molecular diagnostic tool for skeletal dysplasias

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Skeletal dysplasias comprise a large group of more than 450 clinically di-


distinct and genetically heterogeneous diseases associated with mutations in more than 300 genes. Clinical and radiological findings are used to diagnose these diseases. However, due to the genetic heterogeneity of these disorders, the diagnostic process is complex. With the advances in molecular genetics and molecular characterization of the molecular basis of these disorders, molecular tests have become useful in the diagnosis of these dysplasias. Since many dif-

ferent genes have been associated with these disorders, standard diagnostic approaches using Sanger sequencing can be expensive and time consuming. To overcome these limitations, we used targeted exome sequencing and de-

signed a 1.4Mbp panel for simultaneous testing of more than 4800 exons in 309 genes involved in skeletal dysplasias, and 28 genomic regions which were previously associated with these diseases. DNA from 96 individuals with previous clinical diagnosis of skeletal dysplasia was sequenced in 8 multiplexed runs using an Illumina MiSeq sequencer. Reproducibility was tested by repeating the entire procedure for three patients. NGS of the cap-

tured exons resulted in an average coverage of 140X. Causative mutations were characterized in 13 patients so far including de-novo mutations in 7 cases. Analysis of the rest of the data is ongoing. Confirmation with Sanger sequencing was performed for these variants and all were confirmed. Our NGS panel provides a fast, accurate and cost-effective molecular diagnostic tool and can assist in the diagnosis of genetically heterogeneous diseases like skeletal diseases.
Optimization of an imaging cytometry protocol to determine the absolute mean corpuscular haemoglobin F in F-erythrocytes

Krüppel-like factor 1 (KLF1) plays a central role in the developmental glo- 
bin gene switch; carriers of KLF1 mutations have hereditary persistence of 
foetal haemoglobin (HbF) and show variably elevated (10-40%) HbF. This 
variation may be due to differential expression of other modifier genes. The 
KLF1 intron1c can be further deorated by inserting potential molecular 
targets and observing their expression at the cellular level in comparison 
with HbF expression and distribution in F-cells. The mean corpuscular HbF 
(MchbF) is normally estimated by dividing the amount of HbF; determined 
by HPLC, by the number of F-cells, obtained by flow cytometry. Since HbF may be unequally distributed among F-cells, an imaging cytometry protocol enables quantification of HbF per F-cell by fluo-
rerescent measurement of F-cells; problems previously encountered were 
found lacking in efficacy, mostly due to inefficient fixation and high degree of 
diffusion artifacts. A new intracellular anti-HbF antibody-labelling technique 
was thus optimized for use with the Nikon Eclipse Ti inverted fluorescence 
microscope. This technique resulted in images of superior quality from 
which the MchbF could be determined for each F-cell observed. This proto-
col will be used to study the association of KLF1 and other genes, obtained 
through NGS data analyses, with MchbF in normal and HphF individuals, 
to identify new therapeutic targets for β-haemoglobinopathies.

Development of a specific and sensitive High Resolution Melting 
Analysis for detection of beta globin gene mutations

A large amount of data is available on the functional impact of missense mu-
tations in TP53 and on mutation patterns in many different cancers. TP53 
direct sequencing is a time-consuming method with limitations in detection 
level. Here we describe the development of a next generation 
sequencing (NGS) workflow for beta intestinal DNA. Using the 
Illumina, TruSeq Custom Amplicon - TSCA) on 48 patients, and the other 
using capture (Roche NimbleGen, SeqCap EZ). We verified the panel on 30 breast cancer 
samples previously characterized with Sanger sequencing. Sequencing 
reactions were carried out with the Ion PGM™ Sequencer on Ion 318™ chip. The average % of mapped reads on target was 96.8% and average depth of 
coverage 20,386X. Loading 10 samples on an Ion 318™ chip 95% of the 
sequenced amplicons showed coverage higher than 500X. Data analysis 
was performed on Ion Reporter™ software. We detected all the expected somatic mutations including single 
base substitutions, insertions and deletion and only a complex duplication of 18bp was not detected. The overall analytical sensitivity was 95.23%. The 
results were obtained using 32 samples on an Ion 318™ chip. These 
preliminary data demonstrate that the TP53 Ion AmpliSeq™ panel workflow 
and Ion Reporter™ Software analysis solution meets the requirements of cli-
cal research laboratories.

Development and verification of an Ion AmpliSeq™ TP53 Panel

Karyotype and FISH have been standard diagnostic tools in monitoring re-

dressions in cancer genomes: Multiple Myeloma First 

Around birth, a shift from γ- to β-globin gene expression causes a switch 
from foetal haemoglobin (HbF) to adult haemoglobin (HbA). Residual 
amounts of HbF are synthesized throughout life F-erythrocytes. Increased 
HbF levels ameliorate symptoms of β-haemoglobinopathies.
arrangements in this line compared to whole genome sequencing, RNA-seq and SNP-CN datasets. We will also demonstrate the use of the system with unknown CD138+ enriched bone marrow samples from consenting patients currently on clinical trials compared to FISH, G-band karyotype and SNP-CN arrays. We are confident that these data demonstrate that the Irys system is a disruptive innovation with broad applicability to genome research and refinement of normal variation and disease.

P14.87-S
Mutation analysis of triple negative breast cancer patients using next generation sequencing

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Despite the intense research in the field of breast cancer, this disease still remains the second most common cancer in women worldwide, being the first cause of death in women in Romania. Among the different subtypes of breast cancer, triple negative breast cancer (TNBC) needs a special attention due to its poor response to therapy and high malignancy.

The present study was aimed at the identification of mutations in 46 genes involved in cancer in 31 patients with TNBC at the Institute of Oncology “Prof. Dr. I. Chiricuta”, Cluj-Napoca between 2006-2007, using Next Generation Sequencing. We used FFPE tissue samples which were sequenced using the Ion Torrent Personal Genome Machine and the Ion Reporter 1.6 software for data analysis. After data analysis we obtained 103 mutations in 34 genes of the 46 studied. The clinical assessment of the identified mutations showed that three mutations were benign, one was likely benign, 42 were likely pathogenic, 28 were pathogenic and 29 had no assessment. This study also identified KDR, TP53, PIK3CA, FGFR3 and FGFR2 genes as being the most frequently mutated genes. Our results show that TNBC has specific mutations leading to resistance to therapy and poor outcome of the-se patients.

P14.88-M
Four Decade Old Mummified Umbilicus Making Retrospective Molecular Diagnosis of Ornithine Carbamoyltransferase Deficiency

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Inborn errors of metabolism, sampling and preservation of viable speci mens is critical in reaching the correct diagnosis. Utility of preserved umbilicular tissues has been demonstrated in several occasions, but it remains unclear how long the mummified umbilicus can preserve genomic DNA that tolerates sequence-based genetic analysis. We report a Japanese family of successive early deaths presumably due to clinically diagnosed ornithine carbamoyltransferase deficiency. The exact molecular pathology, c.394T>C (p. S132P), was retrosexively delineated using DNA extracted from the mummified umbilicus that had been kept in a keepsake wooden box at home for four decades after the deaths of affected individuals. This observation illustrates the striking stability of DNA preserved in mummified umbilicus, and supports an efficient way of genetic sample preservation. From cultural standpoint, preservation of mummified umbilical cord is a unique but common tradition in Japan for at least several centuries. The exact molecular diagnosis of this X-linked inborn error of metabolism of the urea cycle had an significant impact on the affected family. Hemygous female carriers are at risk of hyperammonemic crisis, and this disorder is treatable with supplemental dietary interventions. Since naturally mummified umbilicus provides a highly effective way of genetic sample preservation, utilization such material should be considered in the retrospective genetic investigation, particularly in patients with south east Asian cultural backgrounds.

P14.91-S
p.Asp59Gly in the RAB40AL gene is a common allelic variant: no evidence for a causal relationship with Martin-Probst syndrome

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The p.Asp59Gly amino acid change resulting from the same two DNA substitutions A to G (rs145606134) and C to A (rs138133927), leading to the discovery of several new mutations were used for a validation step which demonstrated 100% accuracy, while the remaining 14 were analyzed at diagnostic level. The combination of systematic data analysis using an established bioinformatics pipeline followed by Sanger sequencing confirmation and segregation analysis led to the identification of 15 novel variants (1 in USH2A, 4 in PDCH15, 3 in MYO7A, 3 in CDH23, 2 in CDH19, and 1 in GPR98, 2 in USH1G). All these variants were predicted as pathogenic using several in silico predictor tools such as Mutation Taster, Polyphen-2, SIFT and others. Five known pathogenic mutations were also detected. Overall, these 20 alleles explain 12 patients, one completely negative (a new case of genetic heterogeneity or the presence of mutations in regions not yet analyzed of the USH genes?). In term of molecular pathophysiology, USH2A characterizes 12 patients (40%), MYO7A 6 (20%), CDH23 4 (13%), PCDH15 3 (10%), GPR98 2 cases, USH1C and USH1G one each. In conclusion, this methodology clearly provided a reliable strategy for routine gene diagnosis of USH.

P14.893
Direct trans-differentiation of skin fibroblasts for functional testing of unclassified variants

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Introduction Unclassified variants (UVs) are a common finding in DNA diagnostic testing. The introduction of next generation sequencing, allowing the simultaneous analysis of multiple genes, has enhanced the problem of variants with unknown clinical significance. For in vitro functional testing of UVs we need cells that express the specific gene. Aim The aim of this study is to use direct trans-differentiation of skin fibroblasts into smooth muscle cell (SMC) phenotype in order to study the effect of UVs in genes that are involved in susceptibility to thoracic aortic aneurysms. Methods We used culture media with horse serum and TGFβ1 to induce trans-differentiation of patients’ fibroblasts into SMCs. Gene expression was tested with RT-PCR. Splice errors were studied on cDNA. Contracting of the cells was studied on collagen matrix. Results SMCs with SMC phenotype, derived from fibroblasts from patients with UVs in MYH11 were compared to controls. The differentiated cells expressed SMC markers, including α-SMA, confirming successful differentiation. An intrinsic mutation in MYH11, c.3879+2dup, in a patient with TAA and type B dissection with a positive family history for aortic dissection, does not affect the canonical splice donor site, but was shown in transdifferentiated cells from the patient to result in the use of a cryptic splice site in exon 29, resulting in an in frame deletion of 78 bases. The contraction of the patient derived cells was disturbed compared to control cells. Conclusion Trans-differentiation of skin fibroblasts towards SMC phenotype is a powerful tool for the functional analysis of UVs.

P14.90-M
Targeted re-sequencing and USH syndrome: a robust and accurate protocol overcoming the problem of genetic heterogeneity and genetic modifiers

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Usher Syndrome (USH) is characterized by phenotypic and allelic heterogeneity requiring a powerful and reliable molecular diagnosis. Thus, a targeted re-sequencing (TS) panel of the 10 USH genes has been developed. It covers 96% of the targeted regions being characterized by 872 ampicins using Ion Torrent™ technology (Life Technlogies). This protocol has been applied to the analysis of 30 USH cases divided as follows: 16 with already known mutations were used for a validation step which demonstrated 100% accuracy, while the remaining 14 were analyzed at diagnostic level. The combination of systematic data analysis using an established bioinformatics pipeline followed by Sanger sequencing confirmation and segregation analysis led to the identification of 15 novel variants (1 in USH2A, 4 in PDCH15, 3 in MYO7A, 3 in CDH23, 4 (13%), PCDH15 3 (10%), GPR98 2 cases, USH1C and USH1G one each. In conclusion, this methodology clearly provided a reliable strategy for routine gene diagnosis of USH.
who was diagnosed for a genetic cause of isolated optic atrophy using whole exome sequencing (HiSeq 1500 and TruSeq Exome Enrichment Kit, Illumina). Interestingly, the patient did not present any symptoms of MPS. To further try to validate the pathogenicity of the RAB40AL variants, we screened a set of DNA samples representative of the background population of Central Poland (n=788, female:male ratio 1:1) using allele-specific PCR and confirmed suspicious variants by Sanger sequencing (Applied Biosystems).

The RAB40AL variants were found in 18 out of 788 subjects. It corresponds to an allele frequency of almost 2.3%. Results of our study have demonstrated that the p.Asp59Gly amino acid change in RAB40AL gene is present at a high prevalence in the general population that is typical for common polymorphisms. Our data questions the role of p.Asp59Gly as a disease-causing change for MPS.

P14.92-M

Comparative transcriptome and genome analysis down to the sequence level for individual cells


Cell heterogeneity plays a central role in biological phenomena during normal development or disease (e.g., cancer development). As gene regulation is a fundamental process, the analysis of transcript profiles and genomes of single cells can dissect phenotypic variability that is of key interest to scientists. Deep genome and transcriptome analysis of small biological samples using next-generation sequencing (NGS) is mostly limited by the small amount of sample available (fs gDNA and 0.5 pg mRNA/human cell). We developed two new methods for whole genome amplification (WGA) and whole transcriptome amplification (WTA), based on Multiple Displacement Amplification (MDA) technology, from small samples down to a single cell.

Parallel WGA and WTA reactions begin with lysis of 25–1000 cells from the same sample. Both reactions include a ligation and multiple displacement amplification (MDA) reaction. This results in amplification products (WGA-DNA, WTA-cDNA) of the highest comparability and can be used for comparative studies of the genome and transcriptome from the same sample.

The second method (WTA) amplifies RNA from 1–1000 cells directly and includes an efficient lysis, cDNA synthesis, and amplification strategy that results in minimal bias and errors. gDNA is effectively removed to prevent false-positive results.

We amplified a variety of human cells and checked the resulting genome and transcriptome using NGS or qPCR methods. Discussed are experiments on cell-to-cell variation, GC content in comparison to genomic DNA, percentage and transcriptome using NGS or qPCR methods. Discussed are experiments on cell-to-cell variation, GC content in comparison to genomic DNA, percentage of transcriptome coverage with respective error rates, and genome-wide real-time PCR analysis.

P14.93-S

3Gb-Test: Introduction of Whole Genome Sequencing as diagnostic application

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3Gb-TEST a two year CSA-EU- FP7 project, gathers information on the gaps and needs for implementing Whole Genome Sequencing (WGS) into the diagnostic service. The aim is to prepare a detailed implementation plan or roadmap for the transition to the future of genetic testing WGS included where appropriate and applicable.

As the cost of sequencing is decreasing and the knowledge of our genome is increasing we expect that this transition to the future will take off within the next 5 years. 3Gb-TEST will define needs and gaps using questionnaires, expert meetings and literature to shed light on and obtain insights in the following topics: - Wet lab: innovation - The bioinformatics - Clinical interpretation - Personalized medicine and pharmacogenomics - Quality issues and External Quality Assessment - The clinical utility and cost effectiveness

The 3Gb-TEST project brings stakeholders together and disseminates information with respect to the desirable and desirable developments. The Consortium will inform the healthcare community and make recommendations to the European Commission, the European Society of Human Genetics, and national organizations relevant to this field. The roadmap will address all these mentioned topics and prepare an implementation plan for the transition to the future of genetic testing in which WGS is embedded. Substantial investments may be required and the logistical restructuring of genetic services will be addressed. Monitoring and harmonization at the European level of all developments is therefore of the utmost importance. 3Gb-TEST is actively getting in contact with the genetics community and will present the initial results and opinions at the ESHG conference.

P14.95-S

Design and evaluation of two novel methods for detection of AFTPB gene mutations

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Wilson’s disease is a rare autosomal recessive disorder of copper metabolism (OMIM# 27790) leading to copper accumulation in the liver, brain, kidney and bone. It is caused by mutations in the ATP7B gene with a carrier frequency of approximately 1.2%. We report the design and evaluation of two different screening methods for the detection of ATP7B gene mutations which could be used in conjunction in order to minimize the cost and time for genotyping.

1) A specific mutation detection assay by dry-reagent dipstick biosensors applicable to the 10 most common mutations in the Greek population (H1069Q, R969Q, Q289X, 2536delA, L936X, 2299insC, 11148T, 845delT, 1708G>A, X1466R) covering 80% of disease chromosomes. Fragments flank the 10 ATP7B mutations are amplified by multiplex-PCR, followed by multiplex primer extension (PEXT) reaction using allele-specific primers. The primer extension products are simultaneously detected by visual multi-allele dipstick type DNA biosensor using anti-biotin conjugated gold nanoparticles. Optimization studies on the efficiency and specificity of the PEXT reaction were performed.

2) A gene scanning protocol using High Resolution Melting (HRM) analysis, designed to analyze all coding ATP7B regions. The first method was evaluated by analyzing 50 samples of known genotypes (confirmed by sequencing) along with 50 blind samples. The results were fully concordant with reference methods. HRM was evaluated by testing 50 known genotypes and 100 blind samples.

The proposed methods are simple, rapid, do not require purification of the PCR products and could be particularly useful in diagnostic laboratories. The benefits and drawbacks of each will be discussed.

P14.96-M


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The molecular diagnosis of Y-chromosomal microdeletions is a common routine genetic test that is part of the diagnostic workup of azoospermic and severe oligozoospermic men. Since 1999, the European Academy of Andrology (EAA) and the European Molecular Genetics Quality Network (EMQN) have been actively involved in supporting the improvement of the quality of the diagnostic assays by publication of the laboratory guidelines for molecular diagnosis of Y-chromosomal microdeletions and by offering external quality assessment tests. We aim to present an overview on clinical novelties related to the Y chromosome and provide an update on the results of the quality control programme. The original basic diagnostic testing protocol based on two multiplex PCRs remains fully valid and appropriate for accurate diagnosis of complete AZF deletions requiring only minor modification in populations with a specific Y chromosome background. However, in light of novel data on genotype-phenotype correlations, the extension analysis for the AZFa and AZFb deletions is now routinely recommended. Novel methods and kits with excessively high number of markers do not improve the sensitivity of the test, may even complicate the interpretation of the results and are not recommended. The routine screening for gr/gr deletion, a significant risk factor for impaired sperm production, is under debate and eventually it can be performed in selected populations. Annual participation in an external quality control programme is strongly encouraged. The 12-year experience with the EMQN/EAA scheme has shown a steep decline in diagnostic accuracy (genotyping) error rate and a simultaneous improvement on reporting practice.
P14.97-S
Introduction of a targeted next generation sequencing gene panel for familial cancer in DNA diagnostics and genetic counselling

AIM: Our aim was to develop a targeted next generation sequencing (NGS) gene panel that enables to analyze large numbers of genes in patients with familial cancer at relatively low cost.

Methods: A panel of 70 known tumour syndrome genes based on Agilent Sure Select Target Enrichment® for simultaneous mutation detection was developed. The selected genes were present in series of the HRM/ MMR or the BRCA genes previously identified through Sanger sequencing. In an additional set of twelve patients all genes were analyzed anonymously. The samples were sequenced using 151 base pair paired-end reads on an Illumina MiSeq® sequencer and analyzed using Softgenetics’ NextGene® and Cartagenia’s Benchlab NGS® software. Within the NGS panel, three virtual non-overlapping gene subpanels were designed, based on the levels of preventive options and strength of risk information for the panel genes. In genetic counselling the genes to be tested were discussed as the three subpanels rather than individually. For diagnostic purposes NGS with the targeted panel was performed on 75 patients, in whom a pathogenic mutation in the MMR and BRCA genes has been excluded. Only results from chosen subpanels were reported to patients.

Results and Conclusion: In the validation all pathogenic mutations, unclassified variants and neutral polymorphisms detected previously were identified by the NGS panel. In addition, 40 novel variants could all be confirmed by Sanger sequencing. The results of the first 75 patients will be presented. Genetic counselling was adopted to discuss genes as groups rather than as individual genes.

P15.01-S
Swedegen: Genome-wide association studies of adverse drug reactions

Objective: Swedegen (www.swedegen.se) is searching for genetic and clinical factors that predispose patients to adverse drug reactions (ADRs).

The aim is to minimise the risks of ADRs by finding biomarkers that enable selection of the right drug for a patient. Method: We are assembling a biobank of patients with selected ADRs that are identified from the nationwide ADR reporting system or referred directly from collaborating physicians. Consenting patients are interviewed concerning medical and drug history, and supplementary information is collected from medical records. A blood sample for genomic testing is drawn and kept until a sufficient number of patients is reached. The samples are sequenced using 151 base pair paired-end reads on an Illumina MiSeq® sequencer and analyzed using Softgenetics’ NextGene® and Cartagenia’s Benchlab NGS® software. Within the NGS panel, three virtual non-overlapping gene subpanels were designed, based on the levels of preventive options and strength of risk information for the panel genes. In genetic counselling the genes to be tested were discussed as the three subpanels rather than individually. For diagnostic purposes NGS with the targeted panel was performed on 75 patients, in whom a pathogenic mutation in the MMR and BRCA genes has been excluded. Only results from chosen subpanels were reported to patients.

Conclusion: In the validation all pathogenic mutations, unclassified variants and neutral polymorphisms detected previously were identified by the NGS panel. In addition, 40 novel variants could all be confirmed by Sanger sequencing. The results of the first 75 patients will be presented. Genetic counselling was adopted to discuss genes as groups rather than as individual genes.

P15.02-M
Pharmacogenomics and definition of genetic profiles as predictors of treatment success

OBJECTIVE: In hypertensive population only 1/3 of patients reaches blood pressure (BP) targets with the main classes of antihypertensive drugs, with a heterogeneous BP response.

METHODS: We enrolled newly discovered and never treated (naïve) HT patients with BP office >140/90 and <160/110 mmHg. SNPs were genotyped by i28 SNP array on TagMan OpenArray system, in genes for renal transport of sodium, the dopaminergic and RAAS system, vasodilation, growth factors. Eligible patients were treated with Perindopril 4 mg or Hydrochlorothiazide (HCTZ) 12.5 mg. Genetic associations with General Linear Model and chi-squared; logistic regression analysis for responder/non responder comparison after one-month therapy.

RESULTS: We derived Genetic profiles for systolic BP (SBP) response to HCTZ or Perindopril as a specific combination of variants selected by pathway-based algorithms associated to greater response to these drugs. Carriers of HCTZ genetic profile (215 HT) displayed a SBP fall of -18.21 mmHg compared to those not having the profile [-5.56 mmHg], with an effect size of -1.26 mmHg (p=0.0001), sensitivity 41%, specificity 96%, PPV 86%, Negative Predictive Value (NPV) 75%. Perindopril genetic profile (149 HT) was associated to a SBP fall of -1.63 mmHg compared to all the others (-4.7 mmHg) with an effect size of -1.16 mmHg (p=0.0001, 94% specificity, 33% sensitivity, 72% PPV, 74% NPV).

CONCLUSIONS: We developed two pathway-based algorithms for creating genetic profiles in response to HCTZ and Perindopril. We are currently employing these genetic profiles to a priori choose the first drug in naïve HT patients, as innovative methodology for treating essential HT.

P15.03-S
The APOB insertion/deletion polymorphism (rs17240441) influences the postprandial triacylglycerol and insulin response in healthy Caucasian adults - insights from the DISRUPT cohort
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The concept of personalized medicine is now being extended to the field of nutrigenetics with the ambition of giving personalised/f individual dietary advice with greater efficacy in health promotion and disease prevention. To this end, we investigated the impact of 18 polymorphisms (previously implicated in lipid metabolism) on postprandial lipid, glucose and insulin responses in up to 262 healthy adults. The participants consumed a standard sequential mixed test meal, which included a test breakfast (0 min; 49g fat) and lunch (330 min; 29g fat). Blood was collected at baseline (0 min) and on 11 subsequent occasions until 480 min after the test breakfast. Plasma a total (TC) low density lipoprotein (LDL-C) and high density (HDL-C) cholesterol, triacylglycerol, insulin and glucose was determined. There was a significant impact of APOB insertion/deletion polymorphism (rs17240441) on fasting TC (P=0.003), LDL-C (P=0.003), HDL-C (P=0.004), triacylglycerol (P=0.003) and insulin (P=0.003) with higher concentrations in the deletion allele carriers. A significantly higher area under the time response curve was evident for the triacylglycerol (P=1x10^-6) and insulin (P=0.006) response in the deletion allele carriers (n=93) relative to insertion/insertion homozygotes (n=52). Our findings indicate that the APOB polymorphism is likely to be an important genetic determinant of the large inter-individual variability in the postprandial response to dietary fat intake. Greater understanding of how APOB gene influences postprandial lipaemia will advance the prospects for personalised nutrition, where the deletion allele carriers may benefit from personalized dietary strategies to reduce the marked lipaemia in response to meal ingestion.

P15.04-M
rs37793 in GLCCI1 is associated with asthma treatment response
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Asthma treatment response is highly variable and pharmacogenomic markers that predict treatment response would be one step closer to personalized treatment. Polymorphisms in GLCCI1 could be associated with asthma treatment response. We genotyped for rs37793 in GLCCI in 208 adult asthma patients treated with inhaled corticosteroids (ICS). Change in %FEV1 was analysed after 3 months and after at least 3 years of treatment. Treatment success was defined as goal when FEV1 decreased less than 30 ml/year. After 3 months of treatment, change of %FEV1 was higher in patients with GG genotype than in patients with AG+AA genotype (p = 0.049), and this genotype dependent difference was only evident and even higher in non-smokers (p = 0.037). Further results were found after at least 3 years of treatment when all patients were analysed (p = 0.041) and in non-smokers (p = 0.034). Even though, no differences in treatment success (good vs. poor response) were observed when analysing the entire group of patients, treatment success was highly influenced by genotype and smoking status.
P15.05-S Influence of single nucleotide polymorphisms on deferasirox trough levels and effectiveness
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Deferasirox (DFX) is the only once-daily oral chelator for first-line therapy of blood transfusion-related chronic iron overload. DFX pharmacokinetics has been related with response to therapy. This drug is metabolized in liver by UDP-glucuronosyltransferase (UGT) 1A1 and 1A2, by cytochrome P450 (CYP) 1A1, 1A2 and 2D6 enzymes, and it is eliminated via biliary-enteric circulation through multidrug resistance protein 2 (MRP2).

Our aim was to evaluate DFX plasma concentrations according to single nucleotide polymorphisms (SNPs) in genes involved in this drug metabolism and elimination, in a cohort of non-paediatric β-thalassemic patients. Further aim was to define a plasma concentration cut-off value predicting an adequate response to therapy.

DFX concentrations were determined from plasma samples obtained at the end of dosing interval (TINF) using an HPLC-UV method. Allelic discrimination for SNPs in UGT1A1, UGT1A3, CYP1A1, CYP1A2, CYP2D6, MRP2 and BCRP1 genes was performed by real-time PCR.

DFX TINF levels were significantly influenced by UGT1A1 T>C (rs8017299, p=0.045), CYP1A1 A>C (rs2663645, p=0.007), CYP1A2 A>C (rs762551, p=0.014), CYP2A2 C>T (rs2470890, p=0.004) and MRP2 G>A (rs2273697, p=0.032) SNPs. According to Chirnomas and Galanello efficacy definitions, a DFX plasma cut-off value of 20,000 ng/mL was identified (ROC curve, [p=0.032]).

Our findings suggest that clopidogrel resistance might be triggered or decreased by the haplotypes of CYP2C19*2 and CYP2C19*17 variants.

Clotopilin is an effective inhibitor of platelet aggregation due to its selective and irreversible blockade of the P2Y12 receptor on platelet cell membranes. Antiplatelet treatment with clotopilin and aspirin is a recommended procedure for the reduction of stent thrombosis and a vital strategy for patients undergoing percutaneous coronary interventions. It has been demonstrated that clotopilin decreases Q-wave myocardial infarction rate in appropriate patients. The impact of CYP2C19, CYP3A4, CYP2B6, ABCB1, ITGB3 and PON1 gene variants on the antiplatelet effect of clotopilin in Turkish patients. We evaluated on 223 Turkish patients with acute coronary syndrome underwent percutaneous coronary intervention with stent implantation. Platelet reactivity (PRU) and % inhibition were measured with VerifyNow P2Y12 assay in blood samples collected from patients that took a standard dose of clotopilin (75 mg/day) for at least 7 days. 12 genetic variants were genotyped using the Sequenom MassARRAY system. The PRU and % inhibition values of the genotypes were compared statistically. The CYP2C19*2 (G636A) single nucleotide polymorphism was associated with a reduced antiplatelet response (p≤0.001). Conversely, the CYP2C19*17 (B667G) single nucleotide polymorphism was associated with an enhanced antiplatelet effect (p≤0.025). There was not statistically significant difference between the PRU and % inhibition values of the other genetic variant genotypes.

Our findings suggest that clotopilin resistance might be triggered or decreased by the haplotypes of CYP2C19*2 and CYP2C19*17 variants.
P15.09-S
CYP1A2 gene non-coding region polymorphisms in Roma and Hungarian population samples
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CYP1A2 enzyme contributes to biotransformation of wide range of therapeutically important drugs, including caffeine, clopidogrel, clozapine, warfarin, procarcinogens and endogenous substrates. The purpose of this study was to determine and describe the pharmacogenetic profile and interethnic differences in CYP1A2 gene between Roma and Hungarian population. From genomic DNA, 404 Roma and 396 Hungarian healthy subjects were genotyped for two non-coding variants of CYP1A2, namely -163C>A (*1F) and -3860G>A (*1C). Polymerase chain reaction - restriction fragment length polymorphism (PCR-RFLP) technique was applied. The minor allele frequency for CYP1A2*1F variant was significantly different between Hungarian and Roma samples (2.02% vs. 0%, p<0.001). The AA homozygous genotype was not detectable. For CYP1A2*1F polymorphism we found a remarkable differences in presence of AA genotype in Roma population compared to Hungarians (31.9% vs. 49.5%, p<0.001) and in minor allele frequency (56.9% vs. 68.6%, p<0.025). The following CYP1A2 genotypes were identified in Roma and Hungarian samples, respectively: *1A/*1A (18.1% vs. 12.4%), *1A/*1F (50% vs. 36.9%), *1F/*1F (31.9% vs. 46.7%). In Hungarian population we found the *1C/*1F genotype (4.04%), but it was not present in Roma subjects. In conclusion, analysis of distribution of CYP1A2 gene variants revealed further pharmacogenetic differences between Roma and Hungarian population samples. Hungarians have higher chance for rapid metabolism of CYP1A2 substrates, intensified procarcinogen activation and thereby elevated risk for cancers.
This research was supported by TÁMOP-4.2.3-12/1-KONV-2012-0028.

P15.10-M
CYP2C9*8 frequency distribution in Puerto Ricans: Implications for warfarin dosing
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Warfarin is an oral anticoagulant that requires individual monitoring since serious adverse events are common. CYP2C9 encodes for the enzyme mainly responsible of 5-warfarin’s metabolism. Polymorphisms in CYP2C9 have been previously found to be associated with observed warfarin dose variability in different populations, but not in Caribbean Hispanics. Caribbean Hispanics originated as a result of a complex admixture among Caucasians, Africans and Amerindians -a characteristic that should be considered for warfarin management. The rare loss-of-function CYP2C9*8 allelic variant is reportedly more prevalent among individuals with African heritage. Since Puerto Ricans has a significant contribution of African ancestry in their genetic backgrounds, this cross-sectional study was aimed to determine the frequency of CYP2C9*8 in a cohort of 150 Puerto Rican patients undergoing warfarin therapy. DNA specimens were extracted and genotyped for the CYP2C9*8 using a PCR-based Taqman genotyping assay. We found 3 heterozygous for the CYP2C9*8 variant in our study cohort, corresponding to a minor allele frequency of 1% (95%CI: 0.0026-0.031). The observed frequency met Hardy-Weinberg equilibrium. Allele frequency in our cohort was found to be significantly lower than that from a previous report in African-Americans (0.01 versus 0.047, respectively, p=0.045 by two-tailed z-test), with a carrier frequency of 1 in 50 (Puerto Ricans) versus 1 in 11 (African-Americans). Due to the CYP2C9*8 prevalence found among Puerto Ricans, we concluded that this variant should be included in the pharmacogenetic-guided algorithm for warfarin dose predictions in this population.
Approved by University of Puerto Rico, Medical Sciences Campus Institutional Review Board protocol A4070109.

P15.11.5-S
ExtraPyramidal Disorders while Schizophrenia Therapy - analysis of CYP2D6*4 allele influence
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Introduction
A lot of clinical investigations of the schizophrenia therapy reveal the dependence of medication concentration on the individual metabolism peculiarities. The latter depend to a greater degree on the CYP2D6*4 allelic state of the person. The discrepancy between the metabolic status of the patient and definite antipsychotic drug choice and dosage results in various side effects, among which the extrapyramidal disorders (EPD) are the most invalidating.
Methods
The CYP2D6*4 genotype (rs3892907) was revealed for 21 patients suffering from schizophrenia that were cured with various antipsychotic drugs: Fluphenazine, Trifluoperazine, Haloperidol, Fluoxetine, Zuclopenthixol, Sulpride, Risperidone. PCR with specific primers was used for allele estimation. All the patients from Republican Research and Practice Center for Mental Health where cured with definite (one) medicine for 2 weeks and then examined once for the presence of (EPD) with the help of Extrapyramidal Symptoms Rating Scale (ESRS). The analysis of the results was carried out with the WinPepi package of statistical programs for epidemiology.
Results
In the process of pharmacotherapy the patients fell into two groups: those with (99) or without (112) (EPD). The influence of CYP2D6*4 allele as a risk factor for the EPD development was proved for Fluoxetine (P =0.001), Fluoxetine (0.0001). Conclusion We proved the importance of CYP2D6*4 genotyping for the cohort of the patients we studied that were cured with special antipsychotic medication. The elaboration of informative clinical approaches to the adequate drug choice as well as their dosage must take into account the patient’s CYP2D6 genotype.

P15.12-M
Towards personalized medicine in Type 2 Diabetes (T2DM): a genetic risk score for the response to the first line treatment in T2DM, metformin
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T2DM is a common disease characterized by high blood glucose levels. The goal of T2DM treatment is lowering the blood glucose level and the prevention of complications. For this a stepwise treatment approach is used in which metformin is the first treatment step. Unfortunately the glycaemic response to metformin is highly variable between individuals with a large proportion of patients unable to reach the treatment target, defined as glycated hemoglobin (HbA1c)<53mmol. Genetic factors appeared to be involved in the variability in metformin treatment response (h2=0.36). At the moment only one GWAS (n=1024) for metformin treatment response has been published (Zhou et al) and this revealed a genome wide significant locus near the ATM gene, however, eleven other loci (MAF=0.05) reached borderline significance (p<10-5). In this study we measured these SNPs in Dutch T2DM patients treated with metformin monotherapy (n=600) and we generated a genetic risk score of the five SNPs showing a directionally consistent association in our study (ORs; Ranges for OR achieving treatment success ranged between 0.69 and 0.84). In a logistic regression analysis with baseline HbA1c, eGFR and metformin dose as covariates having more risk alleles lowered the OR for achieving treatment success (ORs for 2/3/4, 5/6, 7/8 or 9/10 risk alleles were 1.0, 0.54, 0.45 and 0.30 resp. p=0.027).
In conclusion, using a cohort of patients with T2DM, we showed that a genetic risk score was associated with metformin treatment response. Our results are a first step towards the introduction of pharmacogenetics in T2DM treatment.

P15.13-S
Variant Server in a box
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Many groups have implemented annotation and analysis pipelines to assess the significance of large numbers of variants observed in next-generation sequencing clinical and research assays. Despite all these efforts only 50-75% of patients receive a negative or uncertain diagnostic report because a small set of time-tested data sources and predictive tools were used and the etiology of many diseases is not known. However, there are hundreds of tools and databases emerging as well as meta-data bases and -services that have information for millions of variants. Moreover, there are many more resources such as drug targets, cellular pathways, tissue specific expression, quantitative trait loci, lab tests and model organism data. Cumulative cost of identification, download, try-out, and quality validation of all these tools and database is huge and even if new tools can be integrated, it is hard to navigate all new information without smart filtering and/or visualization tools, user interfaces for which are not cheap and difficult to create. To enable rapid evaluation of new annotation resources, their
combination in pipelines, and user interfacing in challenging clinical NGS diagnostics, we have created MOLGENIS Variant Service, an open-source web application that can be installed as virtual machine, on local servers, as shared resource, and in a cloud. We envision an NGS data exploration app as well as a sharing platform for unified data formats and pipelines, gold standard data sets, well-curated reference knowledge-bases, and optimal user interfaces, results of which can disseminate into research institutes, clinical software companies, and individual labs.

P15.14-M
Personal genomics in Greece: An overview of available direct-to-consumer genomic services and the relevant legal framework
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1University of Geneva, Geneva, Switzerland, 2University of Patras, Patra, Greece, 3University of Zurich, Zurich, Switzerland.

The aim of this study is to provide an overview of the DTC genomic services available in Greece and the legal framework within which they operate. Based on literature review, a questionnaire was distributed to a genetics conference in Greece and in-depth interviews with human geneticists in Greece, we assess the landscape of the DTC genomic testing market and highlight possible particularities of Greek consumers. Furthermore, we identify the existing legal framework regarding DTC genetic testing. Our interest is not limited only to issues such as consumer protection laws, lab quality accreditation and the provision of genetic counseling. We also explore the role of medical specialties and their respective legal responsibilities in the Greek context, since for example the speciality of Clinical Geneticist does not exist. We also explore the legal authority of the National Organization of Medicines (E.O.F) regarding the approval of genetic tests and specific issues relating to paternity tests. We identify gaps in the current regulatory scheme and conclude with recommendations for a more comprehensive legal framework.

P15.15-S
2020 vision and beyond - educating tomorrow’s clinicians for the genomic era
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Genomic technology is opening-out the clinical applications of DNA from the preserve of clinical geneticists in single-gene disorders, to integration of genomic information in all clinical care by clinicians in all fields. In the UK, research underpinning this, is spearheaded by current genome sequencing initiatives including GeLand PGP (both 100k genomes), worldwide 1K (now 2.5K), vertebrate 10K, DDD 12K, and cancer 25K.

Consequently, genomic education requires basic genetic training for all, plus tailored higher level focus in each specialty, rooted in present practical application, and preparing for the future. The education must be a continuum from medical student to trainee to autonomous clinician, and will require curricular and CPD learning outcomes (LOs), learning resources (LRs) and equipped educators for each level and specialty. Clinical geneticists may need wider genomic training for laboratory interface and educational roles, and will need new structured education, tailored higher level focus in each specialty, rooted in present practical application, and preparing for the future. The education must be a continuum from medical student to trainee to autonomous clinician, and will require curricular and CPD learning outcomes (LOs), learning resources (LRs) and equipped educators for each level and specialty. Clinical geneticists may need wider genomic training for laboratory interface and educational roles, and will need new structured education.

P15.16-M
Functional annotation of Estrogen Receptor binding sites in the light of the 1000 Genomes Project
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Understanding the person-to-person variability in diseases by using the unprecedented amount of available genomic data may be approached as never before. We propose to investigate the impact of individual genetic variations in breast cancers. We performed a meta-analysis of all the publicly available ER ChIP-Seq datasets in four ER positive cell lines: MCF7, T-47D (breast cancer derived) and cell lines and EOC1, Ishikawa (endometrium cancer derived) cell lines. The number of peaks in intergenic regions is roughly 50 to 55% while depending on the cell type 30 to 40% of the peaks are localized in introns. Some of the genes may have several intronic binding sites. Intronic binding sites are most likely to regulate their own genes, therefore based on these statistics and the individual assignment of the binding sites to their genomic locations, we have identified a set of genes that have intronic bind- sites in their introns. The number of SNP-s available in the dbSNP database for the identified intronic binding sites of the identified genes is relatively high, that is more than one thousand. Based on our investigations a significant number of SNP-s could influence directly the estrogen dependent regulation of relevant target genes.

P15.17-S
Circulating levels of FSH in men are genetically determined: study on the combined effect of polymorphisms in the FSHR and FSHB genes
C. Vinanzi1, F. Ganz2, M. Pengo2, M. Menegazzo3, C. Foresta3, A. Ferlin3; University of Padova, Padova, Italy.

Recent study have shown that polymorphisms in the FSHR and FSHB genes can modulate circulating levels of FSH. FSHR variants Asn680Ser and c. 29 G>A have been extensively studied in women, while FSHB variant -211 G>T seems to influence male serum FSH levels. There are no studies on the combined effect of these three polymorphism. We have studies 365 subjects: 39 azoospermic, 177 oligozoospermic and 149 normozoospermic. We evaluated seminal parameters; hormone levels; testicular volume; FSHR and FSHB polymorphisms. FSHB polymorphism -211 G>T was found significantly associated to FSH levels, FSHR polymorphism -29 G>A and Asn680- Ser are not associated to different concentration of FSH. Combined analysis of the three polymorphisms again highlights that the major determinant in FSH levels is -211 G>T polymorphism, but shows also that this effect is modulated by -29 G>A polymorphism, as subjects with -211 GG/ 29 GG genotype have higher FSH level. Total sperm count and testicular volume is also modulated by the genotype: Homozygotes -211 TT are invariably azoo-oligozoospermic with a reduced testicular volume and FSH <8 UI/L. Combined effect of Asn680Ser is negligible. This is the first combined study on the influence of FSHR and FSHB polymorphisms on male reproduction and shows that -211 G>T FSHB polymorphism plays an important role, only slightly modulated by FSHR polymorphisms, on FSH levels, sperm count and testicular volume. FSHB -211 G>T influences transcriptional FSH gene activity, thus causing an isolated FSH deficiency with azoospermia and thus represents the best pharmacogenetic marker to FSH treatment.

P15.18-M
Evaluation of microarray gene expression profiling as response to zearalenone exposure on normal intestinal epithelial cell C. Braciu1, V. Pileczi2, O. Vîrtic3, O. Balacescu1, O. Taranu4, I. Berindan-Neagoe2,5; 1Research Center for Functional Genomics, Biomedicine and Translational Medicine, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania, 2Department of Functional Genomics and Experimental Pathology, Institute of Oncology “Prof. Dr. I Chiricuta”, Cluj-Napoca, Romania, 3Faculty of Pharmacy, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania, 4National Institute for Research and Development in Animal Biology and Nutrition (INBA), Galea Breesticiu, Cluj-Napoca, Romania, 5Department of Immunology, Faculty of Medicine, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania.

Zearalenone (ZEA) is an estrogrenic secondary fungal metabolite produced by several Fusarium species. Several studies presented the cytotoxic or effect of this mycozin, but the specific mechanism of action of ZEA still remains unidentified. In order to decipher the molecular changes that occurred during the exposure ZEA in IPEC cells, we assessed the impact of a single dose on gene expression profile at 24 h posttreatment. 10 µM ZEA was proved to have no effect on cell viability (as displays MTT and xCELLigence data), but the microarray expression profiling data for this dose was lead to the identification of 790 genes overexpressed and 1164 downregulated, consis- tently identified across studies with a p-value of <0.05. Gene Ontology (GO) analysis of expression microarray data was done to identify the key processed altered. Some of these gene class associations as represented by GO terms are as would be predicted (cell proliferation and differentiation, apoptosis or cell cycle), while others are unexpected, like the class of the cell adhesion molecules or cellular invasion. These primarily processes altered are usefuly to predict the negative impact of this toxin, by generating an interaction network analysis for the significant statistic genes. The effects of ZEA are much more complex as we observed from the bioinformatics analysis of the genes list associated with critical disease pathways for the case of a single non-cytotoxic dose of ZEA, being the first step for the acquisition of genetic alteration, particularly in the case of mycozin co-contamination or continuous exposure.
P15.19-S

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Increased knowledge in the field of nutrigenetics led to the development of different genetic risk scores and dietary interventions. We have decided to test the efficacy of a gene-based diet (diet from www.genexme) on 191 obese (BMI>25) attending the same nutritional center. Participants were randomly divided in two groups (87 test and 104 control). For both groups a standard nutritional plan was defined subtracting 600 calories from individual need. DNA from the test group was analyzed for 19 genes known to impact on different metabolic areas and taste. Diet was modulated according to individual genetic profile (i.e. people with a non-favorable lipid metabolism profile were given less lipids in their diet) without varying the overall amount of calories. Physical activities plans were per sonalized in the same way in the two groups. No significant differences in age or sex distribution were present in the two groups. Follow-up took place every six months for 2y and showed, in both groups, BMI loss over time and similar compliance. Very interestingly, people in the test group (gene-based diet) lost 33% more weight than controls corresponding to 0.47 BMI points for the square root of time (-1.36 in the control group vs -1.85 in the test group, p=1.8x10^-10). Similarly, the percentage of lean mass increased more in the test group (+6.2%) than controls (+5.3%) (p=2x10^-4).

In conclusion, present findings indicate that a gene-based diet might be more effective in helping people loosing weight as compared to standard nutritional plans.
Leukodystrophy with brain stem and spinal cord involvement and lactate elevation

DARS2
c.455G>T
(Cys152Phe)
0.5%

Smith-Lemli-Opitz syndrome

DHCRT7

c.452G>A
(Trp151Ser)
0.5%

Mitochondrial DNA depletion syndrome 4B

GAP

c.1196delATC
9%

Galactosemia, duarte variant

GALT
c.1430A>G
(Lys476Asp)
1%

Galactosemia

GALT
c.35delG
0.5%

Hemochromatosis, type 1

HFE
c.187G>C
(His63Asp)
32.3%

Epidermolysis bullosa, junctional, Herlitz type

LAMB3
c.1901C>T
(Leu633Ter)
0.5%

Familial Mediterranean fever

MEFV
c.1791T>C
(Gly597Asp)
1.5%

Phenylketonuria

PAH
c.1208C>T
(Ala403Thr)
1.5%

Mitochondrial DNA depletion syndrome 4B (MINGE type)

POLG
c.752C>T
(Thr251Ile)
0.5%

Hyperphenylalaninemia, BH4-deficient, A

PTS
c.216T>A
(Asn72Val)
1%

Alpha-1-antitrypsin deficiency

SERPINA1
c.863A>T
(Glu284Val)
1.5%

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using a cut-off p-value of $10^{-4}$, 42 SNPs emerged as associated according to Results: No genome-wide significant association signal was detected. When ≥2, on which separate GWAS analyses have been performed. A modest overlap (11.6%) was observed among associations across three phases.

Conclusions: Our study showed new potentials potentially involved in the modulation of the response to IFN-beta treatment. An in silico replication analyses on identified targets is planned on an independent cohort of treated MS patients.

P15.28-M Genetic polymorphisms influence the response to adalimumab in Crohn’s disease. U. Potočnik1,2, K. Repnik1, S. Koder1, R. K. Weersma3;

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Biological therapy using fully humanized monoclonal antibodies against TNF-α (adalimumab) is efficiently used to induce and maintain remission in around 70% of Crohn disease (CD) patients not responding to standard treatment. We investigated whether developing adverse drug effects to corticosteroids. We treated single nucleotide polymorphisms (SNPs) contributing to CD risk could help to predict the response to adalimumab (ADA) treatment in CD patients. We used IBDQ index and biological biomarkers (CRP levels) to monitor therapy response after 4, 12, 20 and 30 weeks after first treatment with adalimumab. The strongest association between CRP levels and treatment with ADA was found for ATG16L1 SNP rs10519302. After 12 weeks of treatment, rs10519302 had a positive response (drop of CRP to normal levels or by more than 25%) to treatment with ADA compared to 37.5% of patients with CC genotype (p = 0.0006).

We also found significant associations between SNP rs10512734 located near PTGER4 gene and ADA treatment response measured with both, IBDQ index and biological response measured with CRP. Average increase in IBDQ index (delta IBDQ) after 12 weeks of treatment was higher in the group of patients with GG genotype for SNP rs10512734 (47.6) compared to those with AA or AG genotype (17.4, p = 0.068). Additional SNPs in 6 out of 33 tested CD associated genes (CASP-9, IL27, C11orf30, CCNY, IL13) showed suggestive association with ADA response. Our results suggest ADA response in CD patients is partially genetically predisposed by SNPs in CD risk genes.

P15.29-S AlleleTyper™ Software: a flexible application for mapping SNP genotype and CNV patterns to pharmacogenic allele nomenclature T. Harthorne, N. Mehmet, E. Shelton, H. Leong

Thermo Fisher Scientific, South San Francisco, CA, United States.

Pharmacogenomic (PGx) studies require genetic testing of individuals for multiple variants in drug metabolism enzyme (DME) and transporter genes. For phenotype interpretation purposes, genotyping results must be translated to star (*) allele nomenclature. Star alleles are gene level haplotype patterns associated with protein activity levels. Genetic variants within a haplotype can include SNPs, InDels, and copy number variations (CNVs). Knowing the combination of variants within a given haplotype, and the resultant content in an individual, is of key importance for studying drug metabolism, response and adverse reactions. To facilitate the translation of results for individuals genotyped in studies using TaqMan® SNP and DME Genotyping Assays and TaqMan® Copy Number Assays, we developed a web-based flexible software tool called AlleleTyper™. This software maps sample genotyping data to genetic pattern information in translation tables to star allele nomenclature. User-defined allele nomenclature buckets containing haplotype genetic information, for the gene variants tested in a study, are automatically converted by the software to biallelic translators containing diploid genetic patterns. AlleleTyper™ matches the sample genotypes in results files from TaqMan® Genotype Software and/or CopyCaller® Software to the patterns in the biallelic translator and reports the star allele genotypes determined for each sample. A review of the software workflow and features will be presented, along with data analysis examples. AlleleTyper™ Software greatly facilitates PGx study data analysis. It can also be used for other genotyping applications that require translation of data from multiple TaqMan® assays, including triallelic SNP data analysis and blood genotyping.

P15.30-M ABCB1 haplotype construction in a pharmacogenetic study of cyclosporine treatment response in Greek patients with psoriasis Y. Vasilopoulos1, C. Sarri1, C. Stamati1, E. Zafiriou1, A. Roussakis-Schulze2, A. Patzios3, D. Spartisidou1, Z. Mamari1, T. Sarafidou1;

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Psoriasis is a chronic, inflammatory skin disorder affecting 2-3% of the population worldwide. While there are a large number of effective treatments for treating psoriasis, response to therapy varies among patients which could be due to genetic factors. Cyclosporine is considered to be a cost-effective first-line systemic therapy for psoriasis, however, the response rate is around 60-70%. The aim of the present study, which is based on a Greek multicentre collaboration, was to target ABCB1, which encodes for P-glycoprotein, by selecting polymorphisms that could influence the absorption and disposition of P-glycoprotein substrate drugs like cyclosporine. In detail, T-129C (rs1231619), G1199A (rs2229109), C1236T (rs1128503) G2677T (rs203258) and C4345T (rs1045642) polymorphisms were selected as candidate markers of response to cyclosporine after 3 months of therapy and genotyped in 84 psoriasis patients under cyclosporine therapy. Fifty-two patients (62%) were defined as responders (ΔPASI ≥75%) and thirty-two (38%) as non-responders (ΔPASI ≤50%). Single-SNP and haplotype construction showed that the haplotype for marker C4345T could account for the prediction of ~20% of the non-responders to cyclosporine therapy. Notwithstanding the importance of this finding, there is a remaining 80% to be identified, which could be illuminated by systems network analysis, whereby additional pharmacogenetic targets that may be involved in cyclosporine transport, processing or metabolism are identified. Overall, systems biology coupled with experimental validation of specific markers in large independent cohorts could lead in the creation of a molecular algorithm for the prognosis of psoriasis patients’ response to cyclosporine as well as for other therapies.

P15.31-S Identification of a new late radiotherapy toxicity locus through a three stage genome wide association study L. Fachal1, A. Gómez-Caamaño2, G. Burnett3, P. Peletier1, A. Carballo1, P. Calvo-Crespo1, S. Kerner1, M. Sánchez-García3, R. Lobato-Busto5, D. Dorling3, D. Dearmaley3, M. Ryder1, E. Half1, N. Burn3, A. Garracchi1, R. Basenstein1, C. West1, A. Dunning1, A. Vogel1;

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Increasing evidence supports the role of genetic variants in the development of radio-induced toxicity. Therefore, we performed a three-stage genome wide association study that involved a Spanish cohort of 741 prostate cancer patients treated with radiotherapy to identify new susceptibility loci. The replication cohorts consisted of 633 prostate cancer patients from the UK, and 368 prostate cancer patients from a North-American Caucasian cohort.

Standardized total average toxicity (STAT) scores were derived from individual toxicity endpoints to assess overall acute and late toxicities. Association tests of genotype with STAT-acute or STAT-late scores were performed with logistic regression. Residuals from multivariate linear regression models, including associated non-genetic covariates, were calculated for each patient to quantify the toxicity not accounted for by the available non-genetic covariates. To obtain per allele ORs by logistic regression, patients were stratified into combinations ≤ or > than 1 standard deviation of the acute and late residuals. Seven and 42 loci were associated (p-value≤10-5) with overall acute and late toxicity, respectively. Only one locus associated with overall late toxicity, 2q42.1 (STATLate p-value = 6.85x10-9; OR=6.67, 95%CI: 2.25-19.80) was replicated in the UK cohort (STATLate p-value=2.08x10-4, OR=6.17, 95%CI: 2.25-16.95; meta-analysis p-value=4.16x10-10). The inclusion of the third
cohort gave a meta-analysis P-value=4.64x10^{-11}. Although it is biologically possible that this locus is involved in the reorganization of muscle previously damaged by radiotherapy, future efforts are needed to identify the causal variant underlying the observed association and determine the molecular mechanisms involved.


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Methotrexate (MTX) is an anti-rheumatic drug used in the treatment of rheumatoid arthritis (RA). However, side events are present in 40% of the patients. The aim of this study was to determine the impact of genetic polymorphisms of 5, 10-methylene tetrahydrofolate reductase (MTHFR C677T and A1298C), Dihydrofolate reductase (DHFR 19-base pair deletion allele), thymidylate synthase (TYMS 2R→3R), methionine synthase (MTR A2756G) and methionine synthase reductase (MTRR A66G) in a group of 143 Tunisian RA patients and evaluate its association with MTX toxicity.

Patients who experienced MTX adverse events were defined "with toxicity", those who did not, as "without toxicity". Genotyping was performed using PCR and Taqman®-RFLP method. Demographic and clinical characteristics were obtained and MTX-related adverse effects were recorded. Allele and genotype association were performed using chi² test, genotype relative risk (GRR) and Odds ratio (OR). The regression logistic was also used to investigate the correlation between patient characteristics (MTX dose, duration of treatment, disease duration, age, sex, route of MTX administration) and toxicity.

The analysis highlighted a significant genotypic association of MTHFR C677T polymorphism with increased MTX toxicity [p=0.004], and the strongest association was shown in the T/T genotype [p=0.006]. However, the MTHFR A1298C, DHFR 19-base pair deletion allele and MTRA2756G polymorphisms were not associated with increased MTX toxicity. While TYMS 2R→3R polymorphism had a protective effect on overall MTX toxicity [p=0.038]. Moreover, our results revealed a positive correlation of both dose and route of administration of MTX with toxicity in RA patients [p=0.027; p= 0.004] respectively.

P15.33-S CYP1A1 variant in Roma population samples.

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Polycythemia vera is a rare bone marrow disorder that leads to an abnormal raising in the number of red blood cells, although the numbers of white blood cells and platelets are also on high levels. In the background of the disease a gene mutation called JAK2V617F can be found, but the cause of this and other possible disorder-causing mutations is undiscovered. Our goal was to examine the rs104943 polymorphism located in CYP1A1 gene in 90 Roma (Gipsy) with polycythemia vera versus 95 Roma individuals without this disease. Genotypes were determined with real-time-PCR method, SPSS 20.0 statistical program was applied for evaluating the results. The preliminary data showed 6.11% G allele frequency in Romans with polycythemia vera, which it was found to be 3.70% in non-affected Roma controls. The CYP1A1 1384 A/G mutation has never been analyzed in Roma population before, but further extended studies are required to test the clinical relevance of CYP1A1 in different populations.

P15.34-M Full resequencing of TRAF3IP2 gene in Mozambican patients with SJS/TEN induced by Nevirapine treatment: a pharmacogenetics study.


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Nevirapine (NVP) is a non-nucleoside reverse transcriptase inhibitor, widely prescribed for type 1 human immunodeficiency virus infection. A small proportion of individuals treated with NVP experience very severe cutaneous adverse events, including Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN).

In the last years TRAF3IP2 gene variants were associated with susceptibility to psoriasis, psoriatic arthritis, systemic lupus erythematosus and cutaneous manifestations in inflammatory bowel disease. We hypothesized that this gene, involved in immune response and in NF-KB activation, could be also implicated in the SJS/TEN susceptibility.

To verify our hypothesis we performed a full resequencing of TRAF3IP2 gene in a population of patients treated with NVP. Twenty-seven patients with NVP-induced SJS/TEN and 78 controls, all from Mozambique, were enrolled. All ten exons of TRAF3IP2 gene, including the intron/exon boundaries, were analyzed by direct sequencing. We performed a case/control association analysis and a multivariate logistic analysis. We identified 8 exonic and 3 intronic variants. We did not find any novel variations. The case/control association analysis highlighted an association between rs76228616 SNP in exon 2 and the SJS/TEN susceptibility. In particular the variant allele (C) was more present in SJS/TEN patients than in controls, resulting significantly associated with a higher risk to develop SJS/TEN [P=0.012 and OR=3.65 [95% CI 1.33-10.01]]. A multivariate analysis by logistic regression confirmed the significant involvement of TRAF3IP2 (rs76228616) in the susceptibility to SJS/TEN [P=0.027]. Of course, further studies on larger sample sizes and implications in other African populations are necessary to confirm our results.

P15.35-S Potentially Functional Single Nucleotide Polymorphisms (pSNPs) associated with Response to Fluorouracil in Metastatic Colorectal Cancer Patients.

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Colorectal cancer (CRC) is amongst the top three most commonly diagnosed cancer in the world. Majority of these patients require treatment for metastatic CRC since a third are at stage IV during diagnosis and another third of the ‘curatively’ resected patients (Stages I-III) will relapse. 5-fluorouracil is a common drug used for the treatment of CRC but the response rate to this drug either alone or in combination is less than 40%. Developing a reliable biomarker that can predict response can facilitate the appropriate tailoring of treatment for these patients.

Here we report a novel potentially functional Single Nucleotide Polymorphism (pSNP) approach to identify SNPs predictive of response to 5-FU in Chinese metastatic colorectal cancer (CRC) patients. 1547 pSNPs and one variable number tandem repeat (VNTR) in 139 genes in 5-FU drug (both PK and PD pathway) and colorectal cancer disease pathways were examined in 2 groups of CRC patients. Shrinkage of liver metastasis measured by RECIST criteria was used as the clinical end point. We identified a total of 9 novel pSNPs including 4 non-responder-specific pSNPs with potential functional significance that may be able to distinguish non-responders from responders to 5-FU. These may thus serve as good biomarkers for response to 5-FU.

P15.36-M Semiconductor Next Generation Sequencing (Ion Torrent) of the ABCB1, CYP3A5, and CYP3A4 genes in kidney transplanted patients treated with Tacrolimus.

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Tacrolimus (Tac; FK-506) is an immunosuppressive drug used to avoid the rejection of solid organs. Tac has a narrow therapeutic range and a high inter-individual variability in dose-requirements. Previous studies have linked common CYP3A4-3A5 and ABCB1 (MDR-1) polymorphisms to Tac dose requirements (CYP3A5*3, CYP3A4*1B, CYP3A4*22, C3435T). Our aim was to identify new rare CYP3A5, CYP3A4 and ABCB1 variants that could influence Tac dose through massive parallel sequencing with the Ion Torrent PGM. We created three pools of 75 patients who differed in Tac dosage. The coding exons of the 3 genes were amplified in only two tubes with a custom AmpliSeq and the three pool reactions bar-coded, library amplified, and sequenced in a semiconductor PGM-318 array. These pooling + multiplex approach would facilitate the rapid screening of the three genes at a low cost and with minimum labor requirements. We identified several rare variants in CYP3A5 and CYP3A4 (P405T in CYP3A5, and S195P and I193S CYP3A4, new reported). These missense changes could affect protein function. No missense ABCB1 variants were found.

In conclusion, we identified new variants in CYP3A5 and CYP3A4 that could have an effect on Tac dose requirements. Our NGS-PGM procedure would help to uncover the variation in these genes at a population scale.
P15.38-M
Development of personalised therapeutics for lattice corneal dystrophy type 1
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Personalised medicine offers the prospect of treating genetic conditions using novel molecular methods, and our current research seeks to establish a viable treatment for Lattice Corneal Dystrophy Type 1 (LCDI) using mutation-specific short-interfering RNAs (siRNAs). Corneal dystrophies comprise a group of debilitating hereditary disorders. Most of these conditions are inherited autosomally dominant and caused by deleterious missense mutations. To date our group has focused on two corneal dystrophies; Meesmann’s Epithelial Corneal Dystrophy (MECD) and Lattice Corneal Dystrophy Type 1 (LCDI), which are caused by mutations in the KRT12 and TGFBR1 genes, respectively. The primary focus of this research was to develop a lead siRNA targeting the TGFBI-Arg124Cys mutation, the most common cause of LCDI. A panel of 19 mutation-specific siRNAs was assessed to select lead candidate siRNAs using a high-throughput dual-luciferase reporter assay and pyrosequencing to determine the lead siRNA. Potency and allele specificity of the lead siRNA was assessed in corneal epithelial cells isolated from a patient with a TGFBI-Arg124Cys mutation, using pyrosequencing, qRT-PCR and a TGFBI ELISA. Treatment with this siRNA resulted in a 44% reduction (p<0.01) of the endogenous Arg124Cys allele in this ex vivo model of LCDI, and was without effect on the wild-type allele, demonstrating allele specificity. This research confirms the potential of siRNA therapeutics as a personalised approach for the treatment of heritable TGFBR1-associated corneal dystrophies and opens the prospect for the translation of this technique to the treatment of other corneal dystrophies.

P15.39-S
Search for potential causal variants in transcription factor binding sites using a database integrating SNPs and human genome information
V. Zazza,† T. Nutile,*, T. Sorice, M. Aversano, D. Ruggiero, S. Nappo, M. Ciutò, Institute of Genetics and Bioinformatics, CNR, Naples, Italy.

Human genetic variation underlies a majority of phenotypic differences between individuals, including susceptibility to disease. Genome wide association studies (GWAS) have identified many susceptibility loci/SNPs for complex traits and diseases. The greatest challenge in the ‘post-GWAS’ era is to understand the functional consequences of these loci. When SNPs occur within a gene or in a regulatory region, they may play a direct role in disease by affecting the gene function. Therefore SNPs may help to predict an individual response to certain drugs or the risk of developing particular diseases. SNPs are found everywhere and those associated to complex diseases are found preferentially in non-coding regions. The recognition and the binding of transcription factors (TF) to specific consensus sequences in the genome are crucial for a successful transcriptional regulation in cells. Therefore, we thought to predict and identify potential causal variants that can alter the binding sites of TF using a database integrating SNPs and human genome information. To this frame, we applied this approach looking for variants altering the Activator Protein 1 (AP1) binding site and we identified 7518 SNPs capturing approximately 4500 genes throughout the whole human DNA sequence. In order to screen for causal SNPs, these genes were associated with functional studies; predicted results were grouped and analysed with a large number of bioinformatics tools. Overall, these findings will give new insights into the value of SNPs in AP1 consensus sequence and it will provide an innovative approach that potentially yields additional new candidate genes for several disorders.

P16.01-S
Searching for circulating epigenetic biomarkers of Alzheimer’s disease
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Increasing evidence points to a possible contribution of folate metabolism in modulating the methylation profile and the expression of disease-related genes in complex diseases, such as Alzheimer’s disease (AD). Impaired folate metabolism could result in altered DNA methylation and expression of genes involved in AD pathogenesis. Indeed, taking into account the inaccessibility of brain DNA samples until death, there is increasing interest in searching for peripheral epigenetic biomarkers of the disease. In this regard we obtained peripheral blood DNA of 25 AD patients, 25 individuals with Mild Cognitive Impairment (MCI), and 25 matched controls and searched for changes in DNA methylation of the promoter/first exon of genes involved in DNA methylation: DNMT1, DNMT3B and MTHFR, and in amylod beta production: BACE1 and PSEN1. DNA methylation analyses were performed by means of Methylation Sensitive-High-Resolution Melting (MS-HRM) technique. Moreover, we searched for correlation between the methylation levels of each of the studied genes and circulating levels of homocysteine, folate and vitamin B12, all involved in one-carbon metabolism in the key pathway for DNA methylation reactions. The MTHFR gene showed an inter-individual variability in methylation profiles, and we observed a significant inverse correlation between plasma homocysteine levels and the methylation status of the MTHFR gene. To further address the link between one-carbon metabolism and DNA methylation profiles, we are currently assessing global peripheral DNA methylation biomarkers by means of the analysis of long interspersed nuclear elements 1 (LINE-1). The study was supported by the Italian Ministry of Health (GR-2009-1606229; F.C. Principal Investigator).

P16.02-M
Not all reference gene annotation is created equal
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Having a good quality geneset is essential for the interpretation of functional genomic, functional transcriptomic, and variation data. The GENCODE geneset represents the reference human gene annotation for the ENCODE project and is produced by merging manual annotation and automated Ensemble gene predictions with extensive computational and experimental QC and validation. Using different reference gene sets will inevitably give divergent results and the absence, truncation or misannotation of a gene, exon, or alternatively spliced (AS) transcript may hinder analysis. We will highlight some significant differences between the GENCODE and NCBI Reference Sequence Database (RefSeq) genesets. Specifically, we will discuss divergence in the annotation of alternative splicing (where GENCODE protein-coding loci have a mean of 7.6 AS transcripts while RefSeq only have 2.1), long non-coding RNAs (GENCODE 2.5 fold more genes and 3.7 fold more transcripts), pseudogenes (GENCODE 10% more loci), genomic coverage of annotated pseudogenes (GENCODE 10% more loci), genomic coverage of annotated exons (GENCODE 1.7 fold greater coverage), degree of manual curation (GENCODE 4.5 fold more manually curated transcripts), experimental validation, and functionally descriptive biotypes. We will detail the continued extension and refinement of the GENCODE geneset, including the integration of RNAseq, CAGE, polyAseq, ribosome profiling and epigenomic data, to
identify novel loci, define 5' and 3' transcript boundaries and identify novel translation initiation sites. Finally, we will explain our use of RNAseq data to determine the expression level of all GENCODE transcripts, allowing us to present a reduced, but biologically meaningful, set of transcripts including only those that are highly expressed or expressed in a particular tissue.

P16.03-S
Jannovar: A Java library for Exome Annotation
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Transcript-based annotation and pedigree analysis are two basic steps in the computation of whole-exome sequencing experiments. Gene-centric genetic diagnostics and disease-gene discovery projects. Here, we present Jannovar, a stand-alone Java application as well as a Java library designed to be used in larger software frameworks for exome and genome analysis. Jannovar uses an interval tree to speed up the identification of all transcripts affected by a given variant, and provides HGVs-compliant annotations both for variants affecting coding sequences and splice junctions as well as UTR sequences and genes of interest. This study aims to compare genome-wide association studies and pedigree analysis with VCF files with data from members of a family segregating a Mendelian disorder. Using a desktop computer, Jannovar requires a few seconds to annotate a typical VCF file with exome data.

P16.04-M
Comparison of GWAS and EWAS results to identify loci and genes of interest in asthma
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GWASs and EWASs were performed on DNA samples extracted from members of a family segregating a Mendelian disorder. Using a desktop computer, Jannovar requires a few seconds to annotate a typical VCF file with exome data. We have previously reported an association between interleukin 1 receptor type 1 receptor (IL1R1) and mRNA level differences in lung tissue of asthma patients. We suggest that this novel tool will facilitate selection of patients with Beckwith-Wiedemann syndrome (BWS), a congenital overgrowth disorder with variable expressivity, results from disordered expression and/or function of imprinted genes at chromosome 11p15.5. There are no generally agreed clinical diagnostic criteria, with molecular studies commonly performed to confirm diagnosis. We have previously reported genotype-phenotype correlations with paternal uniparental disomy. We developed a weighted scoring system (sensitivity - 75.9% and specificity - 80.1%) to prioritize patients presenting with the most common features of BWS, and ROC analysis demonstrated superior performance (area under the curve - 0.85: 95% CI: 0.83-0.87) compared to previous criteria. We suggest that this novel tool will facilitate selection of patients with BWS for routine diagnostic testing and so improve the diagnosis of the disorder.

P16.05-P
DNA methylation signature of IL1R1 and IL1R2 genes in asthma
V. Gagné-Ouellet1, A. Boucheur-LeFur1, V. Tremblay-Vuillaume1, S. Guay2, J. Chakar3, L. Bouchard1, L. Czarapin3; 1Institut Universitaire de Cardiologie et de Pneumologie, Ste-Foy, QC, Canada, 2Department of Computer Science, Katholieke Universiteit Leuven, Kortrijk, Belgium, 3Department of Regenerative Therapies (BGRT), Berlin, Germany.

Loss of imprinting (LOI) through methylation loss (LOM) or gain (GOM) may affect gene expression and, in particular, methylation status analysis at specific epigenetic signature in the context of allergic asthma and that differences in IL1R2 gene expression might be related to DNA methylation level changes.
had multiclonal defects. These comprised hypermethylation at GNAS DMRs and hypomethylation at GNAS, PLAGL1, DIRAS3, PAMS08 and ZNF331 loci, confirming the view of a network of imprinted deregulated genes. MMD were detected in blood and saliva from both affected twins, but complementary molecular and MS-MLPA analyses showed that methylation defects were absent/attenuated in the DNA from saliva in the unaffected co-twins. This finding will enhance clinical practice and epigenotype phenotype correlations. In conclusion 450K genome approach appears a reliable technique to profiling the methylation status of all known imprinted loci and unravel their recurrent simultaneous deregulation Supported by 2009MBZPR0_003(to LL).

The investigation of human imprinting disorders has provided important insights into the role of genomic imprinting in normal health and development. Beckwith-Wiedemann syndrome (BWS) is a congenital overgrowth disorder associated with abnormal function of 11p15.5 imprinted genes. The most common cause of BWS is loss of methylation (epimutation) at the imprinting centre 2 (IC2/ICDMR1). We and others have found that a subgroup of patients with BWS harbours epimutations at other imprinting centres (ICs) outside of 11p15.5. This multiple epimutation (ME+) phenotype has been associated with assisted reproductive technologies births though the clinical significance of these additional epimutations has not been clearly defined. Another human imprinting disorder, Silver-Russell syndrome (SRS) is also linked to the 11p15.5 imprinted gene cluster but, in contrast to BWS, is characterized by pre- and postnatal growth retardation and, most commonly, epimutations (loss of paternal allele methylation) at IC1. In order to comprehensively define potential ME+ epigenotypes in BWS and SRS patients we undertook methylation profiling in 67 and 22 patients respectively with the Illumina 450k methylation BeadChip. Analysis of methylation status at 37 imprinted differentially methylated regions (DMRs) (31 well characterized known DMRs (KDMRs) and 6 recently reported novel DMRs (nDMRs) (PMID:24402520)). The most frequently affected non-11p15.5 DMRs in ME+ BWS patients were on chromosome 1, 6 and 15. In addition, epimutations at nDMRs located on chromosome 8 and 22 were frequent. Further analysis of the ME+ epigenotype patterns and clinical correlates in BWS and SRS patients will be presented in detail.

The ability to detect low-level genetic variants in heterogeneous populations of cells is necessary for identifying postzygotic or somatic mutations underlying human diseases such as mosaic birth defects involving the skin. Existing bioinformatics methods for detecting such variations have been mainly developed for cancer genomics and are usually based on the analysis of paired samples. Furthermore, they have limited sensitivity in detecting certain types of variants such as insertions/deletions. SomaticCaller was developed to systematically detect low-level variants in next-generation DNA sequencing data. It consists in browsing aligned sequence data (BAM files) of pairs or trios (i.e. index case and parents) to systematically identify positions with candidate variants. Statistical tests are first performed between diploid samples to measure the sample’s independence of a candidate position for a given variation. In a second time, allelic ratios from all candidate sites are compared to a series of negative controls by Student’s t-test to discriminate true positive variants from probable sequencing or alignment errors. Applied to targeted deep sequencing of a gene associated with mosaic overgrowth syndromes (PIIHCMA), SomaticCaller led to identification of variants with allelic fractions as low as 0.01. Experiments from trio-based exome sequencing data demonstrated its ability to readily detect variants with allelic fractions as low as 0.05. Compared to standard variant callers, SomaticCaller showed increased sensitivity for genetic variants present in less than 10% of reads. Potential applications of this tool are numerous in the growing field of the genetics of mosaic diseases, both for research and molecular diagnosis purposes.

Comprehensive methylation profiling in Beckwith-Wiedemann syndrome and Silver-Russell syndrome B. Lan-Leung, R. Díaz1, J. Bauer2, E. R. Maher3;1University of Birmingham, Birmingham, United Kingdom, 2University of Cambridge, Cambridge, United Kingdom. The most common cause of BWS is loss of methylation (epimutation) at the imprinting centre 2 (IC2/ICDMR1). We and others have found that a subgroup of BWS patients harbours epimutations at other imprinting centres (ICs) outside of 11p15.5. This multiple epimutation (ME+) phenotype has been associated with assisted reproductive technologies births though the clinical significance of these additional epimutations has not been clearly defined. Another human imprinting disorder, Silver-Russell syndrome (SRS) is also linked to the 11p15.5 imprinted gene cluster but, in contrast to BWS, is characterized by pre- and postnatal growth retardation and, most commonly, epimutations (loss of paternal allele methylation) at IC1. In order to comprehensively define potential ME+ epigenotypes in BWS and SRS patients we undertook methylation profiling in 67 and 22 patients respectively with the Illumina 450k methylation BeadChip. Analysis of methylation status at 37 imprinted differentially methylated regions (DMRs) (31 well characterized known DMRs (KDMRs) and 6 recently reported novel DMRs (nDMRs) (PMID:24402520)). The most frequently affected non-11p15.5 DMRs in ME+ BWS patients were on chromosome 1, 6 and 15. In addition, epimutations at nDMRs located on chromosome 8 and 22 were frequent. Further analysis of the ME+ epigenotype patterns and clinical correlates in BWS and SRS patients will be presented in detail.

The field of human genetics is being revolutionized by exome and genome sequencing. A massive amount of data is being produced at ever-increasing rates. Exome sequencing can be completed in a few days using NGS, allowing for new variant discovery in a matter of weeks. The technology generates considerable numbers of false positives, and the differentiation of sequencing errors from true mutations is not a straightforward task. Moreover, the identification of changes-of-interest from amongst tens of thousands of variants requires annotation drawn from various sources, as well as advanced filtering capabilities. We have developed Highlander, a Java software coupled to a MySQL database, in order to centralize all variant data and annotations from the lab, and to provide powerful filtering tools that are easily accessible to the biologist. Data can be generated by any NGS machine (such as Life Technologies’ Solid or Ion Torrent, or Illumina’s HiSeq) and most variant callers (such as Life Technologies’ LifeScope or Broad Institute’s GATK). Variant calls are annotated using DBNSFP and SnpEff, then imported into the database. The Highlander GUI easily allows for complex queries to this database, using shortcuts for certain standard criteria such as “sample-specific variants”, “variants common to specific samples” or “combined-heterozygous genes”. Users can then browse through query results using sorting, masking and highlighting of information. For example, when exploring the genetic landscape of a given disease, a user may be interested in identifying variants that are present in clinical samples but absent in controls. Within the data browser, users can first select a specific sample or a group of samples, then the data is filtered to display only those variants present in the selected samples. The user can then further refine the query by applying additional filters such as allele frequency, impact on protein coding, and conservation across species. The data browser is highly interactive, allowing for new variant discovery in a matter of weeks. The technology generates considerable numbers of false positives, and the differentiation of sequencing errors from true mutations is not a straightforward task. Moreover, the identification of changes-of-interest from amongst tens of thousands of variants requires annotation drawn from various sources, as well as advanced filtering capabilities. We have developed Highlander, a Java software coupled to a MySQL database, in order to centralize all variant data and annotations from the lab, and to provide powerful filtering tools that are easily accessible to the biologist. Data can be generated by any NGS machine (such as Life Technologies’ Solid or Ion Torrent, or Illumina’s HiSeq) and most variant callers (such as Life Technologies’ LifeScope or Broad Institute’s GATK). Variant calls are annotated using DBNSFP and SnpEff, then imported into the database. The Highlander GUI easily allows for complex queries to this database, using shortcuts for certain standard criteria such as “sample-specific variants”, “variants common to specific samples” or “combined-heterozygous genes”. Users can then browse through query results using sorting, masking and highlighting of information.

Molecular diagnosis of bladder cancer by detecting gene p16INK4a from body fluids by methylation-specific polymerase chain reaction (MSP) R. Dumache, M. Pau, B. Rardon, M. Licker, V. Dumitrascu, D. David;1University of Medicine and Pharmacy “Victor Babes”; Timisoara, Romania. Carcinoma of the urinary bladder is the 5th leading causes of death worldwide. Hypermethylation of the CpG islands of gene promoters is one of the earliest and most frequent epigenetic alterations leading to cancer as well as in its development. The purpose of our study was to investigate if hypermethylation of gene p16INK4a can be used as a serum biomarker for early detection of bladder cancer. Materials and methods: Using the methylation-specific polymerase chain reaction (MS-PCR) method, we analyzed the methylation status of gene p16INK4a from serum and its matched tum or in 42 bladder cancer patients, and 35 samples from cancer-free individuals, as controls. Genomic DNA was extracted from serum and tissue samples. Results: Hypermethylation of gene p16INK4a was found in 38 (90.5%) of the 42 bladder cancer patients, and in only 2 (5.8%) of the 35 cancer-free individuals, as controls. Genomic DNA was extracted from serum and tissue samples. Results: Hypermethylation of gene p16INK4a was found in 38 (90.5%) of the 42 bladder cancer patients, and in only 2 (5.8%) of the 35 cancer-free individuals. Hypermethylation status of gene p16INK4a was found in both serum and its matched bladder biopsy sample. Conclusion: We conclude that hypermethylation of gene p16INK4a is involved in early bladder carcinogenesis and therefore might be used as a noninvasive serum biomarker for early detection of bladder cancer.

Somaticcaller: a somatic and post-zygotic mutation detection software from DNA-seq data Y. Dufour1, J. St-Onge1, J. Courcet1, L. Favre1, P. Vabret2, J. Rivière1;1Université de Bourgogne - Equipe GAD, Dijon, France, 2CHU Dijon - Molecular Genetics Laboratory, Dijon, France, CHU Dijon - FMI/TRANSLAD, Dijon, France, CHU Dijon - Dermatology, Dijon, France.

The ability to detect low-level genetic variants in heterogeneous populations of cells is necessary for identifying postzygotic or somatic mutations underlying human diseases such as mosaic birth defects involving the skin. Existing bioinformatics methods for detecting such variations have been mainly developed for cancer genomics and are usually based on the analysis of paired samples. Furthermore, they have limited sensitivity in detecting certain types of variants such as insertions/deletions. SomaticCaller was developed to systematically detect low-level variants in next-generation DNA sequencing data. It consists in browsing aligned sequence data (BAM files) of pairs or trios (i.e. index case and parents) to systematically identify positions with candidate variants. Statistical tests are first performed between diploid samples to measure the sample’s independence of a candidate position for a given variation. In a second time, allelic ratios from all candidate sites are compared to a series of negative controls by Student’s t-test to discriminate true positive variants from probable sequencing or alignment errors. Applied to targeted deep sequencing of a gene associated with mosaic overgrowth syndromes (PIIHCMA), SomaticCaller led to identification of variants with allelic fractions as low as 0.01. Experiments from trio-based exome sequencing data demonstrated its ability to readily detect variants with allelic fractions as low as 0.05. Compared to standard variant callers, SomaticCaller showed increased sensitivity for genetic variants present in less than 10% of reads. Potential applications of this tool are numerous in the growing field of the genetics of mosaic diseases, both for research and molecular diagnosis purposes.
Extracellular matrix plays a significant role in tumor development. Our study focuses on the epigenetic regulation of 12 laminin-encoding genes (LAMA1, LAMA2, LAMA3A, LAMA3B, LAMA4, LAMAC5, LAMBI, LAMB2, LAMB3, LAMC1, LAMC2, LAMC3), 8 genes of integrins (ITGA1, ITGA2, ITGA3, ITGA4, ITGA6, ITGA7, ITGA9, ITGB9), 2 idiotypic genes (CDH2, CDH3) and the dystroglycan gene DAG1. We have surveyed 109 samples of breast cancer, 109 paired adjacent nonmalignant samples, 6 samples of normal mammary gland from autopsy and 6 samples of breast cancer cell lines for aberrant promoter methylation of all 25 genes. Promoters of 11 genes (LAMA1, LAMA2, LAMBI, ITGA4, ITGA6, ITGA7, ITGA9, NID1, NID2, CDH2, CDH3) have demonstrated abnormal methylation in 1.9% to 35% samples of breast cancer and/or adjacent tissues. Our results may be important for understanding of the dramatic changes in the extracellular matrix during tumor growth and development.

Extracellular matrix proteins genes abnormally methylated in breast cancer

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<th>Gene symbol</th>
<th>Methylation in breast cancer and/or adjacent nonmalignant samples (%)</th>
<th>Methylation in normal mammary gland from autopsy (%)</th>
<th>Presence (+) / absence (-) of methylation in breast cancer cell lines</th>
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P16.14-M
Comprehensive transcriptome profiling of breast cancers

V. del Monaco1, V. D’Angelo2, M. D’Auria2, F.D. De Palma2, D. Montanaro1, G. Liguori1, G. D’Auria1, G. Botti1, A. Baldi1, R.A. Calogero1, E. Salvatore2,3,4

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Breast cancer is one of the most frequent malignancies among women and is a major cause of cancer-related mortality, despite advances in early detection and treatment. Breast cancer is a heterogeneous disease with a poorly defined genetic landscape, which poses a major challenge in diagnosis and treatment. Recent reports have described an intricate interplay among diverse RNA species, including protein-coding mRNAs and ncRNAs (long non-coding RNAs, pseudogenes, miRNAs and circular RNAs) which are also implicated in numerous diseases such as cancer.

By massively RNA-seq-sequencing, we obtained 7.8 billion reads from 55 healthy and tumour breast tissues belonging to Basal, HER2-positive and Luminal breast cancers, which were sequenced to high coverage. At the beginning, we aim to define their comprehensive digital transcriptome. In order to analyse the transcriptome in specific tumour cells, we used Laser Capture Microdissection system. Furthermore, we performed a RiboZero-based RNA depletion in order to focus the sequencing effort on the non-ribosomal portion of the total RNA. Comparative transcriptomic analyses highlighted differentially expressed transcripts between the different breast cancer groups, identifying transcripts which may be possible modulator RNAs. This global transcriptomic profiling can illustrate the intricate internal mechanisms of the transcriptome at a very high resolution, allowing us to explore the distinct nature of these breast cancer subtypes, and could provide a new inventory of diagnostic and therapeutic targets.

P16.15-S
Genetics of Cardiomyopathies: In-silico Analysis of Variants in Cardiomyopathies-Associated Genes

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Cardiomyopathies are genetically heterogeneous group of diseases of the myocardium. Mutations in multiple genes have been associated with cardiomyopathy and many cases of cardiomyopathy have a genetic component. Genetic factors may be responsible for 30-50% of cases of dilated cardiomyopathy (DCM), and about 90% of cases of Hypertrophic cardiomyopathy (HCM) are familial. As clinical genetic testing is rapidly emerging with a high density of pathogenic variants, and no or extremely low variant in the population database. Majority of the identified loci overlap protein domains that play critical roles.

rate of missense variation in the two datasets using a window bin of 50bp. Though most variants are rare (78.7%), and 58.4% (2751/4708) being private, 25.7% (655/2544) of missense variants from 1000 Genomes Project database are predicted to be pathogenic. Of the overlapping variants, 20.4% (42/206) and 42.2% (87/206) are known and predicted pathogenic variations respectively. We identified 104 regions distributed among 15 genes, including LMNA, MYH7, TNN1, and MYBPC3, with high density of pathogenic variants and no or extremely rare variations have been reported in them in the population database.

P16.16-M
Complex Chromosomal Rearrangements In B-cell Lymphoma: Evidence Of Chromothripsis?

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Genomic instability is a well-known hallmark of cancer. Recent genome sequencing studies have identified a novel phenomenon called chromothripsis in which complex genomic rearrangements are thought to be derived from a single catastrophic event rather than by several incremental steps. While chromothripsis is well documented in solid tumors and leukemias, chromothripsis in lymphomas is reported to be an uncommon situation. We report here a case of chromothripsis in a patient presenting with a thyroid mass suspicious for diffuse large B-cell lymphoma. Chromosome analysis showed a complex karyotype with multiple rearrangements, including a translocation of chromosomes 3 and 7 involving the BCL6 gene region, chromosomes 14, 7 and 22 with involvement of the IGH gene region and an unbalanced structural rearrangement involving chromosomes 8 and 18 involving the BCL2 gene region.

The karyotype was interpreted as 51~56,X,Y,+t(3;7)(t;g29.11p12)(der7) t(3;7)(14;7;22)q32.21p11.2.q12)(der8) t(14;7;22)q12.012)q12.012)(der8) (8;18) (p12 q21) t(8;18) +(der 9 t (5;9) (q13 q22) t (14;7;22)q12.012)q12.012)(der8) FISH analysis confirmed the BCL6 gene rearrangement, IGH gene rearrangement/extra copies of IGH and 3 copies of BCL2. Array comparative genomic hybridization studies with Cytochip 60K custom oligo array showed multiple complex copy number variations including a previously unidentified chromosome 12 abnormality. However, array analysis did not reveal any imbalance involving the BCL6, BCL2 or IGH gene regions whose rearrangements were observed by FISH, thus suggesting that these rearrangements are balanced in nature. Our patient’s genomic abnormalities show characteristics suggestive of chromothripsis and provides initial evidence that chromothripsis is not confined to solid tumors, but can also be seen in B-cell lymphomas with well characterized one or two step lymphomagenesis.

P16.17-S
Describing chromothripsis using HGVS sequence variation nomenclature, suggested extensions

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Large chromosomal rearrangements are traditionally described using ISCN nomenclature based on chromosomal banding patterns (1). Due to the limited number of translocation breakpoint sequences identified the sequence variation nomenclature guidelines of Human Genome Variation Society (HGVS, http://www.hgvs.org/mutnomen), which are mainly focused on simple variants, did not require specific rules for detailed description of genetic rearrangements. This changed with the introduction of new technologies allowing rapid discovery of breakpoint sequences from complex structural rearrangements including translocations and chromothripsis.

The description of such complex variants challenges the existing guidelines. Previously, we have proposed new HGVS rules for detailed translocation descriptions (2). Here, we suggest extending the HGVS nomenclature guidelines to facilitate unambiguous descriptions of chromothripsis. The main feature of these descriptions is that the precise combination and order of chromosomal fragments can be derived easily. The suggested format should include sufficient flexibility and consistency, allowing alternative interpretations and ambiguous descriptions. The new rules can be combined with those proposed previously for complex changes, which included: i) nesting those proposed previously for complex changes, which included: i) nesting descriptions (2). Here, we suggest extending the HGVS nomenclature guidelines to facilitate unambiguous descriptions of chromothripsis. The main feature of these descriptions is that the precise combination and order of chromosomal fragments can be derived easily. The suggested format should include sufficient flexibility and consistency, allowing alternative interpretations and ambiguous descriptions. The new rules can be combined with those proposed previously for complex changes, which included: i) nesting those proposed previously for complex changes, which included: i) nesting

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sequence variant nomenclature checkers (e.g. Mutalyzer, https://Mutalyzer.nl).

1) ISCN (2013). 2013. An International System for Human Cytogenetics No-

2) http://www.hgs.org/mutations/SVtrans/HG/HG2513.PTPdf


P16.18-M Concordance of methods for CNV detection from whole-exome sequencing as compared with microarray: 100 sample autism cohort

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Copy number variants have been implicated as drivers of many developmental
disorders, including autism spectrum disorder. The platform of choice to
detect genome-wide CNVs has traditionally been microarray (including SNP
arrays that can also detect copy neutral LOH regions); however, since samp-
les have often undergone sequencing to discover pathogenic sequence vari-
ants, it is desirable to exploit these data to also detect CNVs. Various methods
have been proposed to detect CNVs from NGS data, but little information is
available which compare the success of these approaches to orthogonal meth-
ods. Using a large data set of constitutional samples from an autism cohort
that have been subjected to both whole-exome sequencing (WES) and to a
genome-wide SNP microarray, we compare the ability to detect CNVs be-
tween these platforms. Several algorithms for CNV detection from the NGS
data are evaluated, and the results are compared with an established HMM-
based method for detection of CNVs from the microarray platform.

P16.19-S De Novo Assembly and Structural Variation Discovery in Complex Genomes Using Extremely Long Single-Molecule Imaging

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De novo genome assemblies using only short read data are generally incom-
plete and highly fragmented due to the intractable complexity found in most
genomes. This complexity, consisting mainly of large duplications and repe-
titions within haplotype blocks. We also resolve and measure long tandem
repetitions within haplotype blocks. Also resolve and measure long tandem
repetition regions that are likely impossible to assemble by other methods.

P16.20-M Understanding complex gene-environment interactions leading to impaired DNA methylation in colorectal cancer

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We recently applied Methylation Sensitive-High Resolution Melting (MSHRM) technique to evaluate the methylation levels of APC, MGMT, hMLH1, RASSF1A and CDKN2A genes in over 100 colorectal cancer (CRC) samples and in healthy adjacent mucosa specimens (Coppede F et al. Epigenetics 2014, DOI: 10.4161/epi.27956).

Our analysis revealed a correlation between hMLH1 methylation, a marker of the CIMP high phenotype, with low folate levels, tumor location, increa-
sing age, female gender, high number of methylated genes, and the TYMS 1494 6bp ins/del polymorphism. Increasing age correlated also with MGMT methylation levels in CRC and both tumor stage and the MTR 2756A>G polymorphism with RASSF1A methylation.

The APC gene results were methylated in both CRC tissues and healthy adjacent mucosa specimens. APC methylation levels correlated with the TYMS 1494 6bp ins/del polymorphism in CRC samples and with both increasing age and the MTR 2756A>G polymorphism in healthy mucosa.

In order to shed some light on complex gene-environment interactions lead-
ing to impaired DNA methylation in CRC we are currently elaborating those
data by means of artificial neural networks (ANNs) searching for correlati-
ons and lifestyle factors evaluated by means of a detailed questionnaire on lifestyles and dietary habits filled in by each CRC patient. ANNs are able to understand non-linear relationships among studied variables and to high-
light through a graph the complexity of connections among the studied vari-
bles.

The study was supported by Istituto Toscano Tumori (ITT).

P16.21-S Copy Number Calling for Gene Panels with Next-Generation Sequencing (NGS)

A. Vadapalli, J. Ghosh;

Agilent Technologies, Santa Clara, CA, United States.

Detection of copy number variation (CNV) from NGS data is an important
mechanism for understanding and identifying genetic factors in constitu-
tional diseases and cancer. Our evaluation of existing public questionnaires
revealed that they did not reliably detect short length CNVs from NGS gene
panel data. A new CNV detection algorithm is introduced in SureCall v2.0
software for target enriched NGS data generated from SureSelect and Halo-
plex. This algorithm is able to predict breakpoints using the low genomic
covrage of small panels. This feature makes it different from other algorithms
based on read depth that require adequate genomic coverage to normalize
the data and model copy number of different regions. A diploid reference
is used to compute log ratio values from the normalized read depths with
respect to the sample that minimizes the need to make additional correc-
tions with respect to GC content and mappability. Reference and sample
can either be run together or taken from different sequencing experiments.
An internal read depth normalization step handles the difference in the number
of sequencing reads between sample and reference. The resolution of de-
tection is that of a single interval where the interval boundaries are defined
from the baits or amplicons that cover the regions of interest in the genes.
Ablerrant intervals are labeled as significant after applying an iterative meth-

Our analysis revealed a correlation between hMLH1 methylation, a marker of the CIMP high phenotype, with low folate levels, tumor location, increa-
sing age, female gender, high number of methylated genes, and the TYMS 1494 6bp ins/del polymorphism. Increasing age correlated also with MGMT methylation levels in CRC and both tumor stage and the MTR 2756A>G polymorphism with RASSF1A methylation.

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tions with respect to GC content and mappability. Reference and sample
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An internal read depth normalization step handles the difference in the number
of sequencing reads between sample and reference. The resolution of de-
tection is that of a single interval where the interval boundaries are defined
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Ablerrant intervals are labeled as significant after applying an iterative meth-

P16.23-S Variable R.Msp1 fragmentation in genomic DNA due to DNA hypomethylation in CRF patients with MTHFR C677T genotype: polymorphism from genetics to epigenetics.
M. Urfali1, C. Scapoli2, M. Neri1, C. Scotton1, C. Passarelli1, A. Carrieri2, T. Castrignanò3, G. Pesole4, E. Kotelnikova5, E. Schwartz5, F. Gualandi1, A. Ferlini1, 1Department of Medical Sciences, Section of Medical Genetics, University of Ferrara, Ferrara, Italy, 2Department of Life Sciences and Biotechnology, University of Ferrara, Ferrara, Italy, 3Epidemiology and Prevention Unit, Fondazione IRCCS Istituto Nazionale dei Tumori, Milano, Italy, 4CNR Institute of Biomembranes and Bioenergetics, CNR, Bari, Italy, 5ARIADNE Genomics Inc, Rockville, Maryland, US, Rockville, MD, United States.

DNA methylation changes at the 24-dehydrocholesterol reductase gene are associated with High Density Lipoprotein serum levels
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P16.24-M DNA methylation analysis of representative SNP markers in Duchenne muscular dystrophy carriers and controls
F. R. Di Raimo1, C. Scapoli2, M. Neri1, C. Scotton1, C. Passarelli1, A. Carrieri2, T. Castrignanò3, G. Pesole4, E. Kotelnikova5, E. Schwartz5, F. Gualandi1, A. Ferlini1, 1Department of Medical Sciences, Section of Medical Genetics, University of Ferrara, Ferrara, Italy, 2Department of Life Sciences and Biotechnology, University of Ferrara, Ferrara, Italy, 3CINECA Interuniversity Consortium, Roma, Italy, 4Institute of Biomembranes and Bioenergetics, Bari, Italy, 5ARIADNE Genomics Inc, Rockville, Maryland, US, Rockville, MD, United States.

P16.25-S DNA methyltransferases and acute inflammatory pain
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DNA methylation modifications may occur in response to a wide variety of exposures, including diet and metabolic status. In the context of the EPICOR study aimed at identifying relationships between DNA methylation status and myocardial infarction risk (292 cases, 292 controls from the EPIC-Italy cohort). We have associated loci with 16 CpG sites to the DNA methylation in a sample of 277 subjects (153 cases, 124 controls) not treated for diabetes nor under cholesterol/blood pressure-lowering treatments. DNA methylation was assessed with the Illumina HumanMethylation450 BeadChip, and data analysed according to standard procedures (Me-thlyumi, Biocomputer), correcting for gender, BMI, season and centre of recruitment, estimated white blood cells percentage, chip position and batch. At a linear regression analysis, we found an interesting association between a CpG site in the promoter region of the 24-dehydrocholesterol reductase gene (DHCR24) and serum HDL levels (effect size 1.21-10^0, p=3.93-10^0). The result was strengthened and overcome the Bonferroni threshold (1.03-10^0) when including in the analysis further 186 EPIC-Italy subjects belonging to other non-case groups. (N=463, effect size 1.463, p=6.2*10^-7). Among the CpG beadchip probes in the DHCR24 gene region, only one is clearly and significantly associated with HDL levels, making it a putative biomarker of HDL serum levels. Interestingly enough, recent papers report an anti-inflammatory effect of HDLs in vascular endothelial cells through the upregulation of DHCR24 expression. Our preliminary findings are thus worthy of further investigation and may contribute to better describe the inflammatory role of DHCR24 in the HDL mediated inhibition of vascular inflammation.

P16.26-M SNPs identification in duchenne muscular dystrophy female carriers as biomarkers to discriminate symptomatic/asymptomatic phenotypes
F. R. Di Raimo1, C. Scapoli2, M. Neri1, C. Scotton1, C. Passarelli1, A. Carrieri2, T. Castrignanò3, G. Pesole4, E. Kotelnikova5, E. Schwartz5, F. Gualandi1, A. Ferlini1, 1Department of Medical Sciences, Section of Medical Genetics, University of Ferrara, Ferrara, Italy, 2Department of Life Sciences and Biotechnology, University of Ferrara, Ferrara, Italy, 3CINECA Interuniversity Consortium, Roma, Italy, 4Institute of Biomembranes and Bioenergetics, Bari, Italy, 5ARIADNE Genomics Inc, Rockville, Maryland, US, Rockville, MD, United States.

Duchenne muscular dystrophy female carriers are clinically asymptomatic. Nevertheless a small group, defined as manifesting carriers, can develop symptoms, varying from a mild muscle weakness to a DMD-like phenotype. Manifesting carriers may also present cardiac involvement (dilated cardiomyopathy), either alone or in addition to the muscle weakness. Lack of relationship between X-inactivation, transcription balancing and asympto-matic/symptomatic phenotype was recently observed. To date, the molecular mechanism underlying the clinical heterogeneity in female carriers is unknown. In this study, the combined approach of high-throughput technologies and novel statistical analysis methods has allowed us to identify a group of SNPs which could discriminate the symptomatic/asymptomatic phenotypes. We performed Whole Exome Sequencing and RNA-seq analysis (Illumina GAIIx, Agilent Sure Select enrichment) on a symptomatic DMD carrier. The interrogation of the NGS output for a list of 883 genes correlated with the dystrophin pathway (MedScan Pathway Study, Ariadne Genomics), resulted in 29 candidate SNPs: 17 in coding regions, 4 in the 3'UTR and 8 in non-coding regions. These selected SNPs have been investigated in two cohorts of carriers: 18 symptomatic (muscle weakness) and 18 asymptomatic (family history of dystrophinopathy or mild myopathic signs, but absence of muscle weak-ness). Novel statistical approaches on selected SNPs based on multidimensional scaling and membership probability, allowed us to identify three SNPs in 3'UTR region which could discriminate the symptomatic/asymptomatic phenotypes. Finally, the combined approaches of NGS and novel statistical analysis could be a very useful approach for the investigation of small cohorts of patients affected by rare diseases.

P16.27-S Effects of Duplex-specific nucleic acid human lesions profiling using RNA-seq
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RNA-seq is a next-generation sequencing method able to characterize thoroughly gene expression profiles and to perform very accurate differential gene expression analysis. High expression of some genes could both influ-
 Effects of the culture condition and chromatin remodelling agents on the epigenetics of organotypic cultures

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1Department of Health Sciences, University of Milano, Italy; 2Division of Pathology, Fondazione IRCCS Ca’ Granda Ospedale Maggiore Policlinico, Milan, Italy; 3Department of Pathophysiology and Transplantation; University degli Studi di Milano, Milan, Italy; 4Division of Pathology, Ospedale San Paolo, Milan, Italy; 5Division of Thoracic Surgery and Lung Transplantation, Fondazione IRCCS Ca’ Granda Ospedale Maggiore Policlinico, Milan, Italy.

Alterations of the epigenetic pathways are established as hallmarks of tumorigenesis, together with genetic/genomic variations. Tumor epigenetic alterations are of increasing relevance to clinical practice, because they are important “druggable” targets for cancer therapy using chromatin remodelling agents (CRAs). New evidences highlight the relevance of microenvironment on the epigenetics and the need to use culture models that preserve the tissue morphology, to better understand the mode of action of CRAs. We studied the epigenetic response induced by culture condition and CRAs treatment, in a preclinical model based on organotypic culture from normal and neoplastic lung specimens, that preserves ex vivo the original tissue microenvironment and morphology. We assessed the expression pattern of histone deacetylases (HDACs) and methylation profile of “long interspersed nuclear elements” LINE1s and a panel of tumor suppressor genes. We observed that behavior of the organotypic culture respect to that reported for other ex vivo models. Interestingly, culture induced an overall increase of LINE1s methylation, whereas CRAs caused LINE1s demethylation. Differently, culture and CRAs induced opposite effects on the genes: the samples responded specifically, showing demethylation for some promoters and increased methylation for others. Moreover, we noted that the culture caused alterations in the HDACs expression pattern.

These overall data reveal the importance of the maintaining the cells in their original organ architecture to study the mode of action of the CRAs, and suggest that CRAs do not work only in a non-specific way, as previously thought, in particular on the gene promoters.

Benign copy number variations in the human exome: focusing on intellectual disability genes

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The human exome seems to represent a dynamic system in terms of interindividual variability. However, due to the lack of widely accepted criteria for determining copy number variation (CNV) pathogenic value, the creation of a map depicting the wide spectrum of benign CNV in the human exome is hindered. To make a step forward on the road to the map of human exome variations, we have evaluated 151 unrelated individuals using high-resolution CNV analysis by Affymetrix 2.7M SNP-array and an original bioinformatics technology. CNV affecting exons of intellectual disability genes were associated with X-linked mental retardation, X-linked dominant conditions and autosomal dominant intellectual disability. Exome CNV affecting X-linked mental retardation genes in males were as follows: duplication of one OPHN1 exon (3 individuals) and four DLG2 exons (3 individuals), duplication of twenty six L1CAM exons (7 individuals). Benign intragenic CNV affecting X-linked dominant genes in females were associated with duplication of twelve SMCA1 exons (Cornelia de Lange syndrome 2) in 8 individuals and duplication of six PORCN exons (Focal dermal hypoplasia) in 3 individuals. Benign exome variations affecting genes associated with autosomal dominant intellectual disability were associated with deletion of one SMARC2 exon (3 individuals) and duplication of one TSCI exon (12 individuals). KANSL1 duplication (18 individuals) and deletion of three NHEJ1 exons (5 individuals) were observed. We have also found that benign exome CNV affecting intellectual disability genes are relatively common in humans representing a pitfall for molecular diagnosis of genomic and single-gene disorders. Supported by the Russian Federation President Grant (MD-4401.2013.7).
A bioinformatics pipeline to identify candidate disease-causing variants from exome sequencing data

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ABSTRACTS POSTERS

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Genome-wide DNA methylation analysis identifies novel differentially methylated regions in patients with imprinting disorders

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Genomic imprinting is the regulation of gene expression by parent of origin. Imprinting is maintained by epigenetic mechanisms including parent of origin-specific DNA methylation, and its disruption leads to imprinting disorders affecting growth, metabolism and predisposition to cancer. Eight ‘classical’ imprinting disorders are known, linked to (epi)mutations of specific loci. However, some individuals have imprinting anomalies affecting multiple imprinted loci (HIL) throughout the genome. Here we used epigenome-wide analysis to investigate aberrant DNA methylation in HIL patients.

We used the Illumina Infinium Human Methylation450 BeadChip array to assay genomic DNA methylation at ~475,000 sites in ten HIL patients with two clinical presentations (Beckwith-Wiedemann syndrome and neonatal diabetes). We developed a novel informatic pipeline capable of small sample number analysis, and statistical criteria to quantify DNA methylation.

The pipeline robustly detected hypomethylation at known imprinted loci, and 25 further candidate imprinted regions (nine shared between patient groups) including one in the Down syndrome critical region (WRB) and another previously associated with bipolar disorder (PPIEL). Targeted analysis of three candidate regions (NHP2L1, WRP and PPIEL) confirmed allelic expression, methylation patterns consistent with allelic maternal methylation, and frequent hypomethylation among HIL patients.

This study has identified new candidate imprinted genes, and shown a remarkable epigenetic similarities between patients with different imprinting syndromes. Our informatic methods make possible epigenomic profiling of small groups or even individual patients, and have potential to expand our understanding of epigenetic regulation in health and disease.

P16.38-M

Genomic imprinting defects: role of cis-acting elements and trans-acting factors

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Gamete-of-origin dependent gene expression, namely genomic imprinting, is controlled by allele-specific epigenetic modifications of Imprinting Control Regions (ICRs). Either hypo- or hyper- DNA methylation abnormalities abolishing the epigenetic asymmetry at the ICRs result in altered gene expression and disease. DNA methylation changes are accompanied by changes in histone modifications and long-range protein interactions. ICR epigenetic alterations result from either mutations acting in cis or lesions affecting factors acting in trans, but in many cases their cause is unknown. A large cluster of imprinted genes lies on a conserved region of chromosome 11p15.5 and is associated with the growth disorders Beckwith-Wiedemann syndrome (BWS) and Silver-Russell syndrome (SRS). By studying several mutations affecting the IGFB2/H19 ICR, we recently observed that the penetrance of the BWS phenotype correlated with the intensity of ICR hypermethylation and that this is dependent on the affinity of the mutant allele for CTG wild-type sequences, leading to the hypothesis that the mutant allele has a stronger binding capacity. We are currently investigating whether this is true in mouse embryonic stem cells (ESCs), and this study is in progress.

P16.40-M

Human genome resources in Ensembl

A. Zadissa1,2, F. I. Rezwan1, L. E. Docherty1,2, D. I. Wilson1, J. W. Holloway1.

Ensembl provides up-to-date annotation for the human genome including comprehensive updates for all of our resources whenever a new assembly is released. Here we present all the available and upcoming human genome resources in Ensembl.

In December 2013, the Genome Reference Consortium released the latest version of the human assembly GRCh38. A fully annotated version of this assembly will be part of Ensembl v76 scheduled for summer 2014. Before the full release, preliminary Ensembl annotation based on GRCh38 will be available at http://pre.ensembl.org. This preview site, available in spring 2014, includes alignments of human-specific cDNA, protein and EST sequence data as well as the current GENCODE gene set, based on GRCh37. p13, projected onto the new assembly alongside variation data.

For the full release, the GENCODE gene set will be reassessed and rebuilt from the component Ensembl and HAVANA annotation sets. The complete set of variation data, regulatory features and all comparative data such as sequence alignments will be recomputed for the new assembly. HumanBloomMap 2.0 RNAseq data will also be reanalysed and remapped. In addition to the primary assembly, the new human genome also contains 35 ‘assembly units’, with a total of 26 alternative loci that encompass all fix and novel patches to the GRCh37 assembly. Ensembl fully annotates all of these alternative sequence regions.

Moving a research project to a new version of the human genome assembly is a major undertaking and so we are committed to supporting the previous GRCh37.p13 annotation resources for use by the scientific community.

P16.41-S

Full deconvolution of clonal populations in recurrent hematological cancer using gaussian mixture model

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We performed Whole Exome Sequencing on five samples collected in eight years during the disease history of a single patient with hematological cancer (Acute Lymphoblastic Leukemia, Remission and therapy-related Myelodysplastic Syndrome at three different time-points). We identified a joint set of 1201 variants that was present at least in two stages of the disease, for which we calculated allele frequencies corrected by the estimate of tumor purity. These were used to train a set of Gaussian Mixture Models (GMM) allowing for increasing number of classes. We selected the best mo-
del using Akaike Information Content criterion and assigned each mutation to a specific GMM clone. We calculated mutational signatures of each GMM clone according to the procedure proposed by Alexandrov (Nature, 2013) and estimated distance between them. We identified a single clone common to all relapses but not LLA, we built parent/child relationships with other clones using distance among signatures. Our analysis reveals a branching evolution of clones that putatively diverge in response to the clinical treatment. We show GMM is an effective and unbiased technique that can be applied to deconvolve clonal populations in cancer data only using allele frequencies. Clones can be then characterized by their mutational landscape, this information is sufficient to build clonal relationships when studying the evolution of the disease.

P16.42-M

H3M2: Detection of Runs of Homozygosity from whole-exome sequencing data

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Runs of homozygosity (ROH) can be defined as sizable chromosomal stretches of homozygous genotypes, ranging in length from tens of kilobases to megabases. ROHs can be relevant for population and medical genetics, playing a role in predisposition to both rare and common disorders. ROHs are commonly detected by SNP microarrays, but attempts have been made to apply Next-Generation Sequencing (NGS) data. Currently available methods developed for the analysis of uniformly spaced SNP-array maps do not fit easily the sparse and non uniform distribution of the WES target design. To meet the need of an approach specifically tailored to WES data we developed H3M2, an original algorithm based on Heterogenous Hidden Markov Model that incorporates inter-marker distances to detect ROH from Whole Exome Sequencing (WES) data. We evaluated the performance of H3M2 to correctly identify ROHs on synthetic chromosomes and examined its accuracy in detecting ROHs of different length (short, medium and long) from real 1000 genomes project data. H3M2 turned out to be more accurate than GERMLINE and PLINK, two state-of-the-art algorithms, especially in the detection of short and medium ROHs. H3M2 is freely available at https://sourceforge.net/projects/h3m2/.

P16.43-S

Hepatitis C Virus genome variability analysis from high-throughput pyrosequencing data

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High-throughput pyrosequencing enables discovery of rare Hepatitis C Virus (HCV) variants and estimation of viral diversity within a host, which may play an important role in understanding patient’s response to personalized therapy. The aim of our study was to estimate intra-host genomic variation of HCV 1b in naïve and Pegylated Interferon-Ribavirin treated patients. Serum viral RNA from 11 patients was reverse transcribed and amplified to produce 7.5 kb-long amplicons that were random-fragmented. A sequencing library was generated by specific adaptor-ligation and analyzed using Roche 454 Titanium chemistry and GS FLX platform. Data were analyzed using software published by The Broad Institute. Reads from each patient were assembled into contigs (Vicuna), oriented and frameshift-corrected (V-Fat). Software published by The Broad Institute. Reads from each patient were assembled into contigs (Vicuna), oriented and frameshift-corrected (V-Fat). Reads from each patient were assembled into contigs (Vicuna), oriented and frameshift-corrected (V-Fat). Reads from each patient were assembled into contigs (Vicuna), oriented and frameshift-corrected (V-Fat). Reads from each patient were assembled into contigs (Vicuna), oriented and frameshift-corrected (V-Fat). Reads from each patient were assembled into contigs (Vicuna), oriented and frameshift-corrected (V-Fat). Reads from each patient were assembled into contigs (Vicuna), oriented and frameshift-corrected (V-Fat).

P16.44-M

Systems biology approaches to the search for disease genes in Hirschsprung’s disease

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The availability of new methodologies of high performance has revolutionized our ability to discover. However, the high resolution of such technologies can become a double-edged sword. Reduced sample sizes available in rare diseases are often an obstacle for the detection of candidate genes or the study of their molecular basis. The limitations of the approaches based on isolated genes can be overcome using systems biology approaches. This study shows as a combination of pathway-based analysis (PBA) and network analysis allowed to discover four new loci (RASGEF1A and IQGAP2, DCL1 CHRNA7) related to signalling and migration processes associated with the disease, which were then validated in a cohort of 106 independent trios.

The further study of an international cohort of 162 HSCR trios allowed us to confirm the molecular bases of disease using PBA. We found a significant association of processes related to signalling and its regulation as well as affected network of proteins were always the same, which reflects the complexity of the disease. This methodology of prioritization of candidate genes can be extrapolated to any technology of high performance (WES, RNA-seq, etc.).

P16.45-S

The impact of antisense transcription on epigenetic signals

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Several histone post-translational modifications are preferentially located in the promoter region of genes and are associated to the promoter activity. It is debated whether the histone modifications regulate the gene expression i.e., the histone code hypothesis or if they are put there as a consequence of transcription.

In an earlier study we observed that the histone 3 acetylation signal upstream of the transcriptional start site (TSS) was lower in unidirectional compared to bidirectional genes. Following this observation, we hypothesized that the transcribed region is modified during transcription and that the observed upstream signal is caused by transcription in the opposite direction from the TSS. Promoters directing transcription in both directions are frequent in the genome, which would explain the common occurrence of upstream signals.

Here we identified bidirectional or unidirectional genes based on TSS identified from cap analysis of gene expression and database searches. We compared histone modification signals between these two classes of genes across several cell types. We have found significant differences for well-known histone modifications, e.g. H3K4me3, H3K9ac and H3K27ac for which the distribution of transcription on i.e., the histone code hypothesis or if they are put there as a consequence of transcription.

In conclusion, our results support the model of histone modifications occur in the promoter region of genes and are associated to the promoter activity. The further study of an international cohort of 162 HSCR trios allowed us to confirm the molecular bases of disease using PBA. We found a significant association of processes related to signalling and its regulation as well as affected network of proteins were always the same, which reflects the complexity of the disease. This methodology of prioritization of candidate genes can be extrapolated to any technology of high performance (WES, RNA-seq, etc.).

P16.46-M

Gene expression profile of human amniotic stem cells xenotransplanted in a sheep Achilles tendon defect model

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Amniotic derived cells are ideal seed cells for regenerative medicine proto-
cols since they constitute a remarkable plasticity to safety properties. This study investigated the role exerted by human amniotic cells during the process of tendon healing, evaluating by microarray technique, the presence of transcriptome variations in human amniotic epithelial (hAECs) and mesenchymal (hAMCs) stem cells when xenotransplanted into the preclinical ovine model of tendon defect, compared to freshly isolated ones. This analysis allowed to understand in which way, after transplantation, hAECs and hAMCs transcripts have been affected.

Functional analysis of the affected transcripts revealed that the main biological functions involved are: Cell Death and Survival, Cellular Growth and Proliferation, Inflammatory Response, Cellular Function and Maintenance, Skeletal and Muscular System Development and Function and Connective Tissue Development and Function.

These results show that hAECs and hAMCs after xenotransplantation produce a modulation of the genes involved in their survival, and in the inflammatory response. Intriguingly, it has been observed also the up-expression of the transcripts related to the connective tissue development and function as COL1A1 which is involved in the synthesis of collagen type I, the most expressed protein in the tendon. In conclusion, the present study demonstrates that human amniotic derived cells have a direct involvement in new tendon matrix remodeling and tissue regeneration. Altogether these results strongly support the idea that human amniotic cells could be effectively proposed as a prompt stem cell-based therapy that does not require any preliminary in vitro differentiation or any genomic transfection.

P16.47-S Genome-wide characterization of imprinted methylation in humans: Implication for patients with multi-locus methylation defects D. Monk1, F. Court1, C. Tayama1, V. Romanelli2, A. Martin-Trujillo1, J. Iglesias-Platas1, C. Simone1, Y. L. Yap1, C. Le Caignec1, D. Guigay1, N. van de Sande2, K. Hata2, K. Nakabayashi2; 1Institut d’Investigació Biomedica de Bellvitge, Barcelona, Spain, 2National Research Institute for Child Health and Development, Tokyo, Japan.

We investigated the global methylation pattern in KS. We hypothesize that melamine methylation defects, including Transient Neonatal Diabetes Mellitus patients with ZP77 mutations.

P16.48-M Computational pipeline to analyze genomic variants with respect to clinical phenotypes by mining literature. Study of genomic regions related to intellectual disability E. Pranckvičienė1, A. Prancužioniene2, E. Prekšaitė1, V. Kučinskas3; 1Department of Human and Medical Genetics, Vilnius University, Lithuania, 2Department of Genetic Medicine, Aarhus University Hospital, Aarhus, Denmark, 3Department of Clinical Genetics, Odense University Hospital, Odense, Denmark.

Whole exome sequencing (WES) in clinical diagnostics is used as a tool to identify genomic variants in a patient’s genome that may possibly give rise to a particular disorder. Variant interpretation partially relies on consulting databases like OMIM and DECIPHER containing known genomic variants causative of the specific disorders and databases of common genomic variants such as dbSNP.

Novel variants identified by WES have to be characterized with respect to a particular clinical context in order to improve the diagnostics. The functional effect of the variant can be assessed by programs such as PolyPhen, SIFT and Evolutionary Rate Profiling. Cross-referencing of novel variants with genomic databases and literature can be challenging mostly because these variants and their impact on the associated genes causing the manifestation of the phenotype of interest are poorly characterized.

We present a computational exome analysis pipeline based on the ABI SOLiD LifeScope platform. The novel added feature in our pipeline is augmenting the ANNOVAR variant annotations by links to genes strongly connected to the disease phenotypes through the MeSH terms. These connections are deduced computationally by utilizing MEDLINE database and gene ontology annotations of the genes.

The utility of our approach is demonstrated by analyzing genomic regions linked to the intellectual disability phenotype identified in Lithuanian patients.

The research leading to these results has received funding from Lithuanian-Swiss cooperation programme to reduce economic and social disparities within the enlarged European Union under project agreement No CH-3-ŠMM-01/04.

P16.49-S IntSplice: A tool to predict the effect on pre-mRNA splicing of intronic nucleotide substitutions A. Shibata1, T. Okuno1, A. Masuda, K. Ohno; Division of Neurogenetics, Nagoya University Graduate School of Medicine, Nagoya, Japan.

Precise spatiotemporal regulation of splicing is mediated by splicing cis-elements on pre-mRNA. The next generation sequencers disclose a large amount of single nucleotide variations (SNVs) in the human genome. Tools to analyze the effects of SNVs on the protein functions have been recently published PolyPhen-2 and SIFT. SNVs affecting intronic cis-elements potentially compromise splicing, but no dedicated tool has been available. We thus sought for a prediction model. According to the effect size analysis of each nucleotide at intronic positions -50 to -3 on alternative splicing, we extracted 34 parameters that possibly dictated the strength of splicing signals. We also partitioned all the alternative 3’ splice sites in the human ENSEMBL annotation into 500 categories and generated a neural network model. The model predicted the efficiency of alternative splicing events with a correlation coefficient greater than 0.8 for a validation dataset. The model discriminated splicing consequences of intronic single nucleotide variations in dbSNP134 and the Human Gene Mutation Database. We next compared the ENSEMBL-based model with models deduced from RNA-seq of normal human brain, cerebral cortex, heart, liver, skeletal muscle, and colon, and found that a colon-based model yielded the best discrimination. We created a web service program, IntSplice. IntSplice is the first tool for predicting splicing consequences of nucleotide variations at intronic positions -50 to -3.

P16.50-M Genome-wide methylation analysis in Klinefelter syndrome A. Skakkebæk1, M. Swinthic1, A. Bøjesen1, J. Hertz1, J. Østergaard2, A. Pedersen1, M. Wallentin3, K. Sørensen1, C. Grønbak1; 1Department of Clinical Genetics, Vejle Hospital, Vejle, Denmark, 2Department of Endocrinology and Internal Medicine (MEA), Aarhus University Hospital, Aarhus, Denmark, 3Department of Molecular Medicine, Aarhus University Hospital, Aarhus, Denmark.

Klinefelter syndrome (KS) is associated with an increase risk of these disorders, however no study to date have investigated global methylation changes in patients with KS. The aim of this study is to investigate the global methylation pattern in KS. We hypothesize that the methylation pattern is changed in KS and that the methylene changes will explain phenotypic characteristics present in KS. Methods: We performed genome-wide DNA methylation analysis on blood leukocytes from 73 patients with KS and 73 age- and gender-matched controls using the Illumina Infinium Human Methylation 450K BeadChip. Results: 70.525 CpG sites covering over 15,000 genes were found to be differentially methylated in patients with KS compared to the age-matched controls. Among these 61.567 were on autosomal chromosomes, 8903 were on the X-chromosome and 55 were on Y-chromosome. One of the genes with promoter associated differentially methylated CpG sites is the NSD1 gene which is involved in the androgen receptor (AR) transactivation. Other genes possibly involved in the phenotype of KS and differentially methylated were ABIBSP, APOB, APOE, C1QTNF5, CACYP, DPAS1, GABRG1, HOOK4, LRRC61, NLRP2, PEX10, RPL1P, SDHAF1, SPEG. Conclusions: For the first time we show that KS is associated with pervasive genome-wide methylation changes, changes which could play a role in the clinical phenotype seen with KS.
Cutaneous melanoma is the most fatal skin cancer and, although some effective molecular therapies exist, novel targets and drugs are still needed. To provide new insights for novel targets discovery, we performed an extensive characterization by next-generation sequencing (NGS) of a collection of melanoma cell lines derived from metastatic cases. Samples were profiled by whole-exome sequencing (WES) and RNA-sequencing using Illumina technology. Starting from WES data, we developed a bioinformatics pipeline to catalogue 2,172 novel mutations affecting genes in melanoma key pathways targeted by current therapies (MAPK and glutamate pathways) as well as genes never described for melanoma [Cifola, 2013]. Moreover, WES data were used to perform copy number alteration (CNA) analysis using a novel software developed by us, called Excavator, which is very sensitive and precise in DNA copies estimation even in situations of great sample heterogeneity [Magi, 2013]. CNA results were concordant with 250K SNP Array data and used to explore CN state of mutated genes. To collect and share these results, we created a Melanoma Exome Database ([https://155.253.6.64/MExDB/]). On the same samples, we also carried out RNA-sequencing and performed both a traditional gene expression analysis and more sophisticated structural evaluations. Focusing on fusion transcripts, we identified 72 putative events generated by either inter-chromosomal translocations or intra-chromosomal rearrangements, recently defined “conjoined genes” and representing an additional gene regulation mechanism. Globally, NGS proved to be extremely powerful to dissect cancer complexity at both genomic and transcriptomic levels, and to identify novel potential targets for personalized treatment of cutaneous melanoma.
and batch effect. Linear regression analysis showed that 10,000 CpG sites were significantly associated with age after Bonferroni correction (p<0.07). Positive correlation with age was found for 5,687 CpG sites (57%), whereas 4,313 CpG sites (43%) were negatively correlated with age. The top four significant loci (p<10-28) were located in the CpG islands of ELOVL2 and FHL2 genes. Methylation levels of the 4 CpG sites were positively correlated with increasing age, according to previously published results. These results confirm that DNA methylation alterations occur during aging and that ELOVL2 and FHL2 genes could be used as biomarker of aging. Moreover, the methylation differences associated with age could help in understanding molecular mechanisms that underlie the development of age-related diseases.

P16.56-M Whole genome methylation analysis in idiopathic generalized epilepsies
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Genetic factors play a predominant role in the etiology of common idiopathic generalized epilepsies (IGE) syndromes. An increased rate of maternal inheritance and excess number of affected females implicating involvement of epigenetic effects in the etiology of IGE syndromes. In this study, we have performed a whole genome methylation analysis for 15 parent-offspring trios with vertical inheritance of the IGE trait. DNA samples were subjected to bisulfite conversion prior to Illumina Human Methylation 450K Beadarray analysis. Beadchip scans were performed through Illumina BeadStudio platform. We used a comprehensive R-Bioconductor package, namely ‘RnBeads v0.9.9.11’, which implements an analysis workflow that can be used for pedigree-based differential methylation analysis.

After data normalization, quality control, filtering and methylation profiling steps, differential methylation analysis revealed 239 genes with p-values smaller than 0.05. As an alternative approach, we have run a pathway-based analysis to see if genes with differential methylation values map to common pathways. This analysis with PANOVA software revealed various pathways particularly with synaptic and immunological functions.

Analysis of high throughput methylation data has proven to be challenging where various pipelines with alternative normalization and filtering methods should be applied. Two approaches presented herein have resulted in identification of new epilepsy-related genes and pathways that could as well be applied to analysis of other trio based methylation studies.

P16.57-S Molecular characterization of a de novo mutation in a pediatric patient with isolated ectopia lentis as a diagnostic criterion for Marfan syndrome
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Marfan syndrome is an inherited multisystem disorder that affects the connective tissue. The typical onset is in adulthood and often occurs in children with isolated clinical signs. The revised Ghent criteria allow to confirm the diagnosis if ectopia lentis is in concomitance with a mutation in the FBN1 gene definitely associated with Marfan syndrome. Our patient (5 years old) presents a mutation (c.1514Y) never reported in the literature, hence the difficulty in establishing a diagnosis. We perform molecular dynamic simulation to investigate how this mutation affects the protein stability. Analysis was carried out on the mutated form of the N-terminal domain of the human FBN1 protein, (PDB code 4M74). Structural analyses indicate that mutation perturb the secondary structure of the protein, which reduce the folding stability of the system. The C514Y mutation disrupts the C154-C166 disulfide bridge and consequently the two antiparallel beta sheets are impaired. In conclusion, the mutation leads to a destabilization of the native folding of the protein, and therefore a probably reduction of the physiological activity. The patient is followed in follow-up clinics due to all probability of developing the Marfan syndrome. This study emphasizes the utility to characterize a mutation through molecular modeling in all late-onset diseases in order to correct follow up of patients.

P16.58-M Prediction of LHX1-target genes relevant to Müllerian aplasia
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LHX1 is a putative transcription factor widely distributed among vertebrates. In mice, Lhx1 acts as a high-hierarchy regulator during urogenital development, as female Lhx1-null mutants lack Müllerian derivatives. Deletions and point mutations affecting LHX1 have been found in female patients with Müllerian aplasia (MA). However, the low recurrence of these alterations indicates that this disorder is multifactorial, and few reports using high-resolution genome mapping techniques have been published. Hence, the identification of candidate genes for MA demands deeper analysis. In order to identify candidate genes for MA, we used a bioinformatic approach to predict LHX1-target genes, and the results were contrasted against the set of genes with copy-number alterations compiled from the microarray reports on MA patients. Starting from 4 documented LHX1 target regulations in Xenopus and their homologs in 5 other species, a highly conserved 50-nt signature was identified (e-value 1.34e-29). Seven known transcription factor-binding sites were contained in such signature, including those of HOXA5 and HNF8, which have been previously associated to MA. The refined motif was further searched against the human genome promoter regions, and 45 putative target-genes of LHX1 were identified. These, 38 genes are expressed in uterine tissues, 19 during embryogenesis, and 15 in both conditions. Two genes, NPH1 and PIAS3, were found deleted in MA patients. In conclusion, our approach will be useful to prioritize genes relevant to Müllerian development and may help to establish future validation protocols.

P16.59-S Age at onset and disease severity in primary progressive multiple sclerosis: a genome-wide association study, pathway network analysis
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Age at onset and disease severity in primary progressive multiple sclerosis (MS) are currently unresolved. It is hypothesized that a complex polygenetic background could regulate the clinical expression of progressive MS, in particular of the rare primary progressive course (PPMS). Aim: to look for common genetic variants associated to age at onset (AAO) and severity of PPMS, the latter measured as Multiple Sclerosis Severity Score (MSSS). Methods: 51 PPMS patients of Italian origin were genotyped for 296,589 SNPs using Illumina OmniExpress and Human60-Quad chips, and allele association with AAO and MSSS was studied; a protein interaction-based pathway and network analysis was performed using the following tools/database: GEV, GTeX eQTL-browser; STRINGV9.05, Webgestalt, GenoGis, KO, GEGGO, MetaCoreTM. Results: no single association signal exceeded genome-wide significance in both AAO or MSSS analyses. Starting from 4 documented LHX1 target regulations in Xenopus and their homologs in 5 other species, a highly conserved 50-nt signature was identified (e-value 1.34e-29). Seven known transcription factor-binding sites were contained in such signature, including those of HOXA5 and HNF8, which have been previously associated to MA. The refined motif was further searched against the human genome promoter regions, and 45 putative target-genes of LHX1 were identified. These, 38 genes are expressed in uterine tissues, 19 during embryogenesis, and 15 in both conditions. Two genes, NPH1 and PIAS3, were found deleted in MA patients. In conclusion, our approach will be useful to prioritize genes relevant to Müllerian development and may help to establish future validation protocols.

P16.60-M Genetic and epigenetic biomarkers of thymomas in Myasthenia Gravis
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We aimed to identify genetic and epigenetic biomarkers of thymomas in Myasthenia Gravis (MG). To this end, we conducted a genome-wide association study, pathway and network analysis. Results: no single association signal exceeded genome-wide significance in both AAO or MSSS analyses. Starting from 4 documented LHX1 target regulations in Xenopus and their homologs in 5 other species, a highly conserved 50-nt signature was identified (e-value 1.34e-29). Seven known transcription factor-binding sites were contained in such signature, including those of HOXA5 and HNF8, which have been previously associated to MA. The refined motif was further searched against the human genome promoter regions, and 45 putative target-genes of LHX1 were identified. These, 38 genes are expressed in uterine tissues, 19 during embryogenesis, and 15 in both conditions. Two genes, NPH1 and PIAS3, were found deleted in MA patients. In conclusion, our approach will be useful to prioritize genes relevant to Müllerian development and may help to establish future validation protocols.
clodotide synthesis and DNA methylation, have been often linked to aberrant DNA methylation and risk of various types of cancer. We investigated four polymorphisms in genes of this pathway, namely methyltnethyldihydrofolate reductase (MTHFR) 677C>T, thymidylate synthase (TYMS) 28 bp repeats, DNA methyltransferase (DNMT) 3-579TT genotype (P = 0.03) and of the combined DNMT3B -579TT/-149CT genotype (P = 0.001). This was observed in the total cohort of thymoma patients with respect to controls. After gender stratification both associations resulted significant in males. We are currently evaluating the methylation levels of the promoters of tumour-related genes, such as CDKN2A, MLL1, and MGMT, in thymoma cells of MG patients, searching for correlation between DNA methylation levels and DNMT3B polymorphisms.

P16.61-S

Behind the scenes: the hidden challenges of exome sequencing in consanguineous populations

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Our research aims to identify recessive mutations in the endogamous Irish Traveller population using exome sequencing. Through our studies, we have identified issues that may complicate identification of disease mutations in consanguineous populations: (a) increased number of candidate variants due to higher than expected levels of genome homozygosity, (b) the effect of cryptic relatedness on the use of population-matched controls and (c) increased phenotypic variability due to increased likelihood of multiple disorders in the same individual.

Homozgyosity levels in patients from traditionally endogamous populations are often much higher than the predicted levels from first-cousin marriages in non-endogamous populations. This results in a large number of novel/rare homozygous variants per patient exome. Population-matched controls are very useful in this situation to distinguish benign from pathogenic variants. However, due to Traveller culture, key pedigree information is often not forthcoming. Therefore, there is an increased risk of cryptic relatedness between the patients and the controls, which can lead to the erroneous filtering out of pathogenic variants.

Inter- and intra-familial variability in patients with the same disease mutation may be due to variable expressivity. However, in families from endogamous populations there is the increased possibility of patients having more than one recessive disorder; thus distorting the phenotype. When faced with phenotypic variability, we found it useful to analyse each patient’s phenotype individually and have identified patients with up to three different recessive disorders using this approach.

Our findings support the need for customised approaches to exome sequencing in families from endogamous populations.

P16.62-M

Similarity metrics can be used to avoid multiple entries of a single individual in databases of genomic sequence variants

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Removing frequently detected variants is one of the most effective approaches to reduce the number of candidate mutations in the data analysis of next-generation sequencing studies. The incidence of a rare disorder in a population serves as an upper bound for the allele frequency of mutations in the general population. The incidence of a rare disorder in a population serves as an upper bound for the allele frequency of mutations in the general population. The incidence of a rare disorder in a population serves as an upper bound for the allele frequency of mutations in the general population.

However, with a high allele frequency, and by extension a high incidence of a rare disorder, the number of variants in a population will be much higher than the number of variants in a given individual. This is because the number of variants in a population is the product of the allele frequency and the population size, whereas the number of variants in an individual is the product of the allele frequency and the number of chromosomes, which is much smaller than the population size. Therefore, the number of variants in an individual is much smaller than the number of variants in a population, and the number of variants in a population is much smaller than the number of variants in a population.

The Babraham Institute, Cambridge, United Kingdom.

DNA methylation in gametes, particularly in oocytes, marks a subset of genes for appropriate expression in the next generation, as in the case of imprinted genes. DNA methylation has been described as being predominantly over gene bodies. These observations suggest that methylation is governed by the activity of promoters of tumour-related genes, such as CDKN2A, hMLH1, and MGMT, in thymoma cells of MG patients, searching for correlation between DNA methylation levels and DNMT3B polymorphisms.

P16.63-S

A new PCR primer design tool for Sanger sequencing confirmation

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High data quality and accuracy are recognized characteristics of Sanger sequencing projects and are primary reasons that next generation sequencing projects complement their results by capillary electrophoresis data validation. We have developed an on-line tool called Primer Designer™ to streamline the NGS-to-Sanger sequencing workflow by taking the laborious task of PCR primer design out of the hands of the researcher by providing pre-designed assays for the human exome. The primer design tool has been created to enable scientists using next generation sequencing to quickly confirm variants discovered in their work by providing the means to quickly search, order and receive suitable pre-designed PCR primers for Sanger sequencing.

Using the Primer Designer™ tool to design M13-tailed and non-tailed PCR primers for Sanger sequencing we will demonstrate validation of 28-variants across 24-amplicons and 19-genes using the BDD, BDTv1.1 and BDTv3.1 sequencing chemistries on the 3500xl Genetic Analyzer capillary electrophoresis platform.

P16.64-M

Towards integrative family analysis on OMICs data for individual patient diagnostics

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During recent years generating OMICs data became cheaper and faster. Notably DNA sequencing benefited from this change, becoming more popular and frequently used, especially in the domain of diagnostics. As become apparent, the data from DNA sequencing does often not allow for conclusive diagnostics, as it only shows one aspect of what contributes to the patients phenotype. The combination of OMICs data like genomics, transcriptomics and proteomics presents itself as a solution to this problem, especially in the context of family analysis which helps to identify interesting features. While many methods have been developed combining different OMICs sources to create more complete and comprehensive analyses of a patients genotype, doing family analysis in the context of OMICs data with an user friendly visualization of the data is still explored very little. We present our vision of a comprehensive, user friendly application which concentrates on several key features of OMICs data for comparative analysis. Our vision concentrates on the analysis of three key features. Individual SNP comparison on genomics data, gene expression comparison with transcriptomic data and protein variation from proteomics data. While these features are well understood individually, our vision is to create an easy to use tool to combine those three data sources based of GensearchNGS, an application that already provides the required genomics tools and allows for visualization of the analysis and underlying data. Examples will pertain to genetic disorders in myopathies, where we recently could show some progress regarding functional understanding and therapy (Walter et al, 2013).

P16.65-S

Transcription as a key determinant of the DNA methylation landscape in oocytes

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DNA methylation in gametes, particularly in oocytes, marks a subset of genes for appropriate expression in the next generation, as in the case of imprinted genes. DNA methylation has been described as being predominantly over gene bodies. These observations suggest that methylation is governed by the activity of promoters of tumour-related genes, such as CDKN2A, hMLH1, and MGMT, in thymoma cells of MG patients, searching for correlation between DNA methylation levels and DNMT3B polymorphisms.

To investigate the fraction of the methylome linked to transcription, we in- inteegregated genome-wide methylation transcription with deep RNA-seq from different stages of oocyte development. This revealed that most hypermethylated domains precisely matchactive transcription units; this is true especially where transcription units do not match standard genome annotation because of the existence of unannotated upstream transcription start sites (TSS).
in oocytes. Conversely, most hypomethylated domains are transcriptionally silent. We also conclude that methylated CGs are predominantly intragenic, while unmethylated CGs are intergenic or at active TSSs. To test the association functionally, we investigated the imprinted gene Zac1; deletion of its oocyte-specific promoter led to absence of DNA methylation across its entire gene body. These results suggest that perturbations in transcriptional regulation during oogenesis could have profound effects on the oocyte methylation and may provide a link between aberrant methylation and ART.

P16.66.M

The influence of genetics on personality development

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Personality is known as hereditary to a certain extent. In this work we attempt to classify personality traits as binary traits based on genetic information only. For this we used the 60-item NEO-FFI and over 8 million SNPs from 6655 Dutch participants. For feature selection we performed a genome-wide association for each personality trait in a five-fold cross validation setting. All SNPs with a p-value <0.01 were chosen as predictors for a given fold and a given personality trait, amounting to approximately 2,500 associated SNPs for each trait. An artificial neural network was trained with the SNPs as input and the personality scores as output. We found it possible to classify a person's personality to the two sides of the scale significantly better than random. The results of this study prove in a novel way that genetics have an influence on personality. The next step is to identify, which genes these SNPs belong to, which hopefully will lead to a greater understanding of the processes involved in personality development and the onset of personality disorders.

P16.67.S

Analysis of GSTP1 promoter hypermethylation in urine samples of Bulgarian prostate cancer patients and controls

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Introduction: Prostate cancer (PC) is one of the most commonly diagnosed cancer in men of the developed countries. The epigenetic modifications in PC are frequent and extensively studied. One potential noninvasive biomarker in urine is GSTP1 DNA methylation. Materials and Methods: Using HRM technology we have analyzed GSTP1 promoter hypermethylation in urine samples of 64 PC patients, 26 controls with benign prostatic hyperplasia (BPH) and 20 young asymptomatic men (YAM). Our goal was to determine the diagnostic accuracy and correlations of this biomarker with clinicopathological characteristics in Bulgarian PC patients.

Results: Methylation was found in 70.31% of the patients, in 65.38% of the BPH controls and 15% of the YAM. Our results demonstrated that there was not significant difference of GSTP1 methylation among patients and BPH controls but among patients and YAM (p=0.000016). The ability of the biomarker to distinguish patients from BPH controls was evaluated. Serum PSA levels outliers form GSTP1 with estimated AUCs in ROC curve analysis 0.745 and 0.525 respectively. Methylation of GSTP1 correlates with age (Pearson's correlation coefficient 0.225, p =0.018) but not with Gleason score, tumor stage and PSA.

Conclusions: In our study the GSTP1 promoter hypermethylation in urine did not prove to be sensitive and specific diagnostic biomarker for PC and probably is it associated with age. Further investigations are needed to confirm these observations.

Acknowledgements: This work was supported by infrastructural Grant: DIUHK1/2/2009 funded by National Science Fund, Ministry of Education and Science, Bulgaria.

P16.69.S

Solving the cold case - A diagnostic and clinical trial framework for WGS based rare disease discovery


The number of rare monogenic diseases is estimated to be >5000. For half of these the underlying genes are unknown (McKusik V.A., 2011). An increasing proportion of common diseases, such as schizophrenia or autism, previously thought to be due to complex multifactorial inheritance, are now thought to represent a heterogeneous collection of rare monogenic disorders (Mitchell KJ, Porteous DJ, 2011), the large majority of which is still unknown. For the efficient identification of genetic mutations next generation sequencing (NGS) technology has revolutionized molecular diagnostics. Its big advantage is the ability to sequence enormous amounts of nucleic acids in a short time at an affordable cost. However, the benefits come with a number of challenges like NGS data management, quality control, mapping, variant calling and their annotation. We have developed a mutation screening and decision support for analysis of data generated by most common sequencing platforms: Illumina, 454, Ion Torrent, and Sanger Sequencing. The system furthermore allows the clinician to identify causal mutations through NGS data analysis and suggests which regions need to be validated with ABI Sanger sequencing. This makes it much easier to the scientist and the clinician to find causative Mutations. Samples of multiple patients can be processed and their mutations can be compared with ease. Keeping all gathered information of patients together grants for traceability and enables re-use and re-analysis of existing data. For questions where a single causative mutation is to be found in millions of other mutations, comprehensive data management and data analysis is essential.

P16.70.M

From NGS back to Sanger Sequencing: Connecting and Synchronizing NGS and CE Variant Files With the VR Toolkit


Whole exon or panel sequencing projects performed by next generation sequencing technologies typically reveal a large number of variants which may require verification by an orthogonal method. To that end, Sanger sequencing is the method of choice since it is accurate, affordable and easy to perform. To facilitate the re-sequencing of any exon in the human genome we have recently made available to the scientific community a free to use tool called Primer Designer™. The tool provides the designs for over 350.000 PCR primer pairs that cover 99% of all exons in the human genome. Amplicons generated with these primers can be readily sequenced using the BigDye® Direct sequencing kit on the Genetic Analyzer capillary electrophoresis platforms. For variant identification the sequencing files are analyzed with Applied Biosystems Variant Reporter® software which requires the import of a text file with a reference sequence for alignment and comparison. Here we show the utility and workflow of a new on-line tool called VR toolkit that generates a reference file from Primer Designer derived PCR amplicons that contains the chromosomal coordinates. Use of this annotated reference file in Variant Reporter allows the generation of an output file that can be compared and matched to a variant call file (vcf) from an NGS instrument. The VR toolkit enables the connection and synchronization between NGS data and traditional Sanger sequencing data analyzed with Variant Reporter software and should be of benefit for all researchers seeking to validate NGS data by Sanger sequencing.

P16.71.S

Analysis of Genomic Loci in Single Cells on the Fluidigm® C1™ Single-Cell Auto Prep System Without Whole Genome Amplification

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Genomic DNA in single cells can be analyzed using whole genome amplification (WGA) on the Fluidigm® C1™ Single-Cell Auto Prep System, followed by next-generation sequencing [1]. This approach is invaluable when screening for variants, for studying de novo mutations, as well as for looking for defined variants. We present an alternative to WGA for looking at predefined variants, with a simpler workflow, completed in less than eight hours with about three hours of hands-on time. We isolated single GM12752 B-lymphocyte cells, extracted genomic DNA and preamplified [2, 3] 96 specific loci on the C1 System, using a pool of 96 primer pairs that cover 99% of all exons in the human genome. Amplicons from single cells were analyzed on the Fluidigm® C1™ Single-Cell Auto Prep System followed by next-generation sequencing. For variants, for studying de novo mutations, as well as for looking for defined variants. We present an alternative to WGA for looking at predefined variants, with a simpler workflow, completed in less than eight hours with about three hours of hands-on time.

The approach described here can quickly provide insight into specific genes or loci from single cells. We believe this technique will find its applications in single-cell genotyping, variant detection, targeted sequencing and, potentially, copy number variation.
P16.72-M

The influence of structural variants on alternative splicing
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Structural variants (SVs) impact tissue transcriptome by modifying the level and timing of expression of genes that localize within and on their flanks. We used mouse inbred strains to extensively gauge the influence of structural variants on the transcriptome complexity and regulation. We generated extensive RNA-seq data from mice liver and brain and intersected them with the Mouse Genomes project catalog of SVs encompassing insertions, inversions, deletions and copy number gains to assess simultaneously the impact of genome structural changes on both gene expression and alternative splicing.

While large SVs directly impact transcript expression levels, smaller SVs significantly influence splicing diversity in several manners. When lying in an exon, SVs significantly favor the emergence of alternative splicing through usage of multiple donor sites regardless of the preservation or disruption of the open reading frame. Conversely, a deletion or insertion within an intron significantly increases the number of alternative splice acceptor sites of the surrounding exons. Finally, exons that lie just upstream or downstream of a SV-containing intron are significantly more often skipped out by splicing. These impacts are independent from the SV size suggesting that it is a property of the rearrangement per se rather than the consequence of a change of size of the intron or exon. Interestingly, when several SVs are embedded within an exon or intron they appear to have a cumulative effect on splicing events.

We show that SVs do impact tissue transcriptome on a global scale by altering its complexity and diversity through alternative splicing.

P16.73-S

Rapid Detection of Large Structural Variations in a Human Genome Using Nanochannel Genome Mapping Technology
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Large structural variations (SVs) are less common than SNPs and indels in the population but collectively account for a significant fraction of genetic polymorphism and diseases. Base pair differences arising from SVs are on a much higher order (>100 fold) than point mutations, however, none of the existing prevailing methods can comprehensively and effectively detect them. To address these challenges, we first applied a high-throughput cost-effective genome mapping technology using long single molecule (>150 kb) to discover genome wide SVs and structure differences in the YH genome. We detected 278 large SVs (>10 kb), of which 251 of 278 (90%) are interspersed and validated by sequencing based tests, we found that 71 out 78 (91%) are intersected with repeat elements, often the blind spot of re-sequencing and de novo assembly methods. More than 70% of detected SVs are insertion events, known to be difficult to detect by sequencing. In this study, genome mapping also provides valuable information for complex regions (MHC, KIR, TRB/TRA, IGH/IGL etc) with haplotypes.

In addition, for the first time, with long single molecule labeling patterns, inserted exogenous viral sequence and locations can be mapped on a whole genome scale important for understanding virus induced oncogenesis. Inserting exogenous viral sequence and locations can be mapped on a whole genome scale important for understanding virus induced oncogenesis. Mapping also provides valuable information for complex regions (MHC, KIR, TRA/TRA, IGH/IGL et al) with haplotypes.

While large SVs directly impact transcript expression levels, smaller SVs significantly influence splicing diversity in several manners. When lying in an exon, SVs significantly favor the emergence of alternative splicing through usage of multiple donor sites regardless of the preservation or disruption of the open reading frame. Conversely, a deletion or insertion within an intron significantly increases the number of alternative splice acceptor sites of the surrounding exons. Finally, exons that lie just upstream or downstream of a SV-containing intron are significantly more often skipped out by splicing. These impacts are independent from the SV size suggesting that it is a property of the rearrangement per se rather than the consequence of a change of size of the intron or exon. Interestingly, when several SVs are embedded within an exon or intron they appear to have a cumulative effect on splicing events. We show that SVs do impact tissue transcriptome on a global scale by altering its complexity and diversity through alternative splicing.

P16.75-S

EVA: A Completely New Variation Resource at EMBL-EBI
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European Variation Archive (EVA) is a new resource from EMBL-EBI aimed at accepting submissions of and providing access to all types of genetic variation data for all species. To this end, EVA works with partner databases, such as dbSNP, to guarantee free global access to all this genetic variation data. Where available, data submitted to EVA, mainly in VCF format, are closely linked with supporting BAM files in the ENA database. The web portal EVA also aims to provide a data exploration tool or search for a gene or disease topic. Access to VEGA variation data is also provided in a programmatic way by using RESTful web services for a variety of applications, such as annotation pipelines. Performance and functionality are also important goals. EVA backend datastorage is being developed with most advanced computing technologies to scale up to Petabytes of data whilst still being responsive. Some other functionalities developed include data mining and visualisation in order to allow direct access to variation data from a number of entry points including gene, disease, genomics loci, variation type and consequence. Technology implementations are only as valuable as the use cases they serve. EVA also aims to be an important resource for clinical, evolutionary and systems biology researchers by linking and combining regulatory information, pathways and protein-protein interaction data from different resources of EMBL-EBI.

P16.76-M

Whole-exome sequencing reveals novel variants for migraine in Taiwan
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Migraine is a common neurological disorder characterized by recurrent disabling attacks of headache and that affects roughly 14% of the population. Family and twin studies suggest significant genetic basis of migraine. Previous studies have identified several genetic variants by different approaches such as linkage, candidate gene and genome-wide association studies. However, the results remains inconsistent. In order to identify genetic variants for migraine in Taiwan, we performed whole exome sequencing on thirteen individuals from two tetra and one penta migraine families. Except for the father, all subjects in the family are affected. All the three pedigree were compatible with autosomal dominant inheritance. Among these subjects, a total of 3,271,336 variants were identified and an average of 269,865 variants was detected in each individual. Of which 14 variants were identified to be shared by all affected individuals but not by three unaffected fathers after filtering procedures. The variants we identified were mapped to four gene(s) (FRAS1, ABLIM3, FOD4X45, and BAGE2) and three intergenic regions (1p22.2, 4q13.3, and Xq11.1). Eight variants clustered in ABLIM3 were compatible with autosomal recessive inheritance. One novel single nucleotide polymorphism in FOD4X45 gene was detected and was compatible with autosomal dominant inheritance. Our results reveal several novel gene variants for migraine and further studies are required to confirm this finding.
Genetic relationship of European Roma people and eight ethnic groups from the Caucasus area which suggest Romans probably admixed with during their migration

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Historical and genetic studies have suggested that Roma people migrated from India about 1500 years ago and settled in Europe. We investigated the genetic connection of Central European Romans to populations they could be connected or admixed with during their migration through the Caucasus. Populations samples were from Abhkasians, Armenians, Chechens, Kumsks, Kurds, Nogais, North-Ossetians and Tadjiks living in the Caucasus area. We used publicly available Caucasus datasets and our Roma samples genotyped on Affymetrix Genome-wide Human SNP Array 6.0 chip. We also used Stanford-HGDP HapMap Phase 3 and Indian datasets in order to get a better perspective about population relationships. Applying linkage disequilibrium based pruning method, our datasets contained 98701 SNPs, respectively. Using advanced algorithms which are capable of processing large autosomal SNP datasets, we investigated genetic connection of these populations. In order to infer the structure and relationship of populations, principal component analysis (PCA) and ADMIXTURE analysis were applied. PCA results show that Romans, compared to Central European and Indian populations, have more common genetic elements with the 8 investigated populations of the Caucasus area Roma samples were most closely to Tadjiks and Nogais, while the others clustered farther from Romans, approximately in the same distance. ADMIXTURE analysis supports PCA results and shows that the eight populations from Caucasus contain also more Indian ancestry than Central Europeans. The data may also suggest that despite the short interval of contact they still admixed or populations of the Caucasus also have the same strong Ancestral North Eurasian origin as Romas.

This research was supported by TÁMOP-4.2.3-12/1/KONV-2012-0028.
of Parents and Children (ALSPAC) has information on prenatal exposure of father (n=9677) and mother (n=12,707) with detailed follow up (including DXA scans) on their children to age 17y.

With non-smoking mothers, those with prenatal exposure themselves had larger sons at birth, who went on the have increased lean mass and grip strength by 17y; no effects in daughters. By contrast, with non-smoking mothers, paternal prenatal exposure showed no effects at birth but increased growth (weight, BMI, lean and bone mass) in both sons and daughters by 17y; the daughters additionally increased height and fat mass.

With smoking mothers prenatally exposed themselves, there was no effect on sons at birth or later, whilst daughters had later decreases in height, weight, and fat, lean and bone mass. The only transgenerational effect with smoking mothers and paternal prenatal exposure was decreased head circumference at birth in sons which was associated with reduced IQ at 8ys. Taken together, these data evoke the transgenerational plasticity theory of evolved human life history strategy: Interaction analysis with imprinted genes PHLD2A and INS VNTR/AG2 are underway.

P17.06-M
Ancient mtDNA diversity in Bulgaria
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Background: The study of the genetic origin of the Bulgarian population helps for determination of population evolution and further our understanding of changes in the gene pool in space and time. We try to present the formation of our population based on available ancient mitochondrial DNA data.

Materials and methods: Using the main criteria for working with ancient mtDNA we analyzed 122 ancient samples dating from III Millennium B.C. to VIII - X Century A.D. from different regions of Bulgaria. We’ve performed several steps of amplification over-lapping fragments which cover the first hypervariable segment (HVS I) of mtDNA. The amplified fragments were cloned by using specific competent cells (E.coli), followed by sequencing of 360bp of HVS I.

Results: Preliminary results from phylogenetic analysis of 20 ancient samples have shown 18 independent haplotypes. Four of the samples are dating from III Millennium B.C. and 16 of them are dating from VIII - X Century A.D, which coincides with the First Bulgarian state. From the 16 samples dating VIII - X Century A.D eleven have European origin, two - Western Eurasian origin and three- unknown origin. From the 4 samples dating III Millennium B.C. one is with European origin, one with Western Eurasian, one with controversial origin- Western Eurasian or East Asia and one is with unknown origin.

Conclusion: This research presents first original data on ancient and medieval mtDNA samples from individuals who inhabited in time the current Bulgarian territories.

P17.07-S
The ancient genomic DNA (anDNA) and analysis of genetic risk factors related to autoimmune rheumatic diseases HLA linked
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The mountain areas of L’ Aquilia city, such as Barete and Rocca di Cambio are characterized by environmental conditions (low temperature and humidity) that facilitate the preservation of the human remains and anDNA: this factor explains the often tempering of a number of paleoanthropological findings. The migratory events surely resulted in the disappearance of several genes as well as the introduction of foreign alleles, among which some responsible for the development of autoimmune diseases HLA-restricted. In this work we show some preliminary genetic data generated by applying different bone and DNA extraction protocols, innovative and/or suitably modified from commercial available kits for forensic analysis. The immunogenetic and paleopathological assays have showed the positivity for HLA gene HLA-Cw6 (an increased frequency of HLA-Cw3 and HLA-Dw6) is typically observed in Rheumatoid Arthritis. This work has laid the foundation for the design of protocols for extraction and PCR reaction improved and optimized, to be applied to paleogenetics and paleopathology samples on which to assess genetic risk factors related to autoimmune rheumatic diseases ‘HLA linked’.

References

P17.08-M
Association between Azoospermia Factor c (AZFc) rearrangements and Y chromosome lineages
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Complete deletions of Azoospermia Factor c (AZFc) region are common genetic cause of male infertility, while the contribution of partial deletions and duplications to spermatogenic impairment is still controversial. Some studies suggested that Y chromosome background affects the formation of AZFc rearrangements and contributes to spermatogenic failure. The aim of our study was to investigate the association of AZFc rearrangements and Y chromosomal lineages among 474 men from R. Macedonia (328 Macedonians, 110 Albanians and 36 of other ethnicity). AZFc rearrangements were determined by STS markers and gene dosage analysis. Y lineages were assigned using multiple SNApshot of 26 Y-STRs and fluorescent PCR of 17 Y-STRs. Five different AZFc rearrangements (b2/b4, gr/gr and b2/b3 deletions and b2/b4 and gr/gr duplications) were defined for b2/b4 and gr/gr deletions and 16 different AZFc haplogroups were detected, of which five [E1b1b, 12a1(x12a1a), R1a, R1b and J2b] were present in 85% of the studied men. The presence of any AZFc rearrangement was positively associated with R1a (p=3.5x10-19) and negatively with R1b (p=5.88x10-5) and J2b (p=8x10-3) haplogroups. Most of the b2/b4 duplications were found on R1a, with a higher frequency among Albanians than Macedonians. The b2/b4 and gr/gr deletions were detected on different haplogroups with gr/gr present almost exclusively among Macedonians. B2/b3 deletion was present in four men with E1b1b and in the only two men with N haplogroup. In conclusion, determination of both AZFc rearrangements and Y chromosome lineages in ethnically matched populations may improve our understanding of their role on male infertility.

P17.09-S
Association BDNF Val66Met genotype and personality traits in healthy female subjects
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Brain-Derived Neurotrophic Factor (BDNF), a member of the nerve-growth-factor family, plays an important role in the regulation of plasticity synaptic and seems to be involved in the expression of personality traits. The aim of this study was to understand the effects of the BDNF Val66Met functional variant on several personality dimensions assessed using self-reported instruments. We administered the Big-Five-Questionnaire (BFQ-2) and the Temperament and Character Inventory-Revised (TCI-R) for the measure of personality; the Emotional Intelligence Scale (IES) and the Emotional Quotient Inventory (EQ-I) for the measure of Emotional Intelligence construct; a new instrument for the measure of Fluid Intelligence construct (CAT-FIT). We tested the possible interactions between these variables on a cohort of 154 healthy female students recruited at the G. d’Annunzio University, Chieti. A significant and positive correlation has been observed between the BFQ-2 Agreeableness score and the BDNF 66Met carriers compared with the BDNF Val allele genotype (r = 17; p < 0.05). In addition, an association has been evidenced between BDNF 66Met carriers and the TCI-R Reward Dependence subscale (r = -0.20; p < 0.05); furthermore, the BDNF 66Met carriers was significantly associated with the TCI-R Self-Trascendence subscale (r = -17; p < 0.05). No significant association was demonstrated for the BDNF polymorphism and for any of the constructs analyzed. Our findings support the association between Agreeableness, Dependence and Self-Trascendence personality traits and the BDNF 66Met variant in female healthy subjects.
P17.10-M 
Radboud Biobank: a central facility for prospective clinical biobanking in the Radboud university medical center, Nijmegen

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Biobanking is crucial for science-based health care solutions in the 21st century. In order to improve the diagnosis, prevention and treatment of complex, multifactorial disorders and diseases a better understanding is needed of the underlying genetic and environmental pathways. Therefore, there is a growing need for large-scale biobanks to provide samples for bioresearch. The Radboud university medical center is building a central biobank facility to support disease-based biobanks to optimize the use of biomaterial. The Radboud Biobank aims to collect high quality biomaterial and its descriptions (patient, disease specific and phenotypic data) and subsequent data (genotypic data, microarray gene expressions).

For all patient groups included in the Radboud Biobank different sample types are being stored, however, DNA is stored for all groups. The isolation and storage of DNA-samples takes place at the department of Human Genetics of the Radboudumc. An automated sample flow for sample handling, storage and retrieval of all DNA-samples is present. DNA is stored in an automated -20 storage system. This system will assure sample security over the long term, sample integrity and efficient and quality based sample management. At the moment DNA-samples are available of among others, the following patient groups: individuals with a confirmed form/an increased risk of colorectal cancer, type 2 Diabetes Mellitus, Crohn’s disease/ulcerative colitis, venous thrombosis, or with rheumatoid arthritis/arthrosis, neurodegenerative disorders, type 2 Diabetes Mellitus, CROHNS disease/ULCERATIVE colitis, leukemia/multiple myeloma/lymphoma and related disorders, chronic (progressive) renal failure and patients who have had a cerebrovascular vascular accident (18-50 years). There are also DNA-samples available of healthy individuals.

P17.11-S 
Establishing of national birth defects registry in Thailand

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Background: Deaths attributed to birth defects are a major cause of infant and under-five mortality as well as a lifetime disability among those who survive. In Thailand, birth defects contribute to 21% of neonatal deaths. In 2012, Queen Sirikit National Institute of Child Health has initiated a Birth Defects Registry to capture birth defects among newborn infants.

Methods: The birth defects data come from 4 main sources: National Birth Registry Database, National Health Security Office’s reimbursement database, Online Birth Defect Registry Database designed to capture new cases detected later; and birth defects data from 20 participated hospitals. All data are linked by unique 13-digit national identification number and International Classification of Diseases (ICD)-10 codes. This registry includes 19 common structural birth defects conditions and pilots in 20 hospitals. The registry is a hospital-based, hybrid reporting system, including only live births whose information will be collected up to 1 year of age. Results: During the first year of implementation, 20 hospitals from 16 provinces participated. A total of 3,696 infants were diagnosed as having congenital anomalies among 67,813 live births. The prevalence rates (per 1,000 live births) of major anomalies were 26.12. The 5 most common birth defects were congenital heart defects, limb anomalies, cleft lip/ cleft palate, Down syndrome and congenital hydrocephalus.

Conclusions: Information obtained from the birth defect surveillance is the essential in the planning for effective intervention. We suggest that this program should be integrated in the existing public health system to ensure sustainability.

P17.12-M 
Results of exome sequencing of familial patients with non-HHT Brain Arteriovenous Malformations

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Background: Brain arteriovenous malformations (BAVMs) are a tangle of poorly formed blood vessels. Patients with Hereditary Hemorrhagic Telangiectasia (HHT), a disease caused by mutations in AKTI, ENG or SMAD4, often have BAVMs. We sought to identify novel functional variants that may contribute to non-HHT BAVMs.

Methods: Exome sequencing was performed on 5 unrelated families (8 non-HHT BAVM samples, 2 BAVM-free controls). Illumina Trueseq exome capture kit was used for exome capture and sequencing was performed on the Illumina HiSeq2000. Reads were mapped to the reference human genome with (BWA). Variants were called using (GATK) and annotated using (Annovar).

Conclusions: We identified several genes bearing novel potentially pathogenic variants in multiple families in pathways relevant to BAVM. Experiments are needed to verify these findings and determine the potential role of these genes in BAVM pathogenesis.

P17.13-S 
Variation in mutation spectrum partly explains regional differences in breast cancer risk of female BRCA mutation carriers in the Netherlands

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Background: Previously, relatively high cancer risks were observed in BRCA2 mutation carriers (BRCA2 carriers) over 60 in the Northern Netherlands. We aimed to quantify these regional differences in the breast cancer risk, and analyzed whether they could be explained by mutation spectrum or population background risk.

Methods: This consecutive cohort study included all known BRCA1/2 carriers in the Northern Netherlands (N = 1,050). Carrier and general reference population were: BRCA1/2 carriers in the rest of the Netherlands (N = 2,013) and the general population in both regions. Regional differences were assessed with hazard ratios (HR) and odds ratios (OR). HRs were adjusted for birth year and mutation spectrum.

Results: All BRCA1 carriers and BRCA2 carriers under age 60 had a significantly lower breast cancer risk in the Northern Netherlands, HR were 0.66 and 0.64, respectively. Above age 60, the breast cancer risk in BRCA2 carriers in the Northern Netherlands was higher than in the rest of the Netherlands (HR = 3.99, 95%CI 1.11-14.35). Adjustment for mutation spectrum changed the HRs for BRCA1, BRCA2 <60 and BRCA2 >60 years by -3%, +5% and +11%, respectively. There was no difference in background breast cancer incidence between the two regions (OR = 1.03, 95%CI 0.97-1.09).

Conclusions: Differences in mutation spectrum only partly explain the regional differences in breast cancer risk in BRCA2 carriers, and for an even smaller part in BRCA1 carriers. Impact: The increased risk in BRCA2 carriers over 60 may warrant extension of intensive breast screening beyond age 60.

P17.14-M 
Immunochip SNP effect on candidate gene expression in Celiac Disease

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Celiac disease (CD) is an immune mediated disorder caused by intolerance to ingested gluten that develops in genetically susceptible individuals. The major genetic risk factor is HLA but it is not sufficient to explain all genetic susceptibility. GWAS and IMMUNOCHIP identified 39 non-HLA regions associated with CD, and a long list of candidate genes mapping these regions has been proposed. We aimed to analyze the influence of the associated SNPs in proposed candidate gene expression in the intestinal mucosa of active/treated CD patients and controls. 14 control individuals and 9 sample pairs from the disease group with genotype and expression data were included in

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Genetic adaptation of the human circadian clock to day-length

The narrow sense heritability of CKD was 0.23 (P < 0.05). We created genetic risk factors to predict the risk of CKD in Korean populations. We genotyped or imputed based on the 1000 Genomes data after adjusting for population stratification and other confounding factors in GWAS. The linear mixed models (LMM) have been proposed to control for population stratification and other confounding factors in GWAS. In this study, Merlin 1.1.2 was used for the association test, performed independently in 17 different countries including universities, research centres and private companies offering their expertise in science, technology, ethical and legal aspects. The partnering biobanks include 20 large cohorts from which high quality biospecimens and data are available. BBMRI-LPC offers funding and administrative support for access to these cohorts to research investigators throughout Europe and EU associated states, in an open call followed by competitive, peer-reviewed selection. The scientific call undertakes study proposals in the field of common chronic diseases including cardiovascular disease, Type II diabetes and cancer, and is open from May till 15 July 2014. The poster will present the specifics of the call, which offers a unique opportunity for scientists to carry out innovative research projects by EU-funded access to the unprecedented collection of European cohorts.

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Risk assessment of chronic kidney disease based on linear mixed models in Korean populations

Some years ago we suggested to estimate total pathogenic allele frequencies in autosomal recessive disorders results in a biased estimate of this frequency. We assume that a hyperparameter q is proportional to a certain observed frequency. The hyperparameter q is used to estimate the proportion of risk SNPs for schizophrenia and/or bipolar disorder correlated with a photoperiod. Notably, many risk variants showing signals of photoperiod-driven selection map to genes with strong evidence of involvement in circadian rhythm regulation. Thus, human populations adapted to life at different latitudes by tuning their circadian clock systems. This process also involved risk variants for neuropsychiatric conditions. Data herein suggest possible genetic modulators for chronotherapies and candidates for interaction analysis with photoperiod-related environmental variables.

Using mean inbreeding coefficient to estimate total pathogenic allele frequency in autosomal recessive disorders results in a biased estimate of this frequency.

Using mean inbreeding coefficient to estimate total pathogenic allele frequency in autosomal recessive disorders results in a biased estimate of this frequency.
High frequency of hypotokietic hygrocystsoma associated CPT1A mutation in Northeast Siberia caused by positive selection

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Cystic Fibrosis (CF) is the most frequent severe inherited disease among Caucasians, having prevalence of 1:3000 and a carrier frequency of 1:27 in Italy. Three mutations in CFTR gene (F508del, N1303K, G542X) represent approximately 60% of mutated alleles, while a multitude of additional mutations exhibit a low prevalence. In addition, the distribution of individual mutations varies throughout the country and several mutations are peculiar to some regional areas. In the literature, the analysis of a unique network involved in cellular development, death and survival. The independent sample. Genes closest to the replicated SNPs were included in GWAS of CRIPTO serum levels in two isolated villages from Cilento area in Southern Italy. The results suggest that cystic fibrosis is a multifactorial disease, with a genetic contribution, which is likely to be found in the variability of serum levels of CRIPTO in humans and the genetic contribution underlying this variability remains still unknown. We performed these studies have shown conflicting results. Therefore, we performed this meta-analysis to derive a precise estimation of this association. Studies were searched from PubMed up to January, 2014 and were eligible if they included case mothers (CM) that have birth to children with abnormality. Pooling odds ratios (ORs) with 95% confidence intervals were estimated by using allelic, dominant, recessive and codominant genetic models, Hardy-Weinberg equilibrium (HWE) and ethnicity. Eight case-control studies (987 cases and 1,334 CM) were included. No association evidence has been found, neither in the overall analysis, nor in the stratified analysis by ethnicity (ORs with P > 0.05). The shape of funnel plots was symmetrical in almost all genetic models. There is no evidence of heterogeneity, the genotype distributions of the controls of all studies were consistent with HWE and sensitivity analysis indicated robustness of our results. Taken together, our meta-analysis suggested that MTR A2756G polymorphism did not contribute as an independent risk factor of DS.
Food preferences are the first factor driving food choice, nutrition and ultimately diet-related diseases. To understand the genetic component of food liking and in understanding the genetics of human nutrition in general.

Results: We have developed a new R-package called Fast Family-Based Sequence Kernel Association Test (FFBSKAT) to perform kernel machine-based regression association analysis of genetic variants with a continuous phenotype. In the development of FFBSKAT, we applied several analytical and algorithmic improvements to speed up the analysis without any loss of accuracy. We compared the performance of our software with that of two existing software for family-based sequence kernel association testing, namely, ASKAT and fastSKAT, using the Genetic Analysis Workshop 17 family sample. Results demonstrated that FFBSKAT is several times faster than other available software packages, while being similarly accurate. With respect to the available analysis modes, we combined the advantages of both ASKAT and fastSKAT and added new options to empower FFBSKAT users.

Conclusion: The FFBSKAT package provides fast, accurate, and easy-to-use method to perform kernel machine-based regression association analysis of quantitative traits in samples of related individuals. The FFBSKAT package, along with its manual, is available for free download at http://mgb.ub.net/nsc.r/soft/FFBSKAT/

This work was supported by RFB grants №13-04-00272-a.
of genetic introgression and were able to differentiate between individuals with a few small exogenous regions in their genome, and those with long exogenous haplotypes covering a large part of the genome. We found that the pattern of homozygosity was very similar to that of other European populations and identified an individual carrying a chromosome 1 uniparental disorder. Overall, there is very limited evidence for geographic differentiation or stratification of the GS-SFHs sample within Scotland. These findings provide a genetic perspective on the history of the Scottish population, and have implications for further analyses, such as studying the contributions of common and rare variants to trait heritabilities and evaluation of genomic and phenotypic prediction of disease.

P17.30-M
Identification of mutations in the GLI2 gene in Combined Pituitary Hormone Deficiency (CPHD) in Italian patients
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The GLI2 transcription factor is a major effector protein of the sonic hedgehog pathway and it is suggested to play a key role in pituitary development. GLI2 mutations cause holoprosencephaly or holoprosencephaly-like features. In some cases heterozygous GLI2 mutations have been associated with hypopituitarism without other anomalies. However GLI2 is not routinely screened in pituitary hormone disorders. The aim of this study was to determine the frequency of GLI2 mutations in patients with combined pituitary hormone deficiency (CPHD) in Italian patients that resulted negative for mutations in other causative genes (PIT1, PROP1, HESX1, LHX3,LHX4).

In this analysis, we screened the entire gene in 75 CPHD patients. Primers were specifically designed for the 13 coding exons that were analysed by direct sequencing in 18 separate fragments. We identified two novel missense mutations: c.731A>T in exon5 leading to the amino acid substitution p.Asp244Val and c.1157C>T in exon7 resulting in the change p.Pro386Leu. These two variants were absent in about 13.000 individual present in the Exome Variant Server. Both these mutations were predicted as probably damaging using the online tool Polyphen v2, with a high score (1.00 for p.Asp244Val and 0.923 for p.Pro386Leu). They fall within the NH2 terminal repressor domain and an in vitro functional analysis will help to clarify their significance.

These preliminary results are encouraging and we are planning to increase the number CPHD patients and extend the analysis also to Isolated Growth Hormone Deficiency (IGHD).

P17.31-S
A Genome-wide Association Analysis of Lipid traits in a sample of indigenous population from Mexico
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Given the high prevalence of different forms of dyslipidemia in Mexican population, in contrast to European populations, it has been suggested that genetic susceptibility in this population is probably related to its indigenous component as a result of adaptive processes related to energy savings. It is therefore of great importance to identify the genetic variation of the native Mexican population. In this study, 319 individuals from four indigenous populations (174, 70, 25 and 50 from Nahua, Maya, Totonac and Zapotec, respectively) were included. Genotyping was performed using Affymetrix SNP 6.0 microarray. The analysis included a local ancestry estimation to remove segments inferred to be of European origin and mixed linear models using EMMAX, adjusted for age, gender and two principal components. Two steps were performed; first a global-FDR to select the most strongly associated SNPs in independent (non-redundant) regions of the genome discording correlated surrogates in the same LD region. We assessed the sensitivity and specificity of our single-step and sequential SNP-selection strategies. The methods were also validated against known associated regions described published GWAS data from the Collaborative Association Study of Psoriasis.

P17.32-M
Selecting significant SNPs in Genome-wide Association Studies using a global False Discovery Rate
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Standard methods for assessing significance in Genome-wide Association Studies (GWAS) are generally too stringent as they are based on controlling the Family-Wise Error Rate. The FDR (False Discovery Rate) approach provides a more realistic and less stringent alternative. However, controlling the FDR for correlated entities is difficult without making specific assumptions on the correlation structure that may not be realistic. In this study, we define an FDR criterion (global-FDR) for assessing the statistical significance of SNPs in GWAS accounting for correlation (Linkage Disequilibrium) between neighboring SNPs. The criterion extends the local-FDR criterion of Efron and ensures control of the False Discovery Proportion (FDP) under arbitrary correlation structure. We develop a fast and easily scalable Gibbs-type iterative algorithm to approximate the posterior class membership probabilities that are otherwise hard to compute in a general. Unlike the non-parametric estimation approach used in local-FDR, we use a parametric three-group normal mixture model for SNP Z-scores with a known correlation matrix. Using simulations under different correlation scenarios, we found that the global-FDR approach performs significantly better than local-FDR, both in terms of power and accurate control of the type-I error (FDP). We also considered a sequential procedure to select the most strongly associated SNPs identified in independent (non-redundant) regions of the genome discording correlated surrogates in the same LD region. We assessed the sensitivity and specificity of our single-step and sequential SNP-selection strategies. The methods were also validated against known associated regions described using published GWAS data from the Collaborative Association Study of Psoriasis.
For a set of basepairs flanking a candidate locus, we calculate Hamming Distance Ratio (HDR, proportion of basepairs differing between two individuals) for all pairs of individuals (an affected family member and 41 control individuals) and distinguish pairs containing the affected (group 1) from those that do not (group 2). We assess the difference in distribution of HDR values between the two groups by the Kolmogorov-Smirnov statistic at sets of variants 10KB to 100KB around each of ~600 candidate variants. In two hypertrophic cardiomyopathy families, known pathogenic mutations were previously detected: c.1737G>A (p.R582Q) in MYL2 gene [rs10489436, family A] and c.7746A>G (p.R249Q) in MYH7 gene [rs3218173, family B]. We combine results over the 10 regions with suitable test statistics evaluated in permutation analyses and are able to narrow down known disease variants as small as 5% (rank as small as 29 out of 606 candidates, p=0.0001). In a negative control, a restrictive cardiomyopathy pedigree with de novo mutation in the TNNT3 gene in one affected sibling, TNNT3 mutation was ranked 22nd 10 out of 631 variants.

Our new statistical method for prioritizing disease region by Hamming distance between affected family member and unrelated controls will be useful for small AD pedigrees and for differentiating AD and de novo mutations.

**P17.35-S Mitochondrial DNA variation analysis in historical provinces of Romania**

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Since the Middle Ages, Romanian population lived in three distinct provinces: Wallachia, Moldavia and Transylvania until the 19th century. Over the centuries, their territories were repeatedly invaded by different peoples and subjected to external political influences resulting in demographic changes that could affect the genetic structure of populations. We performed mitochondrial DNA analysis in order to visualize the relationships between Romanian and other populations Europe based on HVS1 and HVS II mtDNA sequence data using Sanger sequencing. We presented here a large scale mtDNA analysis of 612 Romanians from these historical provinces of Romania. Multidimensional scaling (MDS) plot was constructed from the pairwise Fst values. The results showed that present day Romanians in all provinces share their maternal ancestry with both eastern/central European and Balkan populations. The three populations of Romania analyzed here exhibit slightly different mtDNA lineage compositions, mainly consisting of the haplogroups H, U, J, T, K, N and W, with significant frequency differences corresponding for H, U and W haplogroups. H haplogroup accounts for 47% in Moldavia, 35% in Wallachia and 33 % in Transylvania. Overall, this study provides a first descriptive analysis of unique mtDNA genome variation in Romania revealing the existence of different degrees of provincial differences of haplogroup frequencies.

**P17.36-M Heritability of age related cognition**

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The role of genetic and environmental factors in specific cognitive abilities in the elderly is poorly understood. Here we estimated their contributions to variation in the cognitive domains memory, executive function and fine motor skills and tested the genetic correlations between these domains. The study was embedded in the Austrian Stroke Prevention Study (ASPS), a population-based cohort study (n=479, mean age=64.6 years, 54.9% women) and in the ASPS-Family including relatives of the ASPS participants (n=376, 177 families, mean age=63.6 years, 60.1% women). The neuropathological test battery included Bäumler’s Lern- und Gedächtnistest, Trail Making B, Digit Span Backward, the Wisconsin Card Sorting and the Perdue Pegboard Test. Heritability was estimated by variance component analyses. We found no genetic correlation between executive function and fine motor skills, the environmental correlation was 37%. There is a substantial heritability of memory and fine motor skills and a moderate of executive functions. Half of the heritability of executive function and fine motor skills can be explained by common SNP. Our results support shared genetic factors for executive function and memory as well as fine motor skills.

**P17.37-S Involvement of HMGA2 (High-Mobility Group A2) in Idiopathic Short Stature (ISS).**

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Several lines of evidence point to HMGA2 as a candidate gene in ISS: i) independent GWAS identified some SNPs on chromosome 12q14 in the vicinity of HMGA2 (rs1042725, rs7968682 and rs7968902) as one of the major determinants of height; ii) microdeletions on 12q14 have been identified in syndromic patients with short stature as common feature. Among these, one patient with only severe growth retardation carried the smallest deletion encompassing HMGA2. The aim of this study was to investigate the involvement of HMGA2 in ISS through the search for mutations/deletions and perform an association study between the HMGA2 SNPs and ISS. One hundred-four patients (48 males and 56 females) with height ranging from -3.8 and -2 SDS were analyzed by direct sequencing and MLPA. None of the patients carried mutation/deletion in the coding sequences and intron/exon boundaries. The same 104 patients and 330 normal stature matched controls were analyzed in an association study with rs7968682. The allele frequency was significantly different between patients and controls (p=4×10⁻⁴). When the genotypes were considered the TT genotype showed an even stronger association (p=6×10⁻⁵). In conclusion, our cohort of ISS patients did not show any pathological mutation in HMGA2, suggesting that high penetrance mutations are not a frequent cause of ISS. However our preliminary results suggest that HMGA2 might be involved in the susceptibility to ISS. We are thus planning to replicate the data in an independent cohort and analyze the tag SNPs surrounding HMGA2 to identify the variations directly responsible for the association.

**P17.38-M Thioredoxin reductase 1 (TXNRD1) gene variability influences quality of aging and longevity**

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Oxidative stress is a major determinant of human aging and a common hallmark of age-related diseases. A protective role against free radicals accumulation has been shown for Thioredoxin (Trx) system and in particular thioredoxin-reductase TrxR, a key selenium protein antioxidant enzyme, able to reduce Trx and other substrates, detoxifying cells from oxidative injuries. An effect of this antioxidant system in human aging can be hypothesized from the association reported between TrxR gene (TXNRD1) with late-life survival in a Northern-European nonagenarian cohort (Soerensen et al, 2013). Using a tagging approach, we investigated the association of 14 SNPs with longevity and quality of aging, by analyzing their variability in relation to markers of functional (Activities of Daily Living, ADL; Hand Grip, HG; Walking speed, WS) and cognitive (Mini Mental State Examination, MMSE) status, in a Southern-Italian elderly cohort (626 subjects, age range 65-104 years). The work confirms the association of TXNRD1 gene with human survival, with two intronic SNPs, rs7310055 and rs4964728, showing association with longevity (p<0.04). Furthermore, three other SNPs, two intronic (rs7962423, rs10861203) and one located in the promoter region (rs1128446), were significantly associated with ADL and HG (p<0.05). Haplotype analyses confirmed the single-SNP results. Moreover, bioinformatic analyses indicated the associated SNPs as putative regulatory sites, whose function should be further experimentally investigated. On the whole, this study confirms a role of Trx antioxidant system on human age-related functional decline and longevity, possibly mediated by modulation of oxidative stress.

**P17.39-S Genetic analysis of thyroid peroxidase (TPO) gene in patients whose hypothyroidism was found in adulthood in West Bengal, India**

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Recent research has revealed that genetic defects due to mutation in the Thyroid Peroxidase (TPO) gene can lead to thyroid dysfunction in the population. We aimed to study the association between genetic defects in TPO and hypothyroidism was found in adulthood in West Bengal, India.
P17.40-M

Genes involved in interleukin-1 receptor type II (IL1R2) activities are associated with asthma related phenotypes

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We previously demonstrated that IL1R2 (Interleukin-1 receptor type-II) is overexpressed in bronchial biopsies of allergic asthmatic individuals and we also associated it with atopy (p = 5.50). This study aims to test for the effect of SNPs and interactions between 182 SNPs belonging to nine genes involved in IL1R2 activities on asthma related phenotypes in the Saguenay-Lac-St-Jean familial asthma collection (SLSJ) and in French families of the Epidemiological study on the Genetics and Environment of Asthma (EGEA).

Single SNP analysis was performed using Family-Based Association Tests (FBAT) software (FBAT). In order to correct for multiple tests, we used a potential risk genotype (p = 0.006; Odds ratio = 1.95; 95% CI: 1.2-3.15) for the disease while another SNP Asp666Asp (c.1998T allele) showed protective tendencies towards the disease (p = 0.006; Odds ratio = 0.67; 95% CI: 0.50-0.89).

To our knowledge, this is the first study reporting the role of IL1R2 gene with hypothyroidism in a population of Asian Indian origin. The study threw up the possibility of TPO gene polymorphisms as a possible pathogenic mechanism of hypothyroidism.

P17.41-S

SHOX gene and patients with hypothyroidism found in adult age. Two hundred consecutive treatment naïve hypothyroid patients (age ≥ 18 years) (cases) who were negative for anti TPO antibody and their corresponding sex and age matched two hundred normal individuals (controls) were enrolled. The 17 genomic regions of the TPO gene were amplified and sequenced directly. We identified 6 different previously known single nucleotide polymorphisms (SNPs) and 2 novel deletions in TPO gene. Two of the six SNPs revealed a significant association with hypothyroidism; Thr725Pro (rs732609) and Asp666Asp (rs1126797). The c.2173C allele of the Thr725Pro in TPO showed a significant association among hypothyroid patients compared to controls (p = 0.01; Odds ratio = 1.45; 95% CI: 1.09-1.92) suggesting it to be a potential risk factor for disease predisposition. Analysis of genotype frequencies of the polymorphism between the two groups demonstrated CC as a potential risk genotype (p = 0.006; Odds ratio = 1.95; 95% CI: 1.2-3.15) for the disease while another SNP Asp666Asp (c.1998T allele) showed protective tendencies towards the disease (p = 0.006; Odds ratio = 0.67; 95% CI: 0.50-0.89).

To our knowledge, this is the first study reporting the role of TPO gene with hypothyroidism in a population of Asian Indian origin. The study threw up the possibility of TPO gene polymorphisms as a possible pathogenic mechanism of hypothyroidism.
Molecular screening of major thalassemia mutations observed in 2012–2013 in eastern Sicily

In this study we evaluated the incidence of alpha, beta and delta thalassemia in 2012–2013 in eastern Sicily

P17.44-M
Study homozygosity disequilibrium in human genome using the whole-genome sequencing data

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Homozygosity disequilibrium (HD), a non-random sizable run of homozygosity in the human genome, has been found related to the evolution of populations and able to confer susceptibility to diseases. In this study, we characterized HD in global populations based on the whole-genome sequencing data from 14 populations of the 1000 Genomes Project. The whole-genome homozygosity intensity was estimated by using L0HAS (Yang et al. Genetic Epidemiology, 2011). Using the homozygosity intensity we identified common genomic regions undergoing HD. We found a high proportion of regions of HD to be population-specific but also identified functionally important regions of HD shared by multiple populations. Genetic differentiation of global populations can be characterized using the patterns of HD. In summary, this large-scale whole-genome sequencing study derives the distribution of HD in the human genome and proves that HD carries genetic information of human population important for studying genetic background. The information also provides important clues for the differential disease prevalence and drug responses in populations.

P17.45-S
A signal near FRMD4A is associated with lower extremity arterial disease in patients with type 2 diabetes in GoDARTS

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Lower extremity arterial disease (LEAD) is a common macrovascular complication of type 2 diabetes (T2D). Twin studies of ankle brachial index (ABI), the main diagnostic criterion of LEAD, estimate the heritability of ABI at ~30%. Two genome-wide signals have been identified for LEAD near CHRNA3 and 9p21.3 irrespective of diabetes status. The aim of this study was to identify genetic determinants of LEAD in patients with T2D. T2D cases were patients with T2D and an ABI < 0.9 or ABI>1.3 and or mid-thigh to mid-foot amputations and or corrective procedures related to LEAD and or prescriptions for medication used to treat claudication. Controls were patients with T2D free of LEAD, coronary artery disease and ischaemic stroke. Allelic effects of 5,886,833 SNPs estimated from 1223 LEAD cases and 5638 LEAD free controls were combined in fixed-effects 1000G meta-analysis. A signal represented by rs72780858 near FRMD4A reached genome wide significance (p=3.7E-9). SNPs in FRMD4A have been associated with nicotine dependence which may influence smoking status. Smoking is known to increase the risk of LEAD 10 fold. Other suggestive signals are located near genes that contain genome wide significant hits for risk factors related to LEAD: rs271946 (p=8.1E-6) near PCSK1 (T2D); rs34562 (p=8.2E-6) near EFNAs (Coronary artery disease) and rs75417257 (p=1.2E-6) near GSER1 (Gluomeler filtration rate and albumin excretion rate). This is the first study of the genetic determinants of LEAD in patients with T2D and several signals including one at genome wide significance were identified.

P17.47-S
Molecular screening of major thalassemia mutations observed in 2012–2013 in eastern Sicily

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In this study we evaluated the incidence of alpha, beta and delta thalassemia in the province of Messina, in the past two years. 236 people were analyzed (Medical Genetics Laboratory of Genetics and Immunology Pediatric UOC), after healthy carrier screening of I and II level of Thalassemia by the diagnostic procedure that provides for the CNC, the determination of the hemoglobin fractions and the assessment of iron status. The phenotypic framework (classical and non classical), who had hematological parameters indicative of mutations at the level of alpha clusters and/or non-alpha-globin, were studied by molecular analysis of related genes. This investigation has shown that in the alpha globin gene, the -α/α (genotype frequency 13.1%; allele frequency 7.83%) is the most common genotype found, with the 6914C>A (Bh Fitzory)/WT (genotype frequency 0.42%; allele frequency 0.21%) and the 6112T>W (genotype frequency 0.42%; allele frequency 0.21%) genotypes. These data show the importance of a careful assessment about haematological indices to carry out a correct diagnosis of genetic defects.
A common functional polymorphism in miR-196a2 is associated with risk factors and disease.

One-year increase in the genetic score (range 2.3 - 3.1 years) associated significantly with future diabetes in women (N=2831, hazard ratio=1.46, P=0.0083) but not in men (P=0.3). Interestingly, the genetic score associated nominally (P<0.05) with prevalent diabetes in men (N=5547, odds ratio=0.87, P=0.042) but no association was seen in women. In women nominal association of higher menopausal age score was seen also with higher triglyceride levels and lower HDL cholesterol. Controlling for BMI and smoking did not affect these associations.

Our results suggest that the generic variants associated with the timing of menopause in women have different effects on metabolism in different genders. In women polymorphisms raising menopausal age raise also the risk of future diabetes 1.5-fold whereas in men the same variants have a slight protective effect on prevalent diabetes.

P17.51-S Exome-wide association analysis of 4,522 individuals from the Oxford Biobank study reveals novel low frequency variants influencing human serum metabolite levels

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Small molecule metabolites are intermediates in disease pathways, and identification of genetic variants influencing metabolite concentrations could provide insight into disease pathogenesis. Common variant effects have been thoroughly scrutinised, but the impact of low frequency (LF) variants (minor allele frequency (MAF) <5%) remains largely unexplored. Here we report an exome-wide association study of metabolite concentrations in 4,522 healthy individuals (age 29-53 years, 55% women) from the population-based Oxford Biobank study. Approximately 230,000 markers were genotyped using the Illumina Human1Mome Beadchip and tested for association with 123 metabolites (80 lipoproteins, 15 lipids, 22 low molecular weight metabolites, and 6 ratios related to fatty acid saturation) quantified by nuclear magnetic resonance of serum samples. After log normalisation, inverse normalised residuals were generated for adjusting age, gender, and principal components. A linear mixed model was used assuming an additive genetic effect. In the single marker analyses, we identified 15 loci with genome-wide significant associations (p<5x10^-8) for one or more metabolites. For two of the loci, the strongest signal involved a LF marker. We also performed gene-level tests: using SKAT for protein-altering variants (minor allele frequency (MAF) <1%) we identified two novel genes (p<2x10^-6) associated with alani ne (P116, p=4x10^-7) and glycoprotein acetyl (C8A, p=2x10^-6), both of them driven by single variants with large effects. An analysis focused on protein-truncating variants (using SEGMEM) revealed a significant association between COQ10A and glutamine (log10 (Bayes factor)=4.25). The identification of genetic variants influencing metabolite concentrations will allow us to explore the causal relationships between these metabolites, other metabolic risk factors and disease.

P17.58-S A common functional polymorphism in miR-196a2 is associated with waist to hip ratio

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MicroRNAs (miRNAs) are small non-coding RNAs that function as crucial regulators in a broad range of biological processes. They have recently gai ned extensive attention as mediators of complex disorders and highlighted as promising diagnostic biomarkers for cardometabolic diseases. Given the central role of miRNAs in gene expression, genetic polymorphisms in miRNA genes are expected to alter miRNA processing and function that may contribute to disease susceptibility. Here we retrieved 78 single nucleotide polymorphisms (SNPs) in all pre- and mature miRNA sequences and systematically investigated their association with 17 cardometabolic traits. We used as benchmark the largest meta-analyses of genome wide association studies (GWAS) on glycemic hemostasis indices, lipid traits, blood pressure, coronary artery disease, type 2 diabetes, and anthropometric measures including information up to 133,000 individuals. We found that a common variant in miR-196a2 (rs1164913: T>C) is significantly associated with waist to hip ratio (WHR) (p-value = 3.4x10^-10). To explore whether this miRNA affects WHR, we examined the association of all miR-196a2 target genes with this phenotype and revealed SFMBT2 to be significantly (p-value = 2.8x10^-4) and SMAD6 is suggestively (p-value = 6.9 x 10^-3) associated with WHR. Additionally, a trans-expression quantitative trait loci (eQTLs) analysis in 762 subjects of the Rotterdam Study suggested an increased trend in the expression levels of SFMBT2 and SMAD6 in individuals carrying the risk allele. These findings may help improve our understanding of the regulatory role of miRNAs in fat distribution.
variants contributing to MS susceptibility in an Italian multiplex family. DESIGN: SNP microarray genotyping and whole-genome sequencing (WGS) in 4 MS patients and 4 unaffected individuals belonging to an Italian multiplex family descending from a first cousin marriage were performed. RESULTS: We identified a high number of variants in each individual, two of which underlie one of the two LOD peaks on chromosome 8p21.2 and 11q23.3. The first one is in the OR8G5 gene, an olfactory receptor gene under positive selection, while the second one falls within the GRAM18 gene, causing an aminoacid substitution (S601P). This second variant is not present in dbSNP and in the 1,000 Genome project, and segregates within the family, being homozygote in 3 affected and heterozygote in the left MS patient. Sanger sequencing confirmed the segregation with the disease. GRAM18 is a very conserved gene from yeast to human. It encodes a membrane protein, which is part of the GRAM containing domain family protein. In the mouse it is highly expressed in the CNS and in specific immune cell subtypes, like dendritic cells and neutrophils. CONCLUSIONS: The use of WGS in an Italian MS multiplex family has been successful in identifying novel rare genetic variants. Further investigations are ongoing to explore the role of the variant on protein function and its involvement in MS.

P17.57.6 Simultaneous estimation of the locations and effects of multiple disease loci in case-control studies
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The genetic basis of complex diseases often involves multiple linked causative loci. Under such a disease etiology, assuming one disease locus in linkage disequilibrium mapping is likely to induce bias and lead to efficacy loss in disease locus estimation. An approach is needed for simultaneously localizing the positions of multiple functional loci. However, due to the increasing number of parameters accompanying disease loci, these estimates can be computationally infeasible. To circumvent this problem, we propose to estimate the main and gene-gene interaction effects and a nuisance parameter at the disease loci separately through a linear approximation. Estimates of the genetic effects are estimated using a generalized estimating equation to estimate the disease loci, and the procedure is conducted iteratively until convergence. The proposed method provides estimates and confidence intervals (CIs) for the disease loci, the genetic main effects, and the interaction effects between loci, with the CIs for the disease loci providing useful regions for further fine-mapping. We apply the proposed approach to a data example of case-control studies. Results of the simulations and data example suggest that the developed method performs well in terms of bias, variance, and coverage probability, regardless of the underlying number of disease loci.
Novel mutations described in Saudi Arabia
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Jeddah is the second largest city in the Kingdom of Saudi Arabia with a population of over 5 million. The Genetic Medicine Department at King Abdul-Aziz University is the major referral center for genetic disorders in Jeddah and was established in 2005. Over this period 1842 patients and families with genetic disease were seen. Patients were assessed clinically and where indicated by genetic testing. They were categorized into the following types based on etiology: chromosomal, single gene disorders, multifactorial, mitochondrial and others. They were then further subdivided into categories. Most mutations identified were novel and different from the western literature. We will present the types of genetic diseases present and the novel mutations identified both locally and nationally and put forward recommendations for future studies including the current set up of a data bank will discussed.

Contribution of SNPs (rs9939609 and rs8057044) and haplotypes of FTO gene to the genetic risk for obesity in children from Yucatan, Mexico

The fat mass and obesity-associated (FTO) gene has been identified as a strong candidate for obesity-related phenotypes in several populations. Significant associations of the SNPs rs9939609 and rs8057044 with body mass index (BMI) and with the risk for obesity have been suggested in homozygous A/A. The central role of FTO might be through an effect on central brochogenic insulin sensitivity. Each FTO risk allele increases BMI by 0.26 to 0.66 units (kg/m2), and the odds of being obese by ~1.3. In this study, we evaluated the association of the SNPs (rs9939609 and rs8057044) and haplotypes of FTO gene with the risk for obesity in children from Yucatan, Mexico; where child obesity is the first cause of morbidity. We included 155 obese children, and 189 non-obese healthy children under a case-control association study. Genotype and allele frequencies between cases and controls were compared using SNPstats software. Genotype and allele frequencies were distributed according to Hardy-Weinberg expectations (p>0.05) in cases and controls, except for rs9939609-FTO in controls (p<0.05). Significant differencies were found for the heterozygous AT genotype of the SNP rs9939609 (p= 0.03) between cases and controls, suggesting that the heterozygous AT genotype of rs9939609 might be a genetic risk factor associated with child obesity in the population of Yucatan. No significant associations were found for the SNP rs8057044 nor for the haplotypes of FTO gene (p>0.05). However homozygotes for the A allele showed a higher mean BMI and higher waist circumference than TT or GG allele carriers.

Albinism is a heterogeneous group of inherited genetic diseases present at birth. This disorder can affect all ethnic backgrounds with an overall prevalence of approximately 1/17,000 people but varies between different forms of Albinism. It varies considerably worldwide. It can be classified as Oculocutaneous Albinism (OA1) caused by mutations in the PYPHI genes, in X-chromosome or as Oculocutaneous Albinism (OA1) an autosomal recessive inherited condition. OA1-4 forms are recognized based on the expression of mutations in four different genes: TYR, OCA2, TYRP1 and SLC45A2. A cohort of 158 OCA or OA1 subjects were recruited from the Medical Genetic Unit and Department of Pediatric Ophthalmology of Niguarda Ca’ Granda Hospital of Milan (Italy) and characterized for TYR, OCA2, TYRP1, SLC45A2. Most mutations identified were novel and different from the western literature. We will present the types of genetic diseases present and the novel mutations identified both locally and nationally and put forward recommendations for future studies including the current set up of a data bank will discussed.

Targeting a gene network of ADAMTS genes in the predisposition to PS
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Pediatric stroke (PS) is an important childhood disease and several genetic polymorphisms contributing to PS risk have been established in numerous candidate gene based association studies. Recently, we have reported results of an Italian family-based association study in the predisposition to PS. To investigate possible causative gene variants in the resulting linkage disequilibrium based candidate regions; we performed a next generation resequencing approach in 48 affected children and 48 unaffected siblings. The selected target regions of about 6.8Mb comprise 42 gene regions including ADAMTS2, ADAMTS12, ADAMTS13 and ADAMTS17. Custom target enrichment was performed using the NimbleGen SeqCap EZ Choice technology. The resulting DNA libraries were paired-end sequenced (100 cycles) on an Illumina HiScanSQ instrument yielding in 300 Gb sequence data in total and 87.1% bases with a QScore > 30. Sequence reads were mapped by using the BWA algorithm and analyzed by GATK yielding in 80% median target specificity and median target region coverage of 176x. Variant annotation was done by using SNPEff and Annovar software tools. A sibship disequilibrium test was applied on the 16,586 identified variants, 4060 of which were novel, to compare the
two sample groups. 32 significant (p<0.05) coding non-synonymous or UTR
SNPs in 14 genes were identified and selected for validation using capillary
sequencing and subsequent genotyping within the full cohort of 270 nuclear
families. The resulting data may help to understand the genetic architecture
of ADAMS genes and their impact on PS.

P17.66-M
A genome-wide association study of Agreeableness suggests a novel
association in the NAV2 gene in Korean women
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Data from genome-wide association (GWA) studies have been used to find
the common variants of personality. In a previous study, we reported that
neurotransmitters and the olfactory receptor 1A2 gene are associated with
neuroticism in a cohort of young Korean women. However, many genetic
variants that are highly associated with certain personality traits are still
unknown. Here, we report on a meta-analysis of GWA data for personality
in three cohort samples (2045 individuals). All participants were of Korean
ancestry. Personality traits were measured with the Revised Neuroticism-
Extraversion-Openness Personality Inventory to assess five factors: Neuro-
ticism, Extraversion, Agreeableness, Openness, and Conscientiousness. In
either discovery stage, classical association analyses were performed under
an additive model followed by meta-analysis using the weighted inverse va-
riance method. We observed consistent direction of effect and significant
association of the NAV2 gene and Agreeableness in either the discovery and
combined stage (p=7.85×10^-7, for meta-analysis). NAV2 gene involves in
optic nerve development and sensory perception of smell and sound. We
previously reported that the sensory system may play an important role in
personality, and the present study leads to the same conclusion. The sensory
system affects personality as a filter of the acceptance system, which may
have an advantage to reconstruction.

This study was supported by a grant of the National Project for Personali-
ized Genomic Medicine, Ministry for Health & Welfare, Republic of Korea
(A111218).

P17.67-S
New genetic matching methods for handling population stratification
in genome-wide association studies
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A usually confronted problem in association studies is the occurrence of
population stratification. In this talk, we propose a decisive extension to
the Cochran-Armitage Test trend in order explicitly take into account struc-
tures obtained from matchings and clustering. We employ pairwise and
groupwise optimal case-control matchings and present an agglomerative
hierarchical clustering, both based on a genetic similarity score matrix. By
simulations of genotype data under the null hypothesis we assess our fra-
amework, in order to affirm that it correctly controls for the type-1 error rate.
By a power study we ascertain, that structured association testing using our
framework displays reasonable power. We compare the results from our
methods with those obtained from a logistic regression model with prin-
cipal component covariates. We also highlight and discuss a possible false-
positive association to Alzheimer’s disease using the principal components
approaches, which is neither reproduced by our new methods nor by the
results of a most recent large meta-analysis.

P17.68-M
The causal role of insulin-like growth factors and binding proteins in
prostate cancer: a Mendelian randomization study
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Epidemiological studies reported positive associations of circulating IGF-I
and IGF-II, and inverse associations of IGFBP binding protein 3 (IGFBP-3) with
prostate cancer risk, whilst IGFBP-2 has been associated with low grade dis-
ese. However, systematic review findings have been inconsistent which may
reflect confounding or reverse causality. We examined the causal role of
IGF-I, IGF-II, IGFBP-2 and IGFBP-3 in prostate cancer using multiple genetic
variants to construct allelic scores as instruments for measured circulating
IGFs and IGFBPs, in a Mendelian randomization approach.

We investigated 1131 SNPs, previously reported to be associated with IGFs
and IGFBPs, in ~700 population controls from the ProtecT study. We gene-
rated allelic scores that consisted of the most strongly and exclusively-as-
nociated SNPs with each biomarker. Finally, we used the allelic scores and
instrumental variable analysis to estimate the causal effect of IGFs and IGFBPs
on prostate cancer in ~40,000 cases and controls from 21 studies included in
the international PRACTICAL consortium.

IGF-I and IGF-II were positively associated with prostate cancer risk. The
estimated causal odds ratios for the effect of a standard deviation (SD) in-
crease in circulating IGF-I and IGF-II was 1.15 (95% CI 1.06, 1.26) and 1.14
(95% CI 1.02, 1.26), respectively. Notably, IGF-II increased risk for advan-
taged and high grade disease. Conversely, IGFBP-2 and IGFBP-3 were not as-
nociated with prostate cancer susceptibility. In conclusion, this Mendelian
randomization study provides additional support for a causal relationship
between IGF-I and IGF-II and prostate cancer risk.

P17.69-S
Combining different sources of information to optimise genomic
prediction of complex traits
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P17.71-S
A Script for Linkage Analysis of Rare variants
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For the past years genome-wide association analysis provided progress in detection of common genetics variants contributing to variation in common disorders and quantitative traits. However, for many traits, genome-wide significant associations did not result in the establishment of the causal underlying variants. Now, whole-exome resequencing of large population samples and formation of databases of annotated functional variants may help to solve this problem. Causal functional variants with a large effect on the variation of traits are rare in populations, but can be aggregated in families. Analysis of samples from isolated populations makes possible to detect genetically informative families where rare variants co-segregate with diseases or traits. To facilitate a search for families with aggregation of the rare variants of interest from extended pedigrees or samples from isolated populations and then to test its co-segregation with trait by the methods of linkage analysis we developed a software LARA (Linkage Analysis of Rare Allele). LARA combine commonly used package for linkage analysis MERLIN (Abecasis et al 2002) and PedCut software (Liu et al 2008) for the pedigree splitting into a set of sub-pedigrees. LARA searches for rare allele carriers, automatically generates and transfers the data, necessary for the analysis. LARA can help to test all functional variants located in the genomic regions already detected by linkage or associations methods. The software LARA is freely available at http://mga.biogenet.nsc.ru/cgi/soft/index.html.

P17.72-M
Correcting for population substructure in rare variant - rare disease association studies
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Sophisticated techniques have been developed for genome wide association studies (GWAS) to correct for population substructure that may cause substantial inflation of test statistics and spurious associations. For rare variants in spatially structured populations the existing methods do not effectively control for stratification. We developed an approach to build up a control group that is most similar to the individuals in the case group considering all exonic variants especially also the rare ones. We show with simulations of real exome data that the power of association studies for rare variants can be optimized if case-control groups are similarity-matched. We also found that the power could be further improved when we increased the control-case group ratio by adding additional exome data.

P17.73-S
Functional linear model for regional association analysis of rare genetic variants in family-based samples
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Methods based on the linear regression models are widely used for genome-wide association analysis of family-based samples. To use these models for region-based analysis, a collapsing approach is commonly used. However its power decreases if effects of causal variants have opposite directions. We developed a new method for the region-based association analysis of family-based samples, using techniques of functional data analysis. The individual discrete genotypes and effects of multiple variants in the analyzed region are considered as the continuous data, which can be described by stochastic functions constructed on the physical positions of the variants, using a finite set of basis functions. Thus, under the functional linear model, the genetic effects of the multiple variants are described by coefficients of the basis functions. All hypothesis of zero values for the coefficients are tested by standard statistical tests. We introduced a covariance matrix defined through a relationship matrix in the trait inheritance model to take into account a genetic relationship between individuals. High power of our method is provided by the simultaneous consideration of genetic information on not only genotypes of multiple variants, but also their physical positions, and by taking into account the related status of the samples. Our method is implemented in the software package FFB-FLM available for free download (http://mga.biogenet.nsc.ru/cgi/soft/FFB-FLM/).
This work is supported by RFBR grants 13-04-00272a, 14-04-00126a.

P17.74-M
Genome wide inbreeding estimation within Lebanese communities using SNP arrays
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Consanguineous marriages have been widely practiced, with variable rates, in several global communities depending on religion, culture, and geography. The populations of the Middle East are among those with the highest inbreeding level and frequency of inbred individuals. A genome wide analysis of 165 unrelated Lebanese has been performed either through the estimation of LOH (Loss of Heterozygosity) or through the FEstim algorithm depending on SNP frequencies. Relying on these genome-wide data that identify regions of homozgyosity by descent (HBD), this study was able to estimate total inbreeding levels, remote consanguinity, and population admixture and structure. The inbreeding coefficient value was estimated to be 1.6% in offspring of unrelated parents (over 3 generations) and 8% in offspring of first cousins. In either case, the remote consanguinity (RC) value was approximately equal to 0.6% resulting from genetic drift or recurrent consanguineous unions. This RC value suggests that for any unrelated marriages in Lebanon, the mates could be related as third cousins or as second cousins once removed. Under the hypothesis that 25% of marriages occur between first cousins, the mean inbreeding (F) value of 2.2% found may explain the increased incidence of recessive disease within offspring. The LOH and FEstim genome wide approaches were applied to investigate the genomic similarity of Lebanese communities. Both approaches revealed a unique ancestral population of the four studied communities (Greek-Orthodox, Maronite, Shiite and Sunni).

P17.75-S
Population genomics analysis in whole genome sequencing of 152 rhesus macaques
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1Baylor College of Medicine, Houston, TX, United States, 2Yerkes National Primate Research Center, Atlanta, GA, United States, 3New England NPrRC, Southborough, MA, United States, 4Wisconsin NPrRC, Madison, Madison, WI, United States, 5Tulane NPrRC, Covington, LA, United States, 6Southwest NPrRC, San Antonio, TX, United States, 7California NPrRC, Davis, CA, United States, 8Oregon NPrRC, Beaverton, OR, United States.
Rhesus macaques (Macaca mulatta) are the most widely studied nonhuman primate model species in biomedical, critical to various aspects of disease research. We applied next generation whole genome sequencing for 152 unrelated individuals (144 Indian-origin, 8 Chinese-origin) using either deep (30X) or low coverage (6X) strategies. Analysis using SNPTools identified 51.6 million SNPs. On average, Indian-origin individuals have >9.5 million variants compared to Chinese ancestry. We identified 110,000 SNPs mapped to conserved ENCODE transcription factor binding motifs. We used position weight matrices from the JASPAR database to assess these SNPs and found >25,000 candidate variants that may significantly affect TF binding, and thus gene expression. We mapped rhesus SNPs to the 4% of the genome identified as conserved across 29 mammals, and found reduced SNP density and MAE consistent with negative selection in those regions. We also applied a number of site frequency spectrum tests and found significant new evidence for both positive and negative selection in both coding and noncoding regions in the macaques. Analyses of LD and local recombination rates are in progress.

P17.76-M
Association of five confirmed risk gene polymorphisms with Rheumatoid Arthritis in the Algerian population
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Rheumatoid Arthritis (RA) is a chronic inflammatory condition of the synovial joints, classified as autoimmune disease. RA affects at least 1% of the world population and is the most common inflammatory joint disease. In addition, about 110,000 SNPs mapped to conserved ENCODE transcription factor binding motifs. We used position weight matrices from the JASPAR database to assess these SNPs and found >25,000 candidate variants that may significantly affect TF binding, and thus gene expression. We mapped rhesus SNPs to the 4% of the genome identified as conserved across 29 mammals, and found reduced SNP density and MAE consistent with negative selection in those regions. We also applied a number of site frequency spectrum tests and found significant new evidence for both positive and negative selection in both coding and noncoding regions in the macaques. Analyses of LD and local recombination rates are in progress.

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Association of five confirmed risk gene polymorphisms with Rheumatoid Arthritis in the Algerian population
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Rheumatoid Arthritis (RA) is a chronic inflammatory condition of the synovial joints, classified as autoimmune disease. RA affects at least 1% of the world population and is the most common inflammatory joint disease. In addition, about 110,000 SNPs mapped to conserved ENCODE transcription factor binding motifs. We used position weight matrices from the JASPAR database to assess these SNPs and found >25,000 candidate variants that may significantly affect TF binding, and thus gene expression. We mapped rhesus SNPs to the 4% of the genome identified as conserved across 29 mammals, and found reduced SNP density and MAE consistent with negative selection in those regions. We also applied a number of site frequency spectrum tests and found significant new evidence for both positive and negative selection in both coding and noncoding regions in the macaques. Analyses of LD and local recombination rates are in progress.
firmed gene polymorphisms (PTPN22rs2476601, STAT4rs7574865, IRF5rs2004640, TRAFI/C5rs101818488 and TNFAIP3rs6927172) in Rheuma-
toid Arthritis risk among the Algerian population.
Methods: The study sample comprised 110 patients with RA and 197 ethni-
cally matched healthy control subjects. Each polymorphism was genotyped using a predesignsed TaqMan® assay. Allele and genotype frequencies in patients and control subjects were compared by chi-square test and odds
ratios with 95% confidence intervals CI.
Results: Statistically significant association of all studied polymorphisms
with RA was detected. The strongest signal was obtained for PTPN22
(rs1801276) with an alleleic P value = 10-11 (OR = 9.89, 95% CI [4.28 -22.5])
Conclusion: This case / controls study lead, for the first time in the Algerian
population, to highlight the association of five risk genetic factors with RA.
This contributes to the characterization of RA genetic component in a popu-
lation still under genetic investigation.

P17.78-M
How does this Arab Genome differ from other genome?
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Whole genome from Caucasian, African, Chinese and Korean individuals have been studied and published to date. Here, we successfully generated, assembled and analyzed the first full genome of a 32 Saudi healthy volun-
teers from using NGS technology (5500xl Genetic Analyzer). Alignment of the 32 Saudi genome with H19 references revealed nearly more than 17
million unique SNPs. However, SNPs comparison analysis with HapMap
phase III populations showed the highest share was with ASW population (1,545,053) while the lowest share was noticed to be with JPT population (1,280,257). The SNPs counts and frequencies per chromosome were parti-
tioned into heterozygous and homozygous categories ranging from 258,174
SNPs (chromosome 2) to 1,182 SNPs on Y chromosome. The SNP frequency was calculated by dividing the number of called SNPs by the length of the
covered consensus sequence, omitting unknown regions from the reference
genome. The heterozygous and homozygous SNPs frequency was calculat-
ed based on their share of the total number of SNPs. A de novo assembly of 9,011 contigs sequences was not represented in NCBI reference genome.
This project is pointing to perform a whole genome/exome of 1000 Saudi indi-
viduals towards Establishment Saudi Genome Database for comprehensive
view of genetics variant such as large structural rearrangement and SNPs.
Conversely, the whole genome/exome of some of the common chronic di-
seases in the Saudi population is already started such as multiple sclerosis.

P17.79-S
Soluble CD40 ligand is regulated by membrane CD40 expression in platelet concentrates
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Etienne, France, Saint-Etienne, France.
Introduction: Soluble CD40 ligand (sCD40L) is a platelet-derived proinflammatory media-
tor that accumulates during platelet reactivation. Unfortunately, in some cases, high
levels of sCD40L cause acute transfusion reactions (ATRs). We inves-
tigated 2 polymorphisms previously associated with high levels of plasma
sCD40L in some diseases, in the aim to identify ‘dangerous’ platelet concen-
trates (PCs).
Material and methods: Levels of sCD40L were measured by LumineX® in 142 PCs (EFS Auver-
gne-Loire). We performed a Tetra-primer ARMS-PCR to genotype CD40-
rS1883832 [C>T] and CD40L-rs3092952 [A>G]. Differences of plasma
levels-Loire). We performed a Tetra-primer ARMS-PCR to genotype CD40-
rS1883832 [C>T] and CD40L-rs3092952 [A>G]. Differences of plasma
levels-Loire). We performed a Tetra-primer ARMS-PCR to genotype CD40-
rS1883832 [C>T] and CD40L-rs3092952 [A>G]. Differences of plasma
eutrophils. We hypothesize that those with TT genotype may express a high
amount of CD40 on their surface and thus may capture sCD40L, becoming more
activated, thus secreting more sCD40L.
Further studies are required in larger samples to confirm this result allow-
ing the discard of PCs with high sCD40L amounts and consequently pre-
venting ATRs.

P17.80-M
Inferring rare disease risk variants based on exact probabilities of sharing by multiple affected relatives
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Family based study designs are regaining popularity because large-scale se-
quencing can help to interrogate the relationship between disease and vari-
ants too rare in the population to be detected through any test of association
in a conventional case-control study, but may nonetheless co-segregate with
disease within families. When only a few affected subjects per family are se-
quenced, evidence that a rare variant may be causal can be quantified from the
disparity of sharing alleles. If an affected relative given it was seen in any one family member under the null hypothesis of complete absence
of linkage and association. We present a general framework for calculating
such sharing probabilities when two or more affected subjects per family
are sequenced, and show how information from multiple families can be
combined by calculating a p-value as the sum of the probabilities of sharing
exactly the same alleles (or more) exactly as would be expected. We also examine the impact of unknown rela-
tionships and propose methods to approximate sharing probabilities based on
empirical estimates of kinship between family members obtained from
geno- and phenotype data. We apply this method to a study of 55 multiple
families with apparent non-syndromic forms of oral clefts from four distinct
distributions. Whole exome sequencing was performed by the Center for In-
herited Disease Research (CIDR) on two or three affected members from
each family. The rare single nucleotide variant rs149253049 in the gene
ADAMTS9 was shared by affected relatives in three Indian families (p<2e-6),
illustrating the power of this sharing approach.

P17.81-S
A Founder Effect for PPBP-associiated recessive osteogenesis imperfecta in Acadian and Cajun Populations
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Mutations in PPBP, a gene that encodes cyclin B, a prolyl cis-trans isomerase, cause recessively inherited osteogenesis imperfecta (OI) that
ranges in severity from moderately deforming to perinatal lethal. Among Acadians in New Brunswick and Cajuns in Louisiana, who represent geo-
graphically distinct populations derived from the same ancestral group in
France, we identified 2 families from the Acadian group and 2 from Louisia-
na, both presumed to be from the Cajun group, with severe/lethal OI caused
by a homozygosity for a previously unreported PPBP mutation (c.344-1G>C, IVS3-1G>T). All transcripts from this allele use a cryptic acceptor site 5nt
down in exon 4 that results in a frameshift and mRNA instability. Both Canadian families presented prenatally; the first family had features of a lethal skel-
etal dysplasia on ultrasound and on autopsy was thought to have perinatal
lethal OI. The second family was thought to have a severe moderately de-
forming OI on ultrasound. The two families from Louisiana presented with
clinical diagnoses of perinatal lethal OI. None had mutations in COL1A1 or
COL1A2. There is no known consanguinity within these families or relation-
ships between them.
In the Acadian/Cajun ancestry, the probable founder effect for PPBP-associated recessive OI among Acadians and Cajuns, likely tracing back to early 17th century France,
in pregnancies/children of Acadian/Cajun ancestry (and their antecedents)
preventing severe skeletal dysplasia, PPBP-associated OI should be con-
sidered. Targeted analysis based on ethnic background may help facilitate
more timely diagnosis and subsequent genetic counseling.

P17.82-S
Genetic survival modeling with large-scale population cohorts
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Recent methodological development on linear mixed models has provided a common framework for heritability estimation, multi-locus association
testing and genomic prediction of quantitative traits in population cohorts.
with arbitrary relatedness structures. The possibility to use population cohorts rather than family structures opens up a multitude of new avenues also for genetic epidemiological research on time-to-event outcomes. However, connecting time-to-event outcomes to whole-genome sequencing data has so far not been computationally feasible with hitherto existing software. We introduce an R package ("GSM") for heritability estimation, multi-locus association testing and genomic prediction of time-to-event outcomes that is scalable to sequencing data. The package implements a very flexible piece-wise constant hazard model that contains an individual-specific Gaussian random effect with an arbitrary covariance structure. Computationally, we transform the problem to a Poisson model, which we analyze by fitting interconnected Generalized Linear Models. The underlying computational algorithm is written in C++ to enable analyses with millions of genetic markers and events in thousands of individuals. We demonstrate the runtime efficiency of our implementation and give an example of heritability estimation and multi-locus association testing for cardiovascular disease related events. Our work extends the computational tractability of linear mixed models from quantitative traits to time-to-event outcomes and will prove useful, e.g., for combining information across individuals' genomes and their hospital records.

P17.83-S

Genome-wide association analysis of swallowing symptoms related to dysphagia in a healthy older adult cohort

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Background: Patients with swallowing difficulties (oro-pharyngeal dysphagia) caused by neurological damage (stroke, Parkinson’s disease, aging) show different recovery patterns which may be caused by genetic determinants.

Aim: To find an association between human genetic variations and swallowing impairments within the aging cohort.

Materials and methods: We performed case-control genome wide association study (GWAS) of self-reported swallowing symptoms related to dysphagia. The analysis included 555 community dwelling, unrelated, older adults (mean age of age = 81.4; SD = 5.349) with known phenotype and genetic information consisting of 512,806 single nucleotide polymorphisms (SNP).

Geno-based association analyses of these traits were also conducted. The genetic data underwent quality control procedures prior to the study. This included analysis of population architecture using Multidimensional Scaling of the genome wide genotype data.

Results: Analysed cohort showed European ancestry with no major population stratification. The results showed one genome wide significant SNP rs17601696 (P=4.83x10^-8) from non-coding region of chromosome 10. Analyses of individual genes did not result in any genome-wide significant association.

Conclusion and future work: SNP rs17601696 may have an impact in swallowing impairment among elderly individuals. The results require replication in an independent cohort with appropriate phenotype/genotype data. Presented GWAS results will be replicated in the human model study using Transcranial Magnetic Stimulation (TMS). Identified genetic loci may play a role of potential markers to predict individual’s outcome from swallowing impairments.

P17.85-S

Investigation of hellenic families with microscopic hematuria reveals the frequency of collagen IV mutations and evidence for activation of the unfolded protein response

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Familial hematuria(s) comprise a genetically heterogeneous group of conditions which include heritable glomerulopathies (Alport Syndrome (AS) and thin basement membrane nephropathy (TBMN)). AS is rare, caused by X-linked COL4A5 or autosomal recessive COL4A3/4 mutations (ARAS), while TBMN is frequent. We sought to evaluate the prevalence of COL4A3/4 mutations among patients presenting with microscopic hematuria (MH), belonging to 91 Hellenic families. Also we studied 28 sporadic patients with MH and four patients with ARAS. We performed functional studies in cultured podocytes, focusing on the induction of the unfolded protein response (UPR) after overexpression of wild type or mutant COL4A chains.

Among 91 families, heterozygous mutations were found in 11 (12.1%). Restricting analysis to 68 families with secretory patients with MH, the positive finding is 11/68 (16.2%). Three heterozygous mutations were found in three of the 28 sporadic patients (10.7%). Altogether, among 52 heterozygous patients 17.3% reached end-stage kidney disease (ESKD). Of those aged >50 years, 26% reached ESKD, in keeping with previous findings that TBMN is not always benign. Functional studies showed that mutant COL4A3/4 chains expressed in podocytes are preferentially retained in the cells, compared to wild type chains. Mutant chains differentially triggered activation of the UPR pathway, as evidenced by activation of BiP, a sensitive ER stress marker.

TBMN may emerge as a more frequent cause of ESKD than AS. The ability of mutant chains to elicit the UPR pathway when overexpressed in podocytes may prove of functional significance and prognostic value.

P17.86-M

Variation in BTBD9 gene is associated with Tourette syndrome in the Polish population

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Tourette syndrome (TS) is a childhood-onset neuropsychiatric disorder manifested by tics. The anatomical location, number, frequency, complexity, and severity of tics change over time. The etiology of the disorder is unknown, though the predominant role of genetic factors has been established. Variants of the BTBD9 gene (rs4714156, rs9296249 and rs9357271) variants of the BTBD9 gene were genotyped. Analyzed SNPs indicated a strong linkage disequilibrium (D=1, r2=0.938-1). MAFs, genotype frequency, allelic, genotypic and haplotype association analysis within examined variants of the BTBD9 gene revealed no significant differences between controls and TS patients. However, there were significant associations between the BTBD9 gene variants and a clinical phenotype of TS. Minor alleles of all three SNPs were found significantly less frequently in patients with ADHD and were more frequent in patients with no comorbidities. There was a borderline statistical significance for minor alleles to be less frequent in patients with severe tics. All three SNPs were not found to be associated with the family history and the age of tic onset.

Our results indicate that the examined variants of the BTBD9 gene are not associated with the risk of developing GTS, but may be associated with comorbidity and tic severity in the Polish population.

P17.87-S

The susceptibility of insulin genes/variants related to type 2 diabetes in Turkish

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Rapidly increasing prevalence of diabetes in Turkey as worldwide has made diabetes as a public health problem which mostly uses the sources of health services on personal and social levels. As well as ethnic and geographical differences, contribution of multiple genes on the emergence of the disease makes type 2 diabetes (T2D) even more complicated to understand.

We introduce an R package ("GSM") for heritability estimation, multi-locus association testing for cardiovascular disease related traits and time-to-event outcomes and will prove of functional significance and prognostic value.

P17.88-M

The T allele of rs7903146 in TCF7L2 is associated with type 2 diabetes in Iranian: a large population-based cohort study

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We performed case-control genome wide association study (GWAS) of self-reported swallowing symptoms related to dysphagia in a healthy older adult cohort.
The southern migration route: a supporting clue from aboriginal populations for the perspectives of proto-Bulgarian ancestry.

P17.90-M
The southern migration route: a supporting clue from aboriginal Vedda people of Sri Lanka

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Being located along the proposed southern migration route and the presence of earliest skeletal evidences of anatomically modern human (37,000 B.P.) with aboriginal Vedda population, the island of Sri Lanka could provide the knowledge of genetic variation of modern humans in South Asia. In order to reveal the genetic relationship of the Vedda people with the tribal groups along the southern coastal migration route, the present study compared the mitochondria DNA (mtDNA) hypervariable segment 1 variations from 75 Vedda people from Sri Lanka with 33 world tribal groups from published data bases. The Vedda people signal out as a exceptional tribal group of South Asia having less than 30% of individuals sharing a mitochondrial haplogroup M with 64% of haplogroups R30, U1 and U7. The latter two haplogroups were recognized as West Eurasian ancestry. In principal component analysis (PCA) some Vedda groups occupied separate positions while some were closely related to South Indian tribal groups. Most interestingly, by viewing PCA from the point of view of the Southeast Asian foragers, it is evident that their closely related groups are the Vedda people. This fascinating genetic footprint is suggestive of yet another piece of evidence of the dispersal of anatomically modern human out of Africa via the southern migration route. And also this mtDNA study highlights Sri Lanka’s strategic location along the southern migration route, thereby providing a genetic gold mine, which will offer insight into the initial settlements and peopling of South Asia.

P17.91-S
RNA-Sequencing reveals differential gene expression between visceral and subcutaneous adipose tissue in Greek women undergoing abdominal surgery


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Adipose tissue is a major endocrine organ that contributes to whole body metabolic homeostasis. The role of body fat distribution in the development of obesity-related metabolic consequences, such as type 2 diabetes, hyperlipidemia and cardiovascular disease is of great importance. Fat depots from different areas of the body display distinct structural and functional properties and have specific roles in pathology.

We applied RNA sequencing (RNA-Seq) to quantify transcript levels in visceral and subcutaneous adipose tissue of women who underwent bariatric surgery or surgical treatment for non-inflammatory disease. RNA-Seq was performed on the Illumina HiSeq2000 platform with paired-end 49 bp sequencing. Reads were mapped using the GMAP mapper with an average of 27.4 million reads per sample. We report here results on differential gene expression determined using DESeq. We have also performed genotyping of the above samples on the Illumina Omni 2.5 exome v1 chip and aim to report candidate regulatory variants through association of SNP genotype with mRNA levels for each tissue. Gene expression of the two types of tissues will be further tested with the levels of cardiometabolic biomarkers. We are also performing formaldehyde-assisted isolation of regulatory elements (FAIRE) in order to identify regions of open chromatin. Combining all the above information will contribute to our understanding of adipose tissue biology and as a result to obesity-related pathogenesis.
to compare their frequency in 240 Eurasian (sub-)populations with more than 20,000 samples. The comparison reveals a statistically significant difference in the distribution of the studied haplogroups between Bulgarians and Altai populations as well as between Bulgarians and Eastern Slavic populations. Based on the novel historical studies which point to a substantial contribution of the proto-Bulgarians to the modern Bulgarian gene pool the obtained results suggest that there is no common genetic ancestry between proto-Bulgarians and present day Altai populations as they reject the hypothesis of the Turkic origin of proto-Bulgarians.

P17.93-S

Forensic parameters and allele frequency distribution of 15 autosome STR loci in a Mestizo population from the State of Yucatan, Mexico

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The State of Yucatan is a region with a high density of population with Mayan ascendance at Southeastern, Mexico. Short tandem repeat (STR) polymorphisms are mainly used in forensic fields foraternity tests and personal identification. Since, there are no STR databases from Yucatan, Mexico, a study is planned to determine the frequency distribution of 15 STR loci. These loci were chosen for good quality and high discriminative power. This study was designed to evaluate the genetic diversity of STR loci in blood samples from Yucatan, Mexico, for the evaluation of the population of Yucatan, Mexico.

P17.94-M

Retrospective analysis of live birth prevalence of children with Down syndrome in Denizli, Turkey

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Down syndrome (OMIM: #190685) is the most frequent chromosome abnormality among live births. Its prevalence increases with maternal age, and can be diagnosed by antenatal screening. The average incidence in the USA is 1/700-800 live births and 1-3/1000 births in the EU. We examined prevalence variations of DS in Denizli, Turkey, through a retrospective study. Sixteen years of survey data were retrieved from the two main state hospitals in the city. We identified 113 DS live births in Denizli for 16 years. The prevalence did not change significantly. The population in Turkey has been increasing during this period, therefore, the prevalence during these 16 years was calculated to be 1/700 live births.

P17.95-S

Six novel loci associated with VEGF circulating levels identified by a meta-analysis of genome-wide association studies

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We sought to identify additional loci associated with circulating VEGF levels measured on ~13,000 individuals from six cohorts using genome-wide association data imputed to the Phase I/3 release of the 1000 genomes. A GWAS of VEGF levels was performed in each cohort and the results were meta-analyzed using an effective sample size weighted meta-analysis approach. Five chromosomal regions (5q14.3, 6p21.1, 8q23.1, 9p24.2, 10q21.3) containing SNPs associated at genome-wide significance with VEGF levels (p-value<5x10-8) were identified. Independence was assessed by conditional GWAS in a forward stepwise fashion, including in the association model the most significantly associated SNP at each step, and repeating this process until all SNPs independently associated with VEGF levels had been detected. Ten independent signals were identified including all four previously-reported loci as well as six novel loci. In silico and de novo replication analysis will be carried out in ~20,000 individuals from three additional independent cohorts, as well as functional validation using RNA data and exploration of the association of these VEGF loci with various clinical endpoints.

Further, pathway analysis will be performed to look for biological processes most likely to be associated with the genes located in the identified loci and to help identify additional VEGF loci.
Evaluating a digital information resource for adolescents in genetic research - adolescent and parent perspectives on information requirements

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There is insufficient evidence regarding adolescent participants’ information needs in genetic research. Previous information interventions have yielded little improvement in understanding and confidence in participation decisions, and have not specifically addressed the needs of adolescents. UK adolescents (aged 12-17) and parents with experience of participation in genetic research discussed their knowledge, attitudes, and information needs surrounding genetic research, in focus groups or interviews. A digital information resource (website) was developed following participants’ suggestions, and subsequently evaluated for its feasibility and desirability by adolescents (n=21) and parents (n=5) with and without experience of genetic research. Thematic analysis was carried out on transcripts of focus groups and interviews, and on written feedback. Predominant attitudes to genetic research were that such research is prestigious and complex, and that participation in genetic research is a relatively simple proposition, until potential outcomes are explored. The evaluation of the resource suggested participants favour comprehensive information, presented using modern technology, which is accessible and manageable. Further, participants recommend that information is ‘customisable’ to accommodate individual differences in requirements, concerning specific content and information quantity. Finally, the analysis suggests participant autonomy is an integral aspect of information provision for adolescents, and should be explicitly incorporated into information design. Digital technology can satisfy participants’ preferences, though information resources should be designed with the specific needs of the adolescent population in mind. Questions remain regarding the ‘minimum essential information’ in genetic research, but participants should be afforded as much choice as possible.

Adolescent participation in genetic research - motivations and influences on adolescent and parent participation decisions

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Increasing numbers of young people participate in genetic research following changes in international regulations. There is a recognised need to support children and adolescents in making informed participation decisions, yet there is little evidence concerning how to do so. Moreover, adolescents are a distinct population from children and adults because of their cognitive development and social context. A thematic analysis of focus groups and interviews with UK adolescent patients (aged 12-17) who have previously participated in genetic research (n=7), and their parents (n=7), explored participants’ experiences of decisions regarding research participation. The analysis suggests that participants prioritise subjective and interpersonal factors when making participation decisions, and that genetic aspects of research are subordinate influences on decisions. Adolescents cited primarily altruistic motivations, while parents referred to their children’s illness and healthcare experiences as key to participation decisions. Further, the analysis demonstrates how motivations such as altruism and personal benefit are mediators of other aspects such as trust and personal histories, and highlights differences between adolescent and parental considerations regarding participation in genetic research. The potential influences of subjective or interpersonal factors on adolescent and parent decisions should be taken into account when inviting participation, in order to support autonomous participation decisions. Further, participant information should be tailored to each group’s needs to reflect adolescent and parent priorities, and to ensure that participants are properly informed concerning genetic aspects of research which may not be viewed as a priority.
bank, involving various stakeholders concerned; 
- the definition of criteria and procedures to disclose individual findings to biobank participants, in preparing for future research on data collected.

P18.06-M
Do we still need to follow the traditional model of face to face results disclosure for BRCA predictive testing? An examination of current practice in the Republic of Ireland (ROI)
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The traditional model of cancer predictive testing services is changing. Many genetic centres are now offering a choice to patients in how they receive their results of the typical face-to-face disclosure. Research has shown that it is feasible to change the current 2 visit model of initial consultation followed by results disclosure, without reducing patient satisfaction to a large degree (Sutphen et al, 2010). In view of this and the increasing demand on the ROI Breast Cancer (BCRA) predictive testing service, a 2 year retrospective study on patient preference in how to receive a BCRA predictive result was performed. The aim was to examine those who had been through the BCRA predictive process previously to study if an alternative to face-to-face result disclosure would have been an option they would have preferred. A questionnaire was used to assess this and results showed that 71.7% of respondents would have liked the option of obtaining their results by telephone or by letter. However, when asked about their actual experience of BCRA predictive results disclosure 40.6% did still prefer the face-to-face contact, while 44.9% would have preferred an alternative. Car parking and distance were the top two variables determining whether the surveyed showed a preference towards options or not, followed by sex and test result. This study shows that while the majority expressed a wish to have a choice, it is important not to underestimate the value of a face-to-face encounter. We are now reviewing our practice for BCRA predictive genetic counselling.

P18.07-S
Risk-stratified screening for cancer and response to personalised genetic information in the general population
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There is evidence that risk-stratified population screening based on multiple factors including a polygenic risk profile has the potential to be more efficient than age-stratified screening. Therefore, genetic information is likely to be used for cancer prevention strategies in the general population in the future. To understand issues of acceptability of such genome based screening in the public, we reviewed issues of genetic risk-communication, how disease risk is perceived and the behavioural response of people to genetic risk feedback. A systematic review and meta-analysis was conducted based on inspection of 1948 abstracts and use of 25 studies. We found that though the general population has limited genetic literacy, they are interested in being informed of their genetic risk status. They are generally positive about using genetic information in disease prevention but not about provision of this information to employers and insurers. There is evidence of positive association between perceived risk and cancer screening behaviour such as uptake and mammography, particularly when risk is communicated by categorising into high, medium and low strata. Yet genetic risk feedback particularly that conveying small increases of risk has little or no effect on lifestyle changing behaviour. Also, personalised risk-communication is effective in improving knowledge and risk perception of the population. Personalised risk information is associated with only short term psychological distress including anxiety and fear but not with fatalism. Strategies of preventing cancer using personalised genetic information may potentially be acceptable to the general public. However, before implementation of risk-stratified screening, further empirical evidence is needed.

P18.08-M
Carrier screening for recessive disorders through exome sequencing

Detection of carrier status for certain recessive Mendelian disorders is a well-accepted health-care practice in several countries since the 70s and aims at the prevention of frequent severe monogenic disorders. Exome sequencing in combination with the increasing knowledge of human pathogenic variation provide the possibility to perform a carrier screening for all the known recessive Mendelian disorders. Such a screening would allow for a much more informative genetic counseling and may alter the total prevalence of the known recessive disorders. In order to test this hypothesis we have used exome sequencing data from 104 individuals of European origin and have identified the total number of likely pathogenic variants in the >1600 recessive disorders for which the responsible gene is known. The mean value was 18.2 variants per individual. Consequently we have randomly paired these exomes in order to create 5356 fictive couples. 33.14% of these couples have at least one gene for which both members are heterozygous for a likely pathogenic variant. These preliminary results exhibit an upper estimate of at risk couples but more precise knowledge and definition of the pathogenic potential of each variant will render the carrier detection more accurate and make it a potent test for family planning.

P18.09-S
Call for a Standardized Genetics Clinical Laboratory Speciality Training Across the Globe: An Initiative for Clinical Molecular Genetics Training in Turkey
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Genetics discipline is composed of several clinical laboratory specialties, as defined by the American Board of Medical Genetics (ABMG): Clinical genetics, biochemical genetics, clinical cytogenetics, and clinical molecular genetics. Except for the clinical genetics, each specialty can be practiced by scientists with PhD and an appropriate training in human genetics. ABMG mandates a two-year, highly structured specialty training for the laboratory scientists before they become lab directors who can officially sign out patient reports. The Clinical Molecular Genetics specialty training program involves a preparation of a logbook documenting contribution to the reporting of 150 cases at different levels, i.e. performance and interpretation of certain number and variety of diagnostic test results, and sharing them with physicians and patients. In addition, rotations in Clinical Cytogenetics and Biochemical Genetics laboratories ensure cross-disciplinary exposure. Didactic lectures and hands-on laboratory training provide fellows competency in skills such as variant interpretation, risk estimation, clinical test development and validation, proficiency testing and regulatory aspects of running a certified clinical laboratory. The investigator, an ABMG certified clinical molecular geneticist, aims to discuss the training outline that can be implemented in the rest of the world, especially in an era of rapidly advancing technologies that results in accumulation of variants in clinical laboratories with an unprecedented speed. In particular, the significance of a standardized variant assessment system across different laboratories (as outlined in Düzkale et al, Clinical Genetics 2013) and transferring variant data from clinical reports to public databases such as NCBI’s ClinVar will be discussed.

P18.10-M
Clinical utility guidelines covering diagnostic next-generation sequencing (NGS)
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Clinical Utility Gene Cards (CUGC) are disease-specific guidelines authored by international expert groups. They are dealing with the risks and benefits of the application of genetic tests in the clinical setting. Each document represents a balanced summary of the analytical and clinical validity, the clinical utility and cost-benefit issues. CUGC’s offer quick guidance to all stakeholders, including clinicians, clinical geneticists, referrers, service providers and payers. Each CUGC is peer-reviewed and published by the European Journal of Human Genetics. CUGs are also freely available on the websites of EuroGen تست, the European Society of Human Genetics and Orphanet. In order to adapt CUGC to NGS approaches we have started to build up a NGS panel data collection. It contains data from NGS providers including panel name, tested genes, disease and genetic background. The overlap of tested genes between different providers can be determined by comparison and genes deemed essential by the providers can be easily identified, serving as the first step in the establishment of CUGCs for NGS-based genetic test applications in diagnostics. In a second step we have modified the disease-specific format of the CUG guidelines and invited experts to put it to test. We here present the state of discussion. A prototype of our data collection is available at the EuroGen test website: https://eurogentest.esgh.org/index.php?id=668. So far we have identified 28 laboratories having launched a total of 944 clinical NGS tests covering 2882 genes. We encourage NGS providers to contact us regarding their current services and to include them in the database.
P18.11-S  Dynamic Consent - A Patient Interface for 21st Century Research Networks  J. Koonings, 1, 2E. Whitley, 1, D. Lund, 3, M. Morrison, 1, H. Teare, 1, K. Melham; 1University of Oxford, Oxford, United Kingdom, 2London School of Economics, London, United Kingdom, 3HW Communications Ltd, Lancaster, United Kingdom.

Biomedical research increasingly relies on the application of novel technologies to allow data to be shared on an unprecedented scale. However, the procedures for ethical involvement of participants have not kept pace with these dramatic changes in research capability; mechanisms of informed consent remain static, paper-based, and organised around national boundaries and legal frameworks.

Dynamic consent (DC) is both a specific project and a wider concept that offers a new approach to consent, to meet the needs of twenty-first century research. It is a personalised, communication portal which allows interactions over time, enabling participants to engage in the donation of their tissue samples and personal information for research purposes, as much or as little as they choose. The technical architecture of DC includes components that can securely encrypt sensitive data and allow participant consent choices to travel with their data and samples when shared with third parties. In addition to improving transparency and public trust, this system is of benefit to researchers by streamlining recruitment, and enabling efficient recontact of participants.

The interface facilitates two-way communication of information to stimulate a more engaged, informed and scientifically literate participant population where individuals can tailor and manage their own consent choices. To date, DC has mainly been developed in the context of biobanking, but it also has potential for use in other domains for a variety of purposes.

In this paper we present dynamic consent and show how it can be used as a tool for translational research and personalised medicine.

P18.12-M  Duty to recontact in clinical genetics: Asystematic review of the literature  E. Otten, M. Plantinga, A. V. Ranchor, M. A. Verkerk, E. Birnie, J. M. van Langen; University Medical Center Groningen, Groningen, Netherlands.

Introduction Many findings from NGS diagnostic techniques cannot be interpreted yet, but may provide medically relevant information in the future. However, guidelines on recontacting former patients if new actionable information arises are lacking. Methods As a first step in developing such guidelines, we conducted a systematic literature search on recontacting in clinical genetics in PubMed, Embase, Web of Science, and Google Scholar. Our search strategy identified 974 articles in all four databases. Fifty full-text articles in English language met our inclusion criteria, and were included in the review. Results Most literature is from the US (48%), followed by Canada (22%) and Europe (22%). In the literature recontacting is usually not regarded a legal obligation in clinical genetics. Most authors do consider recontacting to be desirable. Many articles argue that the responsibility for recontacting should be shared with the patient. We found no national or international guidelines, except for the 1999 ACGM policy statement on duty to recontact. Only four of the fifty articles described practical experiences with recontacting. These showed that patients were usually positive about the renewed contact. Conclusion Most authors consider recontacting to be desirable. The limited empirical evidence indicates that patients appreciate recontacting. Practical problems of implementing recontacting in clinical genetics are brought forward most often as argument contra imposing a duty to recontact. One of the challenges for the future will be to create ways to overcome these. Legal issues remain important. We therefore consider it important to develop guidelines on this topic for the NGS-era.

P18.13-S  The ethical dimensions and the tools for data sharing in genetics within evolving frameworks  A. Cambon-Thomsen; 1A. Pigeon, 2G. Chassagny, 1L. Mahul, 1E. Rial-Sebbag; 1Inserm and University Toulouse III Paul Sabatier UMR 1027, Toulouse, France, 2Inserm, US013, National BIOMICANQUES Infrastructure, Paris, France.

Data as well as biological sample international sharing is paramount in health research. While policy declarations from numerous research institutions and funders encourage such sharing a number of difficulties and needs are identified in practice to „make it happen”. This movement in the context of the availability of large scale sequencing technologies for studying human genomics is confronted with legal and ethical aspects regarding privacy, confidentiality, clinically useful information and the duties attached. Issues related to identifiability, consent process and regulation of access challenge the existing framework. The evolving legal framework regarding exchanges of biological samples (no unified legal EU framework for research) and personal data protection (EU Directive in revision) is challenged. Examples from various consortia and projects are analysed to enlighten the different facets into play, from the P3G consortium (Public population projects in genomics and society), the international consortium on cancer genomics, European infrastructures such as BBMRI (Biobanking and Biomolecular Resource Research Infrastructure) or ESGI (European sequencing and genotyping) and other EU projects. The focus will be on Charts, Codes and tools to foster sharing, especially hSERN (human sample exchange regulation navigator) that gives information on theoretical and practical legal aspects for exchanging biological samples across borders and the BRIP initiative (Bioresource research impact factor) that aims at providing ways to recognize the efforts to make available quality bioresources that are used in further research, thus enhancing the use of the whole culture of samples and data sharing is on its move, but not without difficulties.

P18.14-M  General practitioners and direct-to-consumer genomic tests: a survey in Emilia-Romagna region (Italy)  A. Baroncini, 1O. Calabrò, 1E. Calabrò; 1Medical Genetics Unit, Maternal and Child Health Department, Imola, Italy, 2Regione Emilia Romagna, Bologna, Italy.

Personal genomic tests (PGT) for disease risk assessment, based on genome-wide association study variants, have been offered directly-to-consumers (DTC) by several companies since 2007. Concerns regarding their potential negative impact include, among others, lack of counseling, dubious test quality, unnecessary anxiety and medical interventions based on erroneous/ misinterpreted results. To mitigate worries professional education on PGT-DTC has been advocated and the central gatekeeper role of family physicians has been highlighted.

Relatively few studies have been published on awareness, involvement and attitudes of healthcare providers on DTC marketing of PGT and, to the best of our knowledge, none in Italy.

A 2008 CDC survey showed that 42% of healthcare providers were aware of DTC-PGT, that 42% of them had at least one patient asking questions about having such a test and 15% had at least one patient who brought test results in the past year.

The preliminary results of a 2014 survey in the Emilia-Romagna Region (Italy), involving solely general practitioners, show that slightly more than 20% of the respondents are aware of DTC-PGT and that respectively 85% and 15% of them feel unprepared or only partly prepared to answer questions about such tests. About 45% of them have had at least one patient asking questions on purchasing or performed DTC-PGT during 2013. These data are coherent with the limited number of Italian companies directly marketing PGT and underscore the critical need to enhance primary physicians’ information on genomics tests provided outside of the clinical setting.

P18.15-S  The research policy regarding disclosure of genetic research results: A historical perspective in Japan  J. Minari, 1K. Kato; 1Department of Biomedical Ethics and Public Policy, Graduate School of Medicine, Osaka University, Osaka, Japan, 2Institute for Integrated Cell-Material Sciences (iCeMS), Kyoto University, Kyoto, Japan.

Currently, the return of genetic research results to research participants is a hot topic of debate throughout the world, yet no consensus policy has emerged. Regarding genetic research, Japan has government guidelines, entitled Ethical Guidelines for Human Genome/Gene Analysis Research, which have been set by three ministries in March 2001. These guidelines, together with the preceding document formulated by the government in 2000, Fundamental Principles of Research on the Human Genome, set the principle that a research participant has the right to be informed of his/her genetics information resulting from the research. This means that Japan has taken a stance emphasizing the right of research participants to receive their results since 2000. In the recent revision of the 2001 government guidelines, however, the stance of disclosure in principle was also retained, but the newly added stipulations resulted in researchers’ discretion playing a significantly larger role, due to the strong influence of the Act on the Protection of Personal Information. In this study, we have identified two potential ethical issues, which need to be considered in the revised guidelines. One is that they do not clearly require researchers to offer opportunities for participants to express these wishes and opinions. The other is that one of the exemptions, which show the situation that researchers do not have to disclose, considers only the promotion of research activity, but not the interests of research participants. Based on these findings, we discuss their implications for international research community.
P18.16-M Recent situation about rules of sharing, reuse and circulation of personal genome data in Japan
N. Yamamoto;
Osaka University, Osaka, Japan.

Personal genome data is very important resource for biomedical researches. In very recent years, it is going to be used beyond previous research area and increasing in number with launc of a several-large-scale genome cohorts. Although it has been used after acquisition of donor’s informed consents and the review by the institutional ethics review boards, that are defined on the national guideline for the genome researches, ELSI (ethical, legal and social issues) are becoming rapidly in Japan. For example, discussions about broad consents and return of results issues are becoming more severe. Development of more secure technology for data-sharing and data-reuse are also desired as well as moral governance. Since Personal Information Protection Law will be revised in a several years in Japan, various experts began to discuss how these new types of personal information should be protected. In the poster, these situations about the treatment of personal genome data will be reported.

P18.17-S Genetic Counselling, Genetic Diagnostics, Genetic Prevention, Genetic Education in EB Centre Czech Republic in University Hospital in Brno
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Centre for patients with Epidermolysis bullosa congenita (EB) works at the University Hospital Brno since 2001, from 2012 as the highly specialized medical care centre. EB Centrum CZ is a member of the international network of EB centres and clinical experts. The Centre cooperates with DEBRA Czech Republic (member of the Czech Alliance for Rare Diseases) witch supports people with EB and their families and try for 10 years to engage people with EB to a full life. In EB Center CZ works a multidisciplinary team of health specialists which provides comprehensive care to all EB patients in CZ. The DNA analysis is performed for EB simplex (EBS) and dystrophic EB (EBD) (analysis of the genes for keratin 5 and 14, and collagen VII). The mutation was found in 60% patients with EBS, in 67% patients with the dominant EBD and in all patients with the recessive EBD. In one patient was confirmed a rare form of EB caused by a mutation in the gene for plakulin. Genetic counseling in our Centre was performed in more than 90% families with the recessive form of EBD, in about 66% families with the dominant form of EBD and in about 60% families with EBS. EB patients from Slovakia, Russia and Ukraine are interested in consultation, genetic counseling and DNA analysis. Activity of EB Centre CZ and DEBRA CZ is also focused on education and awareness for healthcare professionals, patients, their families and the public.

P18.18-M Enhancing genetic counseling for Familial Alzheimer’s Disease through improved phenotyping
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Familial Alzheimer’s disease (FAD), despite representing a rare condition, is attracting a growing interest. In clinical practice, we noticed that individuals with a family history of AD show considerable interest for the availability of genetic tests for early detection of the disease. When informed about the availability of a genetic test for autosomal dominant mutations (PSEN1, PSEN2, APP genes) some individuals decide to be tested, while others refuse. Those who refuse to take the test are primarily motivated by lack of efficacious therapies. However, both those who decide to take the test and those who refuse it lament lack of clear information about the clinical development of the condition.

Indeed, we found out that, albeit rare, FAD cases are poorly phenotyped. In particular, we performed a systematic review of studies describing the phenotypic features of FAD cases sustained by PSEN2 mutations resulting in largely incomplete and low-quality data. Given the incomplete penetrance of some mutations, their variable phenotypic expressivity, and the lack of systematic and accurate phenotyping of FAD cases, the possibility of implementing genetic counseling procedures is strongly limited. This may also affect the capacity of individuals to react and cope with the communication of the test result should they turn out to be mutation carriers.
Among the subjects afraid enough by hereditary concern (22), only 13 (59%) regarded this problem as very serious. Deleterious gene took second place, so more than 22/29 (75%) of this population finds this problem very serious.

Complete data were available for 29 individuals (22 relatives, 7 probands: 13 males, 39±5.6 years; 16 females, 31±6.8 years). In order to study the relationship between genes and diseases, the increasing availability and sharing of phenotypic and genotypic data has been advanced as an imperative within the scientific community. In parallel with data sharing practices by clinicians and researchers, recent initiatives have been observed in which individuals are sharing personal genomic data. The involvement of individuals in such initiatives is facilitated by the increased accessibility of personal genomic data, offered by private test providers along with availability of online networks. Personal webpages and online data sharing platforms such as Free the Data, Consent to Research and Genomes Unzipped are being utilized to host and share genotypes, electronic health records and/or family history uploaded by individuals. Although personal genomic data sharing initiatives vary in nature, the emphasis on the individuals’ control on their data in order to benefit research and ultimately health care has seen as a key theme across these initiatives. In line with the growing practice of personal genomic data sharing, this paper aims to shed light on the potential challenges surrounding these initiatives. As in the course of these initiatives individuals are solicited to individually balance the risks and benefits of sharing their genomic data, their awareness of implications of personal genomic data sharing for themselves and their family members is a necessity. Furthermore, given the sensitivity of genomic data and the controversies around their de-identifiability, potential privacy risks and harms originating from unintended uses of data have to be taken into consideration.

**P18.22-M**

**Challenges of web-based personal genomic data sharing**

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In order to study the relationship between genes and diseases, the increasing availability and sharing of phenotypic and genotypic data has been advanced as an imperative within the scientific community. In parallel with data sharing practices by clinicians and researchers, recent initiatives have been observed in which individuals are sharing personal genomic data. The involvement of individuals in such initiatives is facilitated by the increased accessibility of personal genomic data, offered by private test providers along with availability of online networks. Personal webpages and online data sharing platforms such as Free the Data, Consent to Research and Genomes Unzipped are being utilized to host and share genotypes, electronic health records and/or family history uploaded by individuals. Although personal genomic data sharing initiatives vary in nature, the emphasis on the individuals’ control on their data in order to benefit research and ultimately health care has seen as a key theme across these initiatives. In line with the growing practice of personal genomic data sharing, this paper aims to shed light on the potential challenges surrounding these initiatives. As in the course of these initiatives individuals are solicited to individually balance the risks and benefits of sharing their genomic data, their awareness of implications of personal genomic data sharing for themselves and their family members is a necessity. Furthermore, given the sensitivity of genomic data and the controversies around their de-identifiability, potential privacy risks and harms originating from unintended uses of data have to be taken into consideration.

**P18.23-S**

**Opinion about reproductive decision-making among MMR mutation carriers of reproductive age**

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The reproductive techniques such as prenatal diagnosis (PND) or preimplantation genetic diagnosis (PGD), although debated, are often legally forbidden in case of HNPCC syndrome, without considering mutation carriers’ opinion about their reproductive options. We conducted a study in 43 individuals (probands and relatives) identified as Mismatch Repair mutation carriers of reproductive age (until 40 years for women, 50 years for men) exhaustively screened during the genetic counseling consultation in our institution. All of them received a closed questionnaire focusing on socio-clinical possible causes of distress and reproductive preferences (natural conception, PND, PGD or adoption) whose answers were ranked from 0 to 4.

Complete data were available for 29 individuals (22 relatives, 7 probands: 13 males, 39±5.6 years; 16 females, 31±6.8 years). Main reasons of distress given in first were fear of transmitting predisposition to 17 individuals (9 men, 8 women) (58%) and fear of premature death for 8 (4 men and 4 women, 27.5%). For 5 persons the fear of passing on deleterious gene took second place, so more than 22/29 (75%) of this population regarded this problem as very serious. PND / PGD and natural conception were equally reported (52% and 48%, respectively).

Among the subjects afraid enough by hereditary concern (22), only 13 (59%) chose in first the new reproductive techniques and 9/22 (41%) gave priority to natural conception.

**P18.24-M**

**Improved hereditary recurrent fevers diagnostics resulting from participation in the European molecular genetics quality network**

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The hereditary recurrent fevers (HRF) are rare rheumatologic diseases characterized by a course of self resolving inflammatory episodes, inflicted by cells of the innate immune system, and mainly affecting connective tissue, skin and/or central nervous system. If untreated, some HRF patients may develop life threatening, secondary amyloidosis. HRF genetic diagnosis is increasingly requested for patients with recurrent inflammatory episodes of unknown origin. More than 10 HRF genes are listed in the Inifers database. An external quality assessment (EQA) program for the primary tested HRF syndromes (FMF, GAPS, TRAPS, MKD) has been provided by the EMQN since 2009. Fifty laboratories, mostly from European countries, participate in the scheme. Data demonstrating improved genetic diagnosis for HRF since 2009 will be presented; genotype error rates have dropped dramatically compared to a prior 3 year survey (2005-2008). Moreover, with low participation outside Europe, best practice guidelines for the genetic diagnosis of HRF have also been written by HRF genetic and clinical experts, addressing the scope of diagnosis and clinical significance of pathogenic, clinically debated, rare, novel or population specific variants. A simple interpretation chart concludes on the contribution of each variant type to the diagnosis of recessive or dominant HRF disease. Guidelines also addressed minimal details and indication for referral, technical quality assurance measures, variant description nomenclature and recommendation for further genetic testing. Our future goal is to expand the scheme to monogenic autoinflammatory diseases, and to implement EQA on new genetic diagnostic methods for HRF such as Next Generation Sequencing (NGS).

**P18.25-S**

**Attitudes of genomic researchers, health professionals and students on returning incidental findings to whole genome research participants**

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At the moment, there is no consent on how to handle incidental findings (IF) in human research. We are investigating the opinions of genomic researchers, health professionals and students on returning IF to research participants using a questionnaire available on line at the Welcome Trust that was kindly sent to us for translation to Spanish and application in our country. The questions explore if the severity of the health problem incidentally found, the level of risk of getting the condition and the usefulness of the information affect whether the person think the result should be returned. Also, it asks the participants to first answer based on their own professional knowledge and second to answer imagining they are the research participants. The preliminary results show that respondents agree that information on preventable conditions should be returned to participants in genomics research; however, they were more likely to receive IF regarding a life-threatening non preventable condition than to return it to participants. More than ninety percent of subjects would return IF related to drug responses or that could be relevant to their children. And 80% think someone should decide which types of IF to share. Also, 80% feel it is acceptable to return IF related to a life-threatening non preventable condition than to return it to participants. Information on preventable conditions should be returned to participants in genomics research; however, they were more likely to receive IF regarding a life-threatening non preventable condition than to return it to participants. More than ninety percent of subjects would return IF related to drug responses or that could be relevant to their children. And 80% think someone should decide which types of IF to share. Also, 80% feel it is acceptable to return IF related to a life-threatening non preventable condition than to return it to participants.

**P18.26-M**

**To know or not to know: Research participants want to know about incidental findings in WES-studies**

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Introduction

In the last years the ethical and legal management of incidental findings in
masse parallel sequencing have been discussed. There is a lack of literature concerning research participant’s perspective. The aim of this study was to investigate whether research participants want disclosure of IF's and what kind of IF's they want to know about. Methods: 127 research participants in a study of gastrointestinal polyps were informed about Whole Exome Sequencing and the risk of IF's. They were asked to decide whether they A) wanted disclosure on IF's no matter whether the mutations were associated with a non-treatable or non-preventable condition, B) wanted disclosure on mutations associated with treatable or preventable conditions or C) wanted no disclosure at all. Results: Participants who wanted disclosure of all IF's (A) accounted for the majority (n=78), 45 of the participants only wanted disclosure of mutations, which could lead to surveillance or treatment (B) and four participants did not want IF's to be disclosed at all (C). Conclusion: The study showed that almost all research participants wanted disclosure of at least some types of IF's. The answers did not depend on age or sex. We suggest that well-defined IF's are to be disclosed in research projects and that the type of IF's (non-treatable and actionable) are categorized, discussed with the participant, and incorporated in the research consent form.


Genetic information is often considered as specific, among other biological information, because of its personal and family dimension. When a person is diagnosed with a serious genetic anomaly, the disclosure of this information can be relevant for other family members when prevention measures or treatment exist. The transmission of this information raises legal issues for professionals: how to preserve confidentiality and privacy of personal medical information? How to ensure the right to know of the relatives when the information to be disclosed can be of interest for their health? The French legislator tried, in 2004, to draw a balance between these principles by implementing the “genetic information procedure to family members. The lack of adoption of enforcement decrees made the law not applicable since a revision occurred in the new bioethics law (2011). This procedure tends to favor information of relatives by creating a primary legal obligation for the index subject to inform his family members. It also creates professional obligations notably in the ways this information is to be formalised and disclosed when the subject do not want to communicate it. The French legal system is almost complete as many texts (legal and good practices) have enriched the procedure throughout 2013 In the light of these legal novelties we will make a comparative analysis to address the equilibrium of the principles referred to in the law. Their adequacy to the practices, the remaining unclear points (responsibilities not to disclose, genetic information relating to minors).

P18.28-M Gynecologic cancer is “sentinel cancer” for Lynch syndrome L. Carnevali1, L. Cimetti2, A. Chiaravalli1, N. Sahnane2, D. Furlan2, A. Viel1, L. Liberio1, T. Rosati1, F. Sessa1, C. Rivo1, M. Tibbett1; 1Ospedale di Circolo - Polio Universitario, Varese, Italy, 2Department of Surgical and Morphological Sciences, University of Insubria, Varese, Italy, 3Oncologia Sperimentale I. CRO, Aviano, Italy, 4Clinica Ostrizia e Ginecologica, Ospedale di Circolo - Polo Universitario, Varese, Italy.

Lynch Syndrome (LS) arises by a germline mutation of MMR genes. Women with LS show a risk for endometrial cancer equal or greater than colorectal cancer. Specific surveillance and risk-reducing strategy could be proposed in LS patients. MSI and IHC MMR protein expression tests in tumor samples could represent an effective strategy for identifying LS in patients with GC (Gynaecological Cancers). Clinico-pathological features, MSI and Immunohistochemical (IHC) expression of MMR proteins were investigated on 78 tumor samples (62 endometrial, 7 ovarian and 9 cervical tumours) of 75 patients affected by GC. All patients referred to genetic Counseling Service of Varese Hospital from 2001 to 2013. Somatic test including IHC and MSI was performed on 38 patients. Fifteen GC showing MSI and loss of IHC MLH1 expression revealed MLH1 promoter hypermethylation. The mean age at GC diagnosis in LS patients was 46 years. Endometrial cavity was the prevalent site, and 28/30 GC tumours showed a pure endometrial histotype. Twenty nine out of 35 patients show GC as first manifestation of LS. MMR germline pathogenic mutation was identified in 20 patients (10 patients were obligate carriers), the remaining five patients showed variants with unknown significance of MSH6 gene. In conclusion IHC, MSI and MLH1 methylation in GC patients under 50 years is an efficient strategy to identify LS. In addition clinico-pathological features including site, histotype and presence of lymphocytes infiltration help to identify an additional subset of LS patients.

P18.29-S New genomic technologies and medical genetics: How much time is needed? A preliminary study R. Sukenik-Halevy, M. Ludman, A. Raas-Rothschild; Meir Medical Center, Kfar Saba, Israel.

Clinical genetics services are time and labor intensive, since they include both counseling meetings as well as extensive patient related activities such as administration, summary letters, and the interpretation of new genomic technologies. With increasing pressure for cost effective medical care, information is needed to evaluate the time and efforts required for providing medical genetics services. An online survey was conducted among 151 professionals who practice medical genetics throughout the world (85.75% medical geneticists, 13.12% genetic counselors). The reported average amount of time required for counseling sessions for pediatric, oncogenetic, pregnancy with a malformed fetus and preamniocentesis counseling was significantly different: ~6,0,42,51.27 minutes respectively. The average time required to write summary letters varied from 30 to 41 minutes. The time required for literature searches was 31-60 minutes and for patient related activities, 42 minutes. The time for patient related bioinformatics search and for test interpretation was 57 minutes. CMA requires an average of 48 minutes for analysis and for genetic counseling each. Professionals with less than 10 years’ experience needed more time than those with more than 10 years’ experience. Time devoted to clinical work received the highest percentage followed by administration, research and teaching. This study emphasizes the complexity and time consuming demands of the practice of medical genetics in the era of advanced genomic testing, further consideration and assessment is required in order to determine how to adapt genetic services to the demands of cost effectiveness, without compromising the quality of patient care.

P18.30-M Project Superhero: the kids are doing it for themselves J. M. Grey, A. Metcalfe1; 1AMEN, Tunbridge Wells, United Kingdom, 2King’s College, London, United Kingdom.

Multiple endocrine neoplasia (MEN) disorders are autosomal dominantly inherited syndromes characterised by multi-glandular adenomas/carcinomas. MEN1 and MEN2 are patient groups providing support and information resources to MEN patients. However, no information about MEN aimed specifically at children and young people currently exists. Inspired by 2011 research by Metcalfe et al1, AMEN started ‘Project Superhero!’ which aims to 1) increase knowledge about the condition within affected families, 2) increase involvement and compliance by affected young people in their healthcare, and 3) provide families with a wider range of information about MEN to aid open discussion. A Family Focus Day (FFD) held in March 2013 involved 13 young people aged <18 [ages 8-18] from 6 families [2 MEN2a, 1 MEN2b, 2 MEN1, 1 control family]. Adults and young people completed self-assessments to test their knowledge about MEN. Adults’ scores ranged from 3-5 (mean 4.3) for identifying five different glands; young people’s scores ranged from 0-5 (mean 3.2). Knowledge levels (0 = no knowledge, 5 = complete knowledge): adults’ scores ranged from 1-4 (mean 2.8) and young people’s ranged from 1-4 (mean 2.3). Questions from the young people included, ‘Do I have cancer?’ and ‘Will this kill me?’. The findings were used to develop resources to answer their questions, which include MEN1 and MEN2 Medikids’TM comic books and 2 website animations. Evaluation of the resources is underway.

P18.31-S The Genome Clinic in Geneva: an example of a multidisciplinary task force for the clinical use of next generation sequencing S. Fokstuen1, E. B. Hammar2, P. Mukrythanasis2, M. Albarca Aguilera2, M. E. Poleggi1, C. Brockmann1, M. Guipponi1, F. A. Santoni1, A. Maurer1, S. A. Hurst1, C. Moret1, S. Gimelli, E. Statzell1, A. Toloiani-Giacoboni1, E. Rabu1, K. Varkningsen1, E. Sibom Béna1, L. Sibomato Sibomana1, M. Mustafaj1, N. Hamamy1, T. Noualhi1, J. Blouin1, S. E. Antonarakis1; 1University Hospital of Geneva, Geneva 4, Switzerland, 2Institut fétique Historique Humanités, Geneva 4, Switzerland.

The advances of next generation sequencing (NGS) technologies enable their application in clinical care. However, beside the clear benefits of NGS, such an implementation faces technical, ethical and financial challenges, in-
cluding data processing, storage and management, variant interpretation, genetic counseling (informed consent, management of variants of uncertain clinical significance, incidental findings), quality control as well as reimbursement questions. In order to optimally integrate the use of NGS into our clinical practice, a data set has been created in a multidisciplinary working group, the Genome Clinic task force. This task force is composed of physicians and scientists, including clinical and molecular geneticists, bioinformaticians, bioethicists and a coordinator. During our weekly sessions, clinical cases of heterogeneous mendelian disorders that could potentially benefit from a NGS approach are presented, results and interpretation of analyzed cases are discussed, as well as issues related to bioethics, management and health policy.

During the pilot phase, we have validated 20 cases for whole exome sequencing followed by targeted bioinformatics analysis of selected genes. In addition, we have collaborated with the Swiss Federal Office of Public Health (SPOH) in order to render NGS a reimbursable genetic test by the health insurance. We will present the results of resolved clinical cases as well as the outcomes of our interactions with the SPOH.

In conclusion, this multidisciplinary task force has enabled us to deal with the multiple issues related to NGS in clinical practice and to ensure a high standard clinical service within this new and exciting field.

Orphanet has established strong quality standards over the years concerning the different situations of the countries part of the consortium. There are defined inclusion criteria for each activity. Information is collected from official sources specific to each country. Once the information is validated by bioethics experts, who are professionals working in the field of rare diseases with expert knowledge in the relevant activity. Orphanet UK has established several partnerships to post-validate its information. Rare Disease UK and Genetic Alliance validate patient organisations. ERNDIM validates EQA accredited metabolic laboratories in the UK. UK centres of expertise are validated by experts that are part of the EUCERD and research activities are validated by relevant patient organisations. Orphanet UK is also working to establish new partnerships to validate data about molecular and cytogenetic laboratories and data about clinical trials too. Every effort will be made to ensure that information is accurate, comprehensive and up to date.

P18.34-M
Attributes of adult patients and parents of children with cystic fibrosis to consider screening for cystic fibrosis
1Center for Medical Genetics, University Hospital Ghent, Ghent, Belgium; 2University Hospital Ghent, Ghent, Belgium, 3Vrije Universiteit Brussel, Brussels, Belgium; 4University of Ghent, Ghent, Belgium. VU University Medical Center Amsterdam, Amsterdam, Netherlands; 5Ghent CF Reference Center, Ghent, Belgium.

Cystic Fibrosis (CF) is a severe autosomal recessive condition with clinical symptoms such as chronic pulmonary disease and pancreatic insufficiency. CF affects approximately one in 2500-4000 Caucasians, while the carrier frequency is estimated at one out of 25 to 30. Carrier screening for CF has been available to individuals without family history of the disease since the early 1990s. However, very few screening programs have been implemented around the world to date. In order to assess social desirability of carrier screening for CF, it is important to study views and attitudes of key stakeholders, such as patients with CF and their family members.

The aim of this study was to assess views of adult patients and the parents of children with CF regarding carrier screening for CF. Participating were recruited from a register of patients at the University Hospital of Ghent. 134 questionnaires were distributed of which 112 were returned (response rate 83%). In overall, the attitudes towards carrier screening for CF were positive, with 80% of respondents thinking the procedure entails more advantages than disadvantages. Eighty-five percent of the respondents believe that the screening should be routinely offered to all couples planning a pregnancy, while 72.9% were of the opinion that the procedure should also be provided prenatally. Regarding future pregnancies, 46.1% would themselves choose for preimplantation genetic diagnosis, 43.6% would prefer to conceive naturally followed by a prenatal diagnosis. Others were ready to accept the risk of having an affected child, or opt for an adoption, 5% each.

P18.35-S
Predictive genetic testing in hereditary heart diseases: a single-center series of 304 subjects
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Hereditary heart diseases are typically characterized by autosomal dominant inheritance and delayed cardiac expression. Predictive genetic testing (PGT) is offered to asymptomatic relatives to allow targeted medical care and early therapeutics in order to reduce the risk of complications. Psychological issues related to PGT are complex and have been poorly studied. To evaluate our practices regarding PGT for hereditary heart diseases and study the behavior of relatives after the first information consultation, offering a waiting period before blood sampling. We retrospectively studied records from 304 consecutive relatives seen in our department and have requested PGT. Underlying diseases in the families were HCM (60%), DCM (17%), ARVC (15%), LQT (5%), Brugada syndrome (2%) and other (1%). There were 260 adults and 44 minors. At the time of the first consultation, the median age was 37 years (5% of the relatives previously had a cardiac checkup. After multidisciplinary consultation, 22 relatives (8%) dropped out of procedure and 11 relatives (3%) performed blood sampling but did not come back to know their results. Blood sample was delayed for 70% of relatives and immediate for 30%. A total of 21 different genes were analyzed and most frequent ones were MYBPC3 (97), MYH7 (77), LMNA (37). A mutation was present in 36% of relatives and absent in 64%. We observed a high level of genetic uptake after initial consultation but a minority of relatives decided to stop or delay the pGT. These results suggest the benefit of a waiting period before blood sampling.
P18.36-M  
Presymptomatic and predictive genetic testing in minors - a mini-review in preparation for new Danish best practice guidelines

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Aim: In preparation for the creation of Danish guidelines regarding predictive and presymptomatic genetic testing in minors, we have reviewed existing guidelines and policy papers as well as international conventions. Furthermore, we have reviewed existing literature concerning the psychosocial impact of testing children.

Materials: Guidelines on the topic from four of the largest English-speaking genetic associations were reviewed. The Convention on the Rights of the Child and the Convention on Human Rights and Biomedicine were also considered.

Results: In the reviewed guidelines it is recommended to offer genetic testing if the test result will be of medical benefit to the child. In some guidelines em phasis is on immediate benefit. If there is no potential medical benefit it is recommended to defer genetic testing for adult-onset disorders until minors are able to decide for themselves. Some guidelines suggest that psychosocial factors in certain cases can justify genetic testing for adult-onset disorders. Requests for genetic testing for childhood-onset disorders can usually be met. Thorough genetic counseling and consideration of timing of the test is of importance in the decision-making process.

Discussion: There is a lack of knowledge about the psychosocial impact of testing children. The recommendations in the reviewed guidelines are based on the classic medical moral principles: Nonmaleficence, beneficence and respect for autonomy. The recommendations are in consistency with international conventions on the field, but statements within these conventions can be subject to interpretation. We call for increased awareness of the difficulties in defining the "best interest of children".

P18.37-S  
Presymptomatic testing in minors: Requests and practices. Evaluation of pluridisciplinary consultations for the last 20 years


department de génétique, paris, France.

Twenty years ago, the first presymptomatic tests (PST) began for Huntington’s disease. Since then, PST have been extended to other diseases, in particular among minors. The practice of these tests is regulated by law within each European country and follows a number of principles, including respect for the «right to not know» and autonomy. In the case of children, performing such tests is complicated because all of these conditions can- not always be met. In the Department of Genetics in the Pitié-Salpêtrière Hospital, several protocols of PST are reported to the French Biomedicine Agency in accordance with French law. We wanted to explore our practice regarding children. All patients under 18 years of age at the first consultation for a PST were included in this retrospective study. 175 children met the inclusion criteria and they were divided into 4 groups (cardiology, myology, neurorlogy, oncology). Medical data but also access to the test or not, motivations, were collected. The average age of minors at the first consultation was 13 years. 69% of children have performed the test but this varies significantly (p<0.05) according to the pathology (46% in neurology and over 90% in cardiology and oncology). 86% of children also said they did not want the test at the first consultation, underlying the importance of parental demand. The study by group of pathologies also notes that the reflexion time, the reasons given for doing the test vary according to the pathology and emphasize the importance of a differentiated care.

P18.38-M  
Promotion of genetic services in the Slovenia-Italy cross-border region

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Aim: In preparation for the creation of Danish guidelines regarding predictive and presymptomatic genetic testing in minors, we have reviewed existing guidelines and policy papers as well as international conventions. Furthermore, we have reviewed existing literature concerning the psychosocial impact of testing children.

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Discussion: There is a lack of knowledge about the psychosocial impact of testing children. The recommendations in the reviewed guidelines are based on the classic medical moral principles: Nonmaleficence, beneficence and respect for autonomy. The recommendations are in consistency with international conventions on the field, but statements within these conventions can be subject to interpretation. We call for increased awareness of the difficulties in defining the “best interest of children.”

P18.39-S  
The French Foundation for rare diseases: accelerating rare diseases research

E. Chanut-de van den Brink; on behalf of the French Foundation for rare diseases (Fondation maladies rares), Paris, France.

The French Foundation for rare diseases is an innovative cooperative framework dedicated to rare diseases research. Flagship of the second French National Rare Diseases Plan, co-founded by University Hospitals, Research organisations and Patients organisations, we act as a federative and strategic hub to accelerate scientific, clinical and social innovation by stimulating cross-sector cooperation to the benefit of patients affected by rare diseases.

With our headquarters at the heart of the French Platform for Rare Diseases and seven regional coordinators, in direct contact with researchers all over the national territory, our priorities are driven by grounded needs and integrated into a national strategy with an international perspective. Our active support, spanning from basic to translational and clinical research, includes enhancing the access to high throughput technologies such as NGS, promoting international collaboration via dedicated partnerships, and accelerating the translation of research into clinical development through targeted links to orphan drug experts. Since rare diseases research is tightly linked to societal challenges, we are also actively supporting studies on social, economic, ethical impacts of rare diseases. We regularly initiate national working groups, open to international perspectives, to specifically target timely issues, including professional training needs, ethical and regulatory issues around data and bio-specimen collections, as well as the impact of new technologies in genetics, patients’ consent to genetic testing and their paths through diagnosis and treatment. Through this unique range of actions, we aim to contribute to acquainted national public health and research policies and to the promotion of international multi-stakeholders cooperation.

P18.40-M  
Rare Diseases week in Timisoara - a campaign with a good start

M. Gafencu1, G. Doros1, D. Danci1, I. Jurca Simina1, L. N. Bogdan2, M. Puis2; 1Victor Babes University of Medicine and Pharmacy, Timisoara, Romania, 2National Alliance for Rare Diseases Romania, Zalau, Romania.

Aim: „Volunteers for Rare Diseases” was created in „Save the Children” Timis in 2007 when students from the University of Medicine Timisoara, supported by teachers, wanted to work with people with special needs. The campaign launched on the International day of rare diseases was the opportunity to spread informations about this topic in community. An extension to a whole week dedicated to this was the next step.

Increase awareness in general population, involving the local authorities, raising the interest in this field for medical services employees and involving our students in volunteer work were main objectives.

In all the 5 years we used internet as a tool and mass-media campaign also. Volunteers made a street march in the dedicated day with flyers distribution in downtown and placing posters. We organised round table at the local TV with specialised medical staff. Each year we met the children in hospitals and settled lessons for parents.

With parents we discussed the common situations of various diseases, following the monitization of their skills yearly. „Rare Disease Day” has taken place on the International day of rare diseases was the opportunity to launch a campaign with a good start. With parents we discussed the common situations of various diseases, following the monitization of their skills yearly.
authorities realise the need for social protection with different ways to support this people. All the student involved can use informations about this pathology and early diagnose cases, encouraging prevention.

**P18.41-S**

Respecting autonomy while reacting to change: A review of current policy and empirical evidence on re-consent in longitudinal biomedical research

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Background: The need to balance the autonomy of participants and their consent to participate in biomedical research with the fast pace of scientific enquiry is becoming difficult as the number of projects grows and pressure increases at national and international levels to link project resources to facilitate access and data sharing.

Methods: We undertook a literature review from the perspective of longitudinal cohort studies and biobanks. We examined existing policy statements, academic literature on re-consent, and evidence from re-consent exercises.

Results: Guidance on policy bodies is vague, suggesting that re-consent should be sought if changes are made to the original protocol or if new research falls outside the scope of the original consent. Stakeholders' attitudes showed different approaches. Broad consent, with or without an oversight body is a popular alternative, while seeking re-consent for every study was also mentioned by a minority. Several alternatives along this continuum have been suggested by commentators and as a result of empirical studies. Actual and potential research participants want re-consent when a new use presents increased risks and is for a new unrelated condition, while commentators also suggest re-consent when the research is moving beyond existing use, such as for next-generation sequencing.

Conclusion: Practical difficulties, potential loss of participants and increased costs make re-consent for every new study unpractical and in some cases impossible. There is a lack of studies investigating actual participants and their positions on re-consent and research is needed to inform best practice guidelines for re-consent in longitudinal studies.

**P18.44-M**

Gonadal mosaicism in split-hand/foot malformation: Implications for genetic counselling

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Split-hand/foot malformation (SHFM) is a congenital limb defect affecting predominantly the central rays of the hands and feet. SHFM is genetically and clinically heterogeneous with inter and intrafamilial variability in the clinical manifestations. Most cases are inherited in an autosomal dominant manner and a causative genetic alteration is detected in approximately 50% of affected cases. SHFM3 accounts for 20% of cases and is caused by tandem microduplications at 1q24. We report 2 cases of recurrent 1 limb ectodactyly with phenotypically normal parents and 1 case of a female with SHFM3 and her difficult attempts at IVF due to an abnormally high number of affected embryos. Case 1: A brother and sister presented with isolated 1 limb ectodactyly. Microarray analysis showed a de novo 1q24.32 microduplication, which was 434 Kb in size. Case 2: A mother with a child with 1 limb ectodactyly was 16.5 weeks pregnant when abnormalities of the hands and feet were identified on fetal ultrasound. Microarray analysis confirmed the 1q24.32 microduplication, previously identified in the affected sibling. Case 3: The proband was a mother with SHFM3 and a microduplication at 1q24.32, approximately 540 Kb in size. Preimplantation genetic diagnosis of 16 embryos identified the microduplication in greater than 80% of the embryos. We speculate that SHFM3 may represent a subtype of ectodactyly that exhibits a higher chance of gonadal mosaicism. Genetic counselling in these cases should reflect this observation. Further research of the complex inheritance of SHFM is essential to providing families with accurate counselling.

**P18.45-S**

Regulation of genetic information

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Background: Genetic information in Norway is regulated by The Norwegian Act of Biotechnology (2003). One of its purposes is to protect people against discrimination and stigmatization. According to the Act § 5 – genetic information in terms of predictive, symptomatic or carrier status shall not be released to insurance companies or employers. Purpose: The purpose of this study was to gain new insight and broader knowledge regarding regulation of genetic information in Norway. The focus was how people from insurance companies, medical genetics, patient organizations and the Norwegian government experience the regulation of § 5-8. Material and methods: A qualitative method was used with in-depth interviews of seven participants. Results: The participants experienced the § 5-8 to be important. They stated that genetic information is private and can easily be linked to a person’s identity. Genetic information can also easily be misused, misinterpreted and can result in healthy people thinking they have a diagnosis. Despite challenges associated with enforcing the paragraph, the participants believed that it has a major role in protecting people from discrimination and stigmatization. In terms of new technology and commercial forces they also thought the paragraph will be important. Conclusion: The paragraph § 5-8 has a high significance for the participants. It will also be important for future research.

**P18.43-S**

50 years anniversary of Smith-Lemli-Opitz Syndrome

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50 years ago the Smith-Lemli-Opitz Syndrome (SLOS, RSH syndrome) was described for the first time in three male patients by David W Smith, Luc Lemli and John Opitz. It was a clinical description showing microcephaly and hypogenitalism. 30 years later Tint and colleagues found the underlying defect in cholesterol biosynthesis (1994). The gene was identified by a group in Innsbruck (Fitzy et al., 1998), first mutations in SLOS patients have been detected by the same group and also by Wassil et al. (1998). Hence SLOS is a metabolic and malformation disorder caused by mutations in the DHCR7 gene. This gene encodes the 7α-stenoreductase. Now more than 130 mutations are known and all affected patients are included in the DHCR7 database (http://databases.lovd.nl/shared/genes/DHCR7). Mutation spectra are different in European populations. Time since establishment of common founder mutations (c.964-1G>C, p.Trp151* and p.Thr93Metc) is long enough (about 100 and 200 generations) to explain frequencies (1:100 for c.964-1G>C) by genetic drift (Witsch-Baumgartner et al., 2007). There are modifiers of clinical severity of SLOS. Depending on SLOS patient’s maternal variants apoe 2, 3 or 4 the severity vary significantly (Witsch-Baumgartner et al., 2004). The fundamental problem of the disease is the lack of cholesterol during embryogenesis. Regarding therapies adding HMGC-CoA reductase inhibitor (simvastatin) might ameliorate the severity (Jira et al., 2000; Haas et al., 2007). After 50 years the pathophysiology of SLOS is still not as clear due to multiple functions of cholesterol. To help patients it is still necessary to continue research on SLOS.

ABSTRACTS POSTERS

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ture perspectives to prevent people from discrimination and stigmatization. In order to protect human rights, it is important to inform patients about the regulation.

P18.46-M
Chromosomal mosaicism in chorionic villi: implications for prenatal diagnosis and genetic counseling
TOMA, Advanced Biomedical Assays S.p.A., Busto Arsizio, Italy.

Chromosomal mosaicism in chorionic villi (CVS) can be either confined to the placenta (CPM) or generalized to the fetus (TFM). The probability of TFM depends mainly on timing and mechanism generating the mosaicism. Due to the variable distribution of the abnormal cell line, when a mosaicism is detected in CVS a confirmatory amniocentesis should be performed to discriminate between CPM or TFM. We present a diagnostic experience on 52,673 CVS combining cytogenetic analysis of cytotrophoblast and mesenchyme and, in case of CVS mosaic, on amniocytes. A CVS mosaicism was found in 1.01% of CVS. The stratification by category of chromosome abnormality indicates that mosaics involving 47, +mar or sex-chromosome aneuploidies have the highest risk of TFM (35.8% and 31.6%, respectively) while autosomal trisomies and 46,der karyotypes have a lower risk (6.9% and 5.2%, respectively). We will present the risk of TFM stratified by type of chromosome abnormalities distinguishing mosaic (MA) and nonmosaic abnormalities (NMA). Genetic counselling is challenging in case of CVS mosaicism and the need for data to calculate a risk of fetal involvement is vital to refine a personalized chromosome abnormality-based strategy of investigation to reduce the need for follow-up amniocentesis. While prenatal diagnosis is destined to become “molecular” with microarrays, NIPS and NGS, the large cytogenetic diagnostic experience on CVS presented in this study is helpful to evaluate limits and advantages of the new technologies that must be integrated in pretest and post-test counseling.

P18.47-S
Realising Genomics in Clinical Practice- Whole Exome Sequencing and the Patient Pathway
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Whole genome sequencing (WGS) and whole exome sequencing (WES) have been described as transformative technologies with potential to revolutionise patient care. As part of a bigger project addressing the ethical, legal, social (ELSI) and operational challenges likely to be raised by these technologies 'Realising Genomics in Clinical Practice', the PHG Foundation convened a workshop to explore the impact of these technologies on patient pathways. Key features such as the point and source of referral for sequencing will influence how WES/WGS will be translated into clinical practice. By comparing patient pathways currently in use in targeted approaches and whole exome sequencing, our aim was to illustrate the key operational differences and translational aspects that are likely to arise. In particular, we focused on three areas which seem to raise distinctive ELSI challenges: the nature and scope of consent processes and the degree to which they might need adaptation; technical aspects including the scope, construction and standardisation of data filters, and requirement for data sharing in order to validate and interpret findings; and the extent, timing and nature of disclosure of test results, including incidental or unsolicited information. We review our findings and evaluate the prerequisites for effective translation, including requirements for additional genetic counselling, and the educational and training needs of health care professionals, clinical scientists, patients and publics. These findings will be contextualised by presenting preliminary results from the entire Realising Genomics Project. Further information on the Realising Genomics project can be found at http://www.phgfoundation.org/pages/realising_genomics.htm

P18.48-M
Revisiting Hook: rates of fetal aneuploidy and other significant genetic aberrations in women presenting for prenatal care
1TOMA Advanced Biomedical Assays S.p.A., Busto Arsizio, Italy; 2Albert Einstein College of Medicine, Bronx, NY, United States; 3Univeristystyczne Centrum Zdrowia Kobiet i Noworodka, Warszawa, Poland; 4Bryn Mawr College, Bryn Mawr, PA, United States; 5Columbia University Medical Center, New York, NY, United States; 6Natera, San Carlos, CA, United States.

To date, health care providers have relied on modelled values to counsel pregnant women regarding their age-related risks for common aneuploidies, particularly in younger maternal age categories. The goal of this study was to provide a contemporary update using actual data from a single laboratory to derive specific age-related risks for common aneuploidies as well as significant non-aneuploidy fetal genetic aberrations. QFQ-banded karyotypes from prenatal samples analyzed at a single biomedical from 1994-2012 were analyzed. Selected for study, were samples in which the only indication for karyotype was either maternal age ≥ 35 or maternal anxiety (patients <35 years) with exclusion of additional risk factors, such as maternal serum markers or ultrasound anomalies. 12,9,263 karyotypes from villi and AF were analyzed. As expected, autosomal aneuploidies and 47,XYY show a statistically significant positive association with maternal age while MX shows a borderline negative association only for CVS. Risk of non-aneuploid anomalies compromise a substantial share of chromosomal abnormalities in younger women. We present the rates of fetal aneuploidies and other significant chromosomal abnormalities stratified by two periods of gestational age and, when applicable, by maternal age, which have been observed in real clinical practice. As standard prenatal screening strategies currently do not detect these non-aneuploidy DNA aberrations, the data presented in this study is critical for informed patient decision-making which should be a routine part of genetic counseling in the prenatal setting.
EMPAG EDUCATIONAL SESSIONS

EES1.1
Responding to guilt and shame in clinical consultations
C. Baguley
Psychological Professions Network - Health Education North West, Manchester, United Kingdom.

Genetic medicine is an area of health care that often deals with delivering profound and difficult information; it brings clinicians directly in contact with individuals and families who sometimes have to make very difficult and life-changing decisions. Genetic counselling involves the skill of giving information, advice and support to help guide patients through this process. Adjusting to information and assimilating new knowledge can have significant implications for the life decisions patients make. It is now recognised as normal for the course of adjustment to involve a range of emotional responses including denial, sadness, anger and guilt before the stage of acceptance is reached. This process can take time, individuals can vary in presentation and sometimes they can get ‘stuck’ often resulting in unhelpful behavioural consequences and increasing distress. In order to be effective clinicians need to understand this process and be equipped to recognise and respond to emotional responses that may occur within routine consultations.

This workshop looks specifically at the nature of guilt and shame in the context of genetic medicine. Drawing upon a cognitive-behavioural model of emotion we will consider how the difficult and corrosive emotions of guilt and shame can be understood as an attempt by the individual to assimilate information and actions into their pre-existing belief system. Drawing on a combination of theory, reflection on clinical examples, and illustration by video, participants will be encouraged to consider how they can develop their clinical interview skills to identify and respond to expressions of guilt and shame in routine clinical settings.

EES2.1
Qualitative and quantitative methods in psychosocial research
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This presentation will provide an overview of qualitative and quantitative methods in psychosocial research in the genetics setting. In the first part of the session, we will cover three distinct types of qualitative research that have been used in psychosocial research in genetics: thematic analysis; discourse analysis; and deliberative approaches. Each approach is useful for particular kinds of research questions and problems. Case studies will be presented to illustrate the utility of each method, and the specific research questions it is able to address. Case studies will be drawn from the domains of genetic testing and counselling, popular representations of genetic risk, and research participation in biobanks.

The second part of the session will cover the key concepts and study types used in quantitative methodology, including survey studies, case control studies, cohort studies and randomised controlled trial, as well as examples of quantitative studies that may be carried out in the genetics setting to illustrate strengths and weaknesses of different types of designs. This will be followed by an introduction to the most commonly used validated instruments suitable for measurement of genetic counselling and testing outcomes. Finally the session will cover several health psychology theories that are particularly relevant to the genetic counselling and testing setting and may be used to provide the basis for formulation of study hypotheses and interpretation and analyses of findings.

EMPAG PLENARY LECTURES

EPL1.1
The impact of total gastrectomy upon e-cadherin carriers: experiences of eating
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Between 30%-50% of Hereditary Diffuse Gastric Cancer (HDGC) are caused by mutations in the E-cadherin gene. CDH1 mutation carriers have an earlier than average age of disease onset, and greatly increased risks of developing stomach cancer. Individuals identified as at-risk, either because of their family history or as a result of DNA testing, need to make decisions about risk management, whether they will have risk-reducing surgery (total gastrectomy - RRG) or continue screening. This retrospective study interviewed 42 patients, 27 of whom had undergone RRG. In this paper we will reflect upon the impact of surgery on bodily integrity and look at people's experiences of living without a stomach. The paper will focus upon eating post surgery, and discuss the ways in which surgery impacts upon identity. We will demonstrate that following surgery, hunger and satiety are constructed as disembodied experiences or desires that need to be re-embodied. Finally, we will argue that the process of re-embodying these supposed 'physiological' states raises a number of issues about the nature of hunger and satiety. These will be interrogated using an analytic framework in which internal states are understood as grounded within public criteria.

EPL1.2
Impact of rapid genetic counselling and testing on primary surgery and psychosocial well-being in newly diagnosed breast cancer patients: Findings from a randomized controlled trial
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Aims Female breast cancer patients carrying a BRCA1/2 mutation have an increased risk of contralateral breast cancer. We investigated the impact of rapid genetic counselling and testing (RGGCT) on treatment decisions and psychosocial well-being. Methods Newly diagnosed breast cancer patients from 12 Dutch hospitals with at least 10% risk of a BRCA1/2 mutation were randomized to an intervention group (offer of RGGCT) or a usual care control group (ratio 2:1). Study outcomes included uptake of direct bilateral mastectomy (BLM), cancer-specific distress, anxiety, depression, and health-related quality of life (HRQOL). Assessments took place at study entry and at 6 and 12 months follow-up. Results Between November 2008 and December 2010, we recruited 265 women. Based on intention-to-treat analyses, no significant group differences were observed in percentage of patients opting for a direct BLM (14.6% (RGCT group) versus 9.2% (control group); OR 2.31; CI 0.92-5.81; p=0.08). Per-protocol analysis indicated that patients who received DNA test results before surgery (59/178 women in the RGCT group) opted for direct BLM significantly more often than patients who received usual care (22% versus 9.2%; OR 5.09; CI 1.15-8.31; p=0.03). No statistically significant differences were observed between groups over time on any of the psychosocial or HRQOL outcomes. Conclusions These results suggest that RGCT can be safely offered to newly diagnosed high-risk breast cancer patients. However, DNA test results need to be made routinely available pre-surgery in order to play a more significant role in surgical treatment decisions.

EPL1.3
Disclosure of psychosocial research results: a randomized study among GENEP50-Ψ cohort participants
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Background: The disclosure of aggregate research results to long-term cohort participants is rarely done and no recommendation specifies the best way to achieve this ethical obligation. GENEP50-Ψ cohort follows both BRCA1/2 carriers and non-carriers to study their psychosocial and preventive behaviour. Our aim was to study the impact of different formats of disclosure on participants' views about disclosure of research results. We compared three methods of disclosure: 1) a letter accompanied by a telephone call, 2) a letter only, and 3) an online survey.
EPL1.4 Prevalence and detection of psychosocial problems in cancer genetic counseling


Introduction: Although only a minority of individuals undergoing cancer genetic counseling experience high levels of distress, many experience a range of specific psychosocial problems related to genetic counseling. The aim of this study is to evaluate the prevalence of these problems, and to investigate which method for detecting psychosocial problems is most optimal during counseling. Methods: Individuals undergoing genetic counseling for cancer were invited to complete a questionnaire including the Psychosocial Aspects of Hereditary Cancer (PAHC) questionnaire, the Hospital Anxiety and Depression Scale (HADS) and the Distress Thermometer (DT) prior to, or immediately following, their counseling session. Results: The most frequently reported problems of the 137 participants were on the PAHC-domain 'living with cancer' (84%), 'family issues' (46%), 'hereditary predisposition' (45%), and 'child-related issues' (42%). Partial correlations between the PAHC, the HADS and DT were low. Previous contact with a psychosocial worker, and a previous cancer diagnosis were significantly associated with higher distress on the HADS, but explained little variance (9%). No variables were associated with the DT. Previous contact with a psychosocial worker, and having children were significantly associated with several PAHC domains, but explained a small percentage of the variance (2-14%). Conclusion: The large majority of counselees experience specific problems related to cancer genetic counseling. No variables were identified as important predictors of distress or psychosocial problems. To detect experienced psychosocial problems, we recommend that all counselees complete a brief problem-oriented questionnaire like the PAHC, and not only a questionnaire measuring distress, as a routine part of cancer genetic counseling.

EPL1.5 Developing a group programme for BRCA1/2 mutation carriers who underwent prophylactic mastectomy

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Introduction - Prophylactic mastectomy in BRCA1/2 mutation carriers reduces the breast cancer risk significantly, but may have a profound impact on body image and self-esteem. In addition, some women report feelings of isolation and stigmatization. Other issues to face are an increased risk of ovarian cancer, potential cancer risk for offspring, intimacy with the partner, communication with family and ongoing grief. Methods - As a result of our long-year studies on impact of prophylactic mastectomy a group intervention was developed with regard to the supportive-expressive needs of these women. With a maximum of 10 members, a closed 8-sessions group programme was developed, consisting of the following sessions: 1) introduction 2) body image 3) social support and coping 4) social support and loss 5) partner relationship or dating 6) ovarian cancer risk 7) communication within the family 8) evaluation and future plans. Results - Seven women participated in the first group aged 26-52. The older women advised the younger and vice versa, for example with regard to mother-daughter communication issues. The group members reported high satisfaction, particularly they felt they were no longer isolated and could share their experiences and learn from each other. One woman who already had mastectomy for previous breast cancer, felt she was different from the others. Conclusion - A next group will focus on a more homogenous population of women to be closer to the experiences of having had cancer yet or not. After refining content and structure of the programme an intervention study will be established.

EPL2.1 Ok for us, not for them: Patients and genetic counsellors’ experiences of NIPT and views on wider use

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In the UK non-invasive prenatal testing (NIPT) is now routinely offered for sex determination of pregnancies at risk of sex-linked conditions, and technology is progressing towards use for diagnosis of single gene disorders and detection of aneuploidy. We present the findings of a two-arm, qualitative study investigating both genetic patients’ and genetic counsellors’ (GCs’) experiences of NIPT for sex-linked conditions and their views on wider use of the technology. Forty (20 patients and 20 GCs) semi-structured interviews were completed and transcripts analysed using modified grounded theory methodology.

While both groups expressed that they felt positive about the use of NIPT for pregnancies at known genetic risk, they had concerns about broadening its use in the routine antenatal setting, particularly relating to the early timing in pregnancy and the apparent ease of using the technology. Shared concerns included the difficulties of ensuring informed consent, potential misuse of the technology for non-medical indications, and a diminished acceptance of disability in society. Patients articulated a strong sense of distinction from the general public, which included their use and perspectives of the technology, based on their lived experience, prior knowledge of a genetic condition and sense of ‘genetic’ responsibility. GCs discussed concerns about availability of necessary knowledge and time to appropriately offer NIPT in stretched routine services to provide the counselling and support they felt patients required. These findings highlight the importance of discussing and considering the issues surrounding wider implementation of NIPT including differences between those with prior genetic risk and the general population.

EPL2.2 Non-invasive prenatal testing (NIPT): opinions and interest among pregnant women in a country with relative low uptake of prenatal screening

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Objective: To study pregnant women’s current uptake of prenatal screening for Down syndrome, and future interest in non-invasive prenatal testing (NIPT). Methods: Online survey on the Dutch pregnancy Fair website, completed by n=389 pregnant women.

Results: Uptake of the combined test (33%) corresponded to the average uptake in the Netherlands. Most important reason to have this test was reassurance that the child is healthy (57%). Reasons to decline this test were: ‘I do not want to know if I have a child with Down syndrome’ (27%) or ‘NIPT results are just a chance’ (49%), ‘fear of miscarriage due to follow-up invasive diagnostics’ (34%), 230 (59%) had heard of NIPT; 48% were interested in having NIPT, 24% were unsure, and 28% were not interested. Safety (3%) and accuracy (30%) were considered most positive aspects, and 70% were willing to pay for it (average 150 euros). 41% expected that women would deliberate less before taking the test, 23% expected more women to feel obliged to take it. Testing for other diseases was considered positive, mostly because this could avoid suffering (75%). However, 38% expected that women cannot foresee the consequences of their choices, and acceptance of children with a handicap will reduce (35%). 42% found that women should be able to choose from a list of diseases, 25% preferred packages with different diseases to choose from, and 30% preferred a fixed list (no choice). Conclusion: The results suggest that more women will have prenatal screening if NIPT is to replace the combined test. However, challenges for counselling are expected if NIPT is introduced, especially when widening the scope of testing.
EPL2.3 Received information and knowledge about Down syndrome among pregnant women and their partners coming for a first trimester combined (CUB) test - Do they have the knowledge to make the decision? C. Ingvoldstad1, E. Ternby2, G. Annerén1, P. Lindgren1, O. Axelsson2; 1Center for fetal medicine, Karolinska University Hospital, Solna, Sweden, 2Department of Women’s and Children’s Health, Uppsala University, Uppsala, Sweden, 3Department of Immunology, Genetics and Pathology, Uppsala University, Uppsala, Sweden, 4Centre for Clinical Research Sörmland, Uppsala University, Uppsala, Sweden.

Fetal diagnostic testing for chromosomal abnormalities such as Down syndrome (DS) is frequently used in Sweden. Prior to testing expecting parents do not routinely receive information about the conditions being tested for. The aims were to assess why expecting parents choose to undergo CUB-testing (combined ultrasound and biochemical test), their perception of information, thoughts about invasive procedures, possible termination of pregnancy and knowledge about DS.

Method: From November 2010 to March 2011, 105 pregnant women and 104 partners answered a questionnaire after completing the CUB-test, at the Fetal Medicine Unit, Uppsala University Hospital.

Results: The most common reason for choosing a CUB-test is “to get a confirmation on a healthy baby” or “because you should do it.” A majority had not been given information on what it means to live with a child with DS and many requested more information. Internet is the most common source for information about DS. A substantial proportion of pregnant women and partners have little knowledge of the medical, cognitive and social consequences of DS. Twenty-four percent had not yet decided about invasive testing if increased CUB-risk and almost half had not decided what to do about the pregnancy if DS was diagnosed.

Conclusions: A majority of expecting parents attending CUB-test had not received information about DS and requested more information. A substantial proportion of expecting parents have varying and in several aspects low levels of knowledge about DS and its consequences. Many had not yet decided what to do if DS was diagnosed.

EPL2.4 Diagnosis Down syndrome: a cross-cultural study of family experiences M. L. Van Riper; University of North Carolina Chapel Hill, Chapel Hill, NC, United States.

Purpose: Much has been written about family-provider interactions surrounding the diagnosis of Down syndrome (DS) and substantial resources have been devoted to helping health care providers feel more prepared to deliver the diagnosis of DS. However reports of parents receiving inaccurate information continue to appear in the literature. Moreover, anecdotal reports of parents feeling pushed to make unwanted choices, such as undergoing invasive testing or terminating a pregnancy following the diagnosis of DS, are becoming more common. In addition, limited attention has been devoted to understanding how family experiences vary from one country to another. Therefore, the purpose of this presentation is to compare family experiences in four countries (Ireland, Portugal, United Kingdom, USA) where differences currently exist in terms of options for prenatal testing and options for families following the diagnosis of DS. Method: 1185 parents of individuals with DS completed a survey which includes a variety of questions concerning family-provider interactions surrounding the diagnosis. In addition, interviews were conducted with a subset of parents. Results: Findings suggest that 50% of the parents were dissatisfied with family-provider interactions and most health care providers are not following the recommended guidelines regarding how to inform parents. Additionally, important cultural differences were noted. Conclusion: Findings from this study will help in efforts to improve parental satisfaction with family-provider interactions surrounding the diagnosis of DS. In addition, they will help ensure that cultural context is considered during the informing process.

EPL2.5 Dynamics of prenatal screening: blurring boundaries between normative frameworks W. Donderg, G. De Wert; Dept Health, Ethics & Society / Research Schools CAPHRI & GROW/Maastricht University, Maastricht, Netherlands.

Historically, distinct prenatal screening programmes are aimed at finding different types of conditions: 1) maternal/fetal diseases or markers requiring intervention or adapted care in order to secure healthy pregnancy outcomes for mother and child; 2) fetal disorders that the prospective parents may regard as a reason for abortion. Ethically, this is an important distinction. There is a widely shared consensus that for screening leading to no other possible interventions but abortion, prevention is a morally problematic category. This is why official documents define such screening in terms of what may be called the ‘autonomy paradigm’, where the aim is to help individual women/couples make autonomous reproductive choices. This is also reflected in counseling guidelines. Therefore, the purpose of this presentation is to compare family experiences in four countries (Ireland, Portugal, United Kingdom, USA) where differences currently exist in terms of options for prenatal testing and options for gene expression profiles that are predictive of pregnancy complications. The expanding scope of prenatal screening will also provide more options for fetal therapy. This presentation will consist of a systematic exploration of the ethical challenges involved in this blurring of frameworks and conclude with ethics guidance recommendations for the future field of prenatal personalized medicine (Bianchi).

EPL2.6 Stigma and reproduction: the place of stigma in reproductive decisions A. J. Clarke; Cardiff University School of Medicine, Cardiff. United Kingdom.

This paper reviews the impact of a genetic disorder on family life, reflecting its mode of inheritance, and then examines the social impact of a specific genetic disorder, hypohidrotic ectodermal dysplasia. The stigmatisation of HED affected males is as important in the accounts given by their womenfolk as the physical effects of the condition; this impacts on their feelings about transmission of the disorder to the next generation. Perspectives may also change over time, with grandmothers expressing more strongly their sense of guilt at having transmitted the condition, despite there being no question of moral culpability.

We then consider the broader impact of stigma on reproductive decisions. They can be impacted by stigma both (i) within families where the practical effects of a specific genetic disorder are well known, and (ii) in couples faced by decisions in pregnancy with no prior expectation of a fetus affected by a genetic disorder. In the former case, any decision made has implications for the self-esteem of affected family members. In the latter case, decisions about continuing or terminating the pregnancy may be affected by the parents’ remembered past and/or imagined future responses to encountering an affected individual. In this way, parents’ fantasies may shape their decisions.

The scope for parental fantasies to influence decisions is greater when technology amplifies the uncertainty attained in genetic investigations or ultrasound imaging. How will parents’ fantasies of uncertainty play out in practice? What can genetics professionals do to promote respect for affected individuals?

EPL3.1 How do research participants perceive “uncertainty” in genomic sequencing? B. B. Bieseker1, W. Klein2, L. G. Bieseker2, P. K. Han3; 1National Human Genome Research Institute, Bethesda, MD, United States, 2National Cancer Institute, Bethesda, MD, United States, 3Maine Medical Center Research Institute, Scarborough, ME, United States.

Introduction: The scope of uncertainty in genomic sequence information has no rival in health care delivery. We present data from adults participating in an NIH genome sequencing cohort study where perceptions of uncertainty are hypothesized to be key in predicting decisions to learn and act on genomic health information. Methods: We conducted six moderated focus groups with 39 randomly selected ClinSeq® participants, varying whether they had coronary heart disease and/or prior receipt of sequence results. We elicited perceptions of the uncertainties associated with genomic sequencing using writing prompts. Results: Participants perceived the uncertainty as a quality of the information. The majority of participants characterized uncertainty of sequencing information as “changing, fluid, developing, or ground breaking.” These responses led to anticipation of more optimistic future outcomes. Fewer participants described uncertainty as “questionable, less accurate, limited, or poorly understood.” These perceptions seemed to undermine participants’ faith in the information, leading to feelings of disillusionment. Discussion: Our findings suggest that perceptions of uncertainty are related to epistemological beliefs and thus expectation of the information. Interventions to promote realistic expectations of genomic sequencing may mitigate adverse responses to uncertainty.
EPL3.2 Discussing clinical utility; The role of patients and their families
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These last years, many studies investigate the clinical utility of whole exome sequencing as a diagnostic method. Clinical utility is most commonly established on the basis of data that show the effectiveness or economic value of an innovation. Given that whole exome sequencing is a very new method, however, a multidimensional approach to clinical utility seems more appropriate, which also investigates its acceptability for patients.

This paper presents how patients and their families assess the utility of whole exome sequencing, and the diagnosis it produces. Our case-study is based on 20 in-depth interviews with patients and their families, who are involved as participants in a research project which aims to assess the clinical utility of whole exome sequencing as a diagnostic tool for children with hitherto unidentified developmental delay.

The case study provides insight into why these patients and their families want a diagnosis, and how they value the diagnosis that they eventually get. Their evaluations reveal not just the acceptability of WES as a diagnostic tool, but provide insight into how diagnoses are evaluated with respect to their daily caring practice and social lifeworld. We will argue that this provides important input to an assessment of the clinical utility of WES, which may inform how WES is to become part of clinical routine, and how it should be accompanied by counseling.

EPL3.3 Variants in Practice Study (VIP): High risk women’s responses to receiving genetic test results for genomic variants associated with breast cancer risk
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Results from genome-wide association studies (GWAS) have identified common genomic variants that form an important component of the heritability of breast cancer risk. Data from Victorian high risk breast cancer families demonstrate that testing for these genetic factors identifies a significant aetiological group known as ‘polygenic families’ and provides clinically important information.

It is important to investigate lay and professional understandings of novel, complex genetic test information generated by SNP testing to identify the most effective ways for genetic health professionals to communicate this information to patients and the treating medical team.

Our qualitative study aimed to assess patient and healthcare professionals’ understandings of genomic variant data. This presentation focuses upon women’s experiences of receiving SNP results following their participation in a GWAS study: Variants In Practice study (VIP).

Forty women attended an appointment at the familial cancer clinic, Peter Mac, Australia. Women had 1 previously been diagnosed with breast cancer 2 underwent BRCA1/2 mutation testing (no mutation found). Subsequently they were genotyped for 22 common genomic variants from which breast cancer risks were calculated.

Analysis of interview transcripts has revealed a number of preliminary themes, including study participation is motivated by feelings of altruism and specifically responsibility for family members. Receiving SNP information was viewed very positively, particularly by those women who had previously undergone risk-reducing surgery, who felt their decision was validated by their polygenic result. In conclusion, this study suggests that SNP results are regarded as useful information for both the research participants and their families.

EPL3.4 To Disclose, or Not to Disclose? The Context Matters
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Progress in understanding childhood disease using next generation sequencing (NGS) portends vast improvements in the nature and quality of patient care. Ethical questions surrounding the disclosure of incidental findings (IF) persist, as NGS and other novel genomic technologies become the preferred tool for clinical research. Thus, the need for multidisciplinary discussions and disclosure practices on the return of results in paediatric research has never been more immediate. The aim of this study is to explore the views of investigators concerning the disclosure of IFs in the paediatric oncology context. Our findings reveal at least four contextual themes underlying the ethics of when and how young participants and their families could be made aware of these unexpected results during the course of their research participation: clinical significance of the result, respect for persons, scope of professional responsibilities and implications for the healthcare/research system. Moreover, this study illustrates heterogeneity of standards and approaches within the broader researcher community, and the need to recognize the multiplicity of contextual factors that characterize paediatric cancer genomic research, specifically. As NGS increasingly becomes a centerpiece for innovative genetic research in paediatric oncology, sober thought should be given to the possibility of discovering IF, and to proactive and anticipatory management of resultant data that conforms to biethics norms. The authors intend to broaden the scope of ethical disclosure practices for paediatric participants, their families and the investigators who recruit them.

EPL3.5 Comparing the views of Australian parents, paediatricians and genetic health professionals about disclosure of genomic results
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Background: Genomic chromosomal microarray (CMA) testing for childhood investigations has increased diagnostic yields. However, CMAs also increase detection of incidental findings (IFs) and variants of unknown and uncertain clinical significance (VUS). Elucidating patient disclosure preferences may help clinicians anticipate the type of results about which patients want to be informed.

Methods: A questionnaire, using hypothetical scenarios, was designed to investigate and compare the perspectives of parents, paediatricians and genetic health professionals for result disclosure. Quantitative data were analysed using ANOVA and Kruskal Wallis tests. Open text data were analysed using content analysis.

Results: 147 parents, 159 paediatricians and 69 genetic health professionals participated and at least 89% of respondents in each category certainly or probably favoured disclosure of VUS as well as variants of certain clinical significance, with the lowest percentage being amongst parents, who were less sure of their disclosure preferences. There was consensus among respondent groups that knowledge of a variant of certain clinical significance would provide more practical and emotional utility compared to VUS. Parents demonstrated some different perspectives to health professionals; for example, they placed more emphasis on using knowledge of a VUS when considering future pregnancies (KWallis:p<0.001).

Conclusion: This study, together with a previous study investigating the opinions of a subset of these respondents for disclosure of IFs, is the first Australian exploration of preferences for genomic result disclosure, with implications for clinical practice.

(1) Turbitt E, et al. Availability of treatment drives decisions of genetic health professionals about disclosure of incidental findings. EJHG 2014;doi:10.1038/ejhg.2014.11

EPL3.6 The experiences and views of health care professionals and researchers regarding the feedback of results in the context of next generation sequencing studies
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Next generation sequencing (NGS) allows the production of large volumes of sequence data (and potentially genetic results) and the ethical and practical issues regarding feedback of results become particularly pertinent to address. Should (any) results be given to research participants? If so, which results and who should they provide? Within two EU funded projects in oncology (CAGEKID and EUROTARGET), in order to gather researchers’ and health care professionals’ views and experiences on providing results we distributed a questionnaire to attendees of genetics meetings in Europe in 2013. Of the 95 respondents, 88% work as researchers and/or clinicians in a field related to oncology and half (52%) use NGS in some aspect of their work; 56% of respondents state that they provide specific information about NGS to participants or patients before enrolling them in a study or using their samples for sequencing. The majority: 83% had never received requests from physicians or patients for access to NGS data to inform treatment decisions. Regarding feedback of results in a research setting, 54% or respondents think that results stemming from NGS studies should be provided to individual participants and 72% think that actionable incidental findings should be disclosed to participants. Finally, 53% of respondents think that specific measures and/or limitations should be implemented for the sharing of NGS data/results with colleagues in the scientific community. Such empirical data from stakeholders is a valuable contribution to the ongoing discussion of how to responsibly handle and feedback results to patients and research subjects.
EPL4.1 Parental influences on decision making in Duchenne/Bekker clinical trials
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Introduction: Parents’ decisions about enrolling children in Duchenne/Bekker muscular dystrophy (DBMD) clinical trials can be influenced by hopes and expectations, which when unrealistic may challenge informed consent. We explore influences on decision-making. Methods: The study used a community-based participatory research approach. Parents of children in DBMD CTs were recruited for an online survey through Parent Project Muscular Dystrophy and clinics. Participants viewed a series of benefit and worry statements and rated each statement’s influence on decision-making, and how much they expected and hoped/worried about each. Results: From the first 61 participants, CT decision-making was influenced by potential altruistic and individual benefits: learning generalizable information (97% slightly to strongly agree), CT resulting in a drug that works (82%), better future for other children (82%), improved quality-of-life for their child (82%), and parent doing everything to help their child (82%). CT worries most affecting decision-making were: child bothered by side effects (39%), eligibility for another trial (36%), and child not liking the trial (29%). Expectation and hope ratings were strongly correlated with decision-making influence ratings (r=0.5 to 0.8, p<0.01). Associations between parents’ expectations and altruistic decision-making influences yielded the highest correlation values; conversely, associations between hopes and individual-benefit influences yielded the highest values. Conclusions: Anticipated CT benefits influenced parents’ decision-making more than worries. The relationships among hopes, expectations and decision-making influences for altruistic versus individual benefits will be further explored, to inform a new conceptualization of opportunities and challenges during informed consent that extends beyond knowledge-oriented concepts such as therapeutic misconception.

EPL4.2 The impact on children and parents of participation in clinical research for Morquio A syndrome and Sanfilippo A syndrome
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Clinical research trials of enzyme replacement therapy for Morquio A syndrome and Sanfilippo A syndrome are underway. This qualitative study explored the impact of participation on 7 children with Morquio A syndrome and Sanfilippo A syndrome (4/6 eligible families) and parents of children with Sanfilippo A syndrome (4/6 eligible families). Face-to-face semi-structured interviews were carried out, the interviews were transcribed and key themes identified through interpretive phenomenological analysis. The main questions explored were: motivations for taking part, impact on life, and views on the information received prior to the trial. Children described the key role their parents, families, and genetic health professionals played in informing them about medical procedures. They talked about what was “good” (e.g. making new friends) and “bad” (e.g. fear of needles) about taking part. Many parents felt there was no real choice as current management options are limited. Some saw the trial drug as treatment rather than potential treatment. Most felt that too much complex information was given during the consent process, but discussions with the clinical trial team facilitated understanding and provided emotional and practical support. Parents described negative impacts on employment and family life. Positive impacts include an improvement in their child’s condition and a more optimistic outlook for the future. Overall, children and parents felt the advantages outweighed the disadvantages, and would recommend taking part in a similar trial to other children/families. These findings provide an insight into the impact trials have on children and parents’ lives and identify potential improvements for future clinical trials.

EPL4.3 Why do parents request carrier testing in their healthy children? A comparison of genetic health professionals’ and parents’ views
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Many parents want to know the carrier status of their other children following the diagnosis of a child with a genetic condition. However, the reasons behind their desires for this information have not been fully investigated. The aim of this study was to explore the reasons parents want carrier testing performed in their healthy children and how genetic health professionals understand these reasons. Semi-structured interviews were conducted with genetic counselors and clinical geneticists (n=17), and parents (n=25) of children with one of three genetic conditions (cystic fibrosis, haemophilia and Duchenne muscular dystrophy). Inductive content and thematic analyses were used to compare genetic health professionals’ and parents’ accounts of the reasons parents want to know the carrier status of their healthy children. Genetic health professionals expressed views about parents’ reasons for requesting, including that parents primarily want carrier testing to reduce their own anxiety and be reassured. Several professionals indicated that some parents ‘just need to know’, and others acknowledged that generally parents request testing with their child’s best interests in mind. In contrast, parents stated they primarily wanted genetic testing in order to convey the information to their children. Parents felt that disclosing carrier status to their children would allow the children to make informed reproductive decisions or prepare themselves for having an affected child. This mismatch in understanding between the genetic health professionals and parents of the reasons parents want carrier testing in their healthy children has implications for genetic counselling practice.

EPL5.1 What is the role of genetic counsellors? A systematic review of evidence
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The role of genetic counsellors has been clearly established. With the increasing burden on genetic cancer clinics. The present study investigates the referral for genetic counselling. However, in Europe, the role and practice of genetic counsellors is at a critical stage of development: in 2014 genetic counsellors will have their first opportunity to formally register via the European Board of Medical Genetics. The Board states that the genetic counsellor must fulfill a range of roles, including providing information and facilitating psychosocial adjustment of the client. To examine the extent to which genetic counsellors fulfill the prescribed roles, we conducted a systematic review of the published relevant scientific evidence using the method described by the Centre for Reviews and Dissemination. We searched five relevant electronic databases (Medline, CINAHL, SocIndex, AMED and Psychnfo) using relevant search terms and handsearched four journals for research-based papers published in English between 1 January 2000 and 30 June 2013. Of 419 potential papers identified initially, only seven satisfied the inclusion criteria for the review and all studies were conducted outside Europe. The findings indicate that where genetic counsellors are utilised in specialist genetic settings, they undertake a significant workload associated with direct patient care and this appears to be acceptable to patients. Genetic counsellors manage cases related to a wide range of conditions, predominantly where the diagnosis has been clearly established. With the increasing burden on genetic counselling services, there is an argument for the increased use of genetic counsellors in countries where they are under-utilised. However, further research on the roles of genetic counsellors in Europe is required.

EPL5.2 Referral for breast cancer genetic counselling among Turkish and Moroccan patients in The Netherlands
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Introduction: Turkish and Moroccans are the largest minority groups in the Netherlands. Migrant breast cancer patients are underrepresented in family cancer clinics. The present study investigates the referral for genetic counselling and DNA testing among Turkish and Moroccan breast cancer patients.
Methods: Breast cancer patients were identified as Turkish or Moroccan using a name-based approach. Data were ascertained from medical registries of six participating hospitals in Amsterdam and Utrecht, the Netherlands. All patients had been diagnosed with a new breast cancer in the years 2007–2012. A control group included non-Turkish/Moroccan patients from the same hospitals.

Results: In total, 156 Turkish/Moroccan patients have been identified. Preliminary results show that no information about the cancer family history was found in 13 (8%) patient files, and insufficient information was found in 77 (49%) medical files (i.e. report of breast cancer family history only). Approximately 35% (n = 55) of Moroccan and Turkish breast cancer patients fulfilled criteria for breast cancer genetic counselling, of whom 40 (73%) were aged < 40 years at diagnosis. A total of 31 (56%) were actually referred for cancer genetic counselling and testing. These results will be compared to a group of non-Turkish/Moroccan breast cancer patients.

Conclusion: Our study shows that a large group of Turkish and Moroccan breast cancer patients are eligible for genetic counselling and testing due to a young age at diagnosis. Over half of the eligible patients are referred for genetic counselling. Attention should be paid to the completeness of the registration of cancer family history in the hospital records.

EPL5.3 Genetic counselling for Indigenous populations: an exploratory study from the perspective of Australian genetic health professionals

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It is well established that cultural factors impact on the provision of genetic health care in a range of populations. Indigenous populations are thought to have particularly low levels of access to genetic health services, and cultural issues may be a contributing factor. We present data from the first study of genetic health service provision to Indigenous Australians. This qualitative study aimed to identify elements of culturally-competent genetic health service provision for this population group. Twelve semi-structured interviews were conducted with genetic counsellors and clinical geneticists from around Australia who had experience delivering services to Indigenous Australians. Participants were asked to describe their experiences and comment on collective cultural needs they identified, as well as training and resources for health professionals working with Indigenous patients.

Interviews were audio-recorded and transcribed with thematic analysis conducted on the data. The findings show that participants were reluctant to generalise the needs of Indigenous peoples. Some participants asserted that Indigenous peoples have needs that differ from the general population, with others that there were no collective cultural needs, instead advocating an individualised approach. However, being flexible and practical, taking time to build rapport, recognising different family structures and decision-making processes, as well as other socio-economic factors were all identified as important factors in participants’ interactions with Indigenous patients. This research has implications for international policy, training and practice, in addressing the needs of global Indigenous populations in the field of genetic health.

EPL5.4 Attitudes toward consumer-targeted genetic testing in Japan

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Background: Development in genomics has radically changed our self-understanding, and “we have become biomedicalized” (Pil纵深 2007). To US, consumers feel the need for tools to estimate the promises and claims of genetic testing services (Green et al. 2011). Ministry of Industry in Japan is preparing best practice guidelines for consumer-targeted genetic testing, but there is no legal regulation against genetic discrimination. However, few studies on public attitudes exist in Japan. Purpose: This paper shows Japanese citizens’ attitudes toward consumer-targeted genetic testing and its regulation and address broad ethical, legal and social implications in East Asia.

Methods: We employed a web-based questionnaire survey to investigate general perceptions in 2012. In total, 14,718 Japanese citizens completed (RR:37.0%). Results: 14% of respondents knew consumer-targeted genetic testing and just 31% had purchased before. 48.6% showed interests to use susceptibility testing on lifestyle-related diseases, 35.9% for congenital disorders and 30.8% for PGx testing. On genetic testing for children, 27-33.3% of respondents agreed to share results with schoolteachers for “personalized education”. They showed fewer interests in talent identification testing. 56.2% wished to ban genetic discrimination by law. Discussion: Compared with past studies in South Korea and Taiwan, Japanese respondents showed fewer interests. We could explore the reasons why Japanese haven’t been “bio-medicalized” in spite of its innovative position in Asian countries. We’ll compare these results with newly obtained data in 2014.

EPL5.5 Predictors of adverse psychological reactions to receipt of direct-to-consumer genome-wide profiling results

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The purpose of this study was to assess predictors of adverse psychological reactions among direct-to-consumer genomic test consumers. We analyzed data from the Scripps Genomic Health Initiative, which studied 2,037 individuals who underwent the Navigenies Health Compass, a commercially available test yielding personalized risk estimates for 28 complex diseases. The participants completed baseline and follow-up survey measures assessing demographics, personal and family health attitudes, attitudes toward genetic testing, anxiety (STAI), test-related distress (IERS), and reactions to receipt of results. One hundred thirty participants (6.4%) were defined as having an adverse psychological reaction based on change in STAI and/or IERS. These participants reported significant changes in emotions (p<0.0005) and the way they thought of themselves (p=0.032) after receipt of results, as well as differences in level of concern about their health (p=0.024) as compared to the rest of the study population. However, this group did not endorse a different profile of pre-test concerns at baseline, including concerns related to learning about personal disease risk or to not knowing how they would feel about their results (p=0.785, p=0.902, respectively). Further, neither were the number of conditions for which participants had elevated risks, nor the actual estimated risks disclosed significantly different in these participants (p=0.696, p=0.123, respectively). In this study population, while participants’ self-report of feelings and concerns upon receipt of results align with their psychological measures, neither self-assessment of pre-test concerns nor genetic risk estimate information disclosed serve as predictors of distress upon receipt of results.

EPL5.6 “It is a very lonely path”: Exploring experiences of establishing a genetic support group in Victoria, Australia

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The diagnosis of a genetic condition can be life changing. Genetic support groups have an important role in providing mutual or peer support, advocacy, and assisting in public and professional education for families affected by a genetic condition. People may be motivated to start a support group for a variety of reasons, but this can be difficult, and there has been little prior research conducted on the process. This research, which was part of the Master of Genetic Counselling program at the University of Melbourne, aimed to explore how members of the Genetic Support Network of Victoria (GSNV) experienced establishing or attempting to establish a genetic support group in Victoria. Seven semi-structured, in-depth interviews were conducted with nine participants. Using a narrative analytic approach, a number of concepts and themes were identified; participants found that setting up and running a support group could be lonely; they experienced being a support person for others as confronting; and felt they needed to acquire skills to help them establish their group effectively. Participants also needed mutual support and information from a genetic support group, and worked in partnership with health professionals and peak organisations to establish their group. These findings have implications for genetic counsellors who have skills in providing emotional support, training, and facilitating the running of, and access to, support groups. This research also suggests a role for the GSNV in offering practical assistance to those who wish to start a genetic support group in Victoria.

EPL6.1 Co-designing an Intervention to facilitate family communication about inherited genetic conditions (IGC)

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Introduction: Many parents experience difficulties in talking to their children about an IGC that affects their family. Parents often want to talk to their...
children but are unsure about what to say, when to say it and are concerned about their child(ren)'s reactions. As a result, children may be given little or no information and they are often afraid of upsetting their parents by asking. The silence that occurs about the IGC can be detrimental to the long-term mental health and well-being of children. Parents and young people agreed that all family members should attend the MFDG although parents only, should attend the first session. Desired outcomes included: a happier home life, design of a communication tool kit for families use and the development of informal networks. Conclusion: The newly designed MFDG's effectiveness will be tested using a randomised controlled trial.

EPL6.2 A randomised controlled trial of a genetic counselling intervention to enhance family communication - the Gf study

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Background: Disclosure of genetic information within families to at-risk relatives is extremely important, but often problematic. A genetic counselling intervention, delivered post consultation, may result in increased access to genetic services by family members. This Australian randomised controlled trial aimed to assess the effectiveness of intense genetic counselling follow-up on numbers of at-risk relatives utilising genetics services. Methods: Participants (n=45) received three telephone counselling interventions while the control group (n=45) received usual care. After 18 months, clinical files were audited to look for differences in the percentage of at-risk relatives who contacted genetics services. Analyses were adjusted for clustering within families. Results: Overall, 142 (25.6%) at risk relatives in the intervention arm contacted genetic services, compared with 112 (20.9%) in the control arm (adj OR 1.30 95%CI: 0.70-2.42). Subgroup analyses of genetic categories revealed substantial differences in contact percentages: cancers - 28% (intervention) vs 15% (control); cardiac - 32% vs 44%; CF carriers - 10% vs 13% and others (including PrAX, translocation carriers, SMA, Duchenne) - 39% vs 10%. Conclusions: The Gf intervention was effective in enhancing family communication was found to:

• remain congruent with principles of genetic counselling practice
• be delivered successfully by different counsellors
• improve the ability of clients to communicate genetic information effectively

Findings have implications for health professionals who wish to assist clients in effectively communicating new genetic information to at-risk relatives.

EPL6.3 "What would you like to know?" Patients' attitudes towards communication of incidental findings emerging from new sequencing technologies


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In the near future, testing of single or few genes is expected to be replaced by whole genome/exome sequencing (WE/GS), which raises controversies on communication and management of incidental findings. However, few studies have analyzed the perspectives of those expected to be more affected by WE/GS: patients undergoing genetic testing (GT). A semi-structured interview was submitted to patients undergoing GT with the aim of exploring their attitudes toward communication of genetic alterations other than those specifically searched for. Eighty-two patients, 22.8% females and 77.2% males, aged 18-83 years, were interviewed between June 2013 and mid-February 2014. Only a minority (36.6%) stated they had heard of WE/GS before. The majority of interviewed (58; 70.7%) stated they would like to be informed of any alteration. This answer was not influenced by previous knowledge, marital status, gender, education, and purpose of testing, while showed a significant correlation with having children: out of 48 with no children, 22 (75%) desired to know any alteration compared to 22 out of 32 (68%) having children (p=0.04) Although less aware (only 18% reported knowledge of WE/GS), younger people (18-35 years) were more likely to want to know any alteration (86% versus 70% and 60% in people aged 36-54 and 55-83, respectively). The main reason for choosing to be informed of any alterations was the willingness to have a clear knowledge of own risks (99.7%), while helplessness and fear of an unpredictable disease were the main motivations for those willing to be informed only of preventable diseases.

EPL6.4 Genomic investigations: healthcare professional (HCP) and family experiences of managing incidental information in clinical practice

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Background: The ability to analyse the genetic code in ever greater detail means that the possibility of clinically relevant findings, that are unrelated to the original reason for a test, is increased. These Incidental Findings (IFs), especially when identified in children, may not bring health consequences for many years. This greater sensitivity of genetic testing poses challenges to the clinical encounter including consent, and disclosure of results. 

Methods: 1.Identify current practice, including consent and disclosure practices, surrounding IFs in a range of clinical settings in the UK 2.Investigate family and professional experiences and views about the ethical and practical issues raised by the discovery of IFs 3.Inform policy on the consent and disclosure practices of IFs in clinical practice

Methods: The findings from 23 clinic observations and 52 in-depth interviews were analysed thematically.

Findings: 4 main findings will be presented:

1. The possibility of IFs is currently not discussed in any systematic way at the time of genetic testing. 2. More results with uncertain clinical significance are being reported. HCPs communicate these to families in different ways 3. There is no clear consensus from HCPs and families on what and how incidental information should be disclosed 4. HCPs believe that current systems do not facilitate the follow up of IFs long term

Conclusion: Further debate is required to integrate genomic technologies into medicine whilst addressing the ethical challenges, particularly as genetics is mainstreamed.

EPL6.5 „Very often the answer’s not black or white”: Exploring communication in paediatric clinical genetic consultations

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Background: A large proportion of clinical geneticists’ workload includes investigations for children with developmental delay where the underlying cause often remains unknown. New diagnostic technologies provide hope for a diagnosis for many of these children. However, results generated through use of these technologies increases the complexity of communication and uncertainty during genetic consultations. To date there has been limited research into the process of paediatric genetic consultations. This project investigates the process and experiences for clinicians and parents during these consultations.

Methods: This qualitative project investigated consultations across four Australian states. Theoretical framework: Symbolic Interactionism - meaning is derived, created and modified through social interactions. Data: audio-recorded consultations (n=32), parent pre-consultation surveys (n=32), and post-consultation interviews with parents (n=32) and clinicians (n=11). Detailed microanalysis (content, thematic and discourse) was completed
EPI6.6 Communicating oncogenetic information: do gastroenterologists and surgeons discuss heredity with their patients and, if so, what and how?

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Background: Doctors need to investigate if there is an indication for DNA-testing and provide their patients with information. We aimed to gain insight in discussion of cancer genetic topics by gastroenterologists and surgeons as part of an intervention study, comprising a checklist for doctors intending to optimize referral to genetic counselling of patients visiting the Gastro-Intestinal Oncology Centre Amsterdam. Insight into physicians’ performance can improve referral and optimize patient understanding.

Methods: Following a pre-post design, both before and after introduction of the checklist, 40 consecutive, new patients completed a short questionnaire assessing the discussion of cancer genetic topics during the initial consultation. Additionally, after introduction of the checklist, initial consultations were audiotaped for a qualitative analysis of the discussion of cancer genetic topics. Data on family history and referral was collected from medical files.

Findings: Discussion of cancer in the family increased from 78% before, to 90% post-intervention (p=0.01). However, doctors infrequently asked about second-degree family members (pre: 50%; post: 65%; p=0.19) and age at which family members got cancer (pre: 57%; post: 70%; p=0.29). Qualitative analysis of the audiotapes indicated the use of multi-interpretative and vague questions.

Discussion: Contrary to expectations, cancer in patients’ family members was discussed in most intake consultations. However, the suboptimal quality of the discussion hampers optimal referral for genetic counselling. Development of an alternative intervention might help better discussion of cancer genetic topics. Education for doctors is needed to improve knowledge and discussion of cancer genetic topics.

EPI7.1 Consent and confidentiality in clinical genetics: a qualitative study

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Background: Should healthcare professionals (HCPs) have a responsibility to ensure patients’ relatives are aware of genetic risk? What kind of consent is required before genetic information is shared? And how might sharing information affect patient confidentiality? The UK has national guidelines regarding these matters. We explored HCPs’ and patients’ views.

Method: We conducted 13 HCP focus groups (n=60) and 31 patient interviews across data sets for enhanced understanding, triangulation and analytical rigour.

Results: Overall, the content of the consultations was similar although clinicians appeared to have different ‘styles’ of communicating and interacting with patients, and understood the information and had realistic expectations regarding diagnoses. Where expectations were not met, parents were often disappointed. Clinicians described many professional challenges working in this area, both practical and emotional, especially the frustration of frequently being unable to answer parents’ questions regarding the cause of the child’s delay.

Conclusion: Detailed analysis of three complementary data sources provided rigorous and unique perspectives on impacts of new genetic technologies for clinicians and patients. Findings from this study will inform best practice in this area of medical communication.

EPI7.2 Autonomy and emotions: Professional challenges in seeking consent to genetic testing

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Informed consent is the ethical and legal bedrock of clinical practice and research involving humans. This paper investigates consent in practice by exploring how professionals account for consent to microsatellite instability and immunohistochemical testing for features of Lynch Syndrome and bio-banking for research purposes.

Twenty eight semi-structured interviews were undertaken with professionals responsible for seeking consent. Thematic mapping was carried out on transcribed data, which led to the identification of themes for more detailed analysis. Data were examined from a rhetorical discourse analysis perspective, which involved micro-examination of the discursive devices drawn on by participants in their talk.

Two themes encompass the communicative difficulties that were described: Autonomy (enabling choice) and handling emotional responses in interaction. Challenges pertaining to enabling choice involve different forms of opposition to respecting the autonomy of the person giving consent, both within and beyond the individuals involved in communication. Issues concerning handling emotional responses in interaction involve accounts of strong emotions both of those asked to consent and also of the professionals involved. These two themes were unevenly balanced between the settings with challenges of handling emotional responses almost entirely confined to biobanks settings. This is likely to reflect the contexts of current and previous family experiences. In both settings, enabling autonomy becomes complex when the professional is not involved in a face-to-face interaction with the person asked to give consent. This work brings an alternative perspective to accomplishing consent, as an interactive process involving complex moral negotiations within relationships.

EPI7.3 Randomized controlled trial of a telephone-based peer support program for female carriers of a BRCA1 or BRCA2 mutation: Impact on psychological distress

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Objective: To assess the effectiveness of a telephone-based peer-delivered intervention, in reducing distress among female BRCA1 or BRCA2 mutation carriers. The intervention consisted of trained peer volunteers contacting women multiple times over a four month period to provide informational, emotional and practical support.

Methods: 337 participants completed the baseline questionnaire and those reporting interest in taking to other mutation carriers were randomised to either usual care group (UCG) (n = 102) or intervention group (IG) (n = 105).

Two follow-up questionnaires were completed: i) four months after randomization (Time 2, intervention end for IG) and ii) two months later (Time 3). Outcomes included breast cancer anxiety (primary outcome), unmet information needs, and cognitive appraisals about mutation testing.

Results: Over the study period, there was a greater decrease in breast cancer anxiety in the IG than UCG (p<0.01) and at Time 2, the IG’s mean breast cancer anxiety scores were significantly lower than the UCG’s. There was a greater reduction in unmet information needs in the IG than UCG (p<0.01), with unmet needs lower in the IG than UCG at Time 2 (p=0.01). There was a greater reduction in cognitive appraisals-stress in the IG than UCG (p<0.01) with significantly lower scores found at Time 2 for the IG compared to UCG (p<0.01). However cross-sectional differences were not found at Time 3 for any outcome measure.

Conclusion: The intervention is effective in reducing breast cancer related anxiety and unmet information needs in the short-term. Identifying strategies for prolonging intervention effects is warranted.

EPL8.1 Women’s experiences following a prenatal diagnosis of fetal abnormality: The PentaS project

ABSTRACTS EMPAG TALKS

EPL8.3 Difficult decisions in prenatal diagnosis - patients' experiences of decision-making under uncertainty, and the implications for expanding the offer of prenatal testing.

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The practice of fetal anomaly detection has progressed tremendously from the first diagnoses of anencephaly by x-ray to modern 4D imaging techniques and chromosomal microarrays. Although these new technologies will increase the range of abnormalities that can be detected prenatally, the amount of uncertain findings will also increase. The current practice of fetal anomaly detection has largely developed as the result of rapid transfer of research developments to practice with limited prior ethical reflection. To date, although “uncertainty” is often cited as a potential drawback to more detailed genetic testing in the prenatal setting, there has been little study of the impact of uncertainty on the decision-making process of patients. In order to gain insight into these processes and to better understand how to approach the ethical evaluation of new fetal anomaly detection technologies, a qualitative study was undertaken. In-depth interviews of 26 participants who had received an uncertain prognosis in their pregnancies were conducted. These interviews identified a number of key themes including managing information, values and decisional context, and trust. The findings raised a number of questions, regarding for example the usefulness of the notion of autonomy as a primary ethical principle in the prenatal context where parents must make decisions based on unclear and often changing information. These findings have implications for how new fetal anomaly detection technologies are evaluated and put into practice to ensure that the maximum benefit is achieved with the minimum harm.

EPL8.4 Offering a choice between 5 Mb and 0.5 Mb prenatal whole genome SNP array analysis: are pregnant couples able of making informed decisions?

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Background: We implemented whole genome SNP array instead of conventional karyotyping (CK) for prenatal diagnosis (PND). Array detects more chromosomal abnormalities and couples facing prenatal diagnosis. Following topics were addressed: 1/ Which issues arose in the decision to have children?; 2/ Which issues arose in the decision to have an abortion (n=33), or continue their pregnancy (n=6). Women commonly experienced significant grief and overwhelming sadness; many described intense feelings of isolation from their partner, family and friends. Women who had an abortion described feeling negatively ‘judged’ and reported that their partners also experienced significant emotional impact.

Conclusions: Women describe variable and sometimes inadequate levels of follow-up bereavement care and support. There is a need for increased support and couples facing prenatal diagnosis. Providing prenatal testing and abortion in the absence of a full range of supportive options may be considered unethical; this is an important area for ongoing research.

EPL8.5 SNP Array in prenatal diagnosis; first impressions on the psychological impact of receiving a susceptibility locus s a test result

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Background: Genomic SNP array as a first-tier prenatal cytogenetic test for all indications has been implemented in our laboratory. Array may also detect susceptibility loci (SL) for neurodevelopmental disorders, with an unquantifiable risk for the fetus. SL may psychologically challenge the couple in their decision whether or not to continue their pregnancy. Since we implemented SNP array in September 2010, our policy was to disclose the presence of SL. We conducted this study to explore the psychological impact of receiving a SL.

Methods: In our pretest counseling was no emphasis on the possible outcome of array, SL, was least understood. More research is necessary to assess what the psychological impact of SL is when couples have not well anticipated this possible outcome.
EPL9.1

Predictive testing for Huntington Disease: Lessons learned from 24 years’ experience
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Predictive testing for Huntington disease commenced in Sydney, Australia, in 1990, at one genetics service. The same counsellor (a social worker) has coordinated and provided counselling for predictive testing at this service for 24 years. Over this time 554 people have been seen through the predictive testing process, with additional provision of social work assistance to the 19% of mutation carriers who have become symptomatic. This long term perspective has provided some valuable insights into dealing with the challenges of the process and impact of predictive testing. This paper presents our recommended procedures, based on lessons learned, for various aspects of the process including pre-result assessments, timing of test clients’ knowledge of result, testing of minors, testing of siblings, protocol flexibility and the therapeutic relationship versus duty of care, and follow up. This presentation of these procedures, together with expert counselling skills, is likely to achieve the aim of maximising client support and autonomy while minimising harm arising from the outcome of predictive testing for Huntington disease and similar late-onset neurogenetic conditions.

EPI9.2

Patient views on the delivery of predictive test counselling services for Huntington’s Disease
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Huntington’s disease (HD) is a progressive neurodegenerative disorder characterised by involuntary movements, cognitive impairment and psychological symptoms and is inherited in an autosomal dominant manner. Predictive testing by direct mutation analysis for individuals at risk of HD has been available since 1993. Internationally agreed guidelines for predictive testing have been published and recently updated but variability exists in how testing is delivered between centres. Whilst a substantive literature exists on the impact of predictive testing, few studies have looked at how the predictive test counselling process is received by tested individuals. This evaluation study sought the views of individuals who had a predictive test for HD at our centre over a 5 year period (2007-2012). 44 of 100 eligible individuals completed and returned a questionnaire designed for the purpose of the study. Descriptive statistics were used to present the quantitative data and a thematic analysis was conducted on the free text comments. Overall, participants were positive about their experience and valued getting information and support as well as building a good relationship with their genetic counsellor. However, 14/44 participants found the testing process too long and for 7/44 participants the journey time to the hospital took > 2 hours. Proposals for improving the service included a more tailored approach that took greater account of prior experience. In addition participants welcomed the inclusion of information resources such as video clips highlighting a range of testing experiences, and also advocated more focus on post test follow up and support.

EPI9.3

Quality issues in genetic counselling practice for presymptomatic testing: a European Delphi study
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Genetic counselling for presymptomatic testing is complex, bringing both ethical and practical questions. There are protocols for counselling but a scarcity of literature regarding quality assessment of such counselling practice. Generic quality assessment tools for genetic services are not specific to presymptomatic testing. Our aims were to identify aspects of effective counselling practice in presymptomatic testing for neurodegenerative disorders as a basis for developing a quality assessment tool. We used the Delphi phi method to ascertain the views of relevant European experts in genetic counselling practice. Ethical approval was obtained and panellists were anonymous to other contributors. Questionnaires were sent by electronic means to a list of 45 experts, who each contributed to 1-3 rounds (Medical Doctors, Geneticists, Genetic Counsellors, Genetic Nurses, and others). In the first round, we provided a list of relevant indicators of quality of practice from a literature review. Experts were requested to evaluate topics in four domains: a) professional standards; b) service standards; c) consultand’s perspective; d) ethical standards. We then removed items receiving less than 65% approval and added new issues suggested by experts. The second round was performed for the refinement of issues and the last round was aimed at achieving final consensus on high standard indicators of quality, for inclusion in the assessment tool. The most relevant indicators were related to (1) consultand-centred practice and (2) advanced counselling and interpersonal skills of professionals. High standard indicators are being used to develop a new tool for quality assessment of presymptomatic testing counselling practice.

EPI9.4

Experiences and implications of young women undergoing predictive BRCA testing under the age of 30
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Background: This qualitative study focuses on experiences of a sample of young female BRCA carriers who had predictive genetic testing before the age of 30 and explores their motivations for testing and implications of receiving a positive (bad news) result.

Methods: Following appropriate informed consent procedures participants were recruited through the Cancer Genetics Service for Wales. Semi-structured interviews were conducted face-to-face with seven participants. Interviews were transcribed in full and analysed using thematic analysis.

Results: The motives for testing and perceived advantages described by participants were similar to those identified in previous studies with older participants, such as increased awareness and knowledge, and feeling more in control. However some of the perceived disadvantages described by participants were specific to young women. These included feeling pressured to make important life decisions earlier than they would have liked, for example decisions about when/if to have children, and about risk reducing surgery. Participants also reported feeling abandoned or forgotten because of the lack of ongoing clinical contact or feeling ‘stuck waiting’ for screening to begin. None of the participants however, felt that these disadvantages were sufficient to regret having had the test at a young age.

Conclusions: Findings in this small study suggest that having BRCA predictive testing can have positive outcomes for young women. However they should be encouraged during pre test counselling to explore the decisions and choices they may be faced with in the event of a bad news test result and may benefit from ongoing support/follow up.
EPL9.5
The experiences of BRCA1/2 mutation positive women in Northern Norway

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This qualitative study explores how women who have been diagnosed with a mutation in BRCA1 or BRCA2 experience living with risk of cancer development, as well as their need for psychosocial and informative follow-up. The study also investigates whether learning and coping seminars (LCS) contribute to a greater sense of coping. Focus group interviews were performed with female BRCA1 and BRCA2 mutation carriers when they attended LCS. The interviews were performed immediately before and after the LCS. 17 women participated in two focus groups in two different LCS seminars (10+7). The following themes were discussed: Their personal reactions after being identified as mutation carriers, experiences with the risk management program, decision making and experiences regarding risk reducing surgery. In addition, the participants expectations and experience with participating at the LCS were discussed. They were also encouraged to expound upon any other issues important to them. The data were analyzed using content analysis as described by Knodel.

Preliminary results indicate that the participants had experienced random and different information regarding risk reducing surgery. They regarded the LCS as a valuable source for information and psychosocial support, and suggested that future LCS should be available for female BRCA1/2 mutation carriers shortly after receiving their unfavourable test results. Other themes as feelings of loneliness and fear of cancer development were also identified in this study.

EPL9.6
Genetic test declining and high personal colorectal cancer risk perception in DNA mismatch repair gene mutation families

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Purpose: About half of people from mutation-carrying families do not undergo genetic counselling and/or testing to identify their mutation status and risk of colorectal cancer (CRC). We studied perceived CRC risk and qualitative analysis of reasons for declining in this group.

Patients and Methods
We studied 26 participants (mean age 43.1 years, 14 women) in the Australasian Colorectal Cancer Family Registry who were relatives of mismatch repair gene mutation carriers; who had not been diagnosed with any cancer at the time of recruitment and who had declined an invitation to attend genetic counselling and/or testing at the time of interview. Bounded estimates of perceived CRC risk over the next 10 years, understanding of genetic testing and CRC risk, reasons for declining testing and self-reported colonoscopy screening were elicited during a face-to-face semi-structured interview.

Results: A sub-group of decliners (31%) unconditionally rejected genetic testing compared to conditional decliners who would consider genetic testing in the future. Mean perceived 10-year risk of CRC was 54% [95% CI 37, 71] in unconditional decliners, compared with the mean perceived 10-year risk of CRC of 20% [95% CI 5,36] in people who conditionally decline genetic testing. This difference remained after adjusting for potential confounding factors (age, gender and reported screening colonoscopy).

Conclusions: The unconditional decliner group perceive themselves to be at 3.26 times higher risk than conditional decliners. Novel interventions in general practice clinics may improve genetic testing uptake and/or appropriate colonoscopy screening for this high-risk and under-serviced group.
Oncogenetics Unit, Sheba Medical Center, Tel Aviv, Israel, 3The Danek Gertner Institute of Prenatal genetics service: the service users’ perspective

This study was funded by the Israel Cancer Association grant.

Although, PGD for BRCA1/2 mutation is allowed in Israel, it is not funded by the women underwent PGD (n=16), the majority (n=12) ended by declining the fertility preservation treatment, done before chemotherapy (n=5); and Family Results: Three significant factors were found to be associated with PGD: Preference related to the issue.

Methods: We used a qualitative-phenomenological approach. Participants were 19 Jewish Israeli women, who requested PGD for BRCA1/2 mutation decisions among Israeli BRCA1/2 mutation carriers or a spouse of a carrier. Methods: We used a qualitative-phenomenological approach. Participants were 19 Jewish Israeli women, who requested PGD for BRCA1/2 mutation decisions among Israeli BRCA1/2 mutation carriers or a spouse of a carrier.

The phenotype and genotype of Bartter syndrome in Maltese patients

Many of the ethical issues echo those for invasive testing, due to the ease of wanted information about his carrier status if the fetus was affected. While many of the ethical issues echo those for invasive testing, due to the ease of wanted information about his carrier status if the fetus was affected. While many of the ethical issues echo those for invasive testing, due to the ease of wanted information about his carrier status if the fetus was affected. While many of the ethical issues echo those for invasive testing, due to the ease of wanted information about his carrier status if the fetus was affected.

Non-invasive prenatal testing (NIPT) using cell-free fetal DNA in maternal serum can be used to test for specific genetic disorders. The aim of this study is, part of the RAPID project, was to explore ethical issues around NIPT for single gene disorders. We used a qualitative cross-sectional design and recruited carriers of four autosomal recessive conditions: cystic fibrosis, thalassaemia, spinal muscular atrophy and sickle cell disease. Data were collected via focus groups or telephone interview and analysed thematically. Parents were overwhelmingly in favour of NIPT: those with deceased children were especially keen to reduce the chance of fetal loss, while many valued knowledge of fetal status in the first trimester. Obtaining written consent was considered important to emphasise the potential impact of the tests, both on the pregnancy and on decision-making, and to avoid possible repercussions as parents reflected that although the test was easy, subsequent decisions could be hard. Parents felt that giving the mother time to discuss the test with her partner was required before blood was taken. Where fathers declined carrier testing, participants felt that mothers should be able to request a test, but the father should be aware the result might convey potentially unwanted information about his carrier status if the fetus was affected. While many of the ethical issues echo those for invasive testing, due to the ease of non-invasive testing, it is important that it does not become routine and that informed decision-making by parents is supported by professionals.

We describe the clinical phenotype and genotype in a Maltese cohort with Bartter’s syndrome. Children with a genetic diagnosis of Bartter’s syndrome until December 2013 were included. Gender, gestation, birth weight, occipito-frontal circumference (OFC), age at presentation and clinical phenotype at diagnosis, were documented.

Three female and one male were identified with age range of 2-15 years. All pregnancies were complicated by polyhydramnios and prematurity. Median gestational age was 33 weeks. Birth weight ranged from 1.5Kgs-2.1Kgs. All OFC measurements were above P90. Two children were diagnosed clinically at birth, one child at 2 years and one at 6½ years. One child has severe spastic diplegia from complications of prematurity and one child has delayed speech. All have a small triangular face, small chin and body weight along a low percentile. Two children have been diagnosed with nephrogenic Diabetes Insipidus. All have borderline low-normal potassium levels, normal eGFR and parathyroid hormone, and bilateral nephrocalcinosis. Molecular genetic analysis in the coding region of the EN1 gene revealed a homozygous c.277T>G mutation in all patients; all parents are heterozygous for the same variation. A random sample of 100 Maltese and a 100 Belgian DNA samples, revealed the homozygous state in 1 Maltese sample and none in the Belgian samples.

To our knowledge the c.277T>G mutation is reported once, in a compound heterozygote Italian patient. It would appear that the c.277T>G variation is a Maltese mutation since it presents in the homozygous state in all patients. Some phenotypic features are shared by all patients.
Non-invasive prenatal testing (NIPT) and its potential to test for multiple disorders has received much attention. This study explores women’s and men’s attitudes towards NIPT, and their views on widening the scope of prenatal testing in a country with a low uptake of prenatal screening (the Netherlands). Five focus groups with low-risk pregnant women (n=28), three focus groups with men (n=19) and 13 interviews with high- and low-risk pregnant women were conducted. Participants felt that current prenatal screening has great disadvantages such as uncertain results and risk of miscarriage from follow-up diagnostics. Characteristics of NIPT (accurate, safe and early testing) could therefore diminish these disadvantages of prenatal screening and help lower the barrier for participation. This suggests that NIPT might allow couples to discuss about prenatal testing based more on their will to test or not, rather than largely based on fear of miscarriage risk or the uncertainty of results. The lower barrier for participation was also seen as a downside that could lead to unrealistic use or pressure to test. Widening the scope of prenatal testing was seen as beneficial for severe disorders, although it was perceived difficult to determine where to draw the line. Participants argued that there should be a limit to the scope of NIPT, avoiding testing for minor abnormalities. The findings suggest that NIPT could enable more conscious decision-making for prenatal screening. However, to ensure voluntary participation, especially when testing for multiple disorders, safeguards on the basis of informed decision-making will be of utmost importance.

Case Illustrations of the utilization and uptake of NIPT-counselling and management issues

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Non-invasive prenatal testing (NIPT) for aneuploidy using cell-free DNA in maternal circulation has had a dramatic impact on prenatal screening and diagnosis. In Melbourne, Australia, NIPT is offered by several different service providers with out-of-pocket costs ranging from $500 - $1,200. Testing is largely performed in the USA and turn around times vary from 10 days to 3 weeks. Some centres provide clients with genetic counselling. At present, clients attending our public hospital may access NIPT as a first tier screening option or after first or second trimester screening. We have observed that many women who receive an increased risk result for Trisomy 21 will utilize NIPT before committing to an invasive test such as amniocentesis. Personal experiences that appear to influence this decision include IVF pregnancies, history of infertility, advanced maternal age, religious/spiritual beliefs and a desire to further clarify their risk. Counselling for this group of women requires significant time to explore the various pathways and possible outcomes, and the limitations and benefits of each. Case examples will be used to illustrate our experiences with clients taking up NIPT, including managing of false positive results, inappropriate use of NIPT and failure to achieve results. Decision making processes and counselling issues arising from NIPT will be explored.

Monitoring of congenital anomalies in the population of the Republic of Moldova

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In the Republic of Moldova, the monitoring of the CA using since 1991, and from 2009 we are working to incorporate our registry in the EUROCAT. We studied the prevalence and sharing of CA on the basis of genetic monitoring for the period from 2008 to 2012. The overall prevalence of the CA for the 5-year period amounted to 18.92 per 1000 newborns. The maximum frequency of the CA was noted in 2008 - 20.3 per 1000 births, the lowest in 2009 - 18.36 to 1000 newborns. Prenatal screening of pregnant women using non-invasive and invasive diagnostic techniques has allowed to reduce the average frequency of the birth of children with CA for 7.2% to 17.56 per 1000 newborns. In the structure of the CA by prevalence leading place is occupied by CA of the musculoskeletal system (22.5 ± 2.58%), multiple malformations (22.0 ± 2.98%) and the CA of the circulatory system (19.12 ± 4.48%). The prevalence of individual nosological forms of CA (esophageal atresia, cleft lip/palate, omphalocoele, Down syndrome) are consistent with those of the international register of EUROCAT. Results of questionnaire of 150 families with children with CA showed that 85.6% of women do not take folk acid in the first trimester of pregnancy. 74.5% of the mothers drank alcohol during pregnancy. Influence of the teratogenic factors noted at 22.2% of cases. Monitoring data allow to plan and carry out preventive measures to reduce the birth rate of the children with CA in Moldova.

The impact of the perceived severity of genetic conditions on the attitudes towards genetic testing and termination of pregnancy

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Introduction: It is known that different factors (e.g. education, religion, severity of genetic conditions) may influence decisions regarding genetic testing and/or termination of pregnancy.

Objective: The aim of the present study was to investigate the extent to which the perceived severity of various conditions moderates the decision to opt for genetic testing and/or termination of pregnancy.

Methods: The study was conducted on adult couples from the general population in Romania. Participants were asked to complete questionnaires assessing the perceived severity of 30 conditions, the attitudes towards genetic testing and the attitudes towards termination of pregnancy.

Results of the study are being analyzed and presented in detail.

Discussion & Conclusions: This study was aimed at integrating these three concepts in order to have a better understanding of the factors associated with the decision to opt for genetic testing or terminate a pregnancy, in the general population. Implications of the impact of perceived severity of genetic conditions on the decision to opt for genetic testing and termination of pregnancy are being discussed.

Development of Myriad Individualized Medicine Units

Myriad Genetics, Madrid, Spain.

Since its foundation 20 years ago, Myriad genetics has become a leading molecular diagnostic company dedicated to making a difference in patient’s lives. Through the discovery and commercialization of transformative tests to assess a person’s risk of developing disease, guide treatment decisions and assess risk of disease progression and recurrence. These test services achieve top-quality assessment enabling top-quality information for the patient based on test results. Myriad laboratory operates under the highest standards of lean efficiency allowing result reporting much faster than average. In its aim to improve patient’s quality of live and deliver top-quality information to the patients, Myriad Spain has developed a plan to create Individualized Medicine Units (IMUs). These multidisciplinary units located into the health system offer professional cancer counseling services. Different professionals including nurses, geneticists, genetic counselors, oncologists and psychologists will provide genetic testing education to patients and medical specialists. Thus, IMUs will become single organization structures which will permit a degree of coherence in providing genetic tests to counseled individuals. With the creation of IMUs access to an integral service with added value will benefit individuals affected of cancer as well as individuals at risk to develop it. IMUs are patient centered structures which intend to implement equal opportunity, help specialists to interpret genetic test results as well as get genetic guided medical and preventive options closer to patients.

The disclosure of direct to consumer genetic testing: how to regulate them?

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Direct to the consumer genetic testing (DTC GT) service is an indisputable and increasing phenomenon which portray internet society. It lets hypothesize that common people search for more health information and more sense of responsibility on their health behaviors. Indeed, proponents of DTC GT argue that making consumers able to calculate the relative risk of developing certain diseases may result in improved compliance with health-screening practices and more healthful lifestyle choices. The advertisements have a tendency to highlight these benefits and minimize any possible limitations. Moreover, consumers purchase these tests without the obligatory involvement of the health care provider, leaving free interpretation and use of genetic data. Despite these limitations, there is no concrete evidence about the attitudes towards, knowledge and use of DTC GT tests by members of the general population exist. Findings of previous researches indicate a low level of awareness of direct-to-consumer genetic testing impact, because of the hypothetical nature of many studies, use of not representative samples of the population and too little evidence from users. It is necessary to provide a theoretical framework of DTC tests use and to collect systematic data on
cognitive and behavioral DTC GT effects in the general population, in order to achieve progress in the policy arena, regulatory oversight, insights for consumers to make aware decisions and reduce the potential for misinterpretation of genetic test results.

EP12-M
From NICE guidance to clinical practice: the challenge of setting up a service for Familial Hypercholesterolaemia

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In 2008 NICE published guidelines for the identification and management of Familial Hypercholesterolaemia (FH). A key priority was the identification of people with pathogenic DNA cascade testing.

This led to a scoping exercise in how to implement this service in the south central region. This included who, where, when & what kind of service. More importantly who would pay? The Wessex FH Cascade Testing is about to be launched in April 2014, commissioned by 12 Clinical Commissioning Groups (CCGs) within the new NHS structure with pump priming by the South Central Cardiovascular Network (SCCVN) and support from the British Heart Foundation (BHF).

This is a narrative on how this was achieved, including the challenges of commissioning a new service and the opportunities afforded and how this has impacted thus far on the Genetic Service as a whole.

In March 2013, the Cardiovascular Disease Outcomes Strategy (CVDOS) specifically highlighted the need to improve the detection and management of people with Familial Hypercholesterolaemia (FH).

Action 5: The NHS Commissioning Board will take the lead, working with the Chief Coronor as appropriate; to improve the processes for identifying inherited cardiac conditions. The National Clinical Director for Heart Disease will work with all relevant stakeholders to develop and spread good practice in relation to FH and sudden cardiac death.

EP13-S
Developing genetic counselling in Portugal: education, practice and the growing of the profession

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As high-throughput genomic technologies became widespread and genetic healthcare workload expands, there is a call for education and training professionals to translate this changing landscape into appropriate care as well as for the health education of general population. In that sense, genetic counsellors are fully skilled professionals that could play an important role on the provision of safe and ethically adequate practice.

The first Portuguese genetic counsellors have completed their formal training in 2012. The Portuguese course in genetic counselling is accredited by the European Board on Medical Genetics (EBMG), in accordance with the proposed standards for education and core competences for professional practice and, in line with the harmonizing efforts of professional organizations across different countries and healthcare settings. Moreover, a national association of genetic counselling professionals have been created. With this report we aim to provide evidence on the development of genetic counselling in Portugal, its educational program and current challenges on the establishment of the profession.

A growing body of research focusing diverse settings of the genetic counselling provision has been undertaken recently in Portugal. This includes the professionals’ training needs for effective practice, quality issues of counselling, or the set of constraints affecting service delivery. Emergent challenges to continue the development of genetic counselling in Portugal are the recognition of the genetic counsellor profession, the harmonization of practice at national level, and the development of a clinical and counselling supervision network.

EP14-M
Reaction of maternity generations to human genetics in Japan

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Background: Demand for prenatal genetic testing has been increasing in general citizens, despite that they have less literacy on human genetics.

This study aimed to educate human genetics to maternal generations and evaluate the reactions to the education and their understandings. Method: We recruited 10 mothers of pre-school children who accepted the program. We designed a lecture program, followed by focus group interviews (+FGI), which were conducted twice. After having an event on genetics with children, further planning and operation were carried out together with the mothers, then FGI were conducted again. Main theme of this program was to understand and accept a concept, “everybody is different therefore everybody is different”. The transport protocol was approved by the ethical committee of the affiliated institution. Results: After series of genetic education, participants naturally accepted “everybody is different”. Accordingly, some of them changed their attitude to have more confidence to themselves, and some have more tolerance to the children. Most importantly, participants start thinking that learning genetics is useful for children to stop bullying and discrimination, and want nearby friends to learn about it. Discussion: Through genetic education, negative images of heredity that originally participants possessed were drastically disappeared. Therefore, it is important to educate human genetics and to give messages to recognize human diversity simultaneously.

EP15-S
If and when announce a genetic rare disease, are there particular recommendations? That must I know?

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For some people, the disease announce is a shock. This announcement is going to change the course of life of the patient and its perception of the future. We know that is not only the subject which is going to receive the impact of the announcement but a whole family system. Numerous measures implemented have encouraged the improving how the disease is announced to the patient. Indeed, in our presentation, we shall see in what an announcement of genetic rare disease is a particular announcement. We speak about: the representation of the disease rare genetics, the extreme rarity of these diseases, the absence of “name” for many, the notion of orphan disease, the consequences of the dysmorphology, the difficulty giving of the information. It’s essential that the doctors who announce the genetic rare diseases know what is has the work psychically for a patient, a family confronted with the genetic rare disease. It will be there a facilitator for the announcement and the care of the families.

EP16-M
Informing best practice in presymptomatic genetic testing for Huntington disease

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Introduction: International guidelines for offering pre-symptomatic genetic testing for Huntington disease (HIV) first developed in 1994 have been recently reviewed (MacLeod et al, 2012). In light of these recent recommendations, the experience and views of clients who had undergone testing at an Australian HD genetics service operating since 1991 were explored.

Method: Semi-structured telephone interviews with 14 participants were transcribed, de-identified and coded for thematic analysis using NVivo data management software. Results: Six main themes were identified: (1) motivations for testing; (2) access to services; (3) information and client-centred care; (4) perspectives on the guidelines; (5) support person role and impact; and (6) the afterwards. Motivations for testing were similar to previous studies including reducing uncertainty, family planning and to inform risk status of existing children. Participants felt they were seen promptly by the service, that they were provided with sufficient information about testing, their care was client-centred and their needs were met at the time of testing. Some participants wanted testing immediately, but understood the rationale in the guidelines for delaying testing until the second counselling session. Involvement of a support person varied, and those who involved their support person only at the results-giving session described a better experience. Patients were not always clear about what was going to happen post-test, and some were disappointed that the results did not change the course of their life.

EP17-S
Myotonic dystrophy type 1 families: anticipation as a decision making behavior for presymptomatic DNA testing of asymptomatic children

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Myotonic dystrophy (dystrophia myotonica, DM) is the most frequently inherited neuromuscular disease of adult life. The classical DM, known as...
Stenheit disease or DM type 1, is a multisystem disorder with an autosomal dominant inheritance associated with the presence of abnormal expansion of CTG trinucleotide repeats in DMPK gene on chromosome 19q13.3. Anticipation is a well-known phenomenon in DM 1. The greater size of the expansion of CTG repeats is associated with earlier onset and more severe symptoms in the following successful generations. This study presents different decision making choices of parents for presymptomatic -DNA testing of their asymptomatic children in DM1 families. This decisions are defined by the following medical and psychological aspects: genetically verified DM1 diagnosis in one of the parents, family history data for a relative with DM1 symptoms, making a choice about the asymptomatic sibling after receiving an information for affected fetus in second pregnancy of DM1 patient.

**EP18-M**

**Ten years' experience of pre-symptomatic genetic testing for late onset neurodegenerative diseases**

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**Introduction:** Pre-Symptomatic genetic testing (PST) for late onset monogenic neurodegenerative diseases, such as Familial Amyloid Polyneuropathy (FAP), Machado-Joseph Disease (MJD) and Huntington's Disease (HD), is available to at-risk healthy individuals. The aims of this study were: 1) to analyze the motivations for a PST; 2) to access the present clinical situation of the subjects; 3) to inquire about the individual treatment and preventive options; 4) to evaluate the reproductive options; 5) to analyze feelings of regret after PST.

**Methods:** This retrospective study consisted of telephone interviews of 168 carriers who underwent PST at our Medical Genetics Unit between 2000 and 2013 (145 FAP, 11 MJD, 12 DH).

**Results:** Most subjects failed to formulate, retrospectively, a motivation for PST, either than mentioning a positive family history (46%). From all participants 56% did not experience positive effects such as an increase in knowledge and nuance. Their feelings are recognized by their peers and thereby contributing to normalization and sense of release. The participants and their reactions endorsed us to continue these meetings.

**Conclusion:** The purpose of the educational-support groups is that participants feel recognized in their particular situation and can share their experiences with peers. This helps them in learning how to deal with the (possible) genetic predisposition and being able to make their own decisions thoughtfully.

**EP19-S**

**Educational-support groups for daughters of BRCA mutation carriers: a valuable addition to patient care**

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**Introduction:** The social workers of the department of Medical Genetics of the University Medical Centre Utrecht twice a year organize educational-support groups for young women at risk for hereditary breast and ovarian cancer (either yet untested daughters of BRCA1/2 mutation carriers or mutation carriers).

We started these group sessions in 2008 because we noticed: - parents with a BRCA mutation worrying about their daughters <25 years - young women’s need to be in contact with peers

Although there is no medical benefit of knowing one’s mutation status before the age of 25, the risk and uncertainty of (not) knowing are usually hard to deal with.

**Methods:** The group meetings are separated two-hour psycho-educative support sessions with 5-8 participants. Themes include: -Timing of the DNA testing -Family influence on decision making -Talking about BRCA carriership and breast cancer within your family, with friends, at work or school

**Results:** We have conducted 11 group sessions. Over 60% of the participants joined more than once. Participants experienced positive effects such as an increase in knowledge and nuance. Their feelings are recognized by their peers and thereby contributing to normalization and sense of release. The participants and their reactions endorsed us to continue these meetings.

**Conclusion:** The purpose of the educational-support groups is that participants feel recognized in their particular situation and can share their experiences with peers. This helps them in learning how to deal with the (possible) genetic predisposition and being able to make their own decisions thoughtfully.

**EP20-M**

**Living with uncertainty: the experiences of young healthy Italian women who have undergone genetic testing for hereditary breast and ovarian cancer**

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**Introduction:** Testing of the BRCA1 and BRCA2 genes can identify inherited mutations that predispose to breast and ovarian cancer, and thus provides these women with the opportunity to engage in risk-reducing behaviors and programs. The information derived from the test, however, also exposes women to the responsibility of making difficult decisions and raises important new issues that may affect how they manage their lives.

**Aims and methods:** The aims of this qualitative study was to explore, through in-depth interviews, the experiences and behaviors of 22 young Italian women (11 mutation-positive, 11 mutation-negative, mean age: 35.21) who participated in BRCA testing. All the women had a known BRCA mutation in the family but no personal cancer history. Interview data were collected and analyzed in accordance with the grounded theory approach.

**Results and conclusions:** The following psychosocial themes were found to be affected by the results of BRCA testing: childbearing intentions, future projects, family support, feelings towards children and partners, risk perception, attitudes towards risk management strategies. Test results appeared to have a definite but generally not severe impact on the participants' lives although, in some cases, negative emotions were denied in words but expressed through behaviors. The results also showed that the main predictor of negative feelings was a previous experience of cancer in the family. These findings may help clinicians better understand women's experiences of BRCA testing and thus develop adequate, culturally and ethically sensitive interventions in the areas of effective communication, support and care.

**EP21-S**

**The initiator and timing of referral to breast cancer genetic counselling: an exploration of everyday person-centered practice**

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**Objective:** The referral process for genetic counselling in breast cancer patients may be compromised by patient-related factors, like patient's age, referral initiative or cancer history. This study aimed to characterize this referral process in daily clinical practice.

**Methods:** During genetic counseling a checklist was filled in for each counseling session. All women affected with breast cancer attended educational level, the initiator for referral and the ethnic background as reported by the counsellor. Chi-square tests were used to assess associations between patient-related factors and initiator of referral and timing of genetic counselling.

**Results:** Included were 96 consecutive breast cancer patients referred to cancer genetic counselling: 52% of them were referred on their own initiative versus 48% on their doctor’s initiative. There was no significant relationship between initiator of referral and time elapsed since diagnosis, age at time of diagnosis, number of first-degree female relatives and number of first degree relatives affected by any cancer.

**Discussion:** Patients' interest in genetic testing is not clearly related with time elapsed since diagnosis. Family history seems to play a role in the timing for referral.

**Conclusion:** One out of two breast cancer patients plays an active role in the referral for genetic counselling. However, we did not establish a relationship between initiator for referral and time since diagnosis.

**EP22-M**

**The impact of objective versus subjective risk on emotional distress in breast cancer women**

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**Objective:** The aims of this study were to assess the impact of objective versus subjective risk on emotional distress and to investigate the extent to which cognitions mediate the impact of risk perception on emotional distress in a sample of breast cancer women. **Method:** In a retrospective quasi-experiment, a convenience sample of 53 breast cancer women (mean...
EP23-S

Predictive TP53 testing in adolescence and young adults: a case series
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Aims and objectives: The inaccessibility of genetic testing to young people at high risk of developing cancer is a considerable barrier to their access to early detection and survival. The need for young people to be involved in their health care decisions is increasing. The aim of this study was to explore the experiences of young people who had undergone genetic testing for the TP53 gene.

Methods: A qualitative interview study was undertaken to explore the experiences of young people who had undergone TP53 testing. The unique needs of young people were considered when preparing their consent for the consultation and follow-up. The interviews were transcribed and coded for discussion of lifestyle topics (e.g. physical activity, diet, alcohol, smoking). Counselees completed online questionnaires before the first and after the final consultation.

Results: 198 consecutive counselees for breast cancer genetic counselling were video-taped and coded for discussion of lifestyle topics (e.g. physical activity, diet, alcohol, smoking). Counselees mostly raised the topic (60%). Counsellors provided information about lifestyle risk factors to 19% and lifestyle advice to 6% of the counselees.

Conclusions: Informed consent should be sought before genetic testing, and support for lifestyle modification should be provided to counselees. Genetic testing is an opportunity for young people to take control of their health and, in the context of the consultation, the opportunity to discuss their lifestyle practices should not be missed.
ABSTRACTS EMPAG POSTERS

EP30-M Is communication with relatives discussed in the final consultation for breast cancer genetic counselling? 

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Background Counselors in genetic cancer counselling are responsible for discussing possibilities for DNA-testing, risk estimations and surveillance advice with their relatives. We describe whether communication with relatives is discussed in final genetic counselling consultations, whether counsellors provide advice and whether counsellors share their intention. Methods Consecutive new female counsellors who were the first of their family to seek breast cancer genetic counselling were included from 2008 to 2010. We report on counsellors whom received an indication for DNA-testing for themselves or an affected relative and/or a follow-up consultation. Final consultations were videotaped (n=152). Questions, information and advice about family communication were coded. Counsellors' reactions to the counsellor's advice were scored on the level of agreement. Furthermore, counsellors' intentions were transcribed. Findings Communication with relatives was discussed in 73.0% of the final consultations. In 33.6% the counsellor provided advice about family communication and a large majority (n=47, 92%) of the counsellors responded with agreement. Almost half of the counsellors (46.3%) expressed an intention about discussing risk information and most counsellors stated which relative they would inform (52.7%). Few counsellors expressed when (9.2%), how (5.9%) or where (1.3%) they intended to discuss information with relatives. Discussion Almost half of the counsellors expressed an intention to share risk information with one or more relatives. However, most counsellors were vague about how and when they intended to do this. As risk information may be poorly transferred within families, counsellors could explore counselees' intentions and discuss how best to discuss risk information in consultation with relatives.

EP31-S Genetic counselling in post-genomic era

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With the surge of genetic tests and technologies, genetic counsellors are faced with the challenge of translating emerging scientific knowledge into practical information for patients, clinicians and public health policy makers. The new tests and technologies also are associated with new psychosocial and ethical considerations. New guidelines are needed for each new discovery of the genomic impact on phenotype, pathology and disease while "old" syndromes and "old" pathology, continue to require attention. In the new post-Human Genome Project era, genetic counsellors will be an integral part of translating genomic discoveries into beneficial impact on human disease, health care, and medical benefits. The needs for genetic counselling should be designed into genomic research at the onset. Genetic counsellors need to handle old while rapidly assimilating new information and the principal challenge is to be up to date and updated. [World J Med Genet 2013 May 27; 3(2)]

EP32-M Attitude toward genetic testing of children for common disease risk in Japan

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[Purpose] The purpose of this study was to assess the attitude of Japanese general public toward genetic testing of children for common diseases, and to clarify factors related to the attitude by nationwide opinion surveys conducted in 2009 and 2013. [Methods] In the survey in 2009, 4,000 people (2,000 men and 2,000 women) were selected from the Japanese general population by a stratified two-phase sampling method. In 2013, 2,000 people were selected in the same way. They were queried about the following topics in a mail survey: attitudes toward genetic testing for disease susceptibilities of children for common diseases, interest in medical genomic studies, level of genomic literacy and awareness of the benefits and risks of medical genomic studies. [Results] Factors related to the attitude were examined using logistic regression analysis. [Results] The response rate was 52.5% (2,009/3827) in 2009 and 60.3% (1,161/1925) in 2013. The genetic testing for disease susceptibilities of children was favored by 58.8% people in 2009 and 58.4% in 2013. The interest in medical genomic studies did not so change between in 2009 and 2013; belief of "scientific development has more advantage than disadvantage" was a little increased. Interest in medical genomic studies, belief in science and awareness of risks were significantly related to favorable attitudes.
EP33-S
Opinions Of Hearing Parents About The Causes Of Hearing Loss In Their Deaf Children Compared With GJB2 (Cx26) Genetic Testing Results In Three National Republics Of Russia


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This paper will show the results, part of longitudinal study /more than 5 years, where genetic testing has not yet been widely used in public health. We conducted the first sociological research based on surveys of hearing parents of deaf children in three national republics of Russia: Sakha (n=101), Tuva (n=61), and Bashkortostan (n=21) for analysis of subjective opinions of parents about causes of HI in their children followed by comparison with results of genetic testing of GJB2 gene. Most of respondents (73.8%-86.1%) chose answer "non-hereditary" for question about presumptive causes of HI of children and their opinions are more likely based on the absence of deaf relatives. Subjective opinions of parents are inconsistent with genetic testing results, despite different contributions of GJB2 mutations (16%-71%) in studied regions, and in many cases the announcement of testing results may have severe psycho-emotional influence on parents.

Study was supported by RFBR (#12-04-00342 a, #12-04-98520, r_vostok a, #12-04-97004 r_povolzhye a, #14-04-01741 A), SBRAS Integration project #92 "Ethnography of indigenous peoples in Siberia and North Asia: comparative, historical, ethnosocial and genomic analysis", the Sakha Republic President grant for Young Researchers for 2014 (RP980). RAS Program «Fundamental Sciences for Medicine» (#30 for 2013-2015), and «Scientific and Educational Foundation for Young Scientists of Republic of Sakha».

EP34-M
State anxiety as an affecting factor to the perception of the information during the genetic counseling and the reproductive decision making process

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This paper will show the results, part of longitudinal study /more than 5 years/, of the factors that influence the perception of the information during the GC and the reproductive decision making process. Till now we explored the influence of genetic counseling procedures, advanced maternal age, family reproductive history. This study focuses on the pathologic rates of the SA. SA is induced and is a result of the concrete situation, not a typical personal trait. Purpose: Analyzing the anxiety rate during the GC and the reproductive decision making process. Task: Examination of the SA rate with State-Trait Anxiety Inventory (STAI). The object of the study are 100 women, who were pointed for GC because of biochemical screening results and risk pregnancy. Hypotheses: The rate of making reproductive choice after the GC can increase the rates of SA. Results: In most of the cases of women without higher rates of trait anxiety pathologic rates of the anxiety during the process of GC are observed. Conclusion: The GC process the SA is increased and this affects the subjective perception of the information and the reproductive decision making process. This suggests creating a algorithm for good practices for psychological support in the process of genetic counseling.

EP35-S
Content analysis of informed consent for whole-genome-sequencing offered by direct-to-consumer genetic testing companies

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Due to rapid development and a decrease in the price of high throughput sequencing technologies, whole genome sequencing (WGS) has become increasingly available in the research and clinical settings and is also being offered commercially by direct-to-consumer (DTC) companies. This offer amplifies the already identified concerns regarding informed consent for both WGS and the DTC offer of genetic tests. The aim of this project is to study the websites of companies advertising WGS DTC to analyze whether they address the recommended elements outlined by Agyus and co-researchers (2013) regarding consent for WGS in the clinic, which include, among others, pre-test counselling, description of the test process, expected benefits, possible risks, voluntary nature of participation, confidentiality and privacy, informing relatives or not, the storage and future use of samples, management of incidental findings and specific informed consent. Preliminary analysis reveals that the consent information on the websites of Illumina and Geriarex is limited. Moreover, many companies do not mention the majority of these items. Furthermore, both companies require consumers to obtain the test and/or results through a physician. However, involving a physician, and addressing these aspects on their website do not ensure an adequate informed consent process. Indeed, challenges of informed consent include, among others, the potential overload and complexity of the information regarding the process and the potential results, the limited ability of individuals to completely understand the information given, and biased understanding. This empirical study contributes valuable information toward the ongoing debate on the responsible offer of WGS in the commercial realm.

EP36-M
Return of whole-genome-sequencing results in paediatric Research: A Statement of the P3G International Paediatric Platform


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Background: Whole-genome sequencing (WGS) is expected to have a significant impact on the field of human genetics in the near future. The sheer volume of information generated by WGS raises the question of whether or not to return such results. This dilemma is exacerbated in the context of paediatric research as particular issues are raised: the rights of parents to access their child’s genetic information; the best interests of the child; the right (not) to know; the utility of information; consent, and counseling.

Objective: The aim of this research was to develop guidance on how to address the return of WGS results in paediatric research. We worked with the P3G International Paediatric Platform to build a common tool.

Methods: To formulate the Statement, we: (a) reviewed the legal and ethical norms applicable to the European and Canadian research communities and the relevant literature to identify both existing and emerging guidance; (b) conducted a qualitative study of stakeholder groups; (c) developed recommendations; and (d) validated the recommendations through consultations with a large number of stakeholders (e.g. genetic researchers, community-based organizations).

Results: We propose a Statement to address the issues of when WGS results: i) should be returned; ii) should not be returned (and identify exceptional circumstances); and iii) described the procedures for the communication of results.

Conclusion: It is anticipated that the Statement will not only provide a template for paediatric research using WGS, but also guide researchers and ethics committees.

EP37-S
Disclosure of genetic information within the family: evaluation of the model letter accompanying a new French decree


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In France, the recent publication of the decree of June 20th 2013 (n° 2013-348) concerns professionals in medical genetics. It suggests a protocol regarding the transmission of information to relatives after a genetic diagnosis of a serious condition with the possibility of preventative or care measures. If a mutation carrier refuses to directly inform other members of the family.
the family, one option is to call on the services of a genetic professional for the transmission of this information. This decree includes a specific model letter that can be sent to relatives by genetic professionals to invite them to make an appointment at a genetics center to access genetic counseling. As part of a group comprising a geneticist and six genetic counselors, we aimed to study the impact of this letter, both in terms of patient understanding and feelings. Evaluation was based on a brief interview after reading the model letter. Two groups were recruited either from "classical" genetics consultations or from "the general population." We present here the results of 148 questionnaires. Overall, this type of letter appears to be an acceptable procedure for the majority and participant understanding. Nevertheless, two paragraphs were frequently read twice to be understood. The relevance of a third paragraph was frequently questioned. The main feeling is concern, while anxiety appears to occur with lower frequency. In light of these results, we discuss the need to formulate a new model letter accommodating as many people as possible to standardize practices.

**EP38-M**

**Experiences of being a carrier and mother of a child with hemophilia**

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We have conducted a qualitative study to explore women's experiences of being a carrier of the X-linked disorder hemophilia. We collected data through in-depth interviews with 16 women who were carriers and mothers of a child with hemophilia. In analysing these narratives, we adopted an interpretative approach to highlight the personal experience of being a carrier of an X-linked disorder, how meaning is negotiated, and lived out within a wider social context. Preliminary analysis suggests that carriers experience feelings of worry and guilt after having a son with hemophilia. A striking feature in our study is that those who already knew they were a carrier, and thought this knowledge would prepare them, still experienced the event of having a child with the diagnosis distressing. Those who were diagnosed as carriers after having a son with hemophilia seemed to go through a different trajectory, focussing primarily on the child and not so much on their own carrier status. Although open communication about hemophilia and inheritance within the family makes coping with the disease easier, it might not hinder distressing feelings. More nuanced knowledge into experiences and challenges of mothers of children with X-linked disorders could help understanding their situation and facilitate an empowering approach.

**EP39-S**

**The parenthood and the child rare disease: there is specificities?**

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About 3 million people are concerned by in France and even if the media and the French Foundation of Rare Disease are proactive in discussing these diseases, their increased number and specifications are difficult to keep up with. A preexisting child presenting with a rare genetic disease will also be a source of psychological disturbances. An overview of the current literature showed that many dimensions are involved in parents with children diagnosed with rare genetic disease: social, financial, familial, professional, educational, medical and psychological. However this analysis was done on a little number of studies and very little is detailed on the specificity of the rare genetic disease. Also these elements are usually studied to individual levels and not necessarily at the family level. And, if a child is diagnosed with a rare genetic disease, the whole family will be concerned, will suffer and will need to adapt to this new situation. It seems that parents are showing signs of psychic weakness but no prediction can be made on the impact this will have on the family system nor on the factors of protection of weakness. But are there specificities in the rare genetic diseases?

**EP40-M**

**The impact of carrier identification on children's wellbeing from parents' and children's perspectives**

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The identification of sickle cell disorder carriers is routinely reported to parents following newborn screening in England. Although parents express intentions to provide carrier results to children in late childhood, little is known about when carrier status is provided or how children adapt to knowledge of their status. This is despite advice from European guidance that testing be deferred to avoid negative psychosocial detriment in childhood. This study explored the impact of disclosure of sickle cell carrier results on children's wellbeing via separate semi-structured interviews with 9 mothers and their children (M = 9.50 years, range = 6.92 - 14.56 years). Thematic analysis explored themes within and between each parent-child pair. Parents provided partial carrier information to children at around age 7; some parents acknowledged children's residual anxiety yet others did not report adverse effects following disclosure. By contrast, children reported significant anxiety, nightmares, withdrawal, distraction in school and feared negative responses from peers, which they had not discussed with parents or others. Parents and children reported the desire to have more developmentally appropriate information to improve understanding. Poor parental communication and lack of understanding of a carrier status caused children's maladaptive adaptation to results. Greater emphasis should be placed on aiding discussion between parents and children following carrier status disclosure acknowledging how difficult it can be for children to have these discussions, the availability of resources for families and further research regarding the long-term impact of carrier status on children's wellbeing.

**EP41-S**

**Impact of Hemophilia on the Psychological Health of Hemophilia Patients**

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Purpose: Psychological factors have a significant impact on quality of life for patients with chronic diseases such as haemophilia. The aim of this study was to evaluate psychological aspects of haemophilia. SF-36 is one of the most beneficial instruments for assessment of quality of life in haemophiliacs patients. Methodology: The study included 60 patients with A and B severe haemophilia, with the age between 16-40 years old, during 2012-2013. The results of SF-36 questionnaire were examined on the whole group, on age group respectively: 16-25 years old and 26-40 years old. The health condition declared by the haemophiliac patients was correlated with Beck depression parameter. Results: Evaluation on the whole group showed the lowest scores on the domains D4=45,2+/36,4 of the pain, D1=35,1+/36,6 physical performed functionality and vitality D7=44,2+/38,1. There were statistically significant differences between the two groups on D1 and D4 (p<0.01). We observed significant differences in social relationships, mental health and the general state of health between the two groups (p<0.05). The correlation between health score and Beck depression parameter is not significant. Conclusion: Knowledge of the psychological characteristics of haemophilia patients are useful in developing a plan for social integration and finding a healthy balance.

**EP42-M**

**Creation and started by a therapeutic patient education for gaucher's disease patients: the french experience**

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The therapeutic patient education (TPE) is an essential part of chronic disease care, enabling to achieve autonomy in health-related decisions, a better quality of life and better health outcomes. In order to elaborate an educational program adapted to patients, a working group was made up including physicians, nurses, psychologists and the patient's association. The first step was an evaluation of the patient's needs based on patients and professionals' interviews. This investigation brought to light a plurality of representations and real-life experience of the disease as well as numerous repercussions on the working life, the social life, the balance of the couple and the family. Several preferential themes has been identified to design the educational program which contains:

- A personalized consultation in order to identify the patient needs (educational diagnosis) and to organize his educational training
- Three educational group sessions of approximately 2 hours. The choice of sessions for a given patient will be negotiated by the physician and the patient together. They concern the following themes:
  a) Better understanding of Gaucher's disease and its consequences (definition, symptoms, natural evolution, follow-up and complications)
  b) Living with the disease in everyday life (real-life experience, fatigue and pain management, identification of available resources)
  c) Better appropriation of his (her) treatment (criteria of choice of the specific treatment, aditional treatments, oral treatment and drip administration)
EP43-S
Psychological support of BRCA1/2 carriers: audit of patient satisfaction with clinical psychology appointments, issues discussed and the offer of psychological support for women considering risk reducing mastectomies
Guy's and St Thomas' NHS Trust, London, United Kingdom.
BRCA1 and BRCA2 carriers who live in the southeast of England are offered an appointment in a one-stop BRCA clinic at Guy's hospital following identification of a gene mutation. Carriers are offered a choice of appointments with specialists in genetics, breast surgery, gynaecology, oncology and psychology. In addition regular support groups and patient education days are provided. Dedicated psychological support is provided by a clinical psychologist. We will discuss the types of interventions offered and the range of issues addressed.
In addition we will present data from a satisfaction survey carried out in patients who opted to see the Clinical Psychologist in the BRCA follow up clinics between January and March 2014. This survey found that 92% of patients felt completely listened to and understood, 85% felt completely able to talk about the things they needed to and 85% felt that the appointment helped them feel they could move forward a great deal or to some extent with their issues.
UK guidelines recommend that all women considering risk-reducing mastectomies are offered psychological support. The local protocol is that all women seen in the BRCA multidisciplinary clinic are offered an appointment with the Clinical Psychologist. An eighteen month audit of the offer of these appointments (by the psychologist) to the women is currently underway. Preliminary data show that 46% (12) of the total number of women eligible for an appointment (26) were offered an appointment. Possible reasons for this discrepancy and tentative recommendations for practice will be discussed.

EP44-M
Using Patient Reported Outcome Measures in clinical genetics services: Pilot studies in six UK clinical genetics centres.
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Background: Patient Reported Outcome Measures (PROMs), short self-completion questionnaires capturing aspects of health or health-related quality of life, have recently gained prominence in healthcare evaluation worldwide. Historically, the quality of UK clinical genetics services has been evaluated using process measures eg. patient waiting times, numbers of patients seen. Whilst current approaches to quality are important, little attention has been paid to patient outcomes because these have been difficult both to specify and to measure. This paper reports on pilot studies using a new clinical genetics-specific PROM, the Genetic Counselling Outcome Scale (GCOS-24) in six UK clinical genetics centres.
Methods: Patients were asked to complete GCOS-24 before and after clinic attendance. Satisfaction data were also collected in some centres. Data were analysed using analysis of variance and bivariate correlation. Centres provided feedback on feasibility of PROMs data collection and lessons learned.
Results: Five centres demonstrated statistically significant improvement in patients' GCOS-24 scores following clinic attendance with usable samples sizes of 41, 45, 55, 55 and 74 patients respectively (p<0.001). One centre had insufficient matched pre-clinic and post-clinic questionnaires to enable a useful analysis. GCOS-24 improvement scores correlated significantly with patient satisfaction.
Conclusions: Findings demonstrated that participating clinical genetics centres can deliver significant measurable patient benefits and that GCOS-24 has potential to be a useful supplement to existing methods of evaluating quality of routine clinical genetics services. Useful next steps could include developing methods for optimising patient response rates and for integrating PROMs information into continuous quality improvement cycles in clinical care.

EP45-S
Fitting the Pieces Together - Adoption Issues in Genetic Counselling
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Adoption is a unique situation which can present a number of challenges within a genetic counselling session. Primarily, adoption may limit the communication of information between at-risk relatives, with difficulties in notifying either a child who was adopted out at birth, or the biological family of an adopted individual, of genetic risk information. However, many other clinical and psychosocial issues can also be raised in these cases which likewise need to be addressed.

EP46-M
"BRCA isn’t all about boobs...” or is it? Lessons from a BRCA support group
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Within the Plymouth region of the Peninsula Clinical Genetics Service we identified 71 women who had tested positive for a BRCA1 or BRCA2 mutation. Letters were sent to these, inviting them to attend a support group. Twenty-seven women responded with twenty-five attending the group. Numbers were almost precisely equal between BRCA1 and BRCA2, with age ranging from early twenties to over 65. Most attendees had known about the gene change in their family for 4+ years. Motivation for attending focussed on sharing experiences and meeting other women in a similar position. Reporting of beneficial aspects of the group talked with this, with women particularly appreciating the chance to see the outcome of surgery. While distressing stories were shared, all participants reported feeling positive about the event. It was noted that, with appropriate facilitation, groups achieved an effective balance; sharing personal stories while also appreciating individual circumstances. Professional concerns about causing distress to other women who were earlier in their journey were not realised, as participants took an active role in reassuring and supporting one another. Some women listed “helping others” as their main motivation for attending. Discussion at the end of the session helped provide a plan for future groups. It was identified that group support should be two-fold; combining continuity through consistent small groups, as well as the opportunity to divide according to stage of treatment / topic of interest. It is notable that, while women with cancer felt well supported, those undergoing prophylactic mastectomy felt that they lacked a clear pathway.

EP47-S
Differential binding of CREB, USF, and c-Myc to the Calreticulin Human Promoter -220C may be linked with the evolution of higher brain functions in human
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We have previously reported a human-specific nucleotide in the promoter sequence of the calreticulin (CALR) gene at position -220C, which is the site of action of valproic acid. Reversion of this nucleotide to the ancestral type, -220A, co-occurs with severe deficit in higher brain cognitive functions. This mutation has since been reported in the 1000 genomes database at an approximate frequency of 0.0009 in humans (rs138452745). In the current study, we compare the pattern of protein binding between -220C and -220A using electrophoretic mobility shift assay (EMSA) by oligonucleotide probes representing 24 base pairs encompassing -220C-A. Antibodies reactive against transcription factors CREB, USF, and c-Myc were used to identify the specific proteins involved in complexes with DNA. Significant increase was observed in the overall protein complexes binding to the -220C allele vs. -220A. The transcription factors, CREB, USF, and c-Myc, were differentially bound to -220C, represented by supershifts. We propose that differential binding of CREB, USF, and c-Myc to CALR nucleotide -220C may be linked with the evolution of higher brain functions in human.
ABSTRACTS EMPAG POSTERS

EP48-M

Association between COMT Val158Met genotype and personality traits in healthy female subjects

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Dopaminergic genes are associated with a broad range of behaviors. The enzyme catechol-O-methyltransferase (COMT) is involved in extrapyramidal dopamine degradation in prefrontal cortical areas. The relatively common methionine (Met) for valine (Val) substitution at codon 158 of the COMT gene (Val158Met) results in an enzyme with 3-4 times lower rates of catalysis, increasing dopamine levels in prefrontal cortical regions. Among the characteristics of dopamine is important to mention the brain reward systems, infant attachment and adult human personality, particularly higher scores in Val carriers were reported for the Extraversiveness scale of the NEO Five-Factor Inventory and the Novelty Seeking and Persistence scales from Giongiong’s TCI questionnaires. The aim of the study was to compare the COMT Val158Met functional variant with personality traits, assessed using self-reported Big Five Questionnaire (BPQ-2) and Temperament and Character Inventory-Revised (TCI-R). We tested a possible interaction among these variables on a cohort of 154 healthy female students, recruited at the “G. d’Annunzio” University, Chieti. A significant correlation has been observed between Val carriers and the BPQ-2 Dynamism subscale (r=-.17; p<.05). Moreover we observed significant correlations among Val carriers and Temperament traits: Reward Dependence (r=-.16; p<.05) and Persistence (r=-.22; p<.01) between the Val carriers and Cooperativeness, a factor of the character side of the personality, measured through the TCI-R.

EP49-S

Psychological aspects of living with EDS

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EDS patients suffer from a loss of control (body, time, environment, emotions). Inevitably this results in a loss of identity. Four elements stand out: the danger of living in a restricted life, the risk of social isolation, the feeling of uselessness and the problem of being discredited. Especially the latter is very hard to endure. Patients are caught in a typical paradox: successful action among these variables on a cohort of 154 healthy female students, recruited at the “G. d’Annunzio” University, Chieti. A significant correlation has been observed between Val carriers and the BPQ-2 Dynamism subscale (r=-.17; p<.05). Moreover we observed significant correlations among Val carriers and Temperament traits: Reward Dependence (r=-.16; p<.05) and Persistence (r=-.22; p<.01) between the Val carriers and Cooperativeness, a factor of the character side of the personality, measured through the TCI-R.

EP51-S

Post-mortem genetic testing requested by a family member: challenges for genetic counseling

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We report a family where post-mortem genetic testing identified a predisposition to melanoma and pancreatic cancer. The index case referred to genetic counseling presented a cancer of unclear origin (pancreatic/jovarian) at age 58. A sister had died of pancreatic cancer at 59; the mother had died of melanoma at 40 and an aunt was diagnosed with melanoma at 52 and breast cancer at 52 and died of a head and neck cancer at 60. Analysis of BRCA 1 and 2 genes was normal. Analysis of CDKN2A gene was discussed but declined by the patient for different reasons among which were the lack of efficacy of surveillance measures and clear guidelines. The patient gave consent for DNA storage for further analysis and research. After her death, one sister requested genetic testing of CDKN2A through a Canadian genetic team. With the consent of the patient’s husband, the medical authority in Switzerland and the ethical committee in Canada, analysis of CDKN2A was performed in Canada and a pathogenic mutation was identified. Subsequently, the patient’s adult children who live in Switzerland requested genetic counseling and opted for testing. Post-mortem genetic testing is commonly performed in the setting of forensic autopsy (for example following sudden cardiac death) but it presents some specific challenges when requested by a family member and performed on a stored DNA sample. This case raised legal and ethical questions about consent, practical questions regarding reimbursement of the test and awareness of psychological aspects of genetic counseling for offspring.

EP52-M

Reciprocity among genetics professionals in Europe - A UK:Dutch experience.

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Recent literature has highlighted that collaborations among genetics professionals in different countries can help to facilitate genetic counselling practice development. Reciprocity is one manner of achieving this. Reciprocity has been identified by the Transnational Alliance for Genetic Counselling (TAVC) as a useful entity in allowing for progression of the international genetic counselling community as it allows for reflection on current methods of genetic counselling in one’s own and other countries. Furthermore, the December 2013 issue of the Journal of Genetic Counselling was dedicated as a special issue concerning a global perspective of genetic counselling, in particular highlighting the value and drawbacks of student exchange experiences as a component of study programs. Currently this seems to be the most common method of gaining such experience. Here, I present the experience of a UK trained and registered genetic counsellor visiting a Netherlands based genetics service and I reflect on the benefits and drawbacks for students and genetics centres engaging in these opportunities. This is the first report of this kind and it is hoped that it may help to facilitate future reciprocity among these two countries. Furthermore, a Dutch perspective of a UK based service is also provided.

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J01.01

Cytogenetic analysis of first trimester missed abortions
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BACKGROUND: Approximately 15% of all clinically recognized pregnancies are spontaneously aborted and ~60-70% of these are attributable to detectable chromosome abnormalities. Cytogenetic analysis is an important component in the management of miscarriage in early failed pregnancies.

METHODS: A total of 32 patients with missed abortion between 7-11 weeks gestation underwent cytogenetic analysis of chorionic villi, using R-banding cytogenetic techniques. RESULTS: Among 32 examined cases, 14 (43.75%) were with abnormal karyotype and rest of 18 (56.25%) were normal. Of the 14 cases with an abnormal karyotype, there were 3 structural abnormalities and 11 numerical aberrations. When analyzed by maternal age, the rate of abnormalities for first-trimester losses was 38.8% in women younger than 35 years, and 42.85% in those 35 years or older. CONCLUSIONS: A total of 43.75% of the cases with missed abortion had an abnormal karyotype and the percentage was a little higher at advanced maternal age. Karyotyping of spontaneous losses in the first trimester beginning with the patient’s second loss provides clinically important etiologic information and decreases the number of evaluations necessary for recurrent pregnancy loss.

J01.02

An analysis of the cells of trophoderm with the use of aCGH within the scope of PGS, pregnancy rate and the effect of maternal age
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Chromosomal abnormalities are a major cause (in 70 %) of miscarriages in the early stage of pregnancy and in the failure of an embryo implantation. Many aberrations are of maternal origin. The number of aberrations increases with the age of the woman. 530 embryos were analysed from 299 patients with the use of aCGH within the scope of PGS, pregnancy rate and the effect of maternal age.

J01.03

Array CGH in prenatal diagnosis
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Following confirmation of array cgh as first tier diagnostic test for individuals with intellectual/ developmental delay, multiple congenital anomalies and autism spectrum disorders there has been a lot of discussions regarding using this test for prenatal diagnosis. In different countries there has been different approach to this case. Considering the possibility on finding CNVs of unknown significance leading to counseling dilemmas the general trend has been to use this technique in cases of ambiguity identified in routine chromosome analysis or where a normal karyotype is reported in a sonographic report of congenital anomalies. In the period of 24 months starting December 2011 till December 2013 we performed array CGH for 32 amniotic or chorionic villi samples. From these 32 samples, 3 were inconclusive and of the 29, 5 showed a significant CNV. 3 of the 5 had problems in their sonography and 2 were being studied for de novo markers. It appears that array CGH is an instrumental tool in clarifying complicated karyotypes and a significant test for fetuses with abnormal sonography findings.

J01.04

The AZFc region: crossroad between male infertility and recurrent pregnancy loss in women
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Introduction: Male factor infertility comprises approximately 30-50% of all infertility. A great proportion of these patients have spermato genesis failure with a genetic cause. After Klinefelter syndrome, Y chromosome microdeletions (YCM) in AZF regions are the most frequent cause of male infertility. There are evidences that male factors can potentially affect fertilization, human embryo development and viability and placental proliferation. According to the recent studies, there is a potential association between Y chromosome microdeletion and recurrent pregnancy loss (RPL). Screening for Y chromosome microdeletion in AZF region in men with non-obstructive infertility and spouses of women with RPL was the focus of this study.

Material and Methods: This study was carried out with a total of 65 male samples which included 35 non-obstructive infertile men, 15 males from couples with RPL and 15 fertile males as a control. Genomic DNA was extracted from blood lymphocytes. Screening of AZF deletion was performed by multiplex polymerase-chain reaction using 19 sequence tagged sites (STS) sets of primers.

Results: In 35 men with non-obstructive infertility only 1 subject was detected to have Y chromosome microdeletions in SY254 and SY157 and SY255. Results showed no Y chromosome microdeletion in 15 males of couples with RPL and their controls.

Conclusion: Amongst the various regions of AZF, often only microdeletion in the AZFc region could end up with fertility, thus mutation screening at the AZFc region is strongly recommended in males whose spouses have recurrent pregnancy loss after ruling out other RPL causes.

J01.05

Chromosomal abnormalities and Y chromosome microdeletion among Azospermia/Oligospermia Saudi Patients
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A prospective cytogenetic and molecular study was conducted on 123 Saudi men with azoospermia to estimate the prevalence of common genetic abnormalities associated with male infertility. Routine cytogenetic studies were performed to screen for chromosomal abnormalities whereas multiplex PCR methods were employed to screen for submicroscopic microdeletion of the AZFa, b and c regions located on the long arm of the Y-chromosome. Chromosomal abnormalities were detected in 21 patients (17%). Among the 21 patients with cytogenetic abnormalities, 15 (71%) had the Klinefelter’s syndrome 47,XYX karyotype, 4 patients showed different large deletions of the long arm of the Y-chromosome with or without 45,X cell line mosaicism, one patient showed low 47,XXY/46,XY mosaicism and one patient had a chromosomal translocation between chromosomes 2 and 5. The molecular studies revealed submicroscopic microdeletions of the Y-chromosome in 3 (3%) of the 102 patients with a normal male karyotype. These Y-chromosome microdeletions involved regions AZF in all three cases and AZFB in two cases.

Our studies revealed that the prevalence of chromosomal abnormalities in azoospermic Saudi patients is similar to that of major international studies. However, the prevalence of submicroscopic Y-chromosome microdeletions in patients with a normal karyotype was noticeably lower than what have been reported internationally but consistent with a few studies performed in Arab countries including Saudi Arabia. Our findings therefore strengthen the assumption that other genetic and/or non-genetic factors may play a major role in male infertility in Arab patients.

J01.06

The Relationship Between The Index and the Blood Disease Thalassemia and a Sample Report
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Background and Aims: Thalassemia is a severe hemoglobin disease, recognizing by CBC and hemoglobin electrophoresis primarily. At first, CBC and hemoglobin electrophoresis was performed for the index case recognized as α-Thalassemia but finally experimental studies clearly demonstrated that it was different type of thalassemia. In the present study, we report a case of β-thalassemia mutations which is called -101 C>T relative to the transcription start site of β-globine gene, and is a β+–Thalassemia case, but it has been observed with different indices.

Methods: The patient was admitted based on hematologic indices as

ABSTRACTS PUBLISHED ABSTRACTS

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J01.08 Non-invasive prenatal diagnosis of fetal sex using multiplex-PCR
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The uncovering of cell free fetal DNA (cfDNA) in maternal serum of pregnant women (1997) has provided an approach for applying of cfDNA as a non-invasive method for prenatal diagnosis (PND). The determination of fetal gender is the first step of the PND. It is especially important in fetuses at risk of sex-linked disorders and in conditions associated with ambiguous development of the external genitalia. Peripheral blood sample (10 ml) was obtained from 100 pregnant women that referred to the diagnostic laboratory for routine pregnancy tests during 6th to 10th weeks of gestational age. Cell-free fetal DNA was extracted from the maternal serum. The cfDNA was not enriched. The conserve sequences of SRY and GAPDH genes as a marker for fetal gender and for internal control respectively were amplified by Multiplex-PCR using specific primers for their reigns. We considered; amplification of just internal control indicates male fetal gender. Latterly, all the collected results were compared with the real gender of the newborns.

Early determination of fetal gender provides the opportunity of deciding and employing early treatment designed for fetuses at risk. However, this study aims to validate a simple, reliable and applicable method for non-invasive prenatal diagnosis of fetal gender. In this presentation we will demonstrate our data regarding fetal gender. Furthermore, we will present sensitivity and specificity of this study.

J01.09 „Filling the gap“: the prenatal phenotype of two cases of rare microdeletion syndromes
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We describe two cases of prenatal detection of unexpected interstitial deletion syndromes that were recognized by GGH-Array after evidence of ultrasound anomalies.

Case 1: A 43 years old woman in her fourth pregnancy referred for genetic counseling because of cystic hygroma with normal fetal biometry measurements at the first trimester. Level II ultrasonography: micrognathia, low-set ears, lower limb abnormalities. GGH-Array was performed revealing a deletion syndrome of 10,6 Mb on chromosome 6 (locus 6q11q14.1). After termination the fetal pathalogy showed frontal hypertoricosis, small nose, long filtrum, microstomia, thin lips, micrognathia, and low-set ears with normal lobes.

Case 2: A 41 year old woman in her third pregnancy. During the second trimester the pregnancy was complicated by intrauterine growth restriction and unilateral fetal club foot. A microdeletion of 496 kb at 17q21.21 was detected by GGH-Array. This mutation has been reported in only a limited number of families and with a variable phenotype. After termination the physical examination of the fetus demonstrated high forehead, hypertelorism, bulbous nose, thin lips, pointed chin, low-set simplified ears, camptodactyly, tall equinoval and reduced musculature in the lower limbs. Both deletions were shown to be de novo. Understanding the pathogenesis of fetal anomalies and correlations between the pre- and post-natal phenotype is difficult and limited because of a lack of prenatal data from cases reported after birth. The clinical spectrum of microdeletion syndromes could be expanded greatly if more prenatal cases would be described and published in the open literature.

J01.10 Chromosomal abnormalities in couples with reproductive failures and/or those included in ART programs
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1Department of Medical Genetics, Medical University, Plovdiv, Bulgaria, “Clinical Institute for Reproductive Medicine, Plovdiv, Bulgaria,”Urology Clinic, Medical University, Plovdiv, Bulgaria.

Chromosomal abnormalities (CA) are among the major genetic causes of reproductive disorders. Aim: to investigate the prevalence and profile of CA among couples with deferent type of reproductive failures, as well as to assess the value of karyotyping in the routine work-up of couples, referred for ART Material and methods: The study included a total of 947 individuals with reproductive failure. They were divided into the following groups: (1) 273 couples with two or more spontaneous abortions; (2) 193 women and 208 men from couples with infertility +/- one or more unsuccessful IVF procedure. All patients were analyzed cytogenetically for detection of major chromosomal abnormalities.

Results: Chromosomal abnormalities were found in 26 (2.74%) of all investigated individuals, 10 (2.08%) males and 16 (3.43%) females. The determined prevalence of CA was - 4.76% among couples with recurrent abortions (4 times more frequently in women than in men) and 3.24% in individuals (more frequently men) with infertility. The most common type of CA in group (1) (92% of these cases) are balanced structural rearrangements, carried mainly (85%) by female; in group (2) - CA of sex chromosomes (62% of these cases), more frequently (62%) in male. Conclusion: The results of current study demonstrate the significance of chromosomal pathology in etiology of reproductive failure and emphasise the need for thorough genetic work-up in couples referred for infertility. Karyotype analysis should be an integral part of diagnostic work up in couples with reproductive problems especially those undergoing assisted reproductive procedures.
J01.11
Study of chromosomal abnormalities involved in couples with reproductive failure
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BACKGROUND Infertility is a medical problem affecting a significant proportion of the population, up to 15.0% of couples of reproductive age. Genetic pathology is an important part of the general human pathology due to the increasing number of genetic diseases. The purpose of this study was to establish the correlation between the presence of structural and numerical chromosomal abnormalities in one of the partners and their reproductive development.

METHODS During the period from August 2007 to December 2011, 2195 patients with reproduction problems, who had received in our hospital, were investigated for this retrospective study, and the frequency of chromosomal abnormalities was calculated. The control group consists of 83 fertile couples who had one or more children in history, was investigated by karyotype.

RESULTS Of the 2195 patients investigated by classical cytogenetic techniques 91.12% had normal karyotype and 8.88% had chromosomal abnormality, the most common chromosomal changes are polymorphisms. Numerical chromosomal abnormalities were detected in the proportion of 0.65% in infertile men and 0.62% in infertile women in the study group.

CONCLUSIONS: Recently a possible association between infertility and chromosomal abnormalities has been reported with a significant statistically association. Our study shows that there is not association between chromosomal abnormalities and infertility problems, but this study needs to be confirmed with further investigations on a larger control group to establish the role of chromosomal aberrations in the etiology of infertility.

J01.12
FISH assessment of chromosomal aneuploidies in infertile males
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Background. Reproductive failure is one of the most important issues for the population at the age of procreation and approximately 15% of the couple encounters reproductive difficulties. In the last years it was taken in consideration the hypothesis that not only somatic chromosomal anomalies but also germ cells chromosomal aberrations could lead to reproductive failure.

Aim: In this study we used multicolor FISH probes for chromosome 13, 18, 21, X and Y to evaluate the aneuploidy incidence in sperm cells from infertile males.

Methods: The study lot included 35 males with infertility and oligoasthenoteratozoospermia (OAT) and 20 males with normal fertility and normal semen characteristics for which the conventional cytogenetic investigation using peripheral blood revealed a normal karyotype. The fluorescent signals for these chromosomes, were analysed at least 10000 cells/patient.

Results: The overall chromosome disomy and nulisomy in OAT group was higher than the one identified in the control group. By comparing the incidence of the disomy in the OAT group, the highest incidence was the sex chromosome disomy, followed by the disomy of chromosomes 13, 21 (equal values) and then 18. The nulisomy incidence in the OAT group was higher for sex chromosomes, followed by the nulisomy of autosomes 13, then 21 and 18.

Conclusion: During these days, for patients with OAT, intra cytoplasmic semen injection (ICSI) is frequently used, and it is important to inform the patients that the use of spermatozoa for fertilization could be associated with an increased risk of aneuploidy in embryos.

J01.13
Cleidocranial Syndrome with Premature Ovarian Failure
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Our patient family had twenty members that five of them (three brothers, one sister and father) suffered from oligoamena, enamel hypoplasia, dental decay, Miödi proportionate short stature, frontal bossing, Ix of recurrent upper respiratory infections, sinusitis, bilateral clavicle bone hypoplasia, pectus carinatum, short & tapering fingers, lumbar lordosis and thoracic scoliosis. My proband was a 33 years old female with premature ovarian failure and chromosomal abnormality in peripheral blood (40 mosaicism of deletion of 6p2 and 45 XO in fifty metaphase ), other family members had not any fertility problem and chromosomal abnormality.

We gussed Our diagnosis in this case is Cleidocranial Dysostosis and occurrence chromosomal abnormality and this syndrome probably resulted from location of this gene (CBFA1) in short arm of chromosome 6 and variability of mosaicism 45 XO phenomenon in ovarian tissue.

J01.14
Mutations in cyto21hβ gene of women with reproductive dysfunction

The interest in non-classical forms of congenital adrenal hyperplasia (CAH) and its impact on human reproduction was increased. The vast majority of all cases of CAH (95%) was due to deficiency of the enzyme of 21-hydroxylation. Defect of this enzyme is able to lead a miscarriage.

In aim to deter the ratio of the number of gene replica (b) and pseudogene (a) on exon 3 in CYC21Hβ was conducted molecular genetic testing at 46 women, among them 26 women with infertility and 20 women control group. The presence or absence of the mutation in the test region was determined by PCR using a specific primer.

In result of analyses was determined that equal ratio of the number of gene replica (b) and pseudogene (a) on exon 3 (b=a) was detected in 20 patients, representing 76.9% as compared with a group of fertile women (95%). In the group of women with the disorder generative function there are in 6 patients noted an altered replica of the gene (b) and pseudogene (a) on exon 3 (b>a) towards the control (23.1% and 5% respectively).

The present changes in the ratio of the number of replica gene and pseudogene were due to deletion a gene (b) and in this case, probably, it is a mutation which leads to development of reproductive disorders in the homozygous condition.

J01.15
Genetic counselling for pregnant woman with CPT II deficiency - a case report
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Carnitine palmitoyltransferase II (CPT II) deficiency is an autosomal recessive disorder of long-chain fatty-acid oxidation. The three clinical presentations are: lethal neonatal form, severe infantile hepatocardiomuscular form, and myopathic form. While the former two are severe multisystemic diseases characterized by liver failure with hypoketotic hypoglycemia, cardiomyopathy, seizures, and early death, the latter is characterized by exercise-induced muscle pain and weakness.

The lady aged 27 was referred to our hospital at 18 weeks of gestation. Her first child was dead at 9 months old triggered by infection. Postmortem revealed that the boy had CPT II deficiency. Hence she and her husband had genetic test showed the boy is carrier as well as his mother.

We monitored the boy in Growing Care Unit to avoid low glucose level which could cause catabolism, and the cord blood was sent for genetic test. The genetic test showed the boy is carrier as well as his mother.

If the mutations have been identified in an affected family member, molecular genetic testing of at-risk relatives can reduce morbidity and mortality through early diagnosis and treatment. In this case, the patient did not choose to take prenatal test, however, it would be worth to know if the fetus is affected to prepare neonatal care.

J01.16
Reduced fertility and sperm DNA fragmentation
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The interest in non-classical forms of congenital adrenal hyperplasia (CAH) and its impact on human reproduction was increased. The vast majority of all cases of CAH (95%) was due to deficiency of the enzyme of 21-hydroxylation. Defect of this enzyme is able to lead a miscarriage.

In aim to deter the ratio of the number of gene replica (b) and pseudogene (a) on exon 3 in CYC21Hβ was conducted molecular genetic testing at 46 women, among them 26 women with infertility and 20 women control group. The presence or absence of the mutation in the test region was determined by PCR using a specific primer.

In result of analyses was determined that equal ratio of the number of gene replica (b) and pseudogene (a) on exon 3 (b=a) was detected in 20 patients, representing 76.9% as compared with a group of fertile women (95%). In the group of women with the disorder generative function there are in 6 patients noted an altered replica of the gene (b) and pseudogene (a) on exon 3 (b>a) towards the control (23.1% and 5% respectively).

The present changes in the ratio of the number of replica gene and pseudogene were due to deletion a gene (b) and in this case, probably, it is a mutation which leads to development of reproductive disorders in the homozygous condition.
J01.17 Association of MTHFD and RFC1 polymorphisms with the risk of Down syndrome in Romanian population
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1Carol Davila University of Medicine and Pharmacy, Department of Genetics, Bucharest, Romania, 2Life Memorial Hospital, Bucharest, Romania.

Introduction Down syndrome (DS) or trisomy 21 is a genetic disease resulting from the presence of an extra copy of chromosome 21. Although advanced maternal age represents the major risk factor for DS, most of DS children are born from women less than 30 years of age and is seems that different mechanisms are responsible for chromosome 21 nondisjunction in young women compared to older ones, each of them potentially affected by an impaired folate/Hcy metabolism. The objective of this study was to evaluate the correlation between gene polymorphisms MTHFD G1958A and RFC1 G80A and the risk of Down syndrome. Materials and methods Our study included 26 women that gave birth to DS babies and 46 control mothers of healthy children. Genomic DNA was isolated from whole blood, using peqGOLD DNA mini kit (A&TI Biotech) following the manufacturer's instructions. The MTHFD G1958A and RFC1 G80A mutations were investigated by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) analysis. Results The combined RFC1 G80(A)/MTHFD G1958(GG) genotypes compared with the reference RFC1 G80(G)/MTHFD G1958(GG) genotype was associated with increased DS risk (OR 0.18 [0.02-0.73]). Polymorphism analysis of the RFC1 G80A showed a significant association with DS (OR 0.18 [0.02-0.73]). Conclusions This is the first study in a cohort of Romanian mothers of DS children in comparison with control mothers, analyzing RFC1 G80A and MTHFD G1958A polymorphisms as a maternal risk factor for meiotic nondisjunction of chromosomes 21, causing DS.

J01.18 Genetic deletion of Pelota in mice leads to embryonic lethality at an early post-implantation stage
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Pelota (Pel) is ubiquitously expressed, and its genetic deletion in mice leads to embryonic lethality at an early post-implantation stage. In our study, we conditionally deleted Pelo after the establishment of embryonic stem cells (ESCs) and showed that PELO depletion did not markedly affect the self-renewal of ESCs or their capacity to form teratomas. However, Pelo-null ESCs differentiation into extraembryonic endoderm (ExEh) was severely compromised during embryoid body (EB) formation studies. Failure of Pelo-deficient ESCs to differentiate into ExEh was accompanied by the retained expression of pluripotency-related genes and alterations in expression of components of the bone morphogenetic protein (BMP) signaling pathway. Further experiments have also revealed that attenuated activity of BMP signaling is responsible for the impaired development of ExEh in Pelo-deficient cells. Collectively, our results convincingly show that PELO plays an important role in the differentiation of ESCs, into ExEh through activation of BMP signaling. Hence, the observed early embryonic lethality in conventional Pelo-deficient embryos could be attributed to the defects in ExEh formation during early embryonic development.

J01.20 The association between endothelial nitric oxide synthase gene Glu298Asp polymorphism and spontaneous pregnancy loss
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1Department of Medical Genetics, Faculty of Medicine, Canakkale Onsekiz Mart University, Canakkale, Turkey, 2Department of Obstetrics and Gynecology, Faculty of Medicine, Canakkale Onsekiz Mart University, Canakkale, Turkey, 3Gynecology and Obstetrics, Kastamonu Inebolu State Hospital, Kastamonu, Turkey, 4Department of Gynecology and Obstetrics, Faculty of Medicine, Canakkale Onsekiz Mart University, Canakkale, Turkey.

Aim: The endothelial nitric oxide synthase gene (eNOS) polymorphisms have been associated with reduced vascular NO production or increased level of homocysteine and related to pregnancy losses. In the current case control study it was aimed to find out the possible role of eNOS Glu298Asp polymorphism in spontaneous pregnancy loss. Results and Methods: Twenty-three spontaneously aborted fetal materials, 22 mothers who had these abortions and 86 healthy control cases were enrolled in the current results. The genomic DNAs were isolated from aborted materials and peripheral blood samples and genotyping for target polymorphic allele was done by real time PCR technique. Results: Current results showed that eNOS (Glu298Asp) polymorphisms were significantly associated with spontaneous abortion (p=0.011). Conclusion: The present study identified the strong association between eNOS gene polymorphisms and spontaneous pregnancy loss risk.

J01.21 18p partial trisomy with fetal ascites inherited from a mother with familial 18p deletion syndrome
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The Catholic University of Korea, Kyunggido, Korea, Democratic People's Republic of.

The deletion 18p syndrome is one of the most common chromosome abnomalities characterized by dysmorphic features, growth and mental retardation with a poorer verbal performance. Until now no reliable phenotype map for the characteristic clinical findings such as mental retardation, post-natal growth retardation and typical facial features has been established yet. Molecular karyotyping holds the promise of improving genotype-phenotype correlations for frequent chromosome conditions such as the 18p-syndrome. Until now, there have been 9 reported families with transmission of del (18p) from a mother to a child (including our study). We describe a female baby, 46,X,XX[de18][18]x[18][p11.32:p11.2] with fetal ascites, transmission of deletion 18p from a mother and her affected families with partial monosomy 18p of different sizes owing to unbalanced translocations.

J01.22 Structure of prenatally-identified polyploidies - a retrospective study
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1University of Medicine and Pharmacy “Victor Babes”, Timisoara, Romania, 2Private Medical Practice and Genetics Laboratory Dr. Cristina Gug, Timisoara, Romania, 3University of Medicine and Pharmacy “Iuliu Hațieganu” Cluj, Romania.

Objective: The objective of this study was to identify the frequencies and types of prenatally-identified polyploidies through amniocentesis or chorionic villus sampling (CVS), and to compare them with polyploidy identified in terminated pregnancies. Materials and Methods: Fetal karyotypes were investigated from cell cultures and handed with GTG. Amniocytes were also screened with FISH and QF-PCR was used in the CVS cases. Results: We report here prenatal diagnostics through amniocentesis for 3 trispliod fetuses.

J01.19 Gene variation of TLR4 in patients with Endometriosis
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Endometriosis, defined as the presence of endometrial tissue outside of the uterus, is an estrogen-dependent chronic inflammatory condition associated with degrees of pelvic pain and infertility. Toll-like receptors play a key role in immune response, by regulating inflammatory reactions and activating adaptive immune response to eliminate infectious pathogens and cancer debris. Polymorphisms in TLR4 have been shown to be associated with increased susceptibility to diseases such as inflammation and cancer. Ectodomain of TLR4 protein consists of 21 leucine-rich repeats (LLRs) that are crucial for its dimerization and signaling. The aim of this study was to determine gene variations of TLR4 in patients with endometriosis. Sixty-three blood samples were recruited from endometriosis patients referring to Royan Institute along 2012-13, who have been confirmed by laparoscopic surgery. Ethical approval forms were obtained before the samples collection. The control group was consisted of fertile women who had no history of inflammatory disease or using any related drugs. DNA was extracted by kit, special primers were designed, the LRR coding region was amplified by PCR and products were analyzed by sequencing. Ectodomain of TLR4 gene polymorphisms (SNP) (rs120475167) G/A which changes aa E to K, and 4 patients had heterozygote SNP (rs120475168) G/A (aa R to K), and 2 patients had heterozygote SNP (rs120475052) G/A(aa D to N). According to our finding we suggest that the three aforementioned new SNPs in TLR4 gene can be associated with endometriosis. Key words: Endometriosis, gene variation, polymorphism, TLR4.
A total of 374 women underwent CVS procedures, and 172 women underwent amniocentesis. Of these, 75 cases were found to be triploidy. The maternal age was between 22-32 years old in cases with triploidy as compared to 28-43 years old in cases with tetraploidy. Conclusions: Polyploidy are relatively frequent in terminated pregnancies, but can also be present in the first or second semester of pregnancy in live fetuses. Obtaining fetal karyotypes in all cases which present severe fetal abnormalities can elucidate the etiology.

J01.24
Prenatal diagnosis of unbalanced translocation 11,18 - pitfalls of FISH rapid prenatal diagnosis
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FISH screening in uncultured amniocytes, is a standard procedure for the rapid detection of chromosomal aneuploidy in prenatal diagnosis, in order to detect the most common aneuploidies, involving chromosomes 13, 18, 21, X and Y. It is easy to perform, don't need cell culture, the risk for misdiagnosis is lower (~0.4%) and, principally, allow results in less than 24 hours. We report the case of a young pregnant woman with an abnormal ultra-sound scan, revealing a foetus with multiple anomalies, including cardiopathy, short femur and clenched hands. It was the first pregnancy of this young, health, non-consanguineous couple with a normal family history. Aneuploidy was performed and FISH aneuploidy technique, in uncultured amniocytes, was applied, with normal results. Cytogenetic analysis of uncultured amniocytes showed 2 copies of chromosome 11 and 18, with deletion of 11q24-qter and trisomy of 18q11.2-qter, confirmed by FISH. The foetus had several anomalies consistent with trisomy 18 phenotype. The FISH rapid prenatal aneuploidy test is, taken in account its possibilities and limitations, a powerful tool for the clinician in the care of pregnant women. It's limitations, it cannot detect cytogenetic abnormalities such as mosaics, translocations or rare aneuploidies, do not allow this technique to be used as an independent, stand-alone technology and must be performed in conjunction with standard cytogenetic testing for clinical diagnosis. So, it seems likely that, for the foreseeable future, banded chromosomes will remain an indispensable tool in the genetic diagnostic laboratory.

J01.25
Analysis of association between the number of CGG repeats in FMR1 gene with IFV failure
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Infertility is a common clinical problem. It affects 13% to 15% of couples worldwide. In vitro fertilization is a common medical treatment which fails in some cases. IFV is not successful for several reasons. FMR1 Gene (Xq27.3) with CGG repetitive sequence -through their impact on autoimmunity - is one of the genetic factors influencing IFV failures. In normal subjects, the number of iterations is 5 to 54 and in relation to normal ovarian function the number of CGG repeats in the FMR1 gene are 26 to 34. The occurrence of these three repetitive sequences is three genotypes as normal, homoygous, and heterozygous. Heterozygous genotype has 2 modes: with the occurrence one of two alleles greater than 34 sub-genotypes creates het-norm/high and less than 26 sub-genotypes creates het-norm/low. In this study, correlation studies are of case - control and long PCR method. Samples are peripheral blood of 50 women with a history of at least 3 failed IVF: karyotype and normal MTHFR gene and 50 matched controlled group. Total Lab Quant software was used to determine the number of iterations and SPSS 17 software was used in data analysis. Analysis done using frequency tables and coefficients determined by measuring the number of iterations shown by p-value equal to 0.002 indicates a significant relationship between het-norm/low sub genotype in FMR1 gene and IFV failure.

J01.26
Analysis of association between the number of CGG repeats in FMR1 gene with recurrent abortion in Iranian patients
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Most frequent anxieties in pregnant women is recurrent abortion with prevalence of 1 per 300 pregnancies which is occasionally caused through autoimmunity disorder. Recurrent abortion has been observed in pregnant women with autoimmune antibody such as Anti-phospholipids and Anti-TPO. The number of CGG repeats in FMR1 gene correlates to autoimmunity. Increasing in FMR1 repeat number is correlated to fragile X syndrome. Normal range is considered 5-54 repeats. The normal function of ovarian sub-genotypes and recurrent abortion.

Pregnancy loss and adverse pregnancy outcomes (APO) result from a complex interaction of environmental and inherited factors. Although the etiology of more than half of such events remains unclear, gene mutations leading to a hypercoagulable state, such as factor V Leiden, factor II prothrombin, methylene tetrahydrofolate reductase (MTHFR) mutation, are often found associated with pregnancy loss.

J01.27
Hereditary thrombophilia and pregnancy loss
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Polymorphisms in genes for factor II prothrombin (20110G→A), factor V Laiden (1691 G→A) and MTHFR (677C→T), were determined in 121 women with history of pregnancy losses and in 51 women with healthy offspring and no history of APO (control group).

Mutations in investigated genes were found in 25.6% women of study group. Analyses showed that inherited thrombophilias were found in women with 2nd and/or 3rd (50%), rather than those with 1st trimester recurrent pregnancy loss. Women with a history of 3rd pregnancy loss showed the highest prevalence of examined thrombophilic genotypes. Group of women with the history of pregnancy loss, as well as fetal chromosomal abnormalities and congenital malformations, also showed a significantly higher presence of thrombophilic genotypes than the control group. Mutation 677C→T in MTHFR gene was the most frequently found polymorphism among the examinees form the study group. Odds ratio analyses showed that women with thrombophilic genotypes have 5 – 10 times greater risk for APO.

Established association of inherited thrombophilias, especially 677C→T mutation in MTHFR gene, to late pregnancy loss, and fetal malformations and chromosomal abnormalities implies that these mutations might be significant risk factors for a broad spectrum of APO.

J01.30 Polycystic Ovary Syndrome and MTHFR C677T polymorphism in Mexican women
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Introduction: Polycystic ovary syndrome (PCOS) is a common endocrine disorder in reproductive age patients, high homocysteine level has been reported in PCOS, the MTHFR-C677T gene polymorphism have been associated with hyperhomocysteinemia and decrease of folate acid. Objective: To determine MTHFR C677T polymorphism frequency in Mexican women with polycystic ovary syndrome. Material and methods: We included 64 patients with PCOS diagnosis according to Rotterdam criteria and 101 Mexican stizos as a reference group (M). The genotyping was performed by PCR/RFLP technique. The M group presented Hardy-Weinberg equilibrium. Results: In the PCOS group (n=64) the MTHFR C677T genotypic frequencies [%] were distributed as follows: CC 33 [(21], CT 48 [(31] y TT 19 [(12). The allelic frequency at maternal and paternal loci was the C allele was 57 [(73)] and TT was 43 [(55)]. The genotypic frequency in the reference group [n=101] was CC 31 [(31], CT 50 [(51], TT 19 [(19) and allelic frequency for C allele 56 [(113) and for T allele 44 [(89). The allelic frequency comparison between both groups were no statistically different (p>0.05). Conclusion: In PCOS group the MTHFR-C677T genotypic frequency was 19 % and the allelic frequency for T allele was 46%. The genotypic and allelic distribution between both groups (group-PCOS vs group-M) was similar. This study showed no association between MTHFR C677T polymorphism and PCOS.

J01.31 Multilocus methylation defects at imprinted genes in miscarriages from women with recurrent and single pregnancy loss
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Genomic imprinting is an epigenetic phenomenon, which is involved in regulation of embryonic development and placental function. Previously we have reported that the multilocus methylation defects (MLMD) at imprinted genes may be among molecular processes, which are responsible for dysfunction of implanted loci in pathology of early embryonic development. We hypothesize that MLMD at imprinted genes may cause karyotypically normal miscarriage, particularly among women experiencing recurrent miscarriage. Differential methylation of 51 imprinted genes were examined in first-trimester spontaneous abortions (SA) from women who have recurrent (group RPL, from 9 (data of GoldenGate Methylation Cancer Panel) to 105 (Methylation-specific PCR (MSP) SA) and single (group SPL, from 6 (data of GoldenGate Methylation Cancer Panel) to 114 (MSP) SA) pregnancy loss. Sixty induced abortions were investigated as a control group. All spontaneous and induced abortions had the normal karyotype. Our results provide evidence that MLMD at imprinted genes in the group RPL was more frequent than that in SPL (15x10 -2 and 5.2x10 -2, respectively, p<0.01) with predominance of somatic epimutations (10x10 -2 and 3.9x10 -2 respectively, p<0.01) and multiple hypomethylation (9x10 -2 and 4.4x10 -2 respectively, p<0.01). Frequency of MLMD in both groups at maternal loci was higher than that at paternal one (1x10 -2 and 6.4x10 -2, respectively, p<0.05 for RPL; 3.6x10 -2 and 1.4x10 -2, respectively, p<0.01 for SPL). Therefore, the RPL is characterized by multilocus somatic hypomethylation of imprinted genes and MLMD at imprinted genes on maternal loci that associated with abnormal maintenance of maternal imprinting in somatic cells.

J01.32 Genetic association of phase II detoxification genes with recurrent pregnancy losses among Moldavian women
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Background: Recurrent pregnancy loss (RPL) is a multifactor and distressing disease. In this study, we aimed to investigate the relationship between the polymorphism of GSTM1, GSTT1, GSTP1 and the pregnancy loss.
Methods: A case-control study of 100 women with RPL and 100 healthy women was conducted. Have been investigated DNA of 100 healthy child-
ated age up to 17 years for comparative analysis of GST polymorphisms in
Moldova with other countries. PCR, PCR/RLFP methods have been used for
polymorphism detection of GSTT1 and GSTP1 gene.

Results: No significant difference was established (p<0.05) The comparative analysis performed in the healthy population of the RM and other states have shown that the frequency of GSTM1 null genotype in Moldova was similar to that in Ukraine, Italy, Russia, Egypt and Brazil (p<0.05), whilst the frequency of GSTT1 null genotype and GSTP1 polymorphism was significantly different to that countries (p<0.05).

Conclusion: The polymorphism of GSTM1, GSTT1, GSTP1 cannot be assi-
ociated with the risk of recurrent pregnancy loss of Moldovan women.

J01.33
Alpha globin gene mutations in Kurdistan and Kermanshah provinces, Iran

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Alpha thalassemia is one of the most common hemoglobin disorders in the world and its severe clinical form (e.g. H disease) could be transfusion depen-
dent. In this study we genotyped cases with history of blood transfusion or suspicious to H disease who referred to blood transfusion centers and or hospitals in Kermanshah and Kermansh province. Alpha thalassemia was diagnosed on the basis of hematologic index and hemoglobin electro-
phoresis of patient (before blood transfusion) or patient’s parents. DNA was extracted using salting out method and alpha globin mutations were investigated using multiplex Gap-PCR for common deletions and direct se-
quencing for point mutations. One hundred and ten thalassemia patients were recruited and screened using hematologic index and hemoglobin electro-
phoresis. 10 patients with diagnosis of alpha thalassemia were tested. -M deletion was the most common allele that was found in 5 chromosome (25%), followed by -α3.7 in 4 (20%), -α2.5 in 3 (15%), polyA in 3 (15%), -dVSI (-5n) in 3 (15%) and -ac59 in 2 (10%). Deletional H disease (-α/-) was diagnosed in 4 cases (40%) and non-deletional H disease (-/αTα) in 4 cases (40%). Two cases (20%) showed dTα/αTα genotype. Two patients were blood-transfusion dependent, from which the first one received regu-
lar blood transfusion and the second patient was -α2.5/αα3.7 genotype received blood occasionally. This survey indicated diversity of alpha globin mutations and also different clinical manifestation of the H disease.

J01.34
Holt-Oram syndrome as result of prenatal exposure of valproic acid

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Holt-Oram syndrome (HOS) is caused by TBX5 gene mutation and it is esti-
\med to affect 1 of 10 000 individuals. This condition is inherited in an auto-
\mnomal dominant pattern, but most cases result from new mutations in the gene. HOS is characterized by skeletal abnormalities of the hands and
\mms (upper limbs) and heart problems. The diagnosis of HOS can be esta-
\nshed clinically. Clinical signs include upper limbs malformations, anom-
a\naous heart structure (e.g. atrioseptal and ventriculoseptal defects or Fallot tetra-
\md) and cardiac conduction disease, which can cause bradycardia or tachy-
\nria. HOS can be confirmed through TBX5 gene mutation molecular genetic testing - DNA sequence analysis or array comparative genomic hy-
bridization (array CGH). A case report of Holt-Oram syndrome in 6 month girl is represented from Kaunas hospital of LHHS, when mother has been taking a valproic acid 500 mg/day during pregnancy for epilepsy treatment. The antiepileptic drug valproic acid has teratogenic side effect and is a po-
tent inducer of neural tube defects and other abnormalities, such as car-
diac, skeletal and limb defects. When valproic acid cannot be avoided in pregnancy, the lowest possible effective dose should be prescribed. Also it is recommended to use folic acid to 5 mg/day. A usage of folic acid should be started before pregnancy, so family planning and prenatal consultation is recommended for women with epilepsy.

J01.35
Stage-dependent expression of HomeoboxA10 gene in human endometrium

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HoxA10 gene belongs to Homeobox gene family and encodes DNA-binding transcription factor that regulates variety of downstream genes. HoxA10 gene has a well-characterized role in uterine organogenesis during embry-
onic development and functional endometrial differentiation and growth in
\mns. It is actively involved in cell proliferation through the regulation of different genes.

To evaluate the expression profile of HoxA10 gene during menstrual cycle, endometrial tissues were collected from 21 healthy fertile women under-
going laparoscopy for tubal ligation surgery, in menstruation, proliferative and
\nctoriary phases. For this respect ethical approval and informed patient consent was gained for the use of tissue sample. Total RNA was extracted from tissues using TRIzol reagent and cDNA was subsequently synthesized. Quantitative expression analysis was performed using the real-time PCR sys-
\mm.

Results showed stage-dependent manner of HoxA10 gene expression in en-
dometrium tissues as notably increase of expression level in the secretory pi-
\ne to comparison to the menstruation and proliferative phases. This finding suggests that HoxA10 gene may have an important role in re-
gulating endometrial cells proliferation and development in menstrual cycle and it is important for establishing conditions necessary for implantation.

Up-regulation of HoxA10 in secretory phase during the window of implan-
tation may be one of the potential molecular mechanisms of fertility. This gene can be noted as one of the candidate genes that its aberrant expression may contribute to the etiology of infertility and gynecological disorders.

J01.36
No alteration in PRLR gene in Iranian women with idiopathic hyperprolactinemia

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Hyperprolactinemia is seen in a group of infertile women. These women are treated for this problem because high amounts of prolactin can affect fertili-
\ty by changing LH and FSH quantity. The increase in prolactin levels could be due to a disorder in the prolactin receptor, so investigating the genome of this receptor which is located on chromosome 5 might be useful in finding the reason for this problem.

In this study, the infertile women referred to Royan infertility institute, dia-
gnosed with idiopathic hyperprolactinemia whose pituitary MRI results were normal, were compared with the control group who were fertile wo-
\men, for any changes in their prolactin receptor gene. The techniques used in this study were PCR-SSCP and Sequencing .the DNA was extracted from the blood samples by salting out method and then amplified by the poly-
\mase chain reaction. The purified PCR products, were sequenced by the sanger sequencing technique in order to confirm the SSCP result.No change in any of the eleven exons of the prolactin receptor gene was detected neither in control group nor in the patients group, consequently it can be concluded that idiopathic hyperprolactinemia in the affected infertile women, is not in association with any change in the genome of the prolactin receptor. To our knowledge this is the first study on this gene in this group of patients.

J01.37
The role of ID gene family in transformation of endometrial lining of uterus during menstrual cycle

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The inhibitor of DNA binding family members (ID1, ID2, ID3, ID4) inhibit activity of basic helix-loop-helix transcription factor and have an important role in cell growth, differentiation and angiogenesis. Due to the high proliferation and angiogenesis in the endometrial lining of the uterus during the menstrual cycle, it seems that this gene family may be involved in the occurrence characteristics of this tissue. 

This study focused on expression of ID gene family by real-time PCR in 21 healthy fertile women undergoing tubal ligation surgery, between 20-45 years old to investigate the possible role of this gene family in endometrial tissue changes during three phases (menstrual, proliferative, secretory) of menstrual cycle. For this respect ethical approval and informed patient consent was gained for the use of tissue sample. Data revealed high levels of mRNA expression for ID1, ID2, ID3 and ID4 in proliferative phase. Also, all members of this gene family showed more significant expression levels during the secretory phase than the proliferative phase of the menstrual cycle. 

These results suggest for the first time that ID gene family has a dynamic role in the proliferation and angiogenesis of endometrial cells. We propose that changes in expression pattern of this family can be reviewed in gynecologic disorder and infertility that related to embryo implantation.

**J01.38** Polymorphisms of Toll Like Receptor 2, 3 and 4 in patients that do and do not enter labour spontaneously at term

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To assess the association of polymorphisms of Toll Like Receptors (TLR)2, 3 and 4 with the delay in onset of labour at term pregnancies, patients delivering at >37 weeks and without preclampsia, IUGR or a history of preterm delivery were prospectively evaluated. TLR2 Arg573Gln, TLR3 (c.1377G>T) and TLR4 Asp299Gly and Thr399Ile polymorphisms were genotyped by using PCR-RFLP. Patients labouring spontaneously before the 41st week were compared with those who did not labour spontaneously until this week in terms of baseline characteristics, TLR 2, 3 and 4 polymorphisms. These polymorphisms were also performed by using 40th week cut-off. Chi-square test, two sample T test or Man-Whitney U test were used for comparisons as appropriate. 79 patients delivering after 37 weeks were evaluated. All had GC genotype for TLR2 Arg573Gln and TLR4 Thr399Ile. There were no significant differences for TLR4 Asp299Gly GA and TLR3 (c.1377G>T) polymorphisms between patients spontaneously entering or not entering labour until the 41st week; the same was true when the 40th week cut-off was used. Delay in onset of labour at term pregnant patients does not seem to be affected by presence of TLR 2, TLR 3 or 4 polymorphisms. Further studies are needed.

**J01.39** Prenatal diagnosis of APL1 related Leber Congenital Amaurosis (LCA) in the Iranian population: application of novel informative markers rs11658369 and rs8066853

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Leber congenital amaurosis (LCA) shows clinically and genetically heterogeneous disorder which is caused by a large number of mutations in at least 17 different genes. In the present study the application of two genetic markers including rs11658369 and rs8066853 markers located in APL1 genetic region were genotyped and their application were investigated in prenatal diagnosis of the disease in the Iranian population. The markers were genotyped using newly designed specific primers by Tetra-primer ARMS PCR in 150 unrelated healthy individuals in the Iranian population. Analysis of the genotyping data using Genofer program, indicated the presence of informative haplotypes (>5%) with strong linkage disequilibrium for the markers and the APL1 gene. The efficiency of these haplotypes as a suitable tool in prenatal diagnosis of LCA was evaluated in two Iranian families with one affected child with an already diagnosed W278X mutation in the APL1 gene, through an APEX microarray screening and sequencing analysis. The transmission of the normal and affected alleles form parents to their affected child and the fetus was confirmed by using the haplotypes obtained by genotyping of rs11658369 and rs8066853 markers. Interestingly, in line with the mutation results, the genotyping data also confirmed the transmission of normal alleles to fetuses. The families now were given birth to children with normal vision as confirmed by ophthalmic diagnosis. The data suggested that rs11658369 and rs8066853 could be suggested as novel informative markers for linkage and prenatal diagnosis of APL1 associated LCA in the Iranian population.

**J01.40** Analysis of pedigrees of families with reproductive losses

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Given the important role of genetic factors in the genesis of reproductive losses in families with miscarriage, we analyzed taking into account the reproductive disorders in families. When analyzing families accounted reproductive losses and infertility among relatives of the 1st, 2nd, 3rd and 4th degree of consanguinity. Analysis was conducted among 226 families with one miscarriage, 277 families with two miscarriages, 117 families with three miscarriages in history. The comparison group consisted of 201 families in history, which marked the birth of a healthy baby. Conducted under the EBM calculations showed that the presence of relatives in the pedigrees of families with women reproductive losses is IP = 2.47 (CI-95% 1.62:3.75) Among the male relatives of the families of miscarriage, this indicator was IP = 2.18 (CI-95% 1.27:3.59) In general, the odds ratio for relatives with reproductive disorders, excluding sex pedigrees in groups with reproductive losses and in the comparison group was IP = 2.79 (CI-95% 1.95:3.98). Thus, in families with reproductive losses 2.5 times more relatives with reproductive disorders, compared with the families of the comparison group. This indicates a hereditary component, leading to reproductive losses. Availability unspecified hereditary factors, both by women and by men contributes to further research to find genetic markers defining gender reproductive disorders.

**J01.41** Methyltetrahydrofolate reductase C677T and A1298C polymorphism is not associated with oligozoospermic and azoospermic infertile male patients in the Turkish population

M. Yazar, M. Hançer, N. Yenmez, B. Asp299Gly and Thr399Ile polymorphisms were genotyped by using PCR-RFLP. Patients labouring spontaneously before the 41st week cut-off. Chi-square test, two sample T test or Man-Whitney U test were used for comparisons as appropriate. 79 patients delivering after 37 weeks were evaluated. All had GC genotype for TLR2 Arg573Gln and TLR4 Thr399Ile. There were no significant differences for TLR4 Asp299Gly GA and TLR3 (c.1377G>T) polymorphisms between patients spontaneously entering or not entering labour until the 41st week; the same was true when the 40th week cut-off was used. Delay in onset of labour at term pregnant patients does not seem to be affected by presence of TLR 2, TLR 3 or 4 polymorphisms. Further studies are needed.

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Folate pathway plays significant role in the cell phsiology by participating in the DNA repair, methylation and genomic stability. Optimal function of the pathway is essential for high metabolic activity of the testis. Methylene tetrahydrofolate reductase (MTHFR), the key enzyme of folate metabolic pathway, was reported to be five times more active in the testes compared to other organs in a study with adult mice. This is the first study researching the association of MTHFR 677C>T (rs1801133) and 1298A>C (rs1801131) polymorphisms with infertility of the idiopathic nonobstructive azoospermic and oligozoospermic patients. Study population included nonobstructive 75 azoospermic and 62 oligozoospermic nonconsanguinous infertile patients referred to Department of Medical Genetics of Trakya University between 01.03.2001 and 01.06.2001 due to the infertility who had been diagnosed based on the clinical examination and spermograms (World Health Organisation standards, 2010). All patients had normal karyotype without Y microdeletion. Melting curve analysis with labeled probes and primers designed by the manufacturer’s (NLM Diagnostics, Italy) and Real Time Polymerase Chain reaction method (Qiagen, Rotor Gene) have been used. There was no statistically significant association of MTHFR C677T and A1298C polymorphisms and infertility in the study population (p>0.05). The differences in the dietary folic acid intake between populations, even between the different regions of the same geographical area may cause the different results. The similarity of our results with other studies on Caucasian population can be explained by the patients included in this study live in the Trakya region of Turkey and they were being exposed to similar environmental factors.

**J01.42** The association study of MSH5 C253T and MLH3 C2531T polymorphisms in fertile men with non-obstructive azoospermia

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Introduction: Genetic factors cause about 10% of male infertility. However, the etiology of the majority of male infertility cases including non-obstructive azoospermia remains idiopathic. Defects in DNA repair during spermatogenesis is thought to underlie some types of testicular failure. Evidence is accumulating the mismatch repair proteins MSH5 and MLH3 play a crucial role in spermatogenesis. In the present study, we investigated the association between MLH3 C2531T and MSH5 C253T polymorphisms and developing non-obstructive azoospermia. Methods: In a case-control study, peripheral blood samples were obtained from 110 non-obstructive azoospermia patients and 102 proven fertile men. DNA was extracted by using salting out method. Y chromosome...
microdeletions were studied using Multiplex PCR. MLH3 C2531T variants were analyzed using the tetra-amplification reagent mutation system-PCR (HP-ARMS-PCR) method in the patients and controls. MSH5 C8T variants were determined by PCR-RFLP assay in the studied groups. SNPSTAT program was used for the detection of allelic and genotype frequencies and the association between non-obstructive azoospermia and the mentioned polymorphisms.

Results: 14 patients (12.7%) showed Y chromosome microdeletions and therefore were excluded from our study. No association was detected between MLH3 C2531T and MSH5 C8T polymorphisms with developing non-obstructive azoospermia in Iranian patients.

Conclusion: We suggest that the mentioned polymorphisms may not be considered as a genetic risk factor for non-obstructive azoospermia at least in Iranian population. Variability of the results in other populations may be explained due to differences in the ethnic backgrounds.

J01.43 Effect of oxidative stress on KDM5D expression in mature mouse testis

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Male factors are important causes of infertility. Two major causative factors of male infertility are oxidative stress (OS) and genetic factors. OS damages the sperm plasma membrane, the genome integrity and expression profile of genes involved in spermatogenesis. KDM5D or SMY is one of these genes which its alteration is associated with male infertility.

In this study the expression profile of KDM5D gene was evaluated in testis tissues of infertile after OS induction. Oxidative stress in adult mice tests was induced by injection of 1:10 concentration of tertiary-butyl hydroperoxide (TBHP). Adult male BALB/c mice were randomly selected. Case group included treated mice by TBHP for 2 weeks and control group treated only by injection of dH2O. ROS levels in testes tissue samples of all mice measured by flow-cytometry. Consequently the expression of KDM5D gene was quantitatively measured in samples of both groups by real-time PCR. According to Flow-cytometry results, an increase of oxidative stress in ROS treated mice in comparison to control group was observed. Moreover, the expression level of KDM5D gene was lower in TBHP treated mice than that of in control mice. Oxidative stress can have detrimental effects on testicular tissue and alters the expression of some genes which are involved in spermatogenesis.

J01.44 The case of prenatal diagnosis Pallister-Killian syndrome in Sverdlovsk region (Russian Federation)

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Pallister-Killian syndrome or Tetrasomy 12p is an uncommon aneuploidy, which may present in the prenatal period with an ultrasonographically detected fetal abnormality. A 30-year-old woman was referred to our center for evaluation of a frontal edema in her fetus. The pregnancy was uncomplicated and there was no significant medical history of the family. The initial screening scan was performed at 12 weeks gestation and an increased nuchal translucency (3, 8 mm) was obtained. At 14 weeks gestation, the expert ultrasound examination demonstrated increased nuchal translucency (4,1mm) and heart defect (tricuspid regurgitation). The couple was counselled concerning the options of invasive tests for karyotyping of fetus.

The parents accepted to perform a placental biopsy at 15 weeks gestation. The cytogenetic analysis has shown that all cells of placental villus had a supernumerary, metacentric marker chromosome. Prenatal molecular assay for detecting aneuploidies and microdeletion syndromes (Prenatal BoBe*) , that was obtained in parallel with cytogenetic analysis has defined a gain of chromosomal copy number in group autosomal probes (region 12p13).

Using the method fluorescence in situ hybridization (FISH) we have confirmed that the extra chromosome was derived from chromosome 12. Using the method fluorescence in situ hybridization (FISH) we have confirmed that the extra chromosome was derived from chromosome 12.

The parental karyotypes were subsequently checked and were both normal. On the basis of this, the given poor prognosis for Pallister-Killian syndrome and after counseling, the couple elected to terminate the pregnancy at 17 weeks gestation.

J01.45 Association FBLN5 gene polymorphism with pelvic organ prolapse.

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Pelvic organ prolapse - multifactorial disorder characterized by a loss of pelvic floor support leading to the herniation of the uterus into or through the vagina. One of the most important genes encoding proteins of elastic fiber matrix assembly and function is FBLN5. We used tagged SNP approach to increase the genetic coverage of the FBLN5 gene (Haploview 4.2 software; Genotype and phenotype data were collected using a PCR-CTPP (polymerase chain reaction with confronting two-pairs primers) and real-time PCR. The study sample set included patients (n = 210) diagnosed with stage III-IV prolapse based on the Pelvic Organ Prolapse Quantification (POP-Q) examination, and controls (n = 292) were women without prolapse and no prior history of prolapse surgery. Multiple logistic regression analysis adjusted for age, body mass index and vaginal parity was applied to evaluate the associations between FBLN5 SNPs and POP in the entire set and in the strata with/without perineal trauma and fetal macrosomia. The top association signal was found for SNP rs2018736 (protective effect for the minor allele A; recessive model) in the entire set: P = 0.0026, OR = 0.42, 95% CI: 0.24-0.75; in the strata with perineal trauma: P = 0.0018, OR = 0.27, 95% CI: 0.11-0.64; and in the strata with fetal macrosomia: P=0.013, OR=0.14, 95% CI: 0.03-0.71. The results of the haplotype analyses were consistent with the single SNP analysis. The results are clinically important providing a rationale for fibrin-5-targeted therapy in women combining genetic and clinical determinants of higher risk for POP.

J01.46 Preimplantation genetic diagnosis (PGD) for beta thalassemia and birth of a healthy boy after 4 times of therapeutic abortion

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A couple who was referred to us for prenatal diagnosis (PND) for beta thalassemia had experienced 4 consecutive pregnancies with diagnosis of thalassemia major. After four times of therapeutic abortions, the couple decided to have healthy child using preimplantation genetic diagnosis (PGD). Ovulation induction and ovum fertilization were performed in Erfan Hospital, Tehran, Iran. In the 3rd day, a single blastomere from each embryo (in total 8) was removed and given to us. For molecular PGD, a multiple nested PCR, using several STRs markers linked to HBB gene and also amplifying part of the β-globin gene, was done. Out of 8 tested cells, 3 blastomers were thalassemia minor, 2 homozygous normal and one thalassemia major. Also two embryos did not give us conclusive result on being affected or normal due to allele dropout (ADO).

The family decided to transfer 3 embryos. Three weeks after embryo transfer, pregnancy test was positive and a single pregnancy was continued up to the 11th week gestation. The family agreed on fetal testing. CVS was followed. Genetic testing for beta thalassemia and chromosomal aneuploidies showed a healthy boy who was carrier of thalassemia minor. A healthy boy was born on the Feb 4, 2014 by cesarean section. Similar test on placental sample confirmed our findings.

J01.47 Genomic variability study: CNVs analysis in women with premature ovarian failure (POF)

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Premature ovarian failure (POF) is defined by amenorrhea of at least 4 to 6 month duration, occurring before 40 yr of age, with two FHS levels in the postmenopausal range. Genetic basis for POF are FMR1 gene premutation and chromosom anomalies detected by karyotype but its etiology remains unknown. More than 95% of cases. Copy Number Variations (CNVs) form an important class of human genetics variants. Array Comparative Genomic Hybridization (Array-CGH) analysis is able to detect submicroscopic chromosomal rearrangements with a higher genomic resolution.

In this study we selected 32 women affected by POF, with normal FMR1 premutation and normal karyotype. This cohort have been analysed by Array-CGH using NimbleGen platform with a resolution of 100Kb, to identify an association between CNVs and POF. We observed 23 CNVs in 12 patients (37.5%); 3CNVs on the X chromosome and 20 on autosomal chromosomes; 14 duplications and 9 deletions; the rearrangements size were between
J01.48 Expression of HIF and VEGF genes and pregnancy loss
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15% of human pregnancies are known to end in spontaneous abortion before 12 weeks of gestation. A disbalance of cytokines and growth factors can affect negatively early stages of human embryogenesis. The aim of the study is to determine the expression level of the growth factors genes in miscarriage patients.

Study was performed on the RNA samples from two groups of women. The first group included women with a missed abortion or spontaneous abortion. The control group included women with normal pregnancy. The samples of RNA were extracted from decidua and chorion. The level of the gene expression was assessed using the two step reverse transcription probe-dependent fluorescent real-time polymerase chain reaction.

Hypoxia inducible factor (HIF) is the primary molecular sensor respond to oxygen tension changes. HIF as transcription factor regulated many cellular processes, for examples angiogenesis, invasion, cell survival. HIF in hypoxia condition provides a potent stimulus for VEGF synthesis and is essential for development of maternal and placental vascularization in early human pregnancy. In the case of miscarriage the level of HIF-1 gene expression was reduced in the chorion. Analysis of gene expression vascular endothelial growth factor in physiological pregnancy showed that mRNA levels of this gene was significantly lower in decidua compared to the chorionic tissue (P = 0.032). In case of miscarriage the level of VEGF gene expression in chorionic tissue was not different from decidual tissue and significantly lower compared with the level of gene expression in the control.

J01.49 Paternal and maternal origin of Primary ovarian insufficiency (POF/POI) caused by FMR1 gene premutation - using Repeat Primed PCR (RP-PCR) method
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Patients and methods: Early ovarian depletion criteria were consistent with international protocols: secondary amenorrhea, ovarian failure up to the age of 40 years, levels of FSH >40 IU/L, and low estrogen levels. We investigated the CGG trinukleotide repeats in the FMR1 promoter region by hybridization with radiolabeled DNA probes. The premutation CGG repeat number was 55±200, and the gray zone was between 45-54 repeats.

Results: In a 38-year-old woman suffering from premature ovarian exhaustion we found deviations on both alleles of FMR1 gene during the Repeat Primed PCR (RP-PCR) tests. On one allele the CGG repeat number was 76. On the other allele the CGG repeat number was located in the so-called gray zone (CGG 52-54). In both cases deviations were found, we examined the family. We found a 29/52 CGG repeat number in her mother, and a 76 CGG repeat number in her father. The menopause of the mother occurred at the age of 46, the neurological examination of the father for the Fragile X Associated Tremor Ataxia Syndrome is in progress. In the brother of the patient and her family also, because the genetic results have serious implications.

The sequencing of the RHD gene of the child at birth can highlight the two copies of the RHD gene: an allele with the standard RHD gene inherited from the father, and the other with RHD variant (type 1 DIV) identical to mother’s. This case shows the possible clinical consequences of anti-RH1 alloimmunization in a mother carrying a variant of the RHD gene.

J01.50 Maternal variant gene RHD and clinical effects during pregnancy and at birth
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During her second pregnancy, a 35 year old patient known with RH:1 status surprisingly has an anti-RH allo-immunization at 24 weeks of pregnancy. Prenatal non-invasive RHD genotyping on fetal maternal plasma was performed using real-time PCR (TAQMAN technology) studying three regions of the RHD gene (exons 4, 5 and 10) and shows amplification of the 5 exons. The maternal DNA sequencing allows to identify the type IVA RHD variant characterized by lesions of exons 2, 3 and 7: RH1 corresponding antigen exposed the patient to the risk of anti-RH1 immunization. The titer of the anti-RH1 increases dramatically in late pregnancy (maximum titer 1024), however, without sonographic fetal anemia. At birth, the child is RH:1 and has a minor anemia 120 g / l but a positive direct antiglobulin test (due to maternal anti-RH1 antibodies on the surface of his erythrocytes). But at the 12th day, the child presents a deep hemolytic anemia at 52 g / l; requiring 4 GUS transfusions (12th day, 13th day and 30th day). The sequencing of the RHD gene of the child at birth can highlight the two copies of the RHD gene: an allele with the standard RHD gene inherited from the father, and the other with RHD variant (type 1 DIV) identical to mother’s. This case shows the possible clinical consequences of anti-RH1 alloimmunization in a mother carrying a variant of the RHD gene.

J01.51 Diagnostic algorithm for differential diagnosis in high-risk pregnancies identified by prenatal ultrasound screening: two case reports
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Hereby, we present 2 case reports of pregnancies with an initially high risk of congenital anomalies, detected by 1st and 2nd trimester prenatal ultrasound screening (US), that was significantly reduced after a complex follow up approach. Case 1: A 34-year-old woman (23rd week of gestation; wg.) was referred due to increased nuchal translucency (NT: 4.5 mm) and suspected hygroma colli cysticum detected by 1st trimester US. Following CVS we performed examinations comprising standard karyotyping, QF-PCR, arrayCGH, MLPA of subtelomeric regions and SMN1 gene, sequencing of DHCR7 gene and TORCH serology with normal outcomes. US performed in the 16th wg. performed exclusion of NT and absence of other congenital anomalies, except for hyperechogenic bowel (CFTR negative). Fetal ECHO and 3D US were normal. In case 2 a 30-year-old woman (22nd wg.) was examined due the absence of nasal bone and renal pelvic dilatation on 2nd trimester US. Following AME and cordocentesis we utilized identical diagnostic algorithm (except MLPA) and furthermore sequencing of FMR1 and FGFR3 genes with negative results. Both families were reassured and thus opted for continuation. In conclusion, the role of prenatal ultrasound screening is an effective tool for the identification of high-risk pregnancies, and the benefit of the proposed diagnostic algorithm is confirmed to identify high-risk pregnancies.

J01.52 The utility of conventional cytogenetic and aCGH analysis in the diagnosis of the products of conception
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During November 2012-January 2014, 42 samples were analyzed by conventional cytogenetic methods and 5 cases by aCGH technique (Array Comparative Genomic Hybridization). The analyses were performed for early miscarriages or for induced pregnancy termination due to severe fetal ultrasound abnormalities. The samples evaluated were from products of conception between 7 and 23 weeks of pregnancy, in patients aged between 24 and 41 years. Depending on the biological sample received, either, chorionic villi, epithelial tissue, amniotic fluid or fetal cord blood was used for analysis. The karyotyping was successfully achieved in 40 cases and the aCGH analysis for all 5 cases.

Of the 40 cases analyzed by conventional cytogenetics, 23 (57.5%) had abnormal karyotype and in 17 cases (42.5%) no structural or numerical chromosomal abnormalities were identified. Among the abnormal cases we identified: 13 homozygous autosomal trisomy, 3 mosaic abnormalities, 2 unbalanced structural chromosomal aberrations, 1 trisomy and unbalanced structural chromosomal aberration, one triploidy, one monosomy X, 2 double autosomal trisomies, one clonal chromosomal instability. ArrayCGH analysis identified trisomies of chromosomes 21 and 22. In one case genomic aberrations were identified, including genes involved in embryonic development. For two cases with high gestational age the abnormala
QF-pcr in Prenatal Diagnosis: preliminary evaluation of advantages and pitfalls

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Our center is responsible for invasive prenatal diagnosis from the 90s, every year we process by conventional cytogenetic analysis about 1200 samples between amniotic fluid (AF) and chorionic villi (CVS), and about 70 samples from recurrent miscarriages. In 2010 we introduced the QF-pcr analysis for the most common aneuploidies (chromosome 21, 18, 13, X and Y) as support to cytogenetics in:

1. AF samples: when there is a need to have a rapid response in cases of advanced maternal age, clinical suspicion of fetal anomaly or in amniocentesis performed late (more than 20 weeks).
2. CVS samples: in all cases, in conjunction with cytogenetic analysis of chorionic villi DNA used in molecular studies. Over the last three years, we extended QF-pcr analysis to recurrent miscarriages because traditional cytogenetic testing is labour-intensive and has a significant failure rate, especially when the sample quality is poor. In this case we investigate a greater number of chromosomes: 13, 15, 16, 18, 21, 22, X and Y whose aneuploidies are the major causes of miscarriage.

In this work we report a summary of our experience, using CE-IVD QF-pcr systems CYS® labeled. In prenatal diagnosis QF-pcr system proved to be an effective and specific support to cytogenetic analysis, and a useful and reliable tool to diagnose aneuploidy in spontaneous miscarriages, reaching a pathology’s diagnosis in 45% of cases and we’re considering to replace cytogenetic analysis with this molecular system.

J01.55
The correlation between sperm DNA fragmentation and recurrent abortion in Iranian population

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We present a de novo balanced Robertsonian translocation rob(13;14) identified in two trimester of gestation.

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We present a de novo balanced Robertsonian translocation rob(13;14) identified in two trimester of gestation.
Sexual dimorphic with genital ambiguity was the first reason of genitor measurement and imaging. A surgical management was offered for some patients with disorders of sexual differentiation (DSD) discovered during the neonatal period and the enfance development (DSD) discovered during the neonatal period and the enfance. The aim of this study was to evaluate the frequency, the genital anatomy and gender role.

Sex chromosome rearrangements are identified in patients with Turner’s syndrome, in mosaic with 45,X cells. In all these cases, it is important to well characterize the derivate chromosomes in order to establish a good genotype-phenotype correlation, to provide information about the role of different X/Y chromosome regions or loci in the clinical manifestations of patients.

For this purpose, we have collected patients with different sex chromosome rearrangements detected with karyotyping: X-autosome translocations (3); X isochromosome (3), X deletions (4); ring X (1); X inversion (1); Yautosome translocations (4); Y isochromosome (1). The rearranged chromosomes have been analyzed using FISH, MLPA or aCGH when appropriate. After a detailed clinical assessment, genotype/phenotype correlations have been performed.

Management of sex differentiation disorders at University Hospital Hassan II Fes

Hassan II Fes

Sex differentiation disorders represent all the abnormalities in development of the gonads, the genital tract, and the external genitalia. Disorders of sexual differentiation are due to genetic defects or endocrine imbalance. Sex-determining genes (SRY gene) dictate the gonadal sex whereas the fetal testicular hormones determine the somatic sex during sex differentiation. Abnormal sexual development causes unification between fetal gender identity and gender role.

The aim of this study was to evaluate the frequency, the genital anatomy appearance, the diagnostic and the surgical management of disorders of sex development (DSD) discovered during the neonatal period and the enfance development (DSD) discovered during the neonatal period and the enfance. Between September 2009 and March 2013, 30 patients with abnormal sexual development were identified in our unit. First-line testing included biological measurement and imaging. A surgical management was offered for some patients. Sexual dimorphic with genital ambiguity was the first reason of consultation. One patient had male breast development. All clinical evaluations suggested genital ambiguity. This presentation points out the need of an accurate diagnosis of sexual differentiation disorder during the neonatal period. The intervention of a multidisciplinary team is essential as well for assignment of sex as for therapeutic guidelines.

Polyomorphic locus 820AG of IGF-2 gene as a possible marker of fetal development disorders


Insulin-like growth factor-2 (IGF-2) is a mitogen, growth and differentiation modulator for many cell types. It is mainly expressed during the prenatal development, and its activity strongly depends on the genomic imprinting. Genomic imprinting in the chorionic tissues of spontaneously eliminated human embryos has been studied on the model of 820AG (Apal) of the IGF-2 gene locus. Methods: Isolation and purification of DNA and RNA, PCR-RFLP, RT Results. Molecular and genetic analysis was performed of the polyomorphic locus 820AG IGF2 in 107 samples of DNA extracted from the chorionic tissues of spontaneously eliminated human embryos within 5-10 weeks of gestation. The loss of imprinting of the IGF2 gene was analyzed in 41 samples of the chorionic villi cells in human. In 90% of cases, the loss of imprinting was detected. Presence of AG genotype at SNP 820AG of IGF2 gene was shown to cause more than a 7-fold increase in the risk of embryo elimination (OR = 7.72, CI 95% 3.31-18.04). Conclusion. The loss of genomic imprinting of the IGF2 gene may be an important cause of the miscarriages in human.

Decreased expression level and chromatin incorporation of histone acetyltransferase CDY1 in testicular biopsies of infertile men with non-obstructive azoospermia

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Background: Many cases of male infertility associated with a severe impairment of spermatogenesis. In the last stage of spermatogenesis (spermiogenesis), haploid spermatids endures complex changes to differentiate into spermatozoa, this process includes chromatin modifications mediated by different histone modifying enzymes. Chromodomain Y (CDY) proteins encoded by the CDY family of genes, are characterized by two functional motifs, a chromodomain and a histone acetyltransferase catalytic domain. A testis specific CDY protein, named CDY1, binds to histone histone regions through the chromodomain, and then causes hyperacetylation of genes involved in sperm chromatin condensation. This study aimed to investigate the relative mRNA expression and chromatin incorporation of chromodomain Y1 (CDY1) protein in the testis tissues of infertile men.

Material & Method: Local ethical approval was gained for this study and informed consent was given by patients. Testicular biopsies were collected from 31 infertile men referred to Royan Institute and underwent testicular sperm extraction (TESE). These samples distributed into 4 groups: obstructive azoospermia (positive control), severe oligoasthenoteratozoospermia, non-obstructive azoospermia and sertoli cell only syndrome. Using qRT-PCR and nucluesome-ELISA methods, the mRNA levels and chromatin incorporation of CDY1 protein in the testis tissues of infertile men.

Conclusion(s): The results showed lower expression and chromatin incorporation of CDY1 protein in all 3 sample groups with spermatogenesis defect in comparison to positive control. This data demonstrated that the defective epigenetic role of the histone acetyltransferase CDY1 may be associated with male infertility.
Approximately 10-15% of clinically recognized pregnancies terminate with spontaneous abortions. Half of them are associated with chromosomal abnormalities (CA). Cytogenetic analysis of chorionic villi has limitations such as high rate of culture failure, maternal cell contamination and poor chromosome morphology. FISH method with target probes doesn’t allow to receive full information about fetal genome. CGH is the only DNA-based screening method that can detect chromosomal imbalances in a single experiment. In this study 60 abortion specimens were analyzed by G-banding, FISH and CGH (Table). Overall, CA were detected in 25 specimens. Karyotyping was unsuccessful in 31 samples, while CGH and FISH analyses were successful in all cases. G-banding analysis showed normal karyotype in 21 cases and detected abnormalities in 8 cases (32% of CA). FISH using probes targeting chromosomes 13, 18, 21, X and Y detected CA in 15 samples (60% of CA), but in two of these cases wasn’t able to find double trisomies which were revealed by CGH. CGH detected CA in 21 samples (84% of CA) but missed triploidy in 4 cases. Aneuploidies detected only by CGH were all confirmed on by Identifiler Kit that provides 15 different STR markers and compared. Results: One maternal originated trisomic (trisomy 13) aborted material and one is maternal and the other is paternal originated triploidic aborted materials were identified. One aborted material was showed maternal heterodisomic profile in the current results. Conclusion: These results suggest that it is possible to identify the disomy, chromic profiles and parental originated molecular cause of aborti on by Identifier Kit that provides specific microsatellite STR markers.

**J01.64**

Is apolipoprotein E polymorphisms associated with spontaneous abortion S. Valcinotepe, S. O. Hacivel Voglu1, C. Silan, C. Akurat2, E. Koc, F. Silan1, E. Cosar2, A. Uludag, O. Ozdemir1;  
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Objective: Apolipoprotein E has three major isoforms (Apo E epsilon2, epsilon3, and epsilon4) and has important functions in nerve development and repair. To evaluate the association of Apo E polymorphisms and spontaneous abortion we studied the genotypes of spontaneously aborted fetuses, their mothers and control cases. Methods: In the current case control study, three different groups of aborted materials, mothers and healthy control were compared. Target gene of Apo E epsilon2/epsilon3/epsilon4 alleles were analysed by real time PCR method. Results: The epsilon2 and epsilon4 alleles showed high prevalence in aborted materials comparing both with their mothers and healthy control group (p<0.0001). The epsilon4 allele was higher in fetuses comparing with their mothers (p<0.0001). Conclusion: Apo E epsilon2 and epsilon4 alleles seem to be contributing to the thrombophilic risk factors as a seconder parameter to spontaneous abortions.

**J01.65**

Parental identification of aborted materials for chimerism and other molecular etiological parameters by microsatellite STR profiling F. Silan1, A. Uludag1, C. Akurat2, D. Kankaya1, M. Gencer1, M. Urfali1, S. Valcinotepe1, O. Ozdemir1;  
1Canakkale Onsekiz Mart University School of Medicine Department of Medical Genetics, Canakkale, Turkey.  
2Canakkale Onsekiz Mart University School of Medicine Department of Obstetrics and Gynecology, Canakkale, Turkey.

Aim: Structural and/or numerical chromosomal abnormalities are responsible for 50-60% of the first trimester miscarriages, 20-25% for seconder and 5-10% for the third trimester miscarriages. In the current study it was aimed to use the microsatellite STR markers for the parental identifying of aborted materials. Materials and Methods: Sixteen aborted materials and their parents were included in the current study. Thin skin biopsies from aborted materials and peripheral blood EDTA samples from parents were used for total genomic DNA isolation. All samples were identified by using AmpFℓSTR® Identifiler (Applied Biosystems) PCR Amplification Kit that provides 15 different STR markers and compared. Results: One maternal originated trisomic (trisomy 13) aborted material and one is maternal and the other is paternal originated triploidic aborted materials were identified. One aborted material was showed maternal heterodisomic profile in the current results. Conclusion: These results suggest that it is possible to identify the disomy, chromic profiles and parental originated molecular cause of aborti on by Identifier Kit that provides specific microsatellite STR markers.

**J01.66**

Beta globin gene mutations in Kurdish provinces, Iran A. Darabi1, M. Fahalf,2, F. Keshavarzav,2, B. Sedaghatikhayatz,2, S. Azadmehr1, P. Salehifar1, S. Zareenejad

1Science and Research branch, Islamic Azad University, Kurdistan, Iran, Tehran, Islamic Republic of Iran, 2Kawarv Human Genetics Research Center (KHGRC), Dr. Zeinial's Medical Genetics Lab, Tehran, Iran, Tehran, Islamic Republic of Iran, 3Cellular and Molecular Endocrine Research Center, Research Institute For Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Islamic Republic of Iran, 4Samandaj branch, Islamic Azad University, Kurdistan, Iran, Tehran, Islamic Republic of Iran, 5Blood Transfusion Organization, Kurdistan, Iran, Tehran, Islamic Republic of Iran, 6Department of Molecular Medicine, Biotech Research Center, Pasteur Institute of Iran, Tehran, Iran, Islamic Republic of Iran.

Introduction: More than 95% of beta-thalassemia mutations are point mutations in the beta-globin gene in global population. This study was performed to determine beta globin gene mutation in blood transfusion dependent patients with in Kurd ethnicity in Kurdistan province in west of Iran. Materials and Methods: Transfusion dependent patients were enrolled in the study. Diagnosis of beta thalassemia was confirmed using patients’ parent's hematologic index and Hb electrophoresis. DNA was extracted from 5ml of patient’s blood using standard salting out method. Common mutations in Kurdish population were investigated by ARMS-PCR method. Unknown cases were investigated by direct sequencing of beta globin gene. Genotype frequency and allele frequency were calculated.

Results: Fifty eight transfusion dependent beta thalassemia patients (35 male and 34 female) with mean age of 16±6.98 years old were entered in the study. IVSI-1 had most common allele frequency in 37 (27.21%) chromosomes following by Fr8-9 in 22 (16.18%), IVSI-1 in 13 (9.56%), C36/37(T) in 11 (8.09%) and IVSI-110 in 7 (5.67%). Conclusion: IVSI-1 and Fr8-9 were the most common mutations in beta globin gene in Iranian Kurd thalassemia patients, which is in alignment with previous studies among Kurdish population.

**J01.67**

Results of prenatal tests in pregnancies after assisted reproductive technologies V. Obrikyte, D. Serapinas1;  
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In vitro fertilisation (IVF) and other assisted reproductive technologies (ART) are effective treatments for infertility and are widely provided in developed countries. However recent scientific publications suggest that there is an elevated risk of major structural malformations, imprinting defects and such syndromes as Prader-Willi, Angelman, Wiedeman-Beckwith. The aim of the study was to analyse if ART are associated with increased risk of genotoxicity for chromosomal nondisjunction in mouse. Study included analysis of 50 families that prenatally were diagnosed chromosomal anomalies (Down, Turner, Klinefelter, Edwards, Patau syndrome). And control group included 70 families with healthy children. The results of present study showed, that in chromosomal anomalies group three cases of children with chromosomal anomalies were conceived after ART. In control group all healthy children were conceived naturally (p 35 years old) and the occurrence of trisomy (p<0.05). The number of observed pathological cases is not so big to make exact conclusions, but results of present study supports hypothesis, that ART are associated with greater risk of chromosomal anomalies in conceived children.

**J01.68**

Associations of prenatally detected choroid plexus cysts with biochemical risk for congenital disorders P. Bardzilauskas, D. Serapinas

1Department of Pulmonology and Immunology, Lithuanian University of Health sciences, Kaunas, Lithuania, 2Mykolas Romeris university, Vilnius, Lithuania.

Background: The choroid plexus cysts are one of the fetus ultrasonography
findings which concerns parents about their child’s health. Usually cysts are found in an estimated number 1% all performed ultrasonographies. The aim of the study to evaluate the risk of Down, Edwards syndromes and neural tube defect when choroid plexus cyst is found.


derived from spontaneous abortions in the western regions of Ukraine. Methods: The study population consisted of 100 embryo tissues from women with the final diagnosis spontaneous abortions. Cytogenetic analysis was performed in the uncultured chorionic villi samples (CVS), using interphase FISH on interphase nuclei in the - for Y chromosome. Recently, RSPO1 gene seems to be implicated in Testicular DSD: About four North African cases

T. F. Dammak, R. Louati, R. Frihia, O. Trabelsi, O. Kbaa, T. Rebai, N. B. Abdelmoula; Medical University Sfax, Tunisia.

The XX male syndrome (OMIM 400045) now termed Testicular DSD (Disorder of Sexual Differentiation) is a rare genetic condition (1:20,000 to 25,000 male newborns) characterized by a spectrum of clinical presentations, ranging from ambiguous to normal male genitalia. In males without genital ambiguity, the diagnosis is often made during investigation of infertility or delayed puberty with a frequency of 0.9% among azoospermic males.

Here, we report four observations of 46,XX men detected in our genetic counselling during genetic evaluation of infertility with azoospermia, hypogonadotrophic hypogonadism and testicular hypotrophy. Chromosomal abnormality revealed by two karyotypes in different laboratories was refined by molecular investigation of SRY gene using FISH (n=2) and PCR amplification (n=2) as well as AZF loci by multiplex PCR protocol.

The prevalence of XX male syndrome among our azoospermic men serie is 0.97% (4/412). Molecular analyses demonstrated the presence of Yp SRY gene in the four patients with absence of Yp AZF loci. Review of literature shows that SRY positive Testicular DSD is the common variety (85%) and that PCR amplification of SRY is more appropriate, than FISH, to detect a small amount of Y chromosome translocated on to the X chromosome and to detect the Y-chromosome material in mosaic forms: XX-SRY positive/XX-SRY negative. Moreover, SRY-negative cases (15%) should undergo further molecular testing to explore the presence of S0X9, S0X3 or S0x3 promoter microduplication or microdeletion, or cryptic mosaicism for Y chromosome. Recently, RSP01 gene seems to be implicated in Testicular DSD associated with hyperkeratosis.

J01.69 Frequency of miscarriages twins E. Ginzburg. B. Ginzburg; Regional Hospital of Kaluga, Kaluga, Russian Federation.

Conducted a study to determine the frequency of twins in the structure of abortion among 968 families with miscarriage. Families with one miscarriage were 40.74%, with 2 miscarriages 41.82%, with 3 or more miscarriages 14.4%. Frequency of miscarriages twins was 0.88 %, which corresponds to the population frequency of 0.82% of the Kaluga region. Comparative analysis showed that the differences are not significant at OR = 1.08 (CI- 95%: 0.40-2.97). Thus, the recorded rate of reproductive loss miscarriage consisting of twins does not exceed the prevalence of twin births. This allows us to assert that the twin has no effect on the incidence of reproductive losses in the early stages of pregnancy and is comparable with the frequencies of registraible live births.

J01.70 Molecular management of 46,XX testicular DSD: About four North African cases

T. F. Dammak, R. Louati, R. Frihia, O. Trabelsi, O. Kbaa, T. Rebai, N. B. Abdelmoula; Medical University Sfax, Tunisia.

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J01.71 Fluorescence in situ hybridization (FISH) on interphase nuclei in the uncultured chorionic villi samples from spontaneous abortions

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Background: Approximately 15% of all clinically recognized pregnancies end in spontaneous miscarriage. The most frequent cause of spontaneous miscarriage is fetal chromosome abnormalities such as autosomal trisomy, monosomy X and polyplody. Molecular cytogenetic technique has been introduced in the genetic analysis of miscarriages in addition to the conventional karyotyping and provides new insights into this field. The present study displays frequency and spectrum of chromosomal abnormalities in embryos derived from spontaneous abortions in the western regions of Ukraine. Methods: The study population consisted of 100 embryo tissues from women with the final diagnosis spontaneous abortions. Cytogenetic analysis was performed in the uncultured chorionic villi samples (CVS), using interphase FISH on interphase nuclei in the - for Y chromosome. Recently, RSPO1 gene seems to be implicated in Testicular DSD: About four North African cases

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J01.73 Identification of the novel mutation c.618 C > A in HSD17B3 gene in four additional Tunisian patients with 46, XY DSD and molecular confirmation of a specific founder haplotype in the Tunisian population

B. Ben Rhouma, Nella Belghiti1-2, Fatma Abdelhadi1-2, Mouna Mnif3, Hassen Kamoun1-2, Leila Rekese1, Fatia Fakhfakh1; Faculty of medicine of sfax, Tunisia, Sfax, Tunisia.

HSD17B3 isoenzyme is present almost exclusively in the testes and converts delta 4 androstenedione to testosterone. Mutations in the HSD17B3 gene cause HSD17B3 deficiency and result in 46, XY Disorders of Sex Development (46, XY DSD). This study aimed to search for mutations in HSD17B3 gene in six Tunisian patients with 46, XY DSD by DNA sequencing. Polymerase chain reaction (PCR) amplification and subsequent sequencing of all the coding exons of HSD17B3 gene were performed on genomic DNA from all the patients and some available family members and revealed the presence of the novel nonsense mutation in the exon 9 (c.618 C > A) leading to the substitution p.C206X. The mutation was present in a homozygous state in two patients and in heterozygous state in four patients. The mutation p.C206X abolished a HhaI site, this propriety was used to confirm the mutation’s presence in the patients and family members and its absence in 50 controls. The mutation p.C206X was found in six patients who belonged...
to different families raising the possibility of a common founder. Genotyping using microsatellite flanking the HSD17B3 gene was performed and haplotype study showed that the c.618 C > A mutation occurred in a specific founder haplotype in the Tunisian population. The identification of this founder haplotype is important and might help in genetic counselling in relatives of these families and the antenatal diagnosis. Our results showed also that HSD17B3 deficiency could not be a rare etiology of 46, XY DSD in the Tunisian population.

J01.74 Preliminary diagnosis of trisomy 5 mosaicism

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We report a case of prenatal diagnosis of a fetus with trisomy 5 mosaicism, a rare cytogenetic anomaly. These cases were very rare but pose a definite problem in prenatal cytogenetic diagnosis.

A 37-year-old woman, after two miscarriages, underwent amniocentesis at 18 weeks of gestation because of advanced maternal age. Cytogenetic analysis revealed 26 clones with normal female karyotype and 2 clones from two culture vessels with trisomy 5.

The fetus karyotype was a mosaic: 47XX,+5[2]/46XX[26]. A diagnosis of trisomy 5 mosaicism in amniocytes indicates an increased risk for fetal abnormalities, a confirmatory placental sampling may be helpful, whereas a fetal blood sampling have a very limited value.

Trisomy 5 mosaicism may be another example of tissue-limited mosaicism; then the fetal blood sampling could be falsely reassuring.

No further invasive testing was performed until 21 weeks gestation and level II ultrasound examination showed a fetus with intratureine growth retardation (2¢c), tetralogy of Fallot and hypertelorism. The rest of the fetal anatomy were within normal limits.

The couple interrupted the pregnancy.

The autopsy showed hypertelorism, saddle-backed nose. Exploration of the thoracic viscera showed heart disease complicated by characters of the tetralogy of Fallot. No further malformations were found in abdominal viscer.

At the opening of the skull there was brain colliquation.

J01.75 Reduced gene expression and chromatin incorporation of histone demethylase JMD1A in testicular biopsies of infertile men

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Background: Spermatogenesis is a unique process in male reproductive system, which required precise epigenetic regulation of gene expression. JMD1A is a critical epigenetic modifier element that highly expresses in male germ cells and activates expression of genes by demethylation of H3K9me2/me1 modifications. This study aimed to evaluate the expression and chromatin incorporation of JMD1A protein in testicular biopsies of infertile men.

Material &Method: Ethical approval and informed patient consent was gained for the use of tissue samples. Testicular biopsies were collected from 31 infertile men referred to Royan Institute and underwent testicular sperm extraction procedure. These samples were classified into the following four subgroups: obstructive azoospermia (as positive control, n=8), severe oligoasthenoteratozoospermia (n=7), complete maturation arrest (n=8), and spermatozoa only syndrome (n=8). The expression pattern and chromatin incorporation of JMD1A in testicular biopsies were measured by quantitative real-time PCR and chromatin-ELISA techniques, respectively.

Result(s): Our finding revealed that the expression and chromatin incorporation of JMD1A were significantly decreased in severe oligoasthenoteratozoospermia, complete maturation arrest, and spermatozoa only syndrome groups in comparison to obstructive azoospermia patients.

Conclusion(s): This study indicates that JMD1A deficiency in testis tissues can result in defective spermatogenesis in human infertile males.

J01.76 Sperm head morphology as a predictive marker of DNA fragmentation

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In case of teratozoospermia the most appropriate spermatozoa from those with abnormal morphology have to be chosen. It seems very important to know if there is any relationship between sperm morphology and its genome quality. The objective of this study was to investigate whether specific sperm head morphological abnormalities predict sperm DNA fragmentation. Semen analysis was performed for 84 patients attempting IVF clinic for spermogramm and 8 sperm donors according to WHO criteria. Sperm head morphology was assessed using strict Kruger’s criteria. The following sperm head forms were analyzed: normal, big, small, bulb, amorphous, round, double, with vacuoles. The terminal deoxynucletidyltransferase-mediated dUTP-nick-end labeling (TUNEL) assay used to assess sperm DNA fragmentation. Sperm DNA fragmentation rate was significantly higher in patients than in controls (0.64±0.08 vs. 0.21±0.04; p<0.05). The correlation between sperm DNA fragmentation and vacuolated sperm heads was found (r=0.32; p=0.005). The sperm DNA fragmentation was not associated with the frequency of the spermatozoa with other head abnormalities. Vacuolated sperm head should be treated as a marker of sperm DNA integrity abnormalities and might be considered a useful trait for more efficient selection of appropriate sperm used in fertilization. Supported by Carl Zeiss, RF President’s scholarship and RFBR.
such as H3K9ac and H3K9me2 in regulatory regions of mentioned genes in testicular biopsies of infertile men can represent better insight into molecular mechanisms of infertility.

In this study based on spermogram and pathological features of infertile men referred to our institute, testes tissue samples were collected from four groups including severe oligoasthenoteratozoospermia, complete maturation arrest, total cell only syndrome, and hypospermatogenesis as group positive control. Expression of TNP’s and PRMs were evaluated by qRT-PCR. Also, chromatin immunoprecipitation coupled with real-time-PCR was performed to evaluate the incorporation of H3K9ac and H3K9me2 into regulatory regions of mentioned genes. Consent was obtained from patients according to local ethical approval.

Results showed significant decrease in expression of TNP and PRM genes in all groups compared to positive control. These findings also confirmed by ChiP data revealed decreased incorporation of H3K9ac (activating mark) and increased incorporation of H3K9me2 (repression mark) into regulatory regions of above genes in all groups vs. positive control. The finding implies significant association of histone modifications with altered expression of sperm chromatin condensing genes and impairment of spermatogenesis in male infertility.

J01.79 A Prenatal Ring chromosome 13 with CIR and abnormal genitalia

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Ring chromosomes are unusual chromosomal alterations that occur in 1/50,000 human fetuses, although they have been found from nearly all human chromosomes. Mostly, they are consequence of two breakpoints in both arms, followed by fusion of the proximal ends generating a ring with loss of the distal genetic material and resulting in clinical features mimicking terminal deletion syndromes. Here we report a “de novo” prenatal male case with a ring chromosome 13 (r(13)), which was diagnosed after an amniocentesis performed because a suspicious of ambiguous genitalia and CIR (Intrauterine Growth Retardation). There was no family history of chromosomal anomalies, and the pregnancy evolution was normal with a “non-invasive prenatal DNA test on maternal blood” performed on the 13th gestational week, informed as a normal male. In the 19th gestational week an amniocentesis was performed because a suspicious of ambiguous genitalia and CIR and although the QF-PCR was informed as a normal male, the karyotype showed a r(13) and the array-CGH showed a terminal 13q de

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talia and CIR and although the QF-PCR was informed as a normal male, the karyotype showed a r(13) and the array-CGH showed a terminal 13q de

J01.80 The necessity of specific genetic marker based molecular study of the AZFa region in infertile men

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Introduction: The relation between microdeletions in the Y chromosome and developing male infertility has been studied in several populations. The majority of published studies were designed according to the European Academy of Andrology (EAA) and European Molecular Genetics Quality Network (EMQN) guidelines for the better detection of AZF (Azoospermic factor) microdeletions. However, there are several reports indicating that using the EAA and EMQN suggested sequence-tagged site (STS) markers will result in false positive and false negative results at least in detection of AZFa microdeletions. The aims of the present study was to evaluate the accuracy of recommended STS markers for detection of AZFa microdeletion in Iranian patients with non-obstructive azoospermia. Methods: A total of 100 Iranian non-obstructive azoospermic infertile men and 100 proven fertile men were selected for the molecular study of Y chromosome microdeletions in the AZFa region according to the EAA and EMQN guidelines using sY94 and sY96 STS markers. In addition, the presence of sY176 and sY182 STS markers was investigated using multiplex polymerase chain reaction (M-PCR).

Results: Using sY94 and sY96 primers, we found only one patient who had AZFa microdeletion. However, the use of sY176 and sY182 markers three new patients were detected with AZFa microdeletion. Conclusion: It seems that the primers and STS markers recommended by the EAA and EMQN guidelines may not apply to all populations and it is recommended to design a population based STS panel at least for the study of AZFa region for better detection of microdeletions.

J01.81 An NGS-based test for the identification of individuals carrying “non-classic” male genetic mutations


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We developed qCarrier, an NGS-based approach targeting +4000 known mutations in over 200 genes causing recessive diseases, for testing couples undergoing assisted reproduction treatment in order to reduce the odds of passing a recessive or X-linked disorder to the offspring. In contrast to SNP-genotyping platforms, NGS technologies are not limited to detecting previously known or certain types of mutations (i.e. point mutations or small indels), and can efficiently detect a wider range of disease-causing mutations. The qCarrier is based in sequence capture, followed by high-throughput sequencing Bioinformatic analysis is keystone in the process, as it combines algorithms optimized for the identification and annotation of different types of mutations (point mutations, indels, copy-number and balanced rearrangements). For analytical validation of the test, we obtained DNA from 57 unrelated individuals: 39 patients and 18 previously genotyped controls. The validation set was composed of 49 different known mutations, including 29 SNVs, 13 insertions, and 25 CNVs causing genes such as cystic fibrosis, phenylketonuria, spinal muscular atrophy, hypothryroidism, thalassemia, or Duchenne muscular dystrophy. All but one (48/49) different mutations were correctly scored in the blinded study and only one deletion-type mutation remained undetected. This information allowed us to fine-tune the algorithm to reach maximum sensitivity. All single nucleotide changes were validated and no known recessive mutations were called in the control samples. After initial deployment in the clinical setting we assessed the presence of disease-causing recessive mutations in 52.1% of the analyzed samples (11/21).

J01.82 Association between Cytochrome P450 2A5 (CYP2A5) gene polymorphism and the risk of endometriosis in Iranian population

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1University of Guilan, Rasht, Islamic Republic of Iran; 2Shahid Beheshti University of Medical Sciences, Tehran, Islamic Republic of Iran.

The aim of current study was to investigate the association of rs11592737 (A/G) polymorphism of CYP2C19 gene with the risk of endometriosis in Iranian women. 100 patients with endometriosis and 100 controls with no laparoscopic evidence of endometriosis were included in this study. Samples were analyzed for rs11592737 (A/G) single nucleotide polymorphism (SNP) in CYP2C19 gene using Amplification Refractory Mutation System-Polymerase Chain Reaction (ARMS-PCR).Our data revealed a significant difference in the distribution of rs11592737 genotypes between endometriosis patients and controls (P=0.002). Despite GG genotype, AG Genotype was more frequent in patients than the control samples (P<0.001, OR=3.14, 95% CI 1.66-5.96). Significantly, those cases with A allele showed an increased risk of endometriosis compared to the control group (P=0.02, OR=1.63, 95%CI1.08-2.44). No significant difference in the allele frequency has been demonstrated between cases and controls (P=0.89). The results of this study suggest that rs11592737 (A/G) SNP of CYP2C19 may be associated with a higher risk of endometriosis among Iranian population.

J01.83 Prevalence and distribution of somatic genomic variations in placental tissues from anembryonic pregnancies

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High incidence of somatic mutations is a hallmark of abnormal embryogenesis. Recent data indicate the significant impact of copy number variations (CNV) into etiology of early pregnancy loss. However mechanisms of their origin are still poorly investigated. We aimed to estimate the somatic CNV incidence in placental tissues - cytotrophoblast (CT) and (EM) from 6 anembryonic pregnancies (AP) with normal karyotype using SurePrint G3 Human CGH + SNP 4x180K Microarray Kit (Agilent Technologies). Altogether 54 CNVs were found. Twenty-four rearrangements (21 polymorphic and 3 unique, which are absent from the Database of Genomic Variants) were de-
detected in both tissues indicating their mitotic or early mitotic origin before the germ layers divergence. On the other side, 34 (including 2 unique) and 26 (including 16 unique) variations appeared to be tissue-specific for EM and CT, respectively, originating from mitotic errors after the tissues diverged. CNVs appeared de novo in some cases (30%) of them were unique. Sixteen CNVs from 18 were deletions. Eight of these variations were larger than 1 Mb and all of them were confined to the CT (deH1p12, deH4q13.1-q13.2 -2 cases, de7q11.21, de11q11.23 - 2 cases, de7q12.33-q22 -2 cases). The most interesting potentially pathogenic affected genes were GABRG1, GABRA2, GABRA4, GABRB1 (GABA family is responsible for imprintation and endometrial decidualization, dysregulation which is associated with AP1) EPHA5 (may help to organize developing body plan), and CTNNA3 (controls trophoblast invasion). This study was supported by Russian Foundation for Basic Research, grant 14-04-32047.

J01.84 Detection of MED12 exon 2 gene mutations in Iranian women with Leiomyomas
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Introduction: Uterine leiomyomas are non-cancerous tumors arising from the smooth-muscle layer of the uterus that may grow as a single tumor or in clusters. Despite their benign nature, fibroids can cause a variety of health problems including, abnormal menstrual heavy bleeding, pelvic pressure and pain, pregnancy complications and reproductive problems. It has been suggested that fibroids are inherited in exon 2 of the MED12 gene, located on Xq13.1, are responsible for a majority of uterine leiomyomas. According to the role of ethnicity in developing fibroids, the aim of the present study was to investigate the frequency of MED12 exon 2 mutations in uterine leiomyomas of Iranian patients.

Methods: Genomic DNA was extracted from 50 fresh uterine leiomyomas tissue samples by a phenol-chloroform method. PCR-SSCP was used to detect mutations in the MED12 exon 2 and the flanking intronic regions. Fragments with altered banding patterns were sequenced on an ABI 3730XL automated DNA sequencer.

Results: Five type of MED12 gene heterozygous mutations were detected in 24 (48%) of the leiomyomas samples including, 12 (24%) missense mutations, 5 (10%) in-frame deletions, 4 (8%) small nucleotide variants affecting splicing, 1 (2%) deletions/insertion-deletions spanning the intron 1-exon 2 boundary resulting in exon skipping, and 2 (4%) frame shift deletions. No mutation was detected in normal myometrial tissue.

Conclusion: Our study confirms the role of MED12 mutations in the pathogenesis of uterine leiomyomas, regardless of ethnicity. Therefore the gene could be an appropriate therapeutic target for uterine leiomyomas.

J01.85 Epigenetic analysis of regulatory region of CYP19A1 in granulosa cells of patients with polycystic ovarian syndrome
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Polycystic ovarian syndrome (PCOS) is a complex genetic endocrine disorder among the women of reproductive age. Hyper-androgenemia is one of the main clinical features of PCOS. Aromatase, the key enzyme for estrogen biosynthesis, is encoded by CYP19A1 which is comprised of an unusually large regulatory region including 10 tissue-specific promoters. In human cells CYP19A1 is expressed in gonads via promoter PII. The aim of this study is to evaluate the acetylation and methylation levels of lysine 9 of histone 3 (H3K9ac and H3K9me2), in PII promoter region of CYP19A1 is expressed in gonads via promoter PII.

Methods: Genomic DNA was extracted from 50 fresh uterine leiomyomas tissue samples by a phenol-chloroform method. PCR-SSCP was used to detect mutations in the MED12 exon 2 and the flanking intronic regions. Fragments with altered banding patterns were sequenced on an ABI 3730XL automated DNA sequencer.

Results: Five type of MED12 gene heterozygous mutations were detected in 24 (48%) of the leiomyomas samples including, 12 (24%) missense mutations, 5 (10%) in-frame deletions, 4 (8%) small nucleotide variants affecting splicing, 1 (2%) deletions/insertion-deletions spanning the intron 1-exon 2 boundary resulting in exon skipping, and 2 (4%) frame shift deletions. No mutation was detected in normal myometrial tissue.

Conclusion: Our study confirms the role of MED12 mutations in the pathogenesis of uterine leiomyomas, regardless of ethnicity. Therefore the gene could be an appropriate therapeutic target for uterine leiomyomas.

J01.86 Epimutation of RB1 gene promoter is accompanied by hypermethylation of repeated genome sequences in human miscarriages with aneuploidy

High level of chromosomal mosaicism is observed during global epigenetic reprogramming in early human embryogenesis. Previously, we have shown a high incidence of epimutations at RB1 gene (16.4 ± 10–2) in placental tissues of aneuploid embryos with mosaicism. Moreover, hypermethylation of RB1 promoter was associated with smaller size of aneuploid clone. It was suggested that epimutations of RB1 are signs of global disturbance of epigenetic landscape in aneuploid placenta. To test this assumption we sequenced level of retrotransposon LINE-1 promoter DNA methylation in the extraembryonic mesoderm and the cytotrophoblast cells of embryos with complete and mosaic aneuploid karyotype with (n = 14) and without (n = 20) RB1 promoter epimutation, euploid miscarriages (n = 17) and induced abortions with normal karyotype (n = 19). There were no differences of LINE-1 methylation between extraembryonic mesoderm and cytotrophoblast cells (p > 0.05). We found an increased LINE-1 promoter DNA methylation index of LINE-1 was significantly higher in group of mosaic aneuploid embryos with RB1 epimutation (58%) in comparison with complete and mosaic aneuploid embryos without RB1 epimutation (53%), euploid miscarriages (42%), and induced abortions with normal karyotype (52.5%) (p < 0.01). Thus, for the first time we have shown that epimutation of RB1 gene promoter is accompanied by hypermethylation of repeated genome sequences in first trimester miscarriages with aneuploidy. This study was supported by Russian Foundation for Basic Research, grant 14-04-01003.

J01.87 The evaluation of adiponectin and its receptors (Adipor1 and Adipor2) genes expression in rat polycystic ovary syndrome models
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Background: Strong association between hypoadiponectinaemia and the occurrence of polycystic ovary syndrome (PCOS) suggests a pathogenic role for adiponectin. We aimed to evaluate the expression of the adiponectin and its receptors (Adipor1 and Adipor2) genes in immature and mature PCOS rat model that were exposed prematurely to androgen excess.

Methods: Four pregnant Wistar rats in the experimental group were treated with subcutaneous injection of 5 mg free testosterone on day 20 of pregnancy while the controls (n = 4) received only 500 ml of solvent. Female pups (14 cases and 18 controls) of each mother were randomly divided into 3 groups and sacrificed at 3 stages of life: day 0 (new born, n = 10), day 10 (10-day-old, n = 10), and day 75-85 (adult, n = 12). RNAs were extracted from ovarian tissues and complementary DNA expression levels for adiponectin and its receptors genes were measured using TaqMan Real-Time PCR.

Results: The expression levels of investigated genes were not significantly elevated in newborns (Adiponecin: 1.119, Adipor1: 1.559, Adipor2: 1.112 fold), while a significant decrease was detected in 10-day-old rats (Adiponecin 0.292, Adipor1 0.26, and Adipor2 0.161 fold (p≤0.05)). We also observed a marginally significant increase in adiponectin gene expression at puberty (2.669 fold, p=0.007).

Conclusion: The results of this study showed that the expression of adiponectin and its receptor genes is changed in prenatally androgenized rats. These changes may alter the normal expression of steroidogenesis regulatory genes and consequently impair the normal development of ovaries and follicles.

J01.88 The Role of Early Development in Intra-individual Genetic Variation of Normal Human Fetuses
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Epimutation of RB1 gene promoter is accompanied by hypermethylation of repeated genome sequences in human miscarriages with aneuploidy. This study was supported by Russian Foundation for Basic Research, grant 14-04-01003.
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Age-related events is one of origin of midlife copy number variations (CNVs) between tissues in non-genetic diseases; however mosaicism is prevalent in preimplantation stage and miscarriages. We aim exploring fetal mosaicism and its origins in apparently normal fetuses aborted due to maternal indications.

DNAs from 22 tissues of each fetus were studied by array Comparative Genomic Hybridization (array CGH) using 195,000 probe slides in simple loops separately designed for each of two studied fetuses. Reciprocal CNVs as high confidence CNVs validated by qPCR. Functional analysis was performed by Gene Ontology (GO).

About 60 CNVs was observed in each fetus. The frequency of reciprocal CNVs varied from 2 to 18. Analysis of CNVs by array CGH and qPCR showed that changes were not mostly integer multiples. Some of CNVs were shared between both fetuses, some were found in the same tissues and some in different tissues. GO showed that altered genes are mostly involved in embryonic development. Tissues clustering according to CNVs revealed those from the same embryonic origin in some cases are close together in a cluster; however, there were large disagreements with clustering of embryonic layers derivatives.

According to distribution pattern of frequent CNVs their origin should be early development, some preimplantation and some postimplantation. CNVs with low frequency seem to be occurred in later stages. Each organ inherits CNVs with a unique pattern regarding extensive cell mixing/migration in embryonic development. Shared CNVs between fetuses are mostly known hotspots; those occurred in same tissues might have functional role.

J01.89 Prenatal detection of fetal aneuploidy on the Ion Torrent Proton Platform

Noninvasive prenatal testing (NIPT) for fetal aneuploidy detection via massively parallel sequencing has been successfully implemented in a number of high throughput clinical laboratories. Automation and parallelization of the complex workflow has reduced turnaround time and labor while maintaining high sensitivity and specificity. As these developments have improved workflow efficiency, the availability of new sequencing platforms on the market has introduced additional flexibility in implementation. Platform flexibility should encourage competitive pricing, foster innovation and ultimately, improve patient satisfaction.

We examined the performance of the MaterniT21™ assay using the Ion Torrent™ Proton Sequencer (Life Technologies, San Diego California). One hundred and fifty-four patient samples, including sixteen from women carrying a known trisomy 21 fetus, as determined by fetal karyotyping, were analyzed. Libraries were prepared and sequenced according to manufacturer’s recommendations. Sequenced reads were aligned, filtered, and quality scored according to manufacturer recommendations. Robust statistics were then applied to identify positive samples with a z-score greater than 3. Fetal aneuploidy status was correctly determined for 154/154 pregnant females, including 16 carrying a T21 fetus. Though the current Proton workflow requires more labor than is optimal for a production environment, significant improvements in that respect are anticipated in the launch of the Ion Chef platform. The sequencing time was brief < 3 hours and data analysis consistent with standard platforms. In summary, the performance of the MaterniT21™ assay on the Ion Torrent Proton platform in this large nonselected patient cohort shows that the existence of a product line using the Ion Torrent platform enables the performance of prenatal screening for fetal aneuploidy.

J01.90 DNA methylation and demethylation patterns in human spermatogenesis
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We studied the distribution of 5-methylcytosine (5mC) and 5-hydroxymethylcytosine (5hmC) in human spermatogenic cells from testicular biopsy and ejaculate samples. Among analyzed dividing cells several types were detected: mitotic diploid and polyplody spermatogonia and meiotic spermatocytes at the pachytene, diplotene and diakinesis stages. Chromosomes were identified by QFHR/AdC staining. Cytosine modifications were detected by indirect immunofluorescence.

The distribution of 5mC in mitotic chromosomes from spermatogonia was band-specific: R-bands and pericentromeric heterochromatin of chromosones 1, 9 and 16 were enriched in 5mC. Pachytene chromosome abnormalities showed less obvious 5mC-banding, with the most intensive DNA methylation in the peritelomeric regions. Diploëute and diakinesal cells demonstrated high, but almost homogeneous DNA methylation with increased intensity of signal in chiasmata and heterochromatic regions. Mature spermatid contained 5mC.

5hmC was completely absent in mitotic and meiotic chromosomes of spermatogenic cells. Hydroxymethylation was identified in 8.8% of post-meiotic (haploid) spermatid nuclei and in up to 13.89% of spermatocytes, suggesting that they are actively demethylated.

Thus, mitotic chromosomes from spermatogonia and meiotic chromosomes from spermatocytes demonstrate band-specific methylation patterns, but lack 5hmC. The presence of 5hmC in some post-meiotic spermatids and spermatocytes suggests that genome of these cells undergoes active DNA demethylation.

Supported by RFBR, Administration of St.Petersburg, OPTEC grant and stipend from RF President.

J01.91 Elucidation the chromosomal aberration impact on ovarian reserve: A retrospective clinical report
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Constitutional chromosome abnormalities are among the major contributors to the genetic causes of reproductive disorders. Despite all of worldwide efforts have been made so far, prognosis for mosaic X chromosome aberration below 30% of aneuploidy has yet to be established. The purpose of this study was to assess the quantity and quality of chromosomal aberrations that may negatively affect ovarian health causing premature ovarian failure (POF) and diminished ovarian reserve (DOR). In this retrospective observational study of clinical features and biological parameters was performed. A total of 531 individuals who were referred to our ward from 2007 to 2014 because of amenorrhea and poor responders to gonadotropin stimulation. High resolution chromosome analysis by GTG banding was carried out on peripheral blood lymphocytes cultures. Supplementary tests were also performed when required. Of the 531 cases who were assessed for chromosomal defects, 52 showed abnormal karyotype. 22 cases were found to have cell lines with different level of X chromosome variation.

Seven cases who were sex reverse sex determining region Y (SRY) negative, nine cases with abnormal X chromosomes, three cases with structurally abnormal autosomes and four individuals carrying X-autosome translocation were diagnosed. The overall prevalence of chromosomal abnormalities was 9.8% which 2.1% of it belongs to normal variable chromosome features. The frequencies of chromosomal alterations were 5% and 1.7% in POF and DOR females, respectively. The results confirm previous observations and emphasis on the critical role of chromosome abnormalities as one of the possible etiologies for ovarian follicular attrition.

J01.92 Unique case of fertility in SRY-positive female
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We report a unique case of fertility in 29-year-old female with cryptic Y chromosome mosaicism. At the birth the patient presented female genitalia with clitoral hypertrophy, which was corrected surgically. Moderate virilization of female genitalia and high level of 17-OHP allowed to diagnose of congenital adrenal hyperplasia resulting from 21-hydroxylase deficiency. DNA analysis found heterozygous CYP21B V281L mutation. From newborn to present time the patient is under the supervision and treatment by an endocrinologist. Menstrual cycle appeared at the age of 13 years after the normal stimulation. At the age of 23 years she married and after 4 months of unprotected sexual intercourse pregnancy appeared naturally. Pregnancy ended in a birth of a healthy boy by caesarean section at 38 months. Subsequently, the patient was observed by a gynecologist because of ovarian cysts, and surgical laparoscopy with biopsy was performed. Hystology of right gonad showed dysgerminoma. On this occasion, the patient was admitted at cytogenetic and molecular-genetic examinations. Chromosome analysis of cultured lymphocytes showed mosaic 46.XX[47]/45.X[2]/46.XY[1]
karyotype. FISH analysis with CEPX and CEPY probes on peripheral blood lymphocytes and buccal cells confirmed complex sex chromosome mosaicism. Following cell clones were found in lymphocytes and buccal cells, respectively: 45,X (5% and 0%), 46,XX (94% and 75%), and 46,XY (1% and 25%). SRY is found in mosaic clones for chromosomes 13, 18, 21, X and Y, and multiplex PCR for 18 Yq STSs confirmed minor Y chromosome mosaicism with a presence of SRY, ZFY and AMELY loci, and not detected a chimeraism and Y-microdeletions.

J02.01

Angiotensin-converting enzyme (ACE) I/D and alpha-adducin (ADD1) G460W gene polymorphisms in Turkish patients with tinnitus

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Objective: Tinnitus is the perception of experience of sound in the head or ears in the absence of external source. A number of factor influences tinnitus like hearing loss, age, noise exposure and hypertension. Angiotensin-converting enzyme (ACE) insertion/deletion (I/D) and alpha-adducin (ADD1) G460W polymorphisms have been associated to hypertension previously and this polymorphisms may be related to tinnitus. Therefore we aimed to investigate the relationship between tinnitus and angiotensin-converting enzyme (ACE) I/D and alpha-adducin (ADD1) G460W gene polymorphisms.

Methods: The patient group was composed of 89 individuals and the control group was composed of 104 individuals. ACE I/D polymorphism was carried out using polymerase chain reaction (PCR) method and ADD1 G460W gene polymorphism was analyzed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique. Results: ACE I/D polymorphism did not show any difference between the patient group and the controls. There was a significant difference in genotype (p<0.01) and allele frequencies (p=0.0212) of ADD1 G460W polymorphism between patient group with tinnitus and controls. The odds ratio for the (GW) genotype was 2.56, 95% CI=(1.39-4.71) (p<0.01). Conclusion: Our results demonstrated for the first time an association between ADD1 G460W gene polymorphism and susceptibility to tinnitus. ADD1 G460W polymorphism may play an important role in the pathophysiology of tinnitus.

J02.02

A novel mutation of SGK1 gene in central serous chorioretinopathy

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Purpose: Central serous chorioretinopathy (CSR) is a mystery characterized by leakage of fluid under the retina that has a propensity to accumulate under the central macula. SGK1 which has an important role in many epithelial ion transport system may have a role in retinal pigment epithelial pump function whose disturbance is one of the main mechanisms in development of CSR. The aim of this study is to investigate whether SGK1 gene variants and this polymorphisms may be related to CSR.

Materials and methods: We enrolled patients who were diagnosed with chronic CSR (n=32) and unrelated individuals as a control group (n=32). For DNA extraction and PCR amplification followed standard methodologies. SGK1 gene was sequenced using BigDye® Terminator v3.1 chemistry. Results: We identified a novel mutation M32V (2/32) in the patient group, (6, 25% and AF=0.031) rs1057293 (p.068) is located in the encoder region of the SGK1 gene but not associated with CSR. An intrinsic rs1473996 (p.028) is also not associated. We have also identified 3 more intrinsic mutations: 14395C>A (1/32; AF=0.015), 14717A>T (1/32; AF=0.015) and 14572S del TTAC1 (1/32; AF=0.015).

Discussion: M32V is located on the region of 1-60 amino acids which is necessary for localization to the mitochondria. This mutation is probably important for the energy metabolism and plays an important role in the cellular response to hyperosmotic stress and other stress stimuli. Both rs1057293(p.068) and rs1473996(p.028) are not associated with CSR. Probably these 7 snp are promising but it’s difficult to conclude the association between these intrinsic mutations; 14395C>A, 14717A>T, 14572S del TTAC1 and CSR.

J02.03

The occurrence of the rs61749246, the c.*2G>T of the FZD4 gene, in Slovak patients with retinopathy of prematurity

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Retinopathy of prematurity (ROP) is a complex disease affecting the development of retinal vasculature in premature infants. The International classification of ROP divides the development of the disorder into 5 stages. The environmental factors can influence the development of ROP, so the appropriate care may decrease its incidence. However, a genetic predisposition to ROP is suggested. Recently, mutations in genes FZD4, LRPS, TSPAN12, and NPD were identified in 3 to 12% of the cases with ROP. Nowadays, our cohort consists of 11 premature newborns with different stages of ROP (stage 1-3) treated at the Clinic of Neonatology in Martin. We performed the sequence analysis of the coding exons of the three genes - FZD4, TSPAN12 and NPD and an identified rs61749246 (c.*2G>T of the FZD4 gene) in 3 patients with ROP. Two were heterozygous in stage 1, and one was homozygous in stage 2 (T allele frequency 0.18). The control group (n=50) of premature newborns without ROP contains two heterozygotes for rs61749246 (T allele frequency 0.02). The rs61749246 (MAF = 0.008 in dSNP) is the polymorphism of the second G after the TAA stop codon of the FZD4 gene. It has been shown, that there are preferred nucleotides around the stop codon necessary for efficient translational termination. We suggest that this SNP is involved in the pathogenesis of ROP, probably based on gene expression changes during the eye development. We will enlarge our cohort focusing on higher stages of ROP and develop in vitro translation assay for this SNP.

J02.04

Case report of a patient with sensorineural hearing loss due to compound heterozygosity of 35delG and G200R mutations in gene GJB2

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Hearing loss is the most common birth defect and the most prevalent sensorineural disorder and affects about one in 1,000 neonates. More than half of prelingual deafness cases are due to genetic factors. About 70% of all hereditary hearing loss cases are classified as nonsyndromic and recessive. Mutations in the genes GJB2 and GJB6, that are located in the locus DFNB1, contain two heterozygotes for rs61749246 (T allele frequency 0.02). The rs61749246 (MAF = 0.008 in dSNP) is the polymorphism of the second G after the TAA stop codon of the FZD4 gene. It has been shown, that there are preferred nucleotides around the stop codon necessary for efficient translational termination. We suggest that this SNP is involved in the pathogenesis of ROP, probably based on gene expression changes during the eye development. We will enlarge our cohort focusing on higher stages of ROP and develop in vitro translation assay for this SNP.
analyzed the coding sequence of the gene GJB6 and selected regions of genes MT-RNR1 and MT-TS1 of mitochondrial genome of 321 Slovak patients with non-syndromic hearing loss. In this group, not more than one mutation in the gene GJB6 was detected per person. In the gene GJB6 were detected three cases of polymorphisms and two different frameshift mutations in heterozygote state. A 309 kb deletion in this gene was detected in one patient. Presence of the 309 kb deletion in the gene GJB6 was detected in Slovak population for the first time. In the gene MT-RNR1 was detected one type of pathogenic mutation (A1555G) and four types of potential pathogenic mutations. No pathogenic mutations in the gene MT-TS1 were detected.

J02.06 Genetic background of hearing loss among group polish CI patients A. Pollak1, U. Lachowicz1, A. Podgorska1, M. Mueller-Malesinska1, M. Olak2, L. Korniszewska1, H. Szarzynski1, R. Płuski1; 1World Hearing Center, Institute of Physiology and Pathology of Hearing, Katowice/ Warsaw, Poland, 2Department of Histology and Embryology, Medical University of Warsaw, Warsaw, Poland, 3Department of Medical Genetics, Medical University of Warsaw, Warsaw, Poland.

Hearing loss (HL) is a significant medical problem in Poland and worldwide. The cause of hearing loss can be genetic or environmental. Currently the background of genetic hearing impairment is an area of intensive research conducted by many groups. Recently worldwide intensive studies are conducted to clarify the genetic basis of hearing loss. To date, more than 60 non-syndromic deafness genes and more than 1000 deafness-causing mutations have been described. The most common variants responsible for an isolated HL with recessive type of inheritance are mutations in the GJB2 gene (in particular the deletion of guanine at position 35 (35delG)) and therefore the search for genetic basis of hearing loss for diagnostic purposes usually includes only analysis of GJB2 gene, whereas mutations in each of the remaining genes associated with the process of hearing which can also cause hearing loss are not investigated.

According to the preliminary functional analysis, pathologic changes (caused by mutations of GJB2 and GJB6 genes) did not comprise spiral ganglion cells, which are crucial for the results of cochlear implantation. The aim of our study was to estimate the prevalence of genetically related HL among patients with cochlear implants (CI). We have analyzed 1218 patients diagnosed with congenital hearing loss who received CI. Search for mutations was performed by various molecular methods. Our results show that genetic defects of various genes are the most common reason of HL among patients with cochlear implants.

J02.07 KCNQ4 mutation spectrum in Slovak patients with non-syndromic hearing loss K. Ottonellova1, M. Pecimova1, A. Saltysova1, A. Ficek1, L. Kadaš1; 1Faculty of Natural Sciences, Department of Molecular Biology, Bratislava, Slovakia.

Non-syndromic deafness is one of the most common sensory impairment in humans. Most forms of non-syndromic deafness are associated with permanent hearing loss caused by damage to structures in the inner ear. The severity of hearing loss varies, can change over time and can occur at any age. The cause of non-syndromic deafness is complex, with more than hundred genes so far identified; however, some of these genes have not been fully characterized. Different mutations in the same gene can be associated with different types of hearing loss. All this aspects contribute to the complexity of the disease and markedly hamper DNA diagnostics.

In this study we focused on gene KCNQ4, whose mutations lead to DFNA2, a subtype of autosomal dominant non-syndromic deafness that is characterized by progressive sensor-neural hearing loss across all frequencies. The KCNQ4 gene encodes protein called potassium voltage-gated channel KV7.4; its function is to facilitate the inactivation of Kv7.4, a member of a family of potassium channel proteins. In our work we analyzed 324 NSHL patients without mutations in GJB2 gene, which is the most prevalent disease gene. Up to now we found one new deletion in gene KCNQ4 in exon 10 that causes frame shift, some pathologic mutations and also several frequent polymorphisms.

J02.08 Mutational analysis of MIR184 in Iranian Keratoconus patients A. Farzadfar1, E. Elahi1, N. Nassiri1, H. Paylakhi1; 1School of Biology, College of Sciences, University of Tehran, Tehran, Islamic Republic of Iran, 2Shahid Beheshti University of Medical Sciences, Tehran, Islamic Republic of Iran, 3School of Biology, Damghan University, Damghan, Islamic Republic of Iran.

Keratoconus is a noninflammatory corneal thinning disorder and the major cause of cornea transplantations in the Western countries. Despite intensive biochemical and genetic investigations, its underlying cause(s) remains poorly understood. It has been suggested that mutations in VSX1 and SOD1 may contribute to disease presentation in some cases. Linkage analysis in an affected Irish pedigree ultimately in 2011 led to identification of a mutation in the seed region of MIR184 (+57C>T) as the putative cause of keratoconus in this pedigree. In a subsequent screening of 790 patients of European ancestry, two novel causative mutations (+3A>G, +8C>A) in MIR184 were identified. Notably, mutations in MIR184 have recently been reported in two pedigrees, each affected with ocular diseases that affect the cornea. No pathogenic variants in MIR184 were found in 47 unrelated Iranians affected with keratoconus by direct Sanger sequencing. Only one variant allele (+39G>T; rs41280052) was observed in one patient. The same variation has previously been observed in control and keratoconus affected individuals at similar frequencies, suggesting that it is not a cause of keratoconus. Although the sample size was small, it is evident that mutations in MIR184 are not a common cause of keratoconus among Iranian patients. They were not observed among the 94 chromosomes of the patients screened.

J02.09 Comprehensive analysis of keratoconus genetic factors D. M. Nowak1, J. A. Karolak1, M. M. Kubicka1, M. Gajecka2; 1Department of Genetics and Pharmaceutical Microbiology, Faculty of Pharmacy, Poznan University of Medical Sciences, Poznan, Poland, 2Institute of Human Genetics, Polish Academy of Sciences, Poznan, Poland.

Keratoconus (KTCN) is a thinning and anterior protrusion of the cornea resulting in altered refractive powers, and loss of visual acuity. KTCN is a multifactorial disorder in which both environmental and genetic factors are involved. Among the environmental factors, frequent eye rubbing and contact lenses wearing are mentioned. Family form of the disease and the coexistence with other genetic disorders indicate genetic factor involvement. Genetic studies have led to the identification of several loci on different chromosomes, linked to KTCN. However, only few reports indicated causative genes in these loci. Such examples are Dock9, and MIR184. For most of the remaining KTCN loci, single genes were analyzed. Additionally, majority of previous reports concentrated on sequences variants in exons only. This leaves a gap in the studies of KTCN genetics.

The aim of our project was to analyze the DNA sequence information available in the databases of SNVs located within known KTCN loci. Previous studies in KTCN focused on protein-coding sequences. We extended the analyzes to include miRNA genes located in analyzed loci to investigate another genetic factor toward assessment of KTCN complexity. Additionally, sequencing of mitochondrial genome in KTCN patients from Polish population was performed and the data was incorporated into genetic analysis. The KTCN development does not depend on a single change in the gene, but on the accumulation of numerous sequence variants. The complexity of KTCN etiology caused the need to find appropriate approach to investigate this disease.

Support: National Science Centre, Poland, grant no. 2011/03/N/NS5/01470.

J02.10 Megalocornea should be suspected in cases with hypotonia, mental retardation and macrocephaly: neuhauser syndrome an easily missed diagnosis T. Atki1, S. Atki1, O. Gogu1, F. Orhing1; 1Ege University Faculty of Medicine, Izmir, Turkey, 2Kutup Celebi University, Izmir, Turkey.

Neuhauß syndrome, also known as Megalocornea-mental retardation syndrome (MMR), is a very rare autosomal recessive disorder. It can be diagnosed via clinical features: megalocornea, developmental delay, hypotonia and some dysmorphological signs. Genetic etiology is still unknown. About 40 cases have been reported in the literature. Here we report a further MR case. Proband is a 13 month-old girl. Because of macrocephaly, motor and mental retardation, hypotonia and facial dysmorphic features, she was referred to pediatric genetics subdivision for genetic counseling. She was the fifth child of nonconsanguineous parents from small village and born at 35 week of gestation. In the newborn period she was hospitalized for prematurity. At 9 and 11 month of age, she was rehospitalized because of feeding problems and bronchopneumonia. On admission, her weight was 6,6kg(<3centile), height 76cm(25-50centile) and head circumference 46cm(97centile). Macrocerebrum and broad forehead, hypertelorism, megalocornea, strabismus, low set ears, short columella and long philtrum were present. Corneal diameters were more than 13.5mm bilaterally and intraocular pressures were normal. Laboratory tests revealed hypogammaglobulinemia. Echocardiography showed secundum ASD (4mm). On MRI, bilateral cerebral atrophy and thin corpus callosum were observed. Karyotype and subdeltomic FISH were normal. This case is one of the few MMR cases having immunodeficiency.
The aim of this study was to investigate an association of the VDR (vitamin D receptor) A-3731G gene polymorphism with the occurrence of axial myopia in children. We examined 53 girls and 24 boys aged 4-17 yr from Russia. Survival analysis: 19(38 eyes) with high myopia (D>6 D). We examined 44(88) eyes with medium myopia, and 14(28 eyes) with emmetropia. The gene polymorphism was identified with PCR-RFLP. We have found a higher proportion of carriers of VDR A(-3731) allele in the medium myopia group compared to the emmetropia group (32% and 7%, respectively, OR=4.45, 95%CI 1.13-17.53, p=0.016). It is known that the variation of the A(-3731) allele frequency is significant between various populations, being the highest one in Africans (about 74%) and the lowest one in Caucasians (about 19%). The A(-3731) allele is an ancestral while the allele G(-3731) is mutant. Null hypothesis for explaining the mutant allele frequency raise in Caucasians is that this is a result of a random selection, for example, genetic drift during the period of human migration out of Africa. Alternative hypothesis is that the difference in the polymorphism variation is not random, being the result of natural selection. The latter may be true if the G(-3731) allele gives some benefits for survival outside Africa. We hypothesize that the vitamin D receptor is a key regulator of the eye growth, and G(-3731) allele gives more benefits in preventing against myopic eye growth. The increase in G(-3731) allele rates in non-African populations might be explained by changing of ultraviolet radiation intensity.

Ophthalmologic status of patients with Waardenburg syndrome

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Waardenburg syndrome (WS) is a genetically heterogeneous syndrome characterized by pigmentary abnormalities and congenital sensorineural hearing loss. WS has been classified into 4 main phenotypes: WS1 (OMIM193500) with dystopia canthorum, WS2 (OMIM193510) without dystopia canthorum, WS3 (OMIM148820) with dystopia canthorum and upper perimembranous abnormalities, WS4 (Waardenburg-Shah syndrome, OMIM277580), with the additional feature of Hirschsprung disease. WS1 and WS3 are both characterized by pigmentary abnormalities and congenital sensorineural deafness. WS1 and WS3 have been classified as a result of mutations in the PAX3 and PAX6 genes, respectively. WS2 has been characterized by pigmentary abnormalities and congenital sensorineural deafness. Given that no genetic changes have been described in the PAX3 and PAX6 genes in patients with WS2, it is possible that additional genes might be involved in the pathogenesis of this syndrome. WS1 and WS3 are both caused by mutation in the PAX3 gene. The overall incidence is 1/42,000 to 1/30,000. The incidence of WS1 is 1/30,000 and of WS3 is 1/12,000 to 1/15,000. The incidence is higher in Asians and in those with a family history of the disease. WS has been classified into 4 main phenotypes: WS1, WS2, WS3, and WS4. WS1 is characterized by pigmentary abnormalities and congenital sensorineural deafness. WS2 is characterized by pigmentary abnormalities and congenital sensorineural deafness. WS3 is characterized by pigmentary abnormalities and congenital sensorineural deafness. WS4 is characterized by pigmentary abnormalities and congenital sensorineural deafness. The genetics of WS has been studied extensively, and several genes have been implicated in the pathogenesis of this syndrome. The most common cause of autosomal recessive deafness is a deletion of the PAX6 gene on chromosome 11p13. Other genes that have been implicated in the pathogenesis of WS include the SOX10, MITF, and POU3F4 genes. Mutations in these genes are found in a significant number of patients with WS, and they are thought to play a role in the development of the inner ear. The overall incidence of WS is 1/42,000 to 1/30,000. The incidence of WS1 is 1/30,000 and of WS3 is 1/12,000 to 1/15,000. The incidence is higher in Asians and in those with a family history of the disease. WS has been classified into 4 main phenotypes: WS1, WS2, WS3, and WS4. WS1 is characterized by pigmentary abnormalities and congenital sensorineural deafness. WS2 is characterized by pigmentary abnormalities and congenital sensorineural deafness. WS3 is characterized by pigmentary abnormalities and congenital sensorineural deafness. WS4 is characterized by pigmentary abnormalities and congenital sensorineural deafness. 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A novel mutation of the USH2C (GPR98) gene in an Iranian family with Usher syndrome Type II

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Objective: Usher syndrome (USH) is an autosomal recessive disorder illustrated with retinitis pigmentosa (RP) and sensorineural hearing loss with or without variable vestibular dysfunction. USH is genetically and clinically heterogeneous. At least fifteen loci and eleven genes identified in USH. USH syndromes are divided into three subtypes: (i) type I (USH1); type II (USH2); and type III (USH3). USH2 is the most common form of Usher syndrome, responsible for moderate to severe hearing deficits, retinitis pigmentosa (RP) and sensorineural hearing loss with or without variable vestibular dysfunction. At least fifteen loci and eleven genes identified in USH. USH syndromes are divided into three subtypes: Type II and Type III. Type II with USH2C (GPR98) and Type III with USH2D (WHRN).

Methods: After performing homozygosity mapping using microsatellite (STR) markers, we approached whole exome sequencing (WES) to detect the disease-causing mutation of homozygous regions. To confirm the identified homozygote variant in GPR98, sanger sequencing was performed in all family members. Consequently we sequenced 100 normal control to ensure detected variant would not be a polymorphism. Results: We identified a new mutation in GPR98 segregating with USH2C in this family. This missense mutation c.10019T>G leading to R3340G, has been reported for USH2C in Iranian population by our group. To the best of our knowledge, this is the first report of a genetically confirmed case of USH2C using WES in Iran.

The analysis of the GJA3 gene in patients with hereditary congenital cataract from Bashkortostan Republic (Russia)

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Introduction: Cataracts are one of the leading causes of blindness in humans, and in mutations in the connexin 46 (GJA3) and the connexin 50 (GJA8) genes cause congenital cataract. Different mutations in these genes lead to the development of distinct cataract phenotypes. The aim of the study was to analyze the GJA3 gene in patients from Bashkortostan Republic affected with congenital cataract.

Objective: DNA samples of 40 unrelated patients with isolated form of hereditary congenital cataract from Bashkortostan Republic were analyzed.

Methodology: The analysis was performed by direct sequencing of coding region of GJA3 gene.

Results: Three different nucleotide alterations were detected. In one patient of Tatar ethnic origin with zoonal form of cataract deletion c.del11126,1139 was detected; one patient of Tatar ethnic origin with zoonal cataract and microcornea carried the missense mutation c.398G>A (p.Arg133Gln). Both alterations were found in the heterozygous state, had not been described in literature before, and presumably are functionally significant mutations. In one patient new nucleotide substitution c.231C>T (Phe77Phe) in the heterogeneous state was found.

Conclusion: Thus, three previously undescribed structural changes in the gene GJA3 were detected in hereditary congenital cataract patients from Bashkortostan Republic. To determine their functional significance further investigation are required. The study was supported by RFBR grant (14-04-97017 _p-povolgie_a).

Mutation screening in autosomal dominant retinitis pigmentosa family using targeted next generation sequencing

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Objective: Targeted NGS of 4,913 genes associated with known clinical phenotypes was performed on APEX-negative adRP-patient using the TruSightOne Sequencing Panel and MiSeq system of Illumina. Illumina VariantStudio software was used to filter single nucleotide variants (SNVs) and insertions/deletions (indels).

Results: We identified 9,339 SNVs and indels and after applying filtering criteria the numbers of remaining variants (in parentheses) were as follows: (i) exclude homozygous SNVs (5,857); (ii) exclude minor variant frequency <10% (5,846); (iii) keep nonsynonymous changes and splice variants (2,666); (iv) keep if same variant presents in <1% of the general population (608); (v) exclude dbsNP with minor allele frequency >1% (574); (vi) exclude if allele is present in at least one of 6,500 individuals of the Ethnic Variant Server database (527); (vii) disease phenotype consistent with autosomal dominant retinal degeneration (11). Additional exclusion criteria including lack of segregation of the mutant allele within the affected family members will be applied.

Conclusion: Our results suggest that new adRP-locus exists since no pathogenic changes were found in known adRP-genes.

Deciphering the Genetic Basis of Hearing Impairment in Iran: An Ethnic based Survey

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Hearing loss is the most common sensory disorder worldwide which affects 1 of every 500 newborns. At least 50% of cases can be attributed to genetics, most often resulting in nonsyndromic deafness (70%), which is usually autosomal recessive (80%). Despite the heterogeneity, mutations in GJB2 at DFNB1 locus are the major cause of autosomal recessive nonsyndromic hearing loss (ArnSHL) in many populations, including Iran (20%). The fact that many loci are involved together with the heterogeneity of the status, necessitate studying further loci in various Iranian ethnic groups. In this study, after mutation screening of GJB2 and GJB6, we used homozygosity mapping to identify regions of autosomal-by-descent in 23 large pedigrees all originating from a single province of Iran (South Khosans), using STR markers for STR markers for 7 loci.

In our study, four out of the 23 families showed GJB2 mutations. Interestingly heterogeneity within a family were observed, even in large consanguineous pedigrees GJB6 deletions were not detected. One family showed linkage to DFNB3 and one family showed to be linked to DFNB7/11. The remaining did not show linkage to the studied loci.

Our results once again emphasize the heterogeneity of HL among different Iranian ethnic groups. These results could provide further insight into the etiology of HL and may lead to better genetic diagnostics & counseling.

Targeted next-generation sequencing for identification of ABCA4 gene mutations in Polish patients with retinal dystrophies

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The ABCA4 gene is one of the most important genes of the human retina. It encodes an ATP binding cassette (ABC) transporter that is expressed almost exclusively within the retina. Mutations in the ABCA4 gene cause a wide range of retinal degenerations. Due to the advent of therapeutic options, identification of a genetic cause of retinal diseases is becoming increasingly important. The aim of the project was to implement next-generation sequencing (NGS) for identification of ABCA4 gene mutations in a group of Polish patients with Stargardt disease, fundus flavimaculatus or cone-rod dystrophy. Genomic DNA isolated from peripheral blood of 58 patients served as a template. The introduced variant of NGS is based on the preparation of an amplicon library containing all coding sequences of the ABCA4 gene (50 exons). Next, the amplicons were sequenced using the genomic sequencer GS Junior System (Roche). Presence of allelic variants identified by NGS was confirmed by Sanger DNA Sequencing. To predict possible functional consequences of the identified missense variants, two different computational methods, i.e. SIFT and PolyPhen-2 were used. In the studied group of patients, 32 different known mutations and 20 different novel potentially pathogenic variants were found. Our study enabled identification of a genetic cause of retinal di-
sease in 88% of patients. Three patients (5%) did not carry any mutation in the ABCA4 gene and in four patients (7%) only one mutation was found. Patients with an unknown cause of the retinal disease will be examined using whole genome sequencing.

J03.01
Predictive value of alpha-1 antitrypsin level for Z mutation detection in chronic obstructive pulmonary disease
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Alpha-1 antitrypsin (AAT) deficiency is an under-diagnosed condition in patients with chronic obstructive pulmonary disease (COPD). The aim of our study was to evaluate predictive value of quantitative methods of alpha-1 antitrypsin for Z mutation detection in patients with chronic obstructive pulmonary disease. Ninety-one AAT deficiency genotypes (40 MZ, 39 MS, 1 SS, 3 SZ and 8 ZZ) were analysed. Calculated sensitivity of quantitative alpha-1 antitrypsin measurement by nephelometry for heterozygous Pi‘Z allele was 45% and for homozygous ZZ genotype - 88%. Specificity of quantitative alpha-1 antitrypsin analysis for heterozygous deficiency was 98% and for homozygous deficiency - 100%. Thus sensitivity of quantitative alpha-1 antitrypsin analysis is higher than specificity for both - heterozygous and homozygous deficiency.

The results of the present study support the general concept of targeted screening for AAT deficiency with adequate laboratory methods in European countries with Pi‘Z high frequency and large population of COPD patients with highest diagnostic value - AAT genotyping. A case detection programme of alpha-1 antitrypsin deficiency in patients with chronic obstructive pulmonary disease using quantitative methods could be used only in screening programs and exact diagnosis must be confirmed by determining AAT genotype.

J03.02
Identification of a novel missense COL4A5 mutation in Russian family with X-linked Alport syndrome by next generation sequencing
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Alport syndrome (AS) is a genetically determined glomerulopathy that is caused by mutations in type IV collagen chain genes COL4A3, COL4A4 or COL4A5 and clinically characterized by hematuria, proteinuria and end-stage kidney disease (ESRD). The syndrome is often combined with sensorineural hearing loss and ocular pathology. The coding exons and splice sites of the abovementioned genes were sequenced in four patients and two healthy controls from the family on Ion Torrent platform. Here we describe a novel dominant missense mutation rs2305480 C>T in COL4A5 gene causing complete nephrogenic diabetes insipidus in Russian population. The problem of pathogenicity assessment and distinguishing between polymorphic variants vs malignant mutations is discussed. Different approaches exist including online prediction tools (e.g., PolyPhen, SIFT) and databases (pubmed, HGMD, LOVD, ClinVar etc.). We compare those tools for variant classification in Alport syndrome and other pathologies.

J03.03
Novel deletion in AVPR2 gene causing complete nephrogenic DI
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Nephrogenic diabetes insipidus (NDI) is characterized by the inability of urine concentration leading to a high risk of dehydration. In this study, we report a novel deletion in AVPR2 gene causing complete NDI confirmed by microarray analysis. Methods: A male patient was admitted due to polyuria and polydipsia. NDI was suspected and molecular studies were performed to investigate these clinical problems. Results: Sequencing result showed a total deletion (11535 bp) in AVPR2 gene (NG_009645.2:g.5303_16835del) on X chromosome accompanied by 7-bp microhomology at the breakpoint. Following microarray analysis reconfirmed this complete deletion of AVPR2 preserving LCAM1 and ARHGAP4 gene. Conclusion: With sequencing and microarray analysis, we found the novel deletion in AVPR2 causing complete NDI. As shown in this study, molecular diagnosis has several advantages compared with conventional in vivo test. First, patients unable to conserve water may become critically dehydrated in water deprivation test. There are several contraindications of this test, especially in other causes of polydipsia and polyuria such as DM, hypoadrenalism, or CRF. Second, molecular studies can give more detailed information that cannot be provided by conventional tests. In this patient, the sole deletion of AVPR2 preserving LCAM1 and ARHGAP4 meant NDI excluding central type. In addition, since AVPR2 is completely deleted, it can only be complete NDI, not partial type. Therefore, this study suggests that genetic testing is a safer and more useful laboratory tool than the physiologic test in diagnosing and subtyping NDI.

J03.04
Gene variant rs2305480 C>T in gsdmerin B gene (GSDMB) and the risk of recurrent wheezing and asthma in Bulgarian infants
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Objective: The gsdmerin B (GSDMB) gene is located at 17q21.2 and recent reports suggest that GSDMB is associated with childhood asthma in several populations. We investigated the association of a SNP in GSDMB (rs2305480 C>T) with recurrent wheezing, severity of bronchial obstruction and family history of asthma and allergy.

Materials and Methods: Family history of asthma and allergy, recurrent wheezing and atopy were assessed in 93 infants admitted to the hospital for broncholiths. All children were genotyped for rs2305480 C>T by PCR RFLP analysis. The presence of AovII restriction site was indicated by C-allele and the absence - by T-allele.

Results: Data were analysed as a recessive genetic model. Genotype frequencies did not deviate significantly from expected under the Hardy-Weinberg Equilibrium - T/T genotype was 18%, C/T - 47% and C/C - 35%. Our data show that the genotype T/T is a possible risk factor for recurrent wheezing (OR 3.68, 95% CI: 0.98-13.76). Children homozygous for T-allele are more likely to have a family history of allergy and asthma (OR 5.41, 95% CI: 1.43-20.47) and early age of first wheezing - 6.88 mo compared to 10.4 mo for C/C, p = 0.02.

Conclusions: Our results support the role of GSDMB SNP (rs2305480 C>T) for determining asthma phenotypes in preschool children. The study was financially supported by research grant (2013), Medical University - Sofia.

J03.05
Novel deletion in AVPR2 gene causing complete nephrogenic DI
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1Kyung Hee University, Seoul, Korea, Republic of; 2The University of Hong Kong, Hong Kong, Hong Kong.

Background: Nephrogenic diabetes insipidus (NDI) is characterized by the inability of urine concentration leading to a high risk of dehydration. In this study, we report a novel deletion in AVPR2 gene causing complete NDI confirmed by microarray analysis. Methods: A male patient was admitted due to polyuria and polydipsia. NDI was suspected and molecular studies were performed to investigate these clinical problems. Results: Sequencing result showed a total deletion (11535 bp) in AVPR2 gene (NG_009645.2:g.5303_16835del) on X chromosome accompanying 7-bp microhomology at the breakpoint. Following microarray analysis reconfirmed this complete deletion of AVPR2 preserving LCAM1 and ARHGAP4 gene.

Conclusion: With sequencing and microarray analysis, we found the novel deletion in AVPR2 causing complete NDI. As shown in this study, molecular diagnosis has several advantages compared with conventional in vivo test. First, patients unable to conserve water may become critically dehydrated in water deprivation test. There are several contraindications of this test, especially in other causes of polydipsia and polyuria such as DM, hypoadrenalism, or CRF. Second, molecular studies can give more detailed information that cannot be provided by conventional tests. In this patient, the sole deletion of AVPR2 preserving LCAM1 and ARHGAP4 meant NDI excluding central type. In addition, since AVPR2 is completely deleted, it can only be complete NDI, not partial type. Therefore, this study suggests that genetic testing is a safer and more useful laboratory tool than the physiologic test in diagnosing and subtyping NDI.

J03.06
Reversibility of bronchiectasis: case report of Kartagener’s syndrome
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Kartagener’s syndrome is an autosomal recessive disorder primarily manifesting as ciliary movement disorder. Kartagener’s syndrome is part of the larger group of disorders referred to as primary ciliary dyskinesias (PCD). Although the condition is usually inherited in an autosomal recessive pattern, and some specific gene defects have been recognized, it is clear that
the syndrome shows substantial genetic heterogeneity. The incidence of this genetic disorder is estimated to be between 1 and 2 per 30,000 births. Symptoms result from defective eIF2 activity in the airways. The recurrent pulmonary infections are caused by the grossly impaired mucociliary transport system. The causative mutation of the NPHS2 gene is located in the donor splice-site of intron 9: c.1228+5 G>A in one SRNS girl with normal karyotype. Although the management of patients with Kartagener's syndrome remains uncertain and evidence is limited, it is important to follow up these patients with an adequate and shared care system. The present clinical case demonstrated reversibility of bronchiectasis even in congenital Kartagener's syndrome, thus indicating, that bronchiectasis progression is a complex interrelationship among genetic variation and a proper nonspecific management.

**J03.07**
The role of HLA typing for celiac disease diagnosis among children with autoimmune disorders

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Background: The association of celiac disease (CD) with several autoimmune diseases is well-known. Objectives: To determine the prevalence of CD among children with autoimmune disorders (AITD) and insulin-dependent diabetes mellitus (IDDM) and to assess the diagnostic role of HLA typing among these patients.

Methods: 74 children with AITD (lot 1), 98 children with IDDM (lot 2) and 80 healthy children were screened for CD. In patients with at least one positive serologic test for CD, intestinal biopsy was performed.

All children underwent HLA typing for DQ2/DQ8. Results: CD prevalence in lot 1 was 7%, in lot 2 was 6% and in control lot was 0%. All children diagnosed with CD were screened for DQ2/DQ8 (100%). The control subjects showed no heterozygous DQ2 alleles. From 69 children with AITD without CD, 2 children showed heterozygous DQ2 alleles. From 92 children with IDDM without CD, 25 patients (27%) presented homo or heterozygous DQ2/DQ8 alleles. There were significantly more cases with IDDM without CD but with predisposing haplotype for CD (27%) compared to the number of patients with AITD seronegative for CD and with DQ2/DQ8 alleles (4%) p < 0.005.

Conclusions: Recommending AITD and IDDM as selection parameters for CD screening in asymptomatic children is justified. HLA assessment cannot highlight a significant role of a certain allele in the pathogenesis of autoimmune comorbidity AITD/CD or IDDM/CD. DQ2 and DQ8 alleles are mandatory but insufficient for CD development. The interaction of environmental factors is very important. Performing as first line DQ8 alleles are mandatory but insufficient for CD development. The interaction of environmental factors is very important. Performing as first line HLA typing for celiac disease diagnosis among children with autoimmune disorders is required. Further studies with a larger number of patients are needed.

Acknowledgements This work was funded by Internal Research Grants of the University of Medicine and Pharmacy Tirgu Mures, Romania, contract no.29/11.12.2013.

**J03.08**
NPHS2 and WT1 mutations in a Romanian children Population with nephrotic syndrome

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Mutations in NPHS2 and WT1 genes are a frequent cause of steroid resistant nephritic syndrome (SRNS) and occur in 10-20% of children while mutations are absent from children with steroid-sensitive nephrotic syndrome (SSNS). The frequency and spectrum of mutations in these genes is unknown for the Romanian population. Material and methods: This study comprised 42 pediatric patients with NS. 50 healthy children were enrolled as a control group. NPHS2 R229Q polymorphisms were determined by the PCR and RFLP technique utilizing specific primers. Mutation analysis was performed in WT1 genes, in case of abnormality the corresponding sample was sequenced. Results: In NS group 85.7% were SSNS and 14.2% were SRNS. NPHS2 mutation was studied in 2 patients with congenital nephrotic syndrome. NPHS2 R229Q gene mutation was found in 1 out of 10 cases with homogenous distribution in the donor splice-site of intron 9: c.1228+5 G>A in one SSNS girl with normal karyotype. Conclusion: The incidence of NPHS2 mutations in children with NS is considerably lower than that among European children (10-30%). WT1 mutation was present in girl with focal segmental glomerulosclerosis. Therefore screening of WT1 gene in all females with FSGS is necessary. Because NPHS2 R229Q gene mutation was found in all cases of CNS, a screening of this gene in children with CNS in the adjacent counties is required. Further studies with a larger number of patients are needed.

**J03.09**
The Polymorphisms in the IREB2, CHRNA5, CHRNA3 and HHIP Genes and Risk of Chronic Obstructive Pulmonary Disease in Russian Population

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Chronic obstructive pulmonary disease (COPD) is a multifactorial inflammatory disease primarily affecting distal respiratory pathways and lung parenchyma. Genome-wide association studies have identified gene variants influencing the risk of COPD. The aim of this study was to investigate whether IREB2, CHRNA5, CHRNA3, FAM13A and HHIP polymorphisms would be associated with COPD susceptibility in Russian populations.

Methods: Six single nucleotide polymorphisms: rs13180 (IREB2), rs16969968 (CHRNA5), rs1051730, rs6495309 (CHRNA3), rs7671167 (FAM13A), rs1318928 (HHIP) were genotyped in a case-control study (511 COPD patients and 508 controls from Russia). To estimate the strength of association, odds ratios were calculated and potential confounding variables were tested by using logistic regression analysis.

Results: Statistical analysis revealed that SNP rs13180 (IREB2) was associated with COPD (P = 0.004). Analysis showed an association of rs16969968 (CHRNA5) (P = 0.003) and rs1051730 (CHRNA3) (P = 0.0015) in additive model and the A-A-G haplotype of rs16969968 (CHRNA5), rs1051730, rs6495309 (CHRNA3) genes polymorphisms (P = 0.0078) with COPD. The relationship between the rs1318928 (HHIP) (P = 0.0017 for AG genotype) and COPD risk was found. Significant association with severe COPD was observed for rs13180 (P = 0.009), rs16969968 (P = 0.0071), rs1051730 (P = 0.0013), rs13118928 (P = 0.003) polymorphisms. Early onset COPD (before 40 yrs) were associated with rs13180 (P = 0.00001), rs16969968 (P = 0.0001), rs1051730 (P = 0.0004). The SNP rs13118928 near HHIP locus was significantly associated with pack-years of smoking in COPD patients (P = 0.029). We demonstrated that rs13180, rs13118928 and rs16969968 polymorphisms were associated with COPD only in smoking subjects. We confirmed that SNPs the IREB2, CHRNA5, CHRNA3 and HHIP loci were associated COPD in Russian Population.

**J03.10**
MTHFR polymorphisms role in diabetic neuropathy

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Diabetes Mellitus (DM), the most important public health problem of the 21st century, leads to significant mortality and morbidity due to underlying complications. G677T and A1298C polymorphisms of MTHFR gene elevates plasma homocysteine levels, reduces plasma folic acid levels, and elevates plasma total cholesterol levels. C677T and A1298C polymorphisms of MTHFR gene are associated with hyperhomocysteinemia which is a risk factor for atherosclerosis. These results were achieved: radiological findings and lung function were improved. The incidence of NPHS2 mutations in romanian children Population with nephrotic syndrome is well-known. Objectives: To determine the prevalence of CD diabetes mellitus(IDDM) and to asses the diagnostic role of HLA typing among these patients.

Methods: 74 children with AITD (lot 1), 98 children with IDDM (lot 2) and 80 healthy children were screened for CD. In patients with at least one positive serologic test for CD, intestinal biopsy was performed.

All children underwent HLA typing for DQ2/DQ8. Results: CD prevalence in lot 1 was 7%, in lot 2 was 6% and in control lot was 0%. All children diagnosed with CD were screened for DQ2/DQ8 (100%). The control subjects showed no heterozygous DQ2 alleles. From 69 children with AITD without CD, 2 children showed heterozygous DQ2 alleles. From 92 children with IDDM without CD, 25 patients (27%) associated homo or heterozygous DQ2/DQ8 alleles. There were significantly more cases with IDDM without CD but with predisposing haplotype for CD (27%) compared to the number of patients with AITD seronegative for CD and with DQ2/DQ8 alleles (4%) p < 0.005.

Conclusions: Recommending AITD and IDDM as selection parameters for CD screening in asymptomatic children is justified. HLA assessment cannot highlight a significant role of a certain allele in the pathogenesis of autoimmune comorbidity AITD/CD or IDDM/CD. DQ2 and DQ8 alleles are mandatory but insufficient for CD development. The interaction of environmental factors is very important. Performing as first line HLA typing for celiac disease diagnosis among children with autoimmune disorders is required. Further studies with a larger number of patients are needed.
J03.12 Features of clorine ions concentration in suspected cystic fibrosis patients sweat test and its correlation with C reactive protein

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Background: It is essential to confirm or exclude the diagnosis of cystic fibrosis in time and with accuracy in order to avoid inappropriate testing.

Methods: we investigated retrospectively case files of patients with perforated ulcer disease and characterized demographics, clinical features, and the results of diagnostic tests.

Results: 9 out of 48 patients (18.75%) showed higher than normal Cl ions concentration in sweat test that were suspected having cystic fibrosis and to evaluate correlation between this concentration and C reactive protein levels in serum.

Conclusion: MiR-137 methylation is a frequent event in gastric carcinoma tissues, miR-137 methylation was more frequent in tumors localized in antral and prepyloric region compared to adjacent normal mucosa.

J03.14 Methyltylation of miR-137 in gastric cancer and preneoplastic lesions

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INTRODUCTION: MicroRNA miR-137 is an important regulator of gene expression and functions as a tumour-suppressor gene. Expression of miR-137 is downregulated in gobliostoma and colorectal cancer (CRC) due to Cpg island methylation, however, the role of miR-137 methylation in gastric carcinogenesis remains largely unexplored.

AIM & METHODS: The aim of our study was to characterize the epigenetic regulation of miR-137 in gastric carcinogenesis. We determined miR-137 Cpg island methylation level in 81 patients with primary gastric cancer tissues (T-GC) and corresponding adjacent normal mucosa (N-GC), 20 samples of normal gastric mucosa (N) and 23 gastric tissues from patients with chronic atrophic gastritis (CG) using bisulphate pyrosequencing and compared to 29 colorectal cancers (T-CRC) and corresponding adjacent normal colon mucosa (N-CRC).

RESULTS: We confirmed the higher methylation level of miR-137 promoter in T-CRC tissues compared to adjacent normal colon tissues (p=0.004). In similar fashion, but to a lesser extent, methylation of miR-137 promoter region was observed in T-GC tissues compared to adjacent non-cancerous tissues (N-GC) (p=0.045). When compared to the normal mucosa from controls and mucosa from patients with gastritis, we found gradual increase in Cpg methylation (p=0.043).

In subgroup analyses of gastric cancer tissues, miR-137 methylation was more frequent in tumors localized in antrum compared to cardia and corpus (p=0.07).

CONCLUSION: MR-137 methylation is a frequent event in gastric carcinogenesis. The gradual increase in miR-137 methylation, as demonstrated for normal mucosa, chronic gastritis and tumour tissues, may be an early event in gastric carcinogenesis.
3J03.16 Prevalence and spectrum of CYP21A2 gene mutations in women with symptoms of hyperandrogenism in Uzbekistan

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Worldwide prevalence of hyperandrogenism varied from 3% to 23%. This wide range of variation is associated with difficulties of differential diagnosis between polycystic ovary syndrome (PCOS) and nonclassic congenital adrenal hyperplasia (NCAH). Molecular-genetic analysis plays a significant role in this issue, because NCAH is a genetic disorder which develops due to mutations in the CYP21A2 gene.

Therefore, our study aimed to investigate the prevalence of CYP21A2 mutations in women with hyperandrogenism in Uzbekistan. Real-time PCR using allele specific primers and TaqMan probes were used to detect eight most frequent CYP21A2 gene mutations in 361 Uzbek women with hyperandro- genic symptoms. Our results showed that 11.7% of these women have mutations in the CYP21A2 gene. We found the following spectrum of mutations: C1994T-7.14%, T999A-9.5%, A>C55G-7.2%, deletion of 8 bp (707-714) of the 5’UTR-4.75%, G683T-4.75% and C974T-2.4%. In the investigated group of women we did not find T1530A and C2108T mutations. C1994T mutation was found to be the most common mutation in our cohort. Women who harbored this mutation had abnormal menstrual cycle and recurrent miscarriages. In conclusion, genetic screening for CYP21A2 gene mutations by means of real-time PCR is an effective method for differential diagnosis between polycystic ovary syndrome and nonclassic congenital adrenal hyperplasia at the patients with hyperandrogenic symptoms.

3J03.17 Genetic factors of exercise participation and their association with basal metabolic rate and body mass index in overweight/obese Turkish women

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Introduction: Obesity is one of the fast spreading diseases. Insufficient physical activity is one of the main environmental factors in the etiology of the disease. Twin studies have revealed that genetic factors play an important role in physical activity levels. Although the underlying genetic factors are not clearly understood, studies have shown that the LEPR gene is among the candidate genes affecting physical activity level. In our study, we aimed to show the effect of LEPR gene mutations related with the physical activity in obese women. In addition, we evaluated the impact of LEPR gene on body composition parameters.

Material and Methods: 66 overweight/obese women were included in our study. Patients were categorized into 3 groups due to physical activity index levels. After the extraction of genomic DNA from buccal cells, LEPR gene regions were amplified by PCR and PCR products were sequenced. Physical activity levels and body composition parameters were calculated by using actical accelerometer and impedance products were sequenced. Physical activity levels and body composition parameters were calculated by using actical accelerometer and impedance products were sequenced.

Results and Discussion: Three reported polymorphisms, one in exon 6 and two others in intron 7 of the LEPR gene were detected. No relationship between physical activity levels and LEPR was observed. A correlation was found between resting metabolic rate and rs2405556 mutation. Furthermore, we determined a significant relation between different physical activity levels with fat mass, body mass index and total energy expenditure. Further studies are needed to make an interpretation about the relation between physical activity levels, body composition parameters and gene mutations.

3J03.18 Association and gene-gene interaction analyses of genetic variants in IL1B, IL1RN, IL8, IL10 and TNFA genes for peptic ulcer disease in Volga-Ural region of Russian Federation

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A peptic ulcer disease (PUD) is an area of damage to the inner lining (the mucosa) of the stomach or the upper part of the intestine (duodenum). A bacterium, Helicobacter pylori, is the main cause of ulcers in this area. The genes that encode proinflammatory and anti-inflammatory cytokines are good candidate markers of host susceptibility to gastroduodenal disease. The present study was performed to evaluate association and gene-gene interaction of polymorphisms of cytokines genes IL1B (rs1143634), IL1RN (rs7194186), IL8 (rs4073), IL10 (rs1800872) and TNFA (rs1800629) for PUD in Volga-Ural region of Russia. This study enrolled 264 patients with gastric and duodenal ulcers (112 individual were Hpylori-infected), the control group included 277 unrelated individuals without gastro-duodenal pathology with different ethnic origins (Russians, Tatars, Bashkirs). Genotyping was performed by PCR-RFLP analysis and we investigated gene-gene interactions, we employed generalized multi-factor dimensionality reduction (GMDR) method.

The analysis has revealed a strong association of C allele and CC genotype of the rs1143634 of the IL1B gene with PUD in Bashkirs (χ2=7.61, p=0.006; OR=2.87 and χ2=9.28, p=0.002; OR=4.49; 95%CI 1.78-11.35), confirmed by meta-analysis. We have also detected that Hpylori-positive PUD individuals has significant lower frequency of AA genotype of rs4073 of the IL8 gene compared with healthy donors (χ2=5.29, p=0.02; OR=0.46). The 3-locus GMDR-model of cytokine gene-gene interaction, including rs1143634 of IL1B, rs4073 of IL8 and rs1800872 of IL10 genes, was shown for male subgroup (p=0.0547).

Thus, we have determined that cytokine genes may contribute in genetic susceptibility for peptic ulcer disease.
nase chain reaction (PCR) and next digestion restriction endonucleases RsaI, MspI, TaqI, Hsp92II. NFKb insertion-deletion polymorphism was analyzed by PCR. Calculations were carried out on the programme SNPStats. The deviation from Hardy-Weinberg equilibrium were not found.

A genetic association between ATID and three of the eight genotyped SNPs was found in this study among women under the conditions of occupational hazards. It was determined the association with ATID polymorphism rs2362491 NFKb gene in predominant model OR=0.55 (CI 95% 0.33-0.92), p<0.00001. Association with ATID was identified in polymorphism rs12695951 STAT1 gene OR=1.76 (CI95% 1.03-2.99), 0.04 in dominant model (AG + AA vs. CC). Associations were found between rs310216 Jak1 gene OR=2.06 (CI95% 1.17-3.61) p=0.012 in the dominant genetic model (AG+AA vs. GG).

The work was done under supporting Russian Humanitarian Scientific Fund №13-06-00101.

J03.21
Inherited alpha-1 antitrypsin deficiency and spontaneous pneumothorax: possible causal relationship
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Introduction: Disrupted intestinal barrier function is observed during the development of autoimmune and inflammatory diseases like Crohn’s and celiac disease, and several genetic associations have been detected (PARDS and MAG2). Cell models are necessary for the investigation of the functional implication of associated candidate genes. The Caco-2 subline, C2BBe1 have been used as human derived brush border expressing cells that grow in homogenous monolayers that form tight junctions (TJ) and are potentially a good tool for this research. Aim: To determine the function of TJ-related genes during monolayer formation in C2BBe1 cells. Methods: C2BBe1 monolayers (30000 cells/cm²) were grown in 0.4 μm pore size PET inserts in Dubecco's Modified Eagle Medium (DMEM) with 4.5g/l glucose supplemented with 10% inactivated FBS and 1% non-essential amino acids, and transepithelial electrical resistance (TEER) was monitored every 24h for 6 days. RNA was extracted each day and the expression of the most relevant TJ genes for TJ formation (TJP1, CLDN2 and ACTB) and associated PARDS and MAG2 was analyzed by RT-PCR. Results: All genes except MAG2 were expressed in C2BBe1 and mRNA levels increased during the formation of the monolayers. There was a significant positive being significantly correlation between TEER and CLDN2 (r=0.76; p=0.005), TJP1 (r=0.84; p=0.001) and PARDS (r=0.70; p=0.01) expression, but not in ACTB. Conclusion: Monolayer cultures are valid tools for functional analyses of TJ genes. However, studies to determine those genes that are expressed in each cell line must be performed.

J03.24
Detection of Turner Syndrome by PCR-based approach in patients from Uzbekistan
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Turner syndrome (TS) is one of the most common genetic disorder affecting females, occurring in approximately 1:2500 female births. TS occurs when an X-chromosome is completely or partially deleted or when X-chromosomal mosaicism is present. It is characterized by short stature, gonadal dysgenesis, primary hypogonadism, congenital heart disease, renal anomalies, and a variety of somatic features. Girls with TS benefit from early diagnosis and treatment with growth hormone. However, many girls with TS are not detected until after 10 yr of age, resulting in delayed diagnosis and treatment. This study aimed to apply PCR-based approach for detection of Turner syndrome and elucidation the parental origin of the X chromosome in patients from Uzbekistan. We have amplified by polymerase chain reaction five polymorphic markers along the X chromosome (DXS1283E, DYS II, DMD49, AR and DXS52) and three markers along the Y chromosome (SRY, DY32 and DY31). In addition we analyzed patients DNA samples by SYBR Green and TaqMan probe based real-time PCR assay. The results of our study show that monosomy (45,X) was present in 78% of cases, 45,X/46,XX mosaicism in 18,8%, and 45,XX/46,XY in 3,2%. We also determined the parental origin of the X chromosome in the 2 patients. They had a paternal single X chromosome.

PCR-based approach can be recommended for the screening programs regarding detection of girls with TS in Uzbekistan.

J03.25
Average telomere length as a biomarker in children and adolescents with type 1 diabetes at the diagnosis
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Introduction: Subjects with type 1 diabetes (T1D) an autoimmune chronic disease are prone to oxidative stress, increased levels of free fatty acids, increased levels of advanced glycation end products and other factors leading increased risk for T1D complications. Shorter telomeres are associated with T1D complications and lower serum vitamin D levels. Methods: Average telomere length (ATL), nutritional status (BMI-SDS) and vitamin D level at the onset of T1D were determined in 53 Slovenian T1D children/adolescents (median age 8.7 years, 1:1.3 male to female ratio) with good DNA samples. The ATL was determined with qPCR method, vitamin D levels were determined with HPLC method. Results: There was a tendency between a shorter ATL and a higher BMI-SDS (p=0.241; p=0.08). In addition subjects with ATL in the higher ATL tertile tended to have a higher BMI-SDS when compared to those in the lower ATL tertile [0.259 ± 0.457 vs. -0.583 ± 0.282 SDS; p=0.06]. Subjects in the upper BMI-SDS tertile had a lower serum vitamin D levels when compared to those in the lower BMI-SDS tertile [40.6 ± 3.07 vs. 52.86 ± 4.85 μg/L; p=0.045]. Vitamin D serum levels did not significantly differ between sub-

J03.23
Tight junction gene expression in intestinal epithelial cell monolayers
Acute liver failure (ALF) is a rare form of Wilson disease (WD) developing mostly in young female patients. Use of chelating agents and supportive therapies including MARS (Molecular Adsorbent Recirculating System) in timely result in a remission in some cases making liver transplantation unnecessary. We are presenting here a 47 year old male patient with no alcohol consumption, negative hepatis- and autoantibody markers and with elevated transaminase and ferritin levels. The patient was admitted to our institution with severe ALF and with the potential diagnosis of haemochromatosis, but the patients coeruloplasmin level was low with very high level of liver enzymes. The D-penicillamin test supported the diagnosis of WD, but the result of the genetic analysis of the most frequent disease causing mutation in Hungarian population (H1069Q) was negative (wild type). Targeted NGS-based analysis of the entire coding region of the ATP7B gene showed two different disease-causing alteration in this patient and made the diagnosis clear, showing the clinical potential of semiconductor based next generation sequencing with 36 hours of turn around time.

**J03.29**

**Mutations in genes HFE, SERPINA1, CFTR in Wilson’s disease patients in Latvia**

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Introduction Liver inherited diseases are a group of genetically determined diseases that appear upon the loss of function or inactivation of genes with type 1 diabetes. Therapeutic approaches for these diseases include Wilson’s disease (WD), hereditary haemochromatosis, and alpha-1-antitrypsin deficiency. In addition, cystic fibrosis may cause a severe liver involvement in a significant percentage of cases. Mutations in the genes HFE, SERPINA1, CFTR could modify the pathogenesis and clinical appearance of Wilson’s disease. Aim To detect frequency of the most common mutations causing inherited liver disorders mentioned above in patients with WD suggestive symptoms. Material and Methods The study included 115 patients with WD suggestive symptoms and 295 unrelated healthy individuals. DNA analysis: both groups were tested for mutation C282Y and H63D in the gene **HFE**, **F508del** in the gene **CFTR** and **P1** in the gene **SERPINA1**. Results Frequency of different alleles: allele **F508del** in WD patients - 0.009, in control population - 0.01 (p=0.46); **P1** in WD patients - 0.01, in control group - 0.018 (p=0.50); **P1** in WD patients - 0.019, in control group - 0 (p=0.045); **C282Y** in WD patients - 0.02, in control group - 0.035 (p=0.475); **H63D** in WD patients - 0.188, in control group - 0.121 (p=0.019; OR=1.687). Conclusions 1) Alleles **P1** and **H63D** were more frequent in patients with WD suggestive symptoms that could indicate its significance in more severe and better detectable liver disorder in case of WD. 2) But still the role of iron overload and alpha-1-antitrypsin deficiency in the pathogenesis of Wilson disease is not finally elucidated.

**J03.30**

**Molecular characterization of COL4A5 in young Thai males suspected for X-linked Alport Syndrome**

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Background: Presently, a number of young males affected by end-stage renal disease (ESRD) of unknown causes have been registered for renal replacement therapy. Either genetic or environmental causes cannot be excluded. However, search for monogenic causes is challenging since genetic screening can give the information regarding the approach to other patient’s family members such as carrier screening. X-linked Alport syndrome (XLAS) is one of the suspected monogenic causes in young male patients. This particular syndrome is collagen IV-related nephropathy caused by the mutation of **COL4A5** on **Xq22**. Approximately 80% of XLAS leads to end-stage renal disease before the age of 40 with variable association with sensorineural hearing loss and ocular abnormalities. Objective: To characterize **COL4A5** mutation in young Thai male patients affected by ESRD of unknown causes and to develop the rapid and efficient molecular testing strategies for identifying XLAS in clinical practice. Methodology: DNA extraction was performed from peripheral blood leukocytes of Thai male patients, age 10-40 years, affected by ESRD of unidentified causes. Three common mutations of **COL4A5**, C1564S, L1649R, R1677Q were detected by using PCR and melting curve analysis. Results: Twenty patients were recruited in the study. Of them, **COL4A5** mutation was detected in four patients (20%). C1564S is the most prevalent
DNA from peripheral blood sample, after parental consent, we performed a PCR amplification step followed by analysis by direct sequencing of the coding regions of exons 4, 18, 19, 24 and 25 of\(\text{CUL7}\) gene. Using these methods, we confirmed the presence of the founder mutation, del\(\text{TG 4451-4452}\), in three of our patients. Furthermore, we did not detect either mutation or polymorphism in the five targeted exons of\(\text{CUL7}\) gene in the index case of a second family that encloses two 3M patients. 3M syndrome is characterized by genetic heterogeneity that contrasts with the clinical homogeneity of the syndrome. We plan to complete the molecular diagnosis in the other exons of the major gene\(\text{CUL7}\) before switching to the other two genes (\(\text{OB1L1}\) and \(\text{CCDC9}\)) for cases of the family 02.

### J04.02

**Inherited alpha-1 antitrypsin deficiency and chondrosarcoma - causal relationship?**

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Alpha 1-antitrypsin deficiency is a genetic risk factor for manifestation of COPD and chronic liver diseases. There is an ongoing worldwide discussion concerning the role of serpins (serine protease inhibitors) in tumor genesis. Protease inhibitors such as alpha 1-antitrypsin have generally been considered to counteract tumor progression and metastasis because of their ability to inhibit proteases. In this case report we analyze relationship between inherited alpha-1 antitrypsin deficiency and chondrosarcoma. A 47-year-old woman was admitted to the hospital with relapse signs of humerus chondrosarcoma. The patient also had a history of COPD. After chest X-ray and CT, alpha 1-antitrypsin deficiency was suspected. Severe alpha-1 antitrypsin deficiency (\text{PizZ homozygous genotype}) was confirmed. Alpha 1-antitrypsin deficiency might have facilitated the development of chondrosarcoma. Because of a small incidence rate of such diseases, we presume that there is a slight chance for such rare disorders to manifest concurrently in the same patient.

### J04.03

**The importance of MEVF gene mutations in HLA-B*27 positive Ankylosing Spondylitis patients**

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Ankylosing spondylitis (AS) that is etiologically unknown, can cause back and lumbar pain, and environmental, immunological and genetic factors have a role in its pathogenesis, is a chronic inflammatory disease. HLA-B*27 gene, located on short arm of sixth chromosome, have significant role in susceptibility of AS disease. The predisposition of AS disease is higher in HLA-B*27 positive people than HLA-B*27 negative ones. Also, there are some patients who have both AS disease and MEVF gene mutation. In our study, association of MEVF gene mutations have been analysed in HLA-B*27 positive patients who diagnosed as AS and HLA-B*27 negative healthy controls. 80 patients including HLA-B*27 positive 36 male and 14 female AS patients, HLA-B*27 negative 11 female and 19 male AS patients have been studied in the research. 50 healthy controls including 17 female and 33 male HLA-B*27 negative healthy individuals have been studied. HLA-B*27 allele of both patient and control groups were determined by PCR-SSP method. Pyrosequencing method was used for detection of MEVF gene mutations. HLA-B*27 positive patient group and healthy control group were compared in terms of MEVF gene exon 2 and 10 mutations. Frequency MEVF gene exon 2 and 10 mutation is determined statistically higher in HLA-B*27 positive patient group than healthy control group (p=0.012). We suggest that MEVF gene mutations may have a role in etiopathogenesis of HLA-B*27 positive AS disease. That hypothesis needs to be supported by further studies, involved in different populations including higher number of patient and control.

### J04.04

**Influence of sequence variations in MMP3 and GDF5 genes on risk of the anterior cruciate ligament rupture in the Russian population.**

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**Objectives:** Anterior cruciate ligament (ACL) rupture is a severe multifactorial injury. A familial predisposition toward tearing ACL was demonstrated. Matrix metalloproteinases and growth differentiation factor 5 (GDF5) are important physiological mediators of extracellular matrix degradation, remodeling and chondrogenesis. The aim of this study was to determine impact of MMP3 rs679620 and GDF5 rs414383 variations on the risk of ACL rupture.

### J03.31

**A Retrospective Audit into the Screening for Complications in Patients with Hereditary Haemorrhagic Telangiectasia (HHT) in the North West of England**

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**Background:** HHT affects approximately 1 in 5000 people and has been associated with mutations in\(\text{ENG}\) and\(\text{ACVRL1}\) and\(\text{SMAD4}\). Features of this disease are epistaxis, telangiectasia, a family history and visceral arteriovenous malformations (AVMs). International guidelines on the screening of patients with HHT were published in 2011.

**Method:** Patients with HHT were identified using the Molecular Laboratory database at St Mary’s Hospital, Manchester. Screening on mutation-positive patients was audited against the International guidelines.

**Results:** 54 patients were identified and 35 (65%) were found to have a mutation in either\(\text{ENG}\) or\(\text{ACVRL1}\). Pulmonary and cerebral AVMs screening was undertaken in 92% and 62% of patients respectively. Screening for anaemia in those over 35 years was undertaken in 21%. 2/19 (11%) eligible patients were tested for\(\text{SMAD4}\) mutations (both negative). AVMs occurred in 29% ENG and 21%\(\text{ACVRL1}\) patients. Lung AVMs were more common in the ENG group (24% vs 7%). One cerebral AVM was identified and this occurred in the ENG group. Liver AVMs only occurred in the\(\text{ACVRL1}\) group (21% vs 0%). Overall, AVMs occurred more frequently in women than in men (38% vs 7%).

**Conclusion:** A consensus on screening for HHT patients is needed in the UK. It is globally accepted that screening for pulmonary AVMs and anaemia should be undertaken but cerebral screening is still controversial.\(\text{SMAD4}\) mutations should be sought in those patients who are negative for ENG and\(\text{ACVRL1}\). The breakdown of AVMs in this population adds further evidence to a genotype-phenotype correlation in HHT.
J04.05
Filaggrin mutations and atopic dermatitis in Volga-Ural region of Russia
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Atopic dermatitis (AD) is a chronic inflammatory skin disorder and is often the first step in the atopic march. Mutations in the filaggrin gene (FLG), a key component of stratum corneum, have been identified as a strong predisposing factor for allergic diseases. In this study, we screened three FLG loss-of-function mutations (c.2282del4, p.Arg501X, and p.Arg2447X) in AD patients and controls. The AD group consisted of 448 AD patients (177 Russians, 126 Tatars, 145 individuals of mixed ethnic background). The control group included 408 non-atopic individuals (152 Russians, 109 Tatars and 147 individuals of mixed origin). Genomic DNA was isolated by phenol-chloroform extraction. The genotyping of SNPs was performed by PCR-RFLP. In our study the most prevalent FLG mutation was c.2282del4. The allele frequency in AD patients was 6.61% in general group, 6.03% in Russians (p=2.3*10^-4) and 9.35% in Tatars (p=1.6*10^-5). In controls the frequency of c.2282del4 was significantly lower: 1.12% in total group, 0.55% in Russians and 0.47% in Tatars. In AD subjects and controls it was detected with the use of Polymerase Chain Reaction-restriction fragment length polymorphism. A significant difference was found for the FokI polymorphism between the case and control groups. The f allele frequency of 26% was present in BD patients, compared to only 13% in the control group. In addition, the f/f genotype was significantly associated with BD in Russians, 0.85% and 0.47% in Tatars, respectively. Our results show that FLG mutations are frequent among Russian AD patients and controls. The AD group had a significantly decreased risk of ACL ruptures versus AG+/AA genotypes (p=0.014, OR 0.196, 95% CI 0.040 to 0.763). This study suggests a relationship between FLG rs143383 variation and risk of ACL rupture in the Russian population.

J04.06
Correlation between vitamin D receptor gene polymorphisms and atopic dermatitis in Canakkale population: a study based on, FokI and TaqI RFLP technique
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Aim: The pathogenesis of atopic dermatitis (AD) includes genetic and environmental factors leading to immunological and nonimmunological dysfunctions. The vitamin D receptor gene polymorphisms (SNPs), FokI and TaqI have previously been associated with atopic diseases such as allergic asthma. Methods: In a total of 88 AD patients and 96 healthy controls were included in the current study. The genomic DNA was isolated from peripheral blood EDTA, and target VDR gene was genotyped by PCR-RFLP technique after VDR-FokI (rs2282870) and VDR-TaqI (rs713236) restriction enzymes digestion. Results were compared statistically. Results: We use Arlequin ver 3.5 integrated software for population genetics data analysis. Current results showed lack of association for VDR-TaqI polymorphism in AD but showed association for VDR-FokI in current cohort of AD (P=0.0364), (OR: 1.9526). Conclusion: The current preliminary results identified the association between VDR- FokI gene polymorphism and AD in Canakkale population. Results need to be confirm by large scale of patient groups.

J04.07
Vitamin D receptor gene polymorphisms in Iranian Azary patients with Behçet’s Disease
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The aim of our study was to investigate the association of four polymorphisms of the VDR gene (FokI, BsmI, TaqI and Apal) with their susceptibility to Behchet’s Disease (BD) and their clinical manifestations in respect to the Iranian Azari population. In this cross-sectional study we considered the BsmI, FokI, Apal and TaqI polymorphisms in 50 Iranian Azary patients with BD and 50 healthy controls, with the use of Polymerase Chain Reaction-restriction fragment length polymorphism. A significant difference was found for the FokI polymorphism between the case and control groups. The f allele frequency of 26% was present in BD patients, compared to only 13% in the control group. In addition, the f/f genotype was significantly associated with BD in this study. We found no significant differences between the BD and control groups regarding the distribution of Apal, BsmI, and TaqI genotype frequencies. We found no association between VDR polymorphisms and the clinical manifestations of BD. The VDR Falle and f/f genotype is associated with BD in the Iranian Azari population.

J04.08
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Introduction: Brooke and Spiegler, independently described an epithelioma adenoides cysticum and skin endotelioma, as distinct entities. Brooke-Spiegler syndrome (BSS, OMIM #605041) characterized by benign adnexal neoplasia. Prematurn tumors trichoepithelioma, cylindroma and spiradenoma appear childhood and early adolescence. BSS is autosomal dominant entity. The tumor located on head and neck, and increase throughout life. Trichoepithelioma showed flesh-colored papules on face; cylindromatosis presents erythematous nodules on the scalp; and blue-colored-painful lesions on trunk suggested spiradenomas. Pathogenesis is considered a defect in the differentiation of folliculo-sebacueous-apocrine unit. Mutations have been identified in CYLD tumour suppressor gene, mapped to chromosome 16q12-q13.

Case Report:
Case I: 9 years-old female, birth: weight 3,400gr, height 53cm. Between 5-6 years-old displayed flesh-colored papules on face. Showed clinical and histological criteria of BSS. Biopsy revealed trichoepithelioma, 2) scalp region conclusive cylindroma, 3) third lumbar región conclusive ecrine spiradenoma. Given cryotherapy treatment and cosmetic surgery with good results.

Discussion: Brooke-Spiegler syndrome, sex ratio of F3: M1. include skin appendage tumors such cylindromas, thichoepitheliomas and spiradenomas, share a common genetic basis. May be associated with other skin disorders such, basal cell adenomas/carcinomas. Treatment included dermabrasion, cryotherapy and some cases radiotherapy. We present a Mexican family, mother and daughter similar affected, with the clinical features of the disease. Is the first mexican case reported with this entity. Molecular studies are needed to understand the genetic bases of the disease.

J04.09
First molecular analysis of Ehlers-Danlos kyphoscoliotic type in Slavic population
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Aims: The pathogenesis of Ehlers-Danlos syndrome (EDS) includes genetic and environmental factors leading to immunological and nonimmunological dysfunctions. The first step in the atopic march. Mutations in the filaggrin gene (FLG), a key component of stratum corneum, have been identified as a strong predisposing factor for allergic diseases. In this study, we screened three FLG loss-of-function mutations (c.2282del4, p.Arg501X, and p.Arg2447X) in AD patients and controls. The AD group consisted of 448 AD patients (177 Russians, 126 Tatars, 145 individuals of mixed ethnic background). The control group included 408 non-atopic individuals (152 Russians, 109 Tatars and 147 individuals of mixed origin). Genomic DNA was isolated by phenol-chloroform extraction. The genotyping of SNPs was performed by PCR-RFLP. In our study the most prevalent FLG mutation was c.2282del4. The allele frequency in AD patients was 6.61% in general group, 6.03% in Russians (p=2.3*10^-4) and 9.35% in Tatars (p=1.6*10^-5). In controls the frequency of c.2282del4 was significantly lower: 1.12% in total group, 0.97% in Russians and 0.46% in Tatars. Second mutation p.Arg501X in our groups was rarely found. In AD subjects and controls it was detected with the use of Polymerase Chain Reaction-restriction fragment length polymorphism. A significant difference was found for the FokI polymorphism between the case and control groups. The f allele frequency of 26% was present in BD patients, compared to only 13% in the control group. In addition, the f/f genotype was significantly associated with BD in this study. We found no significant differences between the BD and control groups regarding the distribution of Apal, BsmI, and TaqI genotype frequencies. We found no association between VDR polymorphisms and the clinical manifestations of BD. The VDR Falle and f/f genotype is associated with BD in the Iranian Azari population.
J04.10

Atypical Fibrodysplasia Ossificans Progressiva (FOP) phenotype in a girl carrying uncommon missense mutation (p.G535D) in ACVR1


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Fibrodysplasia ossificans progressiva (FOP, MIM #135100) is a rare genetic condition characterized by progressive transformation of soft tissue into bone. There are approximately 3,000 individuals living worldwide with this severely disabling disease, for which there is still no definitive treatment. Heterozygous mutations in ACVR1 (MIM #102576) has been identified as a cause of this condition. All reported patients of various ethnic backgrounds with classic clinical presentation of FOP have previously been found with the identical heterozygous activating mutation 617G>A (R206H). Recently other types of mutations in ACVR1 gene have been described in patients with variant FOP.

We present a case of FOP in 3.5 year old girl carrying uncommon missense mutation 1067G>A (G535D) in the protein kinase domain of ACVR1. Identical mutation has been identified in seven patients with atypical FOP among which at least in two with mild clinical symptoms. On contrary, our patient experiences a severe course of FOP with already significant restriction of movement. Though it should be emphasized, that retrospectively analyzing evolution of intense heterotopic ossification in our patient, we have discovered, that most of the lesions were directly triggered by soft tissue injuries resulting from diagnostic procedures and inadequate rehabilitation that she underwent prior to recognition of her genetic disease.

Our presentation may add to the understanding of the variability of clinical FOP presentation in patients with atypical ACVR1 mutations, as well as help spreading knowledge of this disease among health care professionals, in hope for the earliest possible diagnosis established in each newly affected child.

J04.11

Genetic characterization of a Portuguese patient with fibrodysplasia ossificans progressiva

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Fibrodysplasia ossificans progressiva (FOP) is a rare autosomal dominant disease with a prevalence of approximately 1 in 2 million worldwide. FOP is characterized by the presence of malignations of the big toes and of postural progressive heterotopic endochondral ossogenesis, especially in the presence of exacerbating factors such as trauma, surgical intervention, lesion biopsy, and intramuscular injection. FOP has been associated with a specific mutation on ACVR1 (c.617G>A; p.Arg206His), which encodes a receptor for bone morphogenetic proteins (BMPs). Our aim was to establish the molecular diagnosis by mutation screening of ACVR1. We report a male patient with progressive ossifications since childhood, showing calcification of the axial line with inability to perform flexion and extension of the scapular and pelvic girdle with neck stiffness. Surgical intervention in adolescence resulted in disease progression. At the moment, patient is bedridden and suffering from significant functional limitation.基因 sequencing was performed by PCR amplification of all coding and flanking regions, followed by bidirectional direct sequencing. We have found one missense mutation in exon 6 (c.617G>A; p.Arg206His), previously described as a FOP disease-causing mutation. The codon 206 is at the end of the highly conserved glycine-serine rich (GS) activation domain at the junction with the kinase domain. To our knowledge, this is the first genetic study of FOP in a Portuguese patient. The mutation screening of ACVR1 distinguishes FOP from other disorders allowing the correct clinical management of the patient. Molecular diagnosis will also allow appropriate genetic counseling to this patient and at-risk relatives.

J04.12

First Italian case of Crouzon-like Craniosynostosis and Dental Anomalies (CRSDA, #614188) with severe scoliosis


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Crouzon-like craniosynostosis and dental anomalies (CRSDA, #614188) is an autosomal recessive disorder due to homozygous mutations of the IL1R1 gene. Our patient (a 16 years-old boy) was referred to our Clinic for oculocephaly with fronto-basal bicipital and basipinum. By the age of 4 years he underwent a surgical correction due to raised intracranial pressure with optic nerve atrophy. He had maxillary hypoplasia with malocclusion, persisting deciduous teeth and supernumerary teeth (1.3, 2.3, 3.1, 4.2) treated by surgical correction, severe dorsal curvature with right convexity and thorax asymmetry. Encephalus and spine MRI showed a Chiari II malformation, and enlarged dural sac.

His parents were first cousins hailing from Southern Italy (Naples); his father underwent coliosis surgical correction by the age of 19 years. Molecular analysis of IL1R1 gene in the proband identified a homoyygous mutation c.598C>A (p.Pro200Thr) in exon 6; bioinformatic analyses by Polyphen2 and SIFT suggested that this mutation is harmful for the protein function. The mutation has been previously described by Keupp et al. (2013) in a non-consanguineous Turkish family in two siblings compound heterozygous c.598C>A/c.710G>C. It is also present in the databases as a rare variant. To our best knowledge, this is the first description of an Italian patient affected by CRSDA. We speculate that severe scoliosis, not previously described in affected patients, could be included in the clinical spectrum of the disease.

J04.13

The role of polymorphisms of inflammatory mediators genes in pyoinflammatory diseases of maxillofacial area

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Despite advances in the treatment of pyoinflammatory diseases of maxillofacial area, the number of diseases is increasing from year to year. In this connection, the study of the pathogenesis of pyoinflammatory diseases is one of the most pressing issues in maxillofacial surgery. The purpose of this study was to investigate the role of the cytokines genes in the development of odontogenic inflammatory processes. To estimate the role of polymorphisms of inflammatory mediator genes in genetic predisposition to pyoinflammatory disease, the allele and the genotype frequencies of IL1B, IL1RA, TNFα, TNFβ and IL10 genes were investigated. The studied groups included 189 patients with pyoinflammatory diseases of maxillofacial area divided into two groups: odontogenic phlegmon (141) and osteomyelitis (48) and 105 healthy controls. Genomic DNA was extracted from peripheral blood leukocytes by standard phenol/chloroform method. Genotyping was performed by the PCR-RFLP technique. Studies have revealed that the C* A* genotype (OR = 1.83; 95% CI 1.09-3.10) of IL10 polymorphic locus was associated with increased risk of odontogenic phlegmon, while the C* A* genotype (OR = 0.29; 95% CI 0.08-1.04) of TNFα polymorphic locus 308 G>A is associated with lower risk of osteomyelitis of maxillofacial area. It is possible to suggest that genotype C* A* of IL10 polymorphism (627C>A) and genotype C* A* of TNF polymorphism (308 G>A) may be genetic predictor of odontogenic inflammatory processes. No significant differences were found between the groups of patients and
healthy control concerning the genotype frequencies of the polymorphisms IL1β (3953 C>T), IL1RA (VNTR) and TNFβ (1069 C>T).

J04.14 Ichthyosis, the XXI century pandemy of Ecuador A. E. Tumanta Ortiz, M. Kartelle Gestal, R. Velez; Faculty of Public Health. ESPCH, Biobamba, Ecuador.

Ichthyosis is a group of inherited keratinizing disorders. There are five different types described. This type of genetic disorder can be autosomal recessive or x-linked. This disorder has been related with inbreeding and new mutations. More than 16,000 babies are born each year with some form of ichthyosis. In Manabi province, Ecuador (population 1,369,780) there are more than 230 cases, compared with rates of other countries this is very high. But when put into context, Ecuador is a developing country, in more isolated areas marriage between closely related family members is still common place. We are presenting a case of twin brothers that suffer from ichthyosis.

Case report: Mother and father are first cousins. They are parents of three children, one girl and two between brothers. The boys present the typical fish scales, alopecia, constant conjunctivitis and skin infections. We found isolates of different microorganisms in all samples taken from eyes, skin and wounds. They present constant weeping of the eyes and intense itching. They do not eat pork due metabolic disorders associated with this disease. After living in several places in Ecuador, they moved to Banos a small city in the Andes (1,815 m) because the moderate climate (moisture and temperature) is more favorable to their condition. Their treatment is: Rocutan (isotretinoin) for 2 weeks and after that Neotigason (retinoids, 25 mg every day or two). Their pathology has improved a lot since. They do not show signs of hyperactivity or any mental problems.

J04.15 Lacrimo-auciculo-dento-digital syndrome, case report A. Mitroi, A. Apostol, M. Aschie, G. Casauro; Emergency Chancery County Hospital of Constanta, Constanta, Romania.

Lacrimo-auriculo-dento-digital syndrome (Levy-Hollister syndrome) (MIM:149730), is an extremely rare genetic disorder (prevalence < 1 / 1,000,000) characterised by abnormalities affecting the lacrimal and salivary glands and ducts, ears, teeth and fingers. Levy-Hollister syndrome may occur sporadically or be inherited as an autosomal dominant trait. We present a 16 years old index male patient clinically diagnosed with this syndrome. Our patient present recurrent obstruction of nasal lacrimal ducts and bilateral agenesis of parotid glands. The auricular feature was cup-shaped pinna. The dental features were represented by hypodontia, microdontia, spaced teeth, enamel dysplasia and early onset caries. The limb defects were fifth finger clinodactyly, hypoplasia of the eminence and absence of thumb flexion creases. No family history was found in our case. Due to agenesis of parotid glands is possible that our case should be the result of GFG10 de novo mutation. We compare the phenotype and findings of our case to previously published cases.

J04.16 A novel mutation in SHOX gene in four members with Langer Mesomelic Dysplasia in three members with Leri-Well Dyschondrosteosis of a family. F. Hazan1, A. Aybat1, H. A. Korkmaz1, B. Ozhan1, F. Ozkaynak2, H. Onay1, O. Cagul1; 1Department of Medical Genetics, Dr. Behcet Uz Children’s Hospital, izmir, Turkey, 2Department of Medical Genetics, Ege University Faculty of Medicine, izmir, Turkey, 3Department of Pediatric Endocrinology, Dr. Behcet Uz Children’s Hospital, izmir, Turkey. Leri-Well dyschondrostosis (LWD; #MIM 127300) is an autosomal dominant hereditary disease, which is characterized by short stature, mesomelic shortening of the limbs, and characteristic bilateral abnormality of the wrists known as Madelung deformity. LWD is caused by mutations in the Short Stature Homeobox gene (SHOX). The other diseases which are associated with SHOX gene mutations are Langer Mesomelic Dysplasia (LMD, MIM #249700) and Turner syndrome as well as nonsyndromic idiopathic short stature (ISS). The SHOX gene is localized within the pseudoautosomal region 1 (PAR1) of the X and Y chromosomes. Both alleles of SHOX gene must be functional for normal growth. While heterogeneous mutations of SHOX gene or its enhancer regions are responsible for both LWD and ISS syndromes, LMD is caused by homozygous or compound heterozygous mutations in this gene. Herein, four LMD and one LWD sibling and their consanguineous parents were studied. All LWD and ISS mutation analyses revealed that the parents and one sibling were heterozygous, and the other 4 siblings were homozygous for the c.42delG (p.Q14QfsX15) in the SHOX gene.

J04.17 A novel mutation in LEMD3 gene in a Turkish family with Buschke-Ollendorff syndrome S. Uzal1, H. Gurtan1, M. Ciftci1, H. Tozkir1; 1Trakya University Faculty of Medicine, Department of Medical Genetics, Edirne, Turkey, 2Trakya University, Faculty of Medicine, Department of Medical Genetics, Edirne, Turkey, 3Trakya University, Faculty of Medicine, Department of Orthopedics and Traumatology, Edirne, Turkey, 4Trakya University Faculty of Medicine, Department of Orthopedics and Traumatology, Edirne, Turkey.

Buschke-Ollendorff Syndrome (BOS, OMIM* 667000) is an autosomal dominant disorder of connective tissue with an incidence of 1 / 20,000. Although it is characterized by multiple subcutaneous new or nodules and osteopoikilosis (OPK), some patients may also have melorheostosis. Expressivity of bone and skin manifestations differs between the cases. Mutations in LEM Domain-Containing Protein-3 (LEMD3, OMIM *607844, chromosome 12q14.3) have been implicated in BOS. 14-year-old girl was directed to our department for an incidentally realised osteopoikilosis in the X-ray exploration. She also has yolishaws plaques on her anterolateral thigh. Complete X-Ray study revealed multiple osteostastic foci around the bones as implicated in the BOS. 35-year-old mother has been X-Ray examined and she also has opsteopoikilosis but she has no skin plaques. LEMD3 gene was sequenced from the DNA of the patient and the mother. Both of them have a heterozygous c.2405_2406insAGT mutation in the 12th exon of LEMD3 gene. This mutation results in a premature stop codon at the 802nd position of the MAN protein encoded by LEMD3 gene. The remaining parts of the gene were also sequenced and confirmed that there was not another mutation. We submit the mutation to MutationTaster and see that it causes loss of Interaction with SMAD1, SMAD2, SMAD3 and SMAD5. To our knowledge, this is the first study implicating the c.2405_2406insAGT mutation in the cases of BOS.

J04.18 HRM scanning of mutations in human structural proteins R. Blatny1, L. Honolova1, J. Vaskú1, V. Krivčaný1, V. Filimonekon2, L. Jarolimová1, I. Nováková1, P. Hozák2, M. Krivjanka1; 1KRD molecular technologies Ltd., Prague, Czech Republic, 2Institute of Molecular Genetics, Prague, Czech Republic.

Background: This project aims at the development of assays for detection of mutations in structural proteins. Both intracellular (cytoskeleton and nucleoskeleton) and extracellular proteins (extracellular matrix) are considered, as well as non-structural proteins which are closely functionally associated with these structural proteins. Mutations in the selected proteins are known to cause rare diseases (RD). The project resonates with current clinical need to improve diagnostics and prognostics of rare diseases as formulated in many running national and European programs (e.g. Czech National Action Plan for Rare Diseases, COST NANOET, EURORDIS, EHRDO, M. EUGRAT, ICBSR, e-Rare ERA). Methods: The main detection method chosen is the scanning high-resolution melting analysis. The primer pairs were designed for 65 selected genes. The amplifications are successively optimized to fit maximally two major PCR/HRM conditions for each gene. The following assays have been technicallly confirmed on control DNA and both major tested platforms (LightScanner 96 and LightCycler 480): LMNA, MYH7, DMD, ACTG1, MYH6, MYH2, MYH3, DES, MYLK2, MYH14, MYH9 and MYO6. LMNA, MYH7 and DMD is currently undergoing testing on positive patient and synthetic DNA samples. Results: Female patient, 35 years old, with suspected Emery-Dreifuss muscular dystrophy was scanned using the LMNA sHRM assay. The scanning revealed putative mutation in the exon 6 of the LMNA gene which was confirmed by the Sanger sequencing. Additional reported cases with mutations in the DMD and MYH7 genes will follow. This work is supported by the FR-TIS-588 grant from the Ministry of Industry and Trade of the Czech Republic.

J04.19 A novel homozygote p.Met540Ile LMNA mutation causes mandibulocacral dysplasia type A V. R. Yasaei1, A. Khojasteh1, F. Hashemi-Gorji1, P. Toossi2; 1Genomic Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Islamic Republic of Iran, 2Dept. of Medical Genetics, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Islamic Republic of Iran.

Mandibulocacral dysplasia with type A lipodystrophy (MADA) is a rare genetic disorder inherited in an autosomal recessive fashion characterized by hypoplasia of the mandible and clavicles, acro-osteolysis and lipodystrophy due to mutations in LMNA or ZMPSTE24 gene. Very few families have been studied for the above genes alteration so far. We have investigated a consan-
guineous family with an affected boy for LMNA alteration. Isolated genomic DNA derived from subjects was amplified using intronic primers. The entire sequence of the LMNA gene, including coding regions and exon-intron boundaries were analyzed by PCR and Sanger sequencing. Molecular analysis ascertaining the genotype (c.1620 G>A (p.M540I)) in the proband and heterozygote alteration in the rest of the family. We have also applied several online tools including PolyPhen2, Pmut, SIFT, Mutation Taster and phyre2 to predict the pMet540Ie substitution effects. All these tools showed reduction the stability of the protein structure. We conclude that M540I mutation may cause disease in homozogous state.

J04.20 Atrophic skin patches with abnormal elastic fibers, as a presenting sign of MASS phenotype associated with mutation in the Fibrillin-1 gene

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Marfan syndrome (MFS) is a dominantly inherited disorder of connective tissue caused by mutations in the Fibrillin-1 (FBN1) gene. The most common skin finding in MFS is striae distensae. Particular individuals referred for suspicion of MFS whom do not completely fulfill the MFS diagnostic criteria are classified as having a MASS phenotype. The acronym represents the phenotype’s apparent manifestations: a prolapsed Mitral valve, Myopia, Aortic root enlargement, Skeletal and Skin manifestations. Mutations in FBN1 have been shown to be associated in a few cases with MASS phenotype. Mutations in FBN1 may be an important clue to the diagnosis of these disorders. We studied a case referred for unusual atrophic skin patches on the buttocks. Histopathology and electron microscopy demonstrated markedly abnormal elastic fibers. Consequent medical genetics evaluation led ultimately to the diagnosis of MASS phenotype, and to the discovery of an underlying FBN1 mutation.

Though the clinical suspicion and diagnosis of MASS and related disorders are usually established by its main associated clinical features including the eye, skeletal and vascular involvement, clinicians should be aware of the associated skin manifestations, including unusual atrophic patches with abnormal elastic fibers that can sometimes be the first noted sign of the genetic disorder.

J04.21 Novel COL9A3 mutation in a family diagnosed with multiple epiphyseal dysplasia

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Background : The clinical and radiographic phenotypes of multiple epiphyseal dysplasia(MED) are heterogeneous according to the genetic mutation. Mutations COMP, MATN3, COL9A1, COL9A2 and COL9A3 result in autosomal dominant MED, and mutations in the DTDST gene is associated with an autosomal recessive MED. Here, we present a family with novel COL9A3 gene mutation.

Case : The proband was a 12-year-old boy born from non-consanguineous parents. He was referred to the pediatric orthopedic clinic for the evaluation of intermittent knee pain that occurred from a few months. The radiographic phenotype of COL9-MED is that the epiphysis of the distal tibia, distal radius and distal ulnae showed lateral shortening, wedge shape and small, phallic phenotype of COL9-MED is that the epiphysis of the distal tibia, distal ulnae showed lateral shortening, wedge shape and small.

The short stature and myopia were observed in 2 patients, speech delay, ptosis, ocipital region cyst, submucous cleft palate, pectus deformity, unilateral cryptorchidism and bleeding abnormalities in 1 patient. The mutations in FBN1 were identified in all patients. No mutation was found in the PTPN11 gene. This and previous studies indicate that there are significant phenotypic differences between NFNS and NF1 or NS. Detailed comparison of NFNS clinical symptoms in described group of patients with data from publications as well as genotype-phenotype correlation will be presented.

J04.23 Expression of the genes that are indicators of young bone growth and histological analysis in evaluation of osteogenic process

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Osteoreparation is a complex, dynamic and still incompletely revealed process. There are different approaches in evaluation of osteogenic process. The purpose of our research is evaluation weather and to what extent relative expression of genes that are indicators of young bone growth are in correlation with histological findings, as commonly used methods for evaluation of osteogenic process. In our research, subcutaneous implantation model on BalB/c mice was used. The implants were composed from depo- teized bone mineral matrix and bone marrow and/or different components of blood. The animals were sacrificed after 1, 2, 4, and 8 weeks after implantation. Analysis of implants included comparison of relative expres- sion profiles of the gene for alkaline phosphatase, osteocalcin, osteonectin, osteopontin and collagen type I (RealTime PCR) and histological analysis of implants after staining with hematoxylin-eosin and Masson’s trichrome method. Our results show: the most informative genetics markers are profiles of expression of genes for osteocalcin and alkaline phosphatase, as indicators of stimulation of young bone growth; histological picture was lagging in comparison to the findings of gene expression in all terms of sacrifice; valid assessment of osteogenic process requires a combination of different methods, because neither gene expression nor histological analysis are suf- ficient for complete evaluation of osteogenic process.

Key words: RealTime PCR, histology, ectopic osteogenesis

J04.24 Bone mineral accrual and fracture outcomes in children with osteogenesis imperfecta treated by pamidronate

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The purpose of this study was to evaluate the bone mineral accrual and fracture outcomes in children with osteogenesis imperfecta (OI) treated by pamidronate (PAM). Material and Methods: In our retrospective study 21 children with different types of OI were included: 7 boys and 14 girls. According to clinical OI classification proposed by D. Silence, patients were divided in 3 types: I type - 11, II type - 7, IV type - 3. We divided patients in 2 groups: mild to moderate (OI 1 type) and moderate-to-severe (II and IV types). The standard protocol with cyclic PAM infusions (3 consequent days 3-4 times in a year) was applied in annual cumulative 9-12 mg/kg. All children received vitamin D and calcium supplementation in physiological doses. Observation period was 36 months. Bone mineralization parameters were detected by dual-energy X-ray absorptiometry of lumbar spine L1-4. Results: There were no differences in bone mineral accrual between types.
of OI. The maximum efficacy in bone mineral accrual was observed in first year (+32.9%) and second year (+22.1%) and no real improvement in BMD in third year. Reduction of fractures in OI1 types was from 0.87 (0.64; 1.08) to 0 (0.0; 0.5) fractures per year (p<0.09). In severe OI group fracture reduct-
on was from 28.5 (4.6; 56.2) to 1.1 (0.86; 2.6) fractures per year (p=0.02). Conclusion: PAT treatment was effective in bone mineral accrual and fracture reduction. The maximum efficacy in bone mineral acc-
ral was observed in first two years.

J04.25 
**Medical approach in a severe case of osteogenesis imperfecta**

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Osteogenesis imperfecta is one of the most common skeletal dysplasias and comprises a group of genetic disorders that are characterized by increased bone fragility, low bone mass and increased susceptibility to bone fractures. The inheritance is autosomal dominant, but it may also result from new dominant mutations. We present the case of a young woman, 27 years old, retired, diagnosed with osteogenesis imperfecta in early childhood. She has negative family history, thus, it was considered to be due to a new mutation. The first fractures, double fracture of tibia and femur occurred during infancy, after minor trauma. Until now more than twenty bone surgical treatments were needed. The patient has very short stature, severe scoliosis, deformed thoracic kyphosis, muscular and poor muscle mass, osteoporosis, dentinogenesis imperfecta, especially severe forms, by a multidisciplinary team, increase psychomotor development, improve quality of life and prevent fractures with major vital risk.

J04.26 
**LRP5 gene polymorphism V667M (rs4988321) in man with osteoporosis and Polish population - a pilot study**

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Many recent reports confirmed, in the disease onset, both genetic and en-
vironmental factors, may be different in osteoporosis for women and men. Mutations in the low-density lipoprotein receptor-related protein 5 (LRP5) gene cause malformations characterized by altered bone mineral density (BMD). LRP-5 is a transmembrane protein encoded by gene located in 6q25.1. LRP-5 protein takes part in a proliferation and differentiation of osteoblasts via Wnt signaling. The Wnt/β-catenin signaling pathway stimulates bone formation through a number of mechanisms such as stimulation of preosteoblast replication, induction of osteoblastogenesis, and inhibition of osteoblast and osteocyte apoptosis. LRP-5 is widely expressed in most trabecular bone surfaces. Common LRP5 variants were associated with the osteoporosis risk in males. The aim of this pilot study was to analyze occurrence of polymorphic variant c.2074A (p. V667M, rs4988321) in a group of 187 male patients with osteoporosis and 203 individuals from Polish population. Genotyping was performed by pyrosequencing technique. Hardy-Weinberg equilibrium (HWE) were examined for selected groups by chi-square distribution and Fisher exact tests. The odds ratios (ORs), 95% confidence intervals (CIs), and p-values were calculated. Statistical signifi-
cance was set at p<0.05. We observed statistically relevant higher frequency of minor allele c.2074A in male osteoporosis patients with p=0.01401 (OR=2.162, CI=[1.154-4.050). In conclusion we state that c.2074A in LRP5 cy-

J04.27 
**Association of gene variants in TLR4, TNF-α, IL-3 and IL-6 genes with Perthes disease**


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Background: Perthes disease is idiopathic avascular osteonecrosis of the hip in children, with unknown etiology. Inflammation is present during develop-
ment of Perthes disease and it is known that this process influences bone remodeling. Since genetic studies related to inflammation haven’t been performed in Perthes disease so far, the aim of this study was to analyze the association of frequencies of genetic variants of immune response genes: toll-like receptor 4 (TLR4), tumor necrosis factor alpha (TNF-α), interleu-
kine-3 (IL-3) and interleukin-6 (IL-6) with this disease.

Methods: The study cohort consisted of 37 patients with Perthes disease and 50 healthy controls from Serbia. Polymorphisms of TLR4 (Asp299Gly, Thr399Ile), TNF-α (G-308A) and IL-6 (G-597A, G-174C) genes were deter-
mined by polymerase chain reaction restriction fragment length polymor-
phism method, while IL-3 gene polymorphisms (C-16T, C132T) were deter-
mined by direct sequencing of PCR product.

Results: TLR4 polymorphisms (Asp299Gly, Thr399Ile) were in complete, while IL-3 (C-16T, C132T) as well as IL-6 (G-597A, G-174C) polymorphisms were in perfect linkage disequilibrium. A statistically significant increase of heterozygote subjects for IL-6-G-174C/G-597A was found in controls in com-
pared to patients (p=0.047, OR=2.49, 95% CI=1.06-5.83). Also, the patient group for IL-6-G-174C/G-597A polymorphisms wasn’t in Hardy-Weinberg equilibrium. No statistically significant differences were found between patient and control groups for TLR4, TNF-α or IL-3 analyzed polymorphisms.

Conclusion: Our results suggest that children who are heterozygous for the IL-6-G-174C/G-597A polymorphisms have a lower chance of developing Perthes disease than carriers of both homozygote genotypes.
aetiology in RTSII remains unknown. According to literature data missense mutations are rare, while frameshift, nonsense mutations and splice-site mutations prevail. We describe 5 years old girl with typical signs of RTSII and indication for molecular genetic testing of REClQ4 mutations. After extraction of genomic DNA from peripheral blood we performed direct sequencing of mutation prone exons of REClQ4 gene. Two different mutations have been detected in exon 9: c.1568C>G (p.Ser523Thr) and c.1573delT (p.Cys525 Ala fsX33). The observed frame shifting deletion is the most common REClQ4 mutation, while substitution is rarely described. This is the first case of RTSII from Serbia confirmed by genetic testing. Genetic testing of RTSII is important in the context of differential diagnosis and genetic counseling for patients and their families.

J04.30
A family with proximal symphalangism (SYM1)
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Background: A 33 year old woman was referred to genetic counselling with a family history of congenital stiffness of the digits. Absence of the proximal interphalangeal joints of all fingers except the pollex, and fusion of the calcaneus naviculare and calcaneus cuboideum had previously been diagnosed radiographically. At the physical examination stiff digits and poorly discernable creases were found along with severe platysyndactyly. The pedigree demonstrated autosomal dominant inheritance with four generations of affected family members presenting with stiff fingers, fusion of foot bones and at least one sister with impaired hearing as a result of stapes fixation. A review of the literature revealed that hereditary fusion of the proximal interphalangeal joints was first described by Harvey Cushing in 1916, by- passed by other case reports. This is - to the best of our knowledge - the first family to be reported in Denmark. The NOG gene (17q22) encodes noggin, a secreted polypeptide important for regulating multiple signaling pathways, particularly in cartilage and bone. A mutation herein is believed to cause Proximal Symphalangism (SYM1) which conforms to the above condition.

Methods: Sequence analysis of NOG1. Results: Test results of NOG1 mutation screening are pending.

J04.31
Investigating of Interleukin-10 (IL-10) family cytokines polymorphisms in patients with psoriasis
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Background: Interleukin (IL)-10 family cytokines IL-10, IL-19, IL-20, and IL-24 have been implicated in autoimmune diseases and we have previously reported that genetic variants in IL10 gene cluster were associated with psoriasis. Objective: To analyze the relationship of genetic polymorphisms in the IL-10 gene cluster with psoriasis for Russian population. This study also explores whether there are gene-gene interactions among these genetic polymorphisms. Methods: A total of 273 patients with psoriasis and 298 matched healthy controls were enrolled to carry out a case-control study for 46 SNPs in IL10 gene cluster. Genotyping for the SNPs was conducted on the Applied Biosystems 3730 DNA Analyzer using SNPlex™ technology. Results: The results showed that the genotype distributions of IL20 T/T (rs1518108) and IL20 A/G (2232363) are significantly different between case and control groups (P = 0.034 and P = 0.047, respectively). Carriers of IL10 A/G (rs1554286) and IL20 A/G (2232363) allele conferred risk to psoriasis (OR = 2.26, 95% CI 1.05-4.88) while those of IL10 T (rs1554286) and of IL20 T (1400986) allele of IL20 A (2232363) allele conferred risk to psoriasis (OR = 1.75, 95% CI 1.05-2.93). Conclusions: Our preliminary data suggest that four polymorphisms (rs1554286, 1400986, rs2232363, rs1518108) located in IL10 gene cluster related to inflammatory and immunity processes showed an association with protection or development of psoriasis in Russians.

J04.32
Mutations in COL2A1 in a Brazilian cohort of 15 patients with SEDC phenotype
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Type II collagenopathies are characterized by a spectrum of different conditions among which the most common phenotype is the spondyloepiphyseal dysplasia congenita - SEDC. More than 30 missense mutations, usually private and involving the change of a glycine, have been described in patients with SEDC. Unfortunately, little is known about severity and follow up of the patients with known mutations. Here we present the molecular results of 15 new patients with SEDC. The molecular analysis was performed by direct sequencing of the COL2A1. Thirteen mutations have been found until now, and the majority is located at the center of the triple-helical domain between exons 23-27. Among the 13 mutations, eight are novel (p.G1516S, p.G526A, p.G570D, p.G670R, p.G498D, p.G668R, p.G1149R, and p.G1080V), and result in the glycine substitution by a bulkier amino acid. Two mutations were recurrent and previously described, p.R989C and p.G539E. In two patients with no mutations the sequencing of COL2A1 is still ongoing. Severe phenotype was associated with the following mutations: p.R989C and p.G570D and p.G668R. The patients with the following mutations G549E and G516S, all died in the first months of life. The mutations p.G1149R and p.G1080V were seen in less severe phenotypes. The remaining mutations could not be associated with severity because children are still very young. In conclusion, p.R989C mutation seems to produce a constant and severe phenotype. The two less severe phenotype were associated with mutations in the extremity of the triple-helical domain.

J04.33
Homozogous shox gene deletion detected by array CGH in a girl with long mesomelic dysplasia
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Langer mesomelic dysplasia (LMD) is characterized by hypomelia with severe hypoplasia of ulna and fibula, and bowed, thickened radii and ulna, causing deformities of the hands and feet. LMD is caused by homozygous mutations in the SHOX/SHOXY (short stature homeobox) gene, of which heterozygous mutations or deletions cause Leri-Weil Dysplasia (LWD). Phenotype of LWD can be incomplete between and within families. We present a 13 year old female with LMD, the second child of healthy first cousin parents. She had micrognathia, disproportionate short stature with various musculoskeletal findings (absence of the distal flexion creases of the 3rd, 4th, 5th fingers on the right, camptodactyly of the 3rd, 4th, 5th fingers on the left, tibial bowing). X-rays revealed hypoplasia of ulna, fibulae and the mandible. Chromosome analysis and FISH investigation by using SHOX gene probe revealed no results. Sequence analysis failed due to unsuccessful PCR amplifications. Array comparative genomic hybridization (a-CGH) study showed a 17 kb homozygous deletion, encompassing the SHOX gene. Probands's parents were heterozygous for the same deletion by a-CGH. FISH was uninformative, because there was no difference between the intensity of the signals on both chromosomes. Since the primers used were located within the deleted region, molecular studies could not be performed. A-CGH proved to be the most powerful diagnostic tool in this case.

J04.34 Papillon-Lefevre syndrome and an autosomal dominant form of palmoplantar keratoderma in the same Indian family: mutation screening and in-depth bioinformatic analyses of the cathepsin C gene
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Papillon-Lefevre Syndrome (PALS, MIM 245000) is a rare autosomal recessive disorder characterized by palmoplantar keratoderma and early onset severe periodontitis affecting both deciduous and permanent dentition. Several recessive families and sporadic cases with variable clinical presentation have been reported. Several loss-of-function mutations have been identified in lysosomal protease cathepsin C gene (CTSC) in BM1 individuals from various ethnic groups. We describe Papillon-Lefevre syndrome and an autosomal dominant form of palmoplantar keratoderma in the same Indian family (UR075) and present the mutation analysis of the cathepsin C gene. Sequence analysis of known exons and splice sites revealed a homozygous nucleotide substitution G to A at nucleotide position 901, resulting in a mutation from glycine (G) to serine (AGC) at amino acid position 301 (G301S). This mutation was observed in homozygous condition in an affected individual, and excluded other affected with severe palmoplantar keratoderma from the same family. We re-evaluated family UR075 and excluded chromosome 11q14-12q1 region by linkage analysis. This mutation was found to be at a condition
highly conserved residue in of CTSC gene. Structure prediction and energetic analysis of wild-type CTSC, comparison with mutant (G340S) revealed that this change in amino acid does not imply any secondary structural change. However, prediction of functional effect(s) of this mutation is possibly dama-
ing the protein structure and/or function. Additionally, based on the energy calculation and the modeled protein structure of the mutant is ex-pected to be energetically unstable.

**J04.35**

**PROGINS progesterone receptor polymorphism in Systemic Lupus Erythematosus**

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Background: Systemic lupus erythematosus (SLE) is a chronic inflammatory autoimmune disease with an unexplained etiology. Several studies have in-
vestigated the role of steroid hormones and their receptor polymorphisms for the development of the disease. However, the clinical significance of the PROGINS receptor polymorphism of the progesterone receptor gene has not been studied yet, despite the known immunosuppressive actions of the pro-

gesterone. Therefore, the present study aimed to investigate the potential influence of the PROGINS haplotype on the SLE onset and clinical manifesta-

tions. Materials and methods: The PROGINS Alu insertion polymorphism was investigated in 122 Caucasian lupus patients and 105 healthy controls by PCR-RFLP analysis. Results: PROGINS variant allele (Alu ins) was found in 58.3% of the patients. No significant differences in the genotype fre-
quencies of progesterone receptor PROGINS polymorphism in patients and controls were observed, although the prevalence of Alu ins/Alu ins genotype was more common in controls than in patients (4.7% vs. 8.2%, p=0.182).

The progesterone receptor polymorphism did not influence the clinical ma-

**J04.36**

**Detection of rs2073618 polymorphism in the Osteoporoteiner gene in Slovak post-menopausal women**

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Osteoporosis is a common complex disease in post-menopausal women, which is characterized by decreased bone mineral density (BMD) and detoration of skeletal microarchitecture leading to increased bone fragility and fracture. Several studies proved that genetic factors play an important role in the pathogenesis of osteoporosis. Osteoporogen (OPG) is a recently discovered member of the TNF receptor superfamily that acts as important paracrine regulator of bone remodeling. In the present study, we screened rs2073618 polymorphism in the OPG exon 1. Study group included 200 post-menopausal women diagnosed by osteoporotes based on clinical features and radiological evidence. Control group included 200 post-menopausal non osteoporotic women. Genotyping for the presence of rs2073618 polymorphism was performed using the Custom TaqMan®SNP Genotyping assays. The frequencies of investigated genotypes for rs2073618 polymorphism in the group of patients with osteoporosis were as follow: GG (21.0%), GA (56.5%), CC (22.5%), the distribution in control groups was: GG (24.0%), GA (56.0%), CC (20.0%). Hardy-Weinberg equilibrium was tested for each group of participants using χ2 test. All statistical analyses were performed using SPSS 16.0. No differences in genotype or allele frequencies in OPG gene rs2073618 polymorphism between patients with osteopo-

**J04.37**

**Severe osteosclerosis in a patient with trichothiodystrophy**

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Trichothiodystrophy (TTD) is rare autosomal recessive disease that affects DNA repair mechanism. TTD demonstrate great variability in severity and clinical involvement of different tissues and organs - skin and hair, bones, gonads, brain. Several attempts are made to classify TTD, according photo-
sensitivity, age of onset and, more recently, genetic findings. We report on a female patient with severe form of TTD. She is the first child in a family. The baby was delivered in 33 week of pregnancy and had low birthweight and poor acocumulations during perinatal period. The baby suffered from photosensitivity, elongated and diaphyseal dystrophic nails. Ichtiosis was present from the begin-

**J04.38**

**Association between vitamin D receptor gene polymorphisms and chronic periodontitis among Libyans**

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Background: Chronic periodontitis (CP) is an oral disease resulting in in-

**J04.39**

**Frequent mutation Ala597Glu in Alox12B gene at autosomal recessive congenital ichthyosis in patients from Russian Federation**

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Autosomal recessive congenital ichthyosis (ARI) is a heterogeneous group of disorders of keratinization. It divides on several types the main of which are lamellar ichthyosis (LI) and nonbullous congenital ichthyosiform ery-

**Erythematosus**

Erythematosus development as well as on the autoimmune disease susceptibility. The phenotype in Bulgarian SLE patients. Further studies in other ethnic groups inclusions: PROGINS polymorphism is not associated with a specific clinical 

**J03.35**

**Investigation of the relation-

**Conclusion:** Vitamin D receptor Apal SNP C/T rs731236 may be related to the risk of CP in the Libyan population.

**J03.49**

**Severe osteosclerosis in a patient with trichothiodystrophy**

E. Sukarova-Angelovska1, N. Jaspere2, M. Kocove3, I. Stefanovska2, V. Anastasovska2, G. Ilievska2,
J04.40 Molecular-genetic analysis in patients with autosomal recessive osteogenesis imperfecta from Russia
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Osteogenesis imperfecta (OI) is a clinically and genetically heterogeneous brittle bone disorder. Whereas dominant OI is mostly due to heterozygous mutations in either COL1A1 or COL1A2 encoding type I procollagen, recessive OI is caused by mutations in genes encoding proteins involved in type I procollagen synthesis or chaperoning. We aimed to study mutations in CR- TAP, LEPRE1, PPBP and SERPINF1 genes in OI patients. We examined 78 patients with OI and 100 healthy controls corresponding by age, gender, ethnicity and place of residence. We sequenced the coding and exon-flanking regions of CRTAP, LEPRE1, PPBP and SERPINF1 genes. We identified two distinct heterozygous mutations, undescribed before. For the first time previously unreported splicing mutation c.1724+4G>A in LEPRE1 gene was identified in one patient from Tatar population and the c.913C>G (p.Leu305Val) mutation in SERPINF1 gene was observed in patient from Bashkir population. We observed 12 SNPs: rs4234239 and rs586178547 in CRTAP, rs2307247, rs2253557 and rs4904 in PPBP, rs3738499, rs3738498 and rs3738497 in LEPRE1, rs58697961, rs2070112, rs1362687 and rs165833 in SERPINF1 previously described; whereas, the c.1153-780G>A intron 6 of CRTAP gene was novel identified in two patients from one family. Interestingly, rs4904 in PPBP gene was identified in a patient with c.1081C>T (p.Arg36X) in COLIA1; one patient with OI was characterized by rs2307247 in PPBP gene and c.579delT (p.Gly194ValfsX71) in COLIA1. Accordingly, for the first time two novel unreported mutations in LEPRE1 and SERPINF1 genes and one novel SNP in CRTAP gene was observed in Russian patients with OI.

J04.41 A clinical report on congenital joint dislocations: A new association
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A clinical report on congenital joint dislocations: A new association

J05.01 Simvastatin affects ABCA1 expression and cholesterol efflux in THP-1 macrophages by a ROR-Alpha-dependent pathway
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The ATP-binding cassette transporter A1, ABCA1, is a ROR-Alpha target gene that participates in the removal of cholesterol from lipid-laden macrophages, a crucial anti-atherogenic mechanism. Statins are currently the most efficacious therapy for the treatment of hypercholesterolemia and cardiovascular diseases. Some studies have shown that statins decrease ABCA1 expression and cholesterol efflux from human macrophages. However, other studies have reported the opposite effect even in a mouse model of atherosclerosis. In this study our aim was to investigate the ABCA1 expression and apolipoprotein A1 (apoAI)-mediated cholesterol efflux after simvastatin treatment in THP-1 macrophages. Cholesterol is one known ligand of ROR-Alpha and is important in cardiovascular diseases like atherosclerosis. Thus, we further explored the effect of simvastatin on activation of the ROR-Alpha in human macrophages by studying the influence of cellular cholesterol efflux. We observed that simvastatin repressed the expression of ABCA1 gene, and that this repression was partially prevented by ROR-Alpha ligands (especially by SR1001). Furthermore, ligand induced activation of ROR-Alpha by CPG 52608 and SR 1001 increased apoAI-mediated cholesterol efflux in THP-1 macrophages. In conclusion, activation of ROR-Alpha not only increased ABCA1 expression and cholesterol efflux in the absence of simvastatin, but also restored these functions in the presence of simvastatin. With the demonstration of the ABCA1 that involve in the pathogenesis of atherosclerosis might be controlled by an inducible transcription factor, this study offers a potential therapeutic treatment for the disease.

J05.02 Expression of two ABCG1 transporter isoforms in macrophages of patients with atherosclerosis
V. Miroshnikova1,2, E. Demina1, A. Panteleeva1, N. Mayorov2, V. Davydenko1, A. Schwarzman1; 1Pavlov State Medical University, Saint-Retersburg, Russian Federation, 2Saint-Retersburg Pavlov State Medical University, Saint-Petersburg, Russian Federation, 3Institute of Experimental Medicine, Saint-Petersburg, Russian Federation.

The ABCG1 transporter plays an important role in reverse cholesterol transport by mediating the efflux of cholesterol from macrophage foam cells to high density lipoproteins. Two major ABCG1 isoforms exist in humans, which differ by the presence or absence of twelve amino acids between the ATP-binding cassette transporter A1, ABCA1, is a ROR-Alpha target gene that participate in the removal of cholesterol from lipid-laden macrophages, a crucial anti-atherogenic mechanism. Statins are currently the most efficacious therapy for the treatment of hypercholesterolemia and cardiovascular diseases. Thus, we further explored the effect of simvastatin on activation of the ROR-Alpha in human macrophages by studying the influence of cellular cholesterol efflux. We observed that simvastatin repressed the expression of ABCA1 gene, and that this repression was partially prevented by ROR-Alpha ligands (especially by SR1001). Furthermore, ligand induced activation of ROR-Alpha by CPG 52608 and SR 1001 increased apoAI-mediated cholesterol efflux in THP-1 macrophages. In conclusion, activation of ROR-Alpha not only increased ABCA1 expression and cholesterol efflux in the absence of simvastatin, but also restored these functions in the presence of simvastatin. With the demonstration of the ABCA1 that involve in the pathogenesis of atherosclerosis might be controlled by an inducible transcription factor, this study offers a potential therapeutic treatment for the disease.

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The ABCG1 transporter plays an important role in reverse cholesterol transport by mediating the efflux of cholesterol from macrophage foam cells to high density lipoproteins. Two major ABCG1 isoforms exist in humans, which differ by the presence or absence of twelve amino acids between the ATP-binding cassette transporter A1, ABCA1, is a ROR-Alpha target gene that participate in the removal of cholesterol from lipid-laden macrophages, a crucial anti-atherogenic mechanism. Statins are currently the most efficacious therapy for the treatment of hypercholesterolemia and cardiovascular diseases. Thus, we further explored the effect of simvastatin on activation of the ROR-Alpha in human macrophages by studying the influence of cellular cholesterol efflux. We observed that simvastatin repressed the expression of ABCA1 gene, and that this repression was partially prevented by ROR-Alpha ligands (especially by SR1001). Furthermore, ligand induced activation of ROR-Alpha by CPG 52608 and SR 1001 increased apoAI-mediated cholesterol efflux in THP-1 macrophages. In conclusion, activation of ROR-Alpha not only increased ABCA1 expression and cholesterol efflux in the absence of simvastatin, but also restored these functions in the presence of simvastatin. With the demonstration of the ABCA1 that involve in the pathogenesis of atherosclerosis might be controlled by an inducible transcription factor, this study offers a potential therapeutic treatment for the disease.

J04.42 Autosomal recessive congenital ichthyosis: identification of a Spanish family with a new PNPLA1 mutation
L. Fachal1, L. Rodríguez-Pazos2, M. Ginarte3, Á. Carracedo1, J. Toribio3, P. O. Simsek-Kiper1, E. Dikoglu1, B. Campos-Xavier2, A. C. Ceylan1, G. E. Utine1, S. Unger3; 1Fundación Pública Gallega de Medicina Xenómica SERGAS, Grupo de Medicina Xenómica-USC, CIBERER, IDIS, Santiago de Compostela, Spain, 2Dermatology, University Hospital Complex of Santiago de Compostela, SERGAS, Santiago de Compostela, Spain, 3Medical Genetics Unit, Lausanne University, Lausanne, Switzerland.

Autosomal recessive congenital ichthyosis: identification of a Spanish family with a new PNPLA1 mutation

J06.01 Expression of two ABCG1 transporter isoforms in macrophages of patients with atherosclerosis
V. Miroshnikova1,2, E. Demina1, A. Panteleeva1, N. Mayorov2, V. Davydenko1, A. Schwarzman1; 1Pavlov State Medical University, Saint-Retersburg, Russian Federation, 2Saint-Retersburg Pavlov State Medical University, Saint-Petersburg, Russian Federation, 3Institute of Experimental Medicine, Saint-Petersburg, Russian Federation.

The ABCG1 transporter plays an important role in reverse cholesterol transport by mediating the efflux of cholesterol from macrophage foam cells to high density lipoproteins. Two major ABCG1 isoforms exist in humans, which differ by the presence or absence of twelve amino acids between the
Cardiac Surgery, Policlinico Sant’Orsola-Malpighi, Bologna, Italy, 3Clinical Genetics Unit, diomyopathy detected 6 months after birth without any familial history of treated 4 years later but with a failed valve repair. P2 had hypertrophic car respectively. Pregnancies were complicated by hydroamniosis. P1 had a con- -

From Bir Ali Ben Khalifa and no consanguineous parents from Sidi Bouzid, second case are 7-years-old girls born to distant consanguineous parents from birth. We recruited 20 patients who underwent surgery for BAV and TAA to inves- -

Germline heterozygous gain-of-function mutations of the BRAF gene that encode a downstream molecule of RAS in the RAS-MAPK signaling pathway. Germline heterozygous gain-of-function mutations of the BRAF gene that encode a downstream molecule of RAS in the RAS-MAPK signaling pathway. 

Mutations in ACTA2 have been reported as major cause of familiar TAA (up to 15%) of sudden infant death syndrome (SIDS) and 9% of intrauterine fetal demise (IUIDF). Recently, mutations in two genes encoding the calcium- binding protein calmodulin (CALM1 and 2), have been associated with recur- rent cardiac arrest in infants with Long QT Syndrome. Catecholaminergic Polymorphic Ventricular Tachycardia and Idiopathic Ventricular Fibrilla- tion. Calmodulin mutations disrupt Ca2+ signalling in the heart, affecting membrane ion channels’ function and kinase-mediated signal transduction. Given the life-threatening arrhythmias described in infants, it seemed logical to expect that calmodulin could play a role in SIDS and in IUIDF. Mutations in the calmodulin genes (CALM1-2-3) were analysed with Sanger sequencing in a cohort of SIDS cases (n=46) and in a population of IUIDF (n=44; gesta- -

Results: Mutational analysis of the 3 CALM genes did not identify any pa-

Methods: Genomic DNA was extracted from frozen tissue. All three genes encoding calmodulin (CALM1-2-3) were analysed with Sanger sequencing in a cohort of SIDS cases (n=46) and in a population of IUFD (n=44; gestational age at death >20 weeks) classified as “unexplained” after a post-mortem evaluation.

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We report on two families seen at our Regional Cardiac Genetic Service in Glasgow. Both demonstrate clinical variability as well as the potential for congenital heart diseases. Molecular analysis of genes involved in the RASopathy genes (PTPN11, KRAS, HRAS, NRAS, BRAF, RAL1, SOS1, MAP2K2, SHOC2, and CBL) by HRM and direct sequencing revealed hetero-

Background: Cardiac channelopathies are responsible for approximately 15% of sudden infant death syndrome (SIDS) and 9% of intrauterine fetal demise (IUIDF). Recently, mutations in two genes encoding the calcium-binding protein calmodulin (CALM1 and 2), have been associated with recurrent cardiac arrest in infants with Long QT Syndrome. Catecholaminergic Polymorphic Ventricular Tachycardia and Idiopathic Ventricular Fibrillation. Calmodulin mutations disrupt Ca2+ signalling in the heart, affecting membrane ion channels’ function and kinase-mediated signal transduction. Given the life-threatening arrhythmias described in infants, it seemed logical to expect that calmodulin could play a role in SIDS and in IUIDF. Mutations in the calmodulin genes (CALM1-2-3) were analysed with Sanger sequencing in a cohort of SIDS cases (n=46) and in a population of IUIDF (n=44; gestational age at death >20 weeks) classified as “unexplained” after a post-mortem evaluation.

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Results: Mutational analysis of the 3 CALM genes did not identify any pa-
ventricular non-compaction. Clinical heterogeneity is an important feature of DES and MHV7 related inherited cardiac disorders. There is a need for careful assessment of individuals at the Cardiac Genetic Clinic to ensure recognition of these conditions, early diagnosis, appropriate treatment, cascade testing and surveillance for unaffected, at risk relatives.

**J05.08** Chromosome 9p21 rs564398 variant is associated with the internal carotid artery stenosis severity

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Carotid atherosclerosis is atherosclerotic stenosis of proximal internal carotid artery (ICA), and is one of the main causes of stroke. Recent genome-wide association studies (GWAS) revealed chromosome 9p21 INK4B-INK4A is a novel locus for susceptibility to type-2 diabetes and coronary artery disease. INK4A/ARF transcript, p16INK4A, arrests cell cycle progression by inhibiting the activities of CDK4/CDK6. Cell cycle regulation may be an important mechanism in vascular smooth muscle cells for atherosclerosis progression. Thus, we aimed to examine the frequency of the single nucleotide polymorphisms (SNPs) on chromosome 9p21 in carotid atherosclerosis (CA).

The study is composed of 50 symptomatic and asymptomatic CA patients and 53 healthy controls. Genomic DNA extraction was performed from peripheral blood leukocytes. Real-time polymerase chain reaction (RT-PCR) was used to analyze 4 SNPs (rs564398 A/G, rs1075727 z74 A/G, rs2383207 A/G, rs1075727 A/G) in CA patients and controls. Analysis of 4 SNPs revealed a significant difference in the genotype distribution for rs564398 and rs1075727 between CA patients and controls (p=0.027 and p=0.01). However no significant relationship was found in genotype frequencies of rs1075727 and rs2383207 when CA patients and controls were compared (p=0.05). There was also a significant relation in allele frequencies of rs564398, rs2383207 and rs1075727 polymorphisms between CA patients and controls (p=0.016, p=0.049, p=0.001). ICA stenosis severity was found to be associated with the AA variant of rs564398 polymorphism in CA patients (p=0.01).

These results indicate that, chromosome 9p21 rs564398 and rs1075727 polymorphisms may be associated with CA. These findings need to be confirmed by further studies.

**J05.09** ADIPOQ variants in patients with coronary artery disease in Turkish population

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Adiponectin, a hormone produced predominantly by adipocytes, is an essential modulator of insulin sensitivity and has anti-atherogenic and anti-inflammatory properties. Several studies have been performed to investigate the association of genetic variations in the adiponectin with obesity, insulin resistance, and type 2 diabetes (T2D), but few studies were performed in association with coronary artery disease (CAD). Adiponectin is coded by ADIPOQ gene located on chromosome 3q27, consisting of 3 exons and 2 introns spanning a 17-kb region. Among the variations of the ADIPOQ gene reported, rs13730539 (-11391G>A), rs2241766 (+45T>G), rs1501299 (+276G>T), and rs2241767 (+349A>G) have been most extensively studied and are thought to be linked to CAD. In our study, the effects of these polymorphisms on ADIPOQ on CAD were investigated and 125 patients and 123 healthy controls were included. Polymorphisms were screened by PCR-RFLP method. Genotyping data and demographic characteristics were analyzed by the SPSS 24.0 program. p <0.05 was considered statistically significant. No effect of these polymorphisms on CAD was detected in association analysis under additive, dominant and recessive models (p>0.05). While Genotype distributions were in Hardy-Weinberg equilibrium (p>0.05) in patient and under additive, dominant and recessive models (p>0.05). While Genotype distributions were in Hardy-Weinberg equilibrium (p>0.05) in patient and healthy controls, respectively. Data analysis (p-value=0.815) revealed no association between Leu125Val polymorphism of ADIPOQ-1 gene and coronary artery disease. Preliminary results do not reveal an association between Leu125Val polymorphism and coronary artery disease. This study is continuing.

**J05.10** Investigation of association between Leu125Val polymorphism of PECAM-1 gene and coronary heart disease in the Iranian population

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Coronary heart disease (CHD) is a major cause of death in Iran and many other countries. CHD is multifactorial disease, which is probably influenced by a combination of environmental and genetic factors. Several studies indicate that conditions leading to myocardial infarction and death can be prevented by controlling environmental factors and screening for genetic risk factors. Atherosclerosis is the most predominant coronary artery pathology and it seems that Platelet Endothelial Cell Adhesion Molecule-1 (PECAM-1) plays a role in creation of atherosclerotic plaques. In this study, we have investigated the association between Leu125Val polymorphism of PECAM-1 gene and coronary artery disease in Iranian population. Blood samples were collected from 85 healthy controls and 126 angiographically confirmed CAD patients with stenosis in at least one coronary artery. After DNA extraction, the PECAM-1 Leu125Val polymorphism detection was carried out by PCR-RFLP method. Frequencies of GG, GC, CC genotypes were 17%, 35%, and 48% in patients and 20%, 35% and 45% in healthy controls, respectively. Data analysis (p-value=0.815) revealed no association between Leu125Val polymorphism of PECAM-1 gene and coronary artery disease. Preliminary results do not reveal an association between Leu125Val polymorphism and coronary artery disease. This study is continuing.

**J05.11** Association of rs7903146 polymorphism in TCF7L2 gene with myocardial infarction in T2DM patients in Uzbekistan

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The Coronary Heart Disease (CHD) has the similar risk factors with type 2 Diabetes Mellitus (T2DM). A polymorphism in the TCF7L2 gene has been found to be associated with type 2 diabetes in several ethnic groups. Possible relationship between the genetic polymorphism of the TCF7L2 gene and CHD was not clear.

We aimed to determine the association between rs7903146 (C/T) polymorphism in TCF7L2 gene with the development of myocardial infarction (MI) in patients with T2DM in Uzbek population. We genotyped 108 patients divided into 3 groups: I - patients without CHD (n = 26), control; II - patients with CHD without MI (n = 42); III - patients with MI (n = 40). All patients were unrelated, aged over 45 years, and had disease duration over 10 years.

Results of genotyping showed that the CC genotype frequency in 1 group was 15.4% and the CC genotype was present in 70% patients of III group. The frequency of the T allele (83.8%) in patients with MI was significantly higher compared to the group without CHD (65.4%, P <0.05, control). The same, we found significantly lower prevalence of allele C (16.3 ± 4.1%) in patients with MI compared with the control group (34.6 ± 6.6%, P <0.05). The results of our study suggest that TCF7L2 rs7903146 polymorphism is significantly associated with development of MI in patients with T2DM. TT genotype of TCF7L2 gene may be a predictor of the risk of myocardial infarction in patients with T2DM.

**J05.12** GLA nonsense mutation (W162X) and cardiac involvement in heterozygous females

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Background. In the past, medical literature stated that Fabry disease affects only male. Based on X-linked pattern of inheritance the heterozygous females are usually asymptomatic. Recent studies describe Fabry disease in heterozygous females but manifestations tend to occur at a later age than in male and are often less severe in females. The aim of our study was to detect the presence of GLA mutation in females of a family with Fabry disease and correlate with the severity of clinical phenotype. Subjects and methods. Five related females of the same family were enrolled and clinically assessed. Enzyme activity levels were evaluated too. Genetic testing included isolated DNA from blood samples and sequence analysis of all coding exons and all intron-exon boundaries of the GLA gene. Results. All five females were found to be heterozygous for a familial pathogenic GLA mutation (c.485C>A). The mutation caused different low levels of enzyme activity in grandmother, mother, daughter and the two fraternal nieces of the grandmother. None of
J05.13 Polymorphisms in FII, FV and PAI-1 genes in Ukrainian patients with atherothrombotic and cardioembolic ischemic stroke

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Background and aim: Imbalance between coagulation and fibrinolysis factors is known to be a predictor of thrombosis and cardiovascular events. The aim of our study was to compare the genotype distribution of the coagulation factor FII and FV and fibrinolysis factor PAI-1 functional polymorphisms in patients with atherothrombotic ischemic stroke and patients with cardioembolic ischemic stroke with atrial fibrillation (AF).

Methods: 54 patients with cardioembolic ischemic stroke with AF and 60 patients with atherothrombotic ischemic stroke from Ukraine were included in this study. The genotypes of FII G20210A, FV G1691A (Leiden) and PAI-1 5G/4G polymorphisms were determined by PCR analysis based on the banding pattern on gel electrophoresis.

Results: No individuals with FII and FV homozygous mutations were found in both study groups. In cardioembolic stroke patients with AF, 1 (2%) FII and 3 (6%) FV heterozygous mutations were found. In patients with atherothrombotic stroke, 5 (8%) FII and 6 (10%) FV heterozygous mutations were found. Among patients with cardioembolic ischemic stroke with AF, PAI-1 5G/5G, 5G/4G and 4G/4G genotypes were observed in 9 (17%), 23 (43%) and 21 (40%), and in atherothrombotic stroke in 14 (24%), 19 (32%) and 26 (44%) patients respectively.

Conclusion: Our findings suggest that there is a tendency toward a higher frequency of FII G20210A heterozygotes in atherothrombotic ischemic stroke patients compared with cardioembolic ischemic stroke patients with AF. We also observed that FV Leiden heterozygotes are more frequent in cardioembolic stroke with AF. PAI-1 homozygous state is equivalent between the two study groups.

J05.14 The importance of genetic profile for thrombophilia detection in patients with pulmonary venous thrombosis after operation for anomalous pulmonary vein return malformations

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Aim: To present two rare cases of pulmonary thromboembolism in vital vessels after surgery for cardiac malformations, late discovered with positive genetic profile for thrombophilia.

Material and methods: A 3 mo old girl presented with signs of cardiogenic shock and myocardial ischaemia due to totally anomalous pulmonary venous return (TAPVR) in coronary sinus. The second case was a 2 yo girl, with recurrent wheezing, discovered with right pulmonary veins draining in superi- rior vena cava, a partial anomalous pulmonary veins return (PAPVR). Both patients were operated.

Results: First case, two years after cardiac surgery presented with discrete bluish discoloration of the skin, but with normal O2Sat. The pulmonary venous return was redirected to the left atrium. Angio CT detected superior vena cava thrombosis, with reverse flow into axzygos system, and complete thrombosis of the right venous brachiocephalic trunk. Second case, was opera- ted two years after surgery for LPA to left at left atrium. After two years she presented with haemoptisia. Angio CT detected right pulmonary venous trunk embolism, with pulmonary edema in the right lung. She was treated with heparin. In both patients we detected positive genetic predisposition to thrombophilia, that changed the recommendation for anticoagu- lant therapy, for life long.

Conclusion: Patients with operated TAPVR and PAPVR have the risk for pul- monary thromboembolism, which is vital when the collector is redirected to left atrium. Genetic profile for thrombophilia is important to be searched in this type of cardiac malformations. If positive, anticoagulant therapy is life long manda- tory, to prevent thromboembolism and even death.

J05.15 Investigation on Mitochondrial DNA deletions in Iranian Dilated Cardiomyopathy and Hypertrophic Cardiomyopathy patients

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Hypertrophic cardiomyopathy is a genetic disorder with autosomal domi- nant inheritance. The disorder has been estimated to occur in 0.05%-0.2% of population. Recently mitochondrial DNA mutations have been associated with cardiomyopathies. Mitochondria are the major site of energy produc- tion in the cell. Thus it is reasonable to assume that energy dependant tissu- es such as heart, brain, skeletal muscle and endocrine system are affected by mitochondrial dysfunction. Many mitochondrial diseases arise from defects of the mitochondrial respiratory chain. mitochondrial disorders have maternal inheritance pattern. The mtDNA mutation rate is much higher than nuclear DNA due to lack of a repair mechanism and also having no intron. Recent studies have reported materially inherited, non-x linked ACMs are associated with defects in mitochondrial oxidative metabolism. Methods: In this study we screened 52 Iranian hypertrophic cardiomyopathy’s patients for mitochondrial DNA deletions. Results: Mitochondrial DNA deletions were detected by PCR using 6 paired primers. Five different deletions were found in 29 patients (55.8%). Eighteen patients (34.4%) showed 8.5 kb deletion. Twelve (23%) patients had 9 kb deletion. Seven patients (13.4%) had a 7.3 kb deletion. Eight patients (15.5%) had 4977 bp common deletion between nt161-nt13640, and 11 patients had 7.4 kb deletion. Multiple deletions have been found in 11 patients (21.1%). Conclusion: Mitochondria DNA deletions may occur as a result of aging; on the other hand mt deletions may affect myocardium and lead to secondary hypertrophy. However, the question regarding primary or secondary role of mtDNA deletions in hyper- thorphic cardiomyopathy remains unanswered.

J05.16 Novel SMA4 mutation causing juvenile polyposis (JP) and hereditary haemorrhagic telangiectasia (HHT)

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HHT is a genetic disorder with autosomal dominant inheritance pattern, causing abnormal blood vessel formation - multifocal vascular telangiecta- ses and/or arteriovenous malformations(AVMs). The majority of patients harbor mutations in members of the transforming growth factor (TGF)-β pathway - either the endoglin (ENG) oractivin receptor-like kinase 1 (ALK1) genes. Mutations in SMAD4 gene, encoding an intracellular mediator of TGF-β signals, are known to cause JP. Both HHT and JP are uncommon, but a combined syndrome JPHHT has been reported.

We present three generations of HHT family with three affected members. All of them were anaemic, and have been diagnosed with multiple pulmona- ry AVMs, cerebral cavernous haemangiomata was documented in one of them. The proband had a history of gastrointestinal (GI) bleeding and few colon polyps documented at 9 year age. No GI bleeding/polyps were documented in remaining family members. No mutations were found in ENG/ALK1 genes on direct sequencing. We performed SMAD4 direct sequencing on the proband and found a novel frameshift mutation (p.R531GfsX536) in exon 11, leading to stop codon and synthesis of truncated protein.

The clinical importance of SMAD4 mutation finding in HHT has been widely discussed as these patients are likely to be at risk of JPHHT and developing gastrointestinal cancer. Based on our findings, ENG and ALK1 mutation negative HHT patients might benefit from SMAD4 genetic testing. We suggest that HHT patients with SMAD4 mutations should be regularly screened for JPHHT.

J05.17 Next generation sequencing - useful tool in molecular diagnosis of inherited cardiomyopathy

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Cardiomyopathy is characterized by mechanical or electrical dysfunction of cardiac muscle and it is a known risk factor of sudden cardiac death. More than hundreds of variant in 84 genes have been associated with inherited cardiomyopathy. A high number and variability of involved genes complica- te diagnosis of cardiomyopathy. However, existence and availability of new
modern method - next generation sequencing enables analysis of high number of genes at the same time - massively parallel approach. Sequencing DNA libraries in this fashion significantly shortens time and reduces costs of analysis, and makes previously cost prohibitive experiments possible. Hence, the presented diagnostic NGS workflow for the analysis of 46 genes involved in pathogenesis of the inherited cardiomyopathy by using True-Sight Enrichment technology (Illumina) followed by sequencing on the MiSeq (Illumina). This pilot study comprised group of 40 unrelated patients with DCM, ARVC and ventricular fibrillation. We detected several different variants, some of these variants were common and already published with proven association with development of cardiomyopathy and some of them were classified as new variants, where co-segregation analysis in pedigrees has to be performed. All detected variants classified as pathological, like pathological and variants of unknown clinical significance (VOUS) have been confirmed by classical Sanger sequencing. Our results demonstrate that this is a sensitive and robust assay with an average of 95% of target regions consistently covered to x20 depth. This diagnostics approach can be a powerful tool for identifying presymptomatic individuals in families.

**J05.18 The relationship between PIA1/PIA2 Glycoprotein IIIa genetic polymorphism and ischaemic stroke in Northern Romania**

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Introduction: Ischaemic stroke is one of the leading causes of disability and death in Romania. Platelets play a key role in generating the protective hemostatic plug that prevents blood loss at sites of vascular injury. The identification of functional prothrombotic polymorphisms during the past decade encouraged the study of common polymorphisms affecting platelet glycoproteins (GP) in stroke. The aim of the present study is to evaluate the relationship between PIA1/PIA2 Glycoprotein IIIa gene polymorphism and ischaemic stroke in a Northern Romanian population group and to determine whether it has an influence on the risk of cerebral events. This is a cross-sectional, randomized, case-control study. The amplification of the relevant gene fragment was performed from both directions on an ABI3500xl autosequencer. Results: Molecular analysis did not reveal an increased frequency of A1A2 mutant genotype in the study group compared to the control group (p = 1.000, OR = 0.951, CI = 0.504-1.797).

Conclusions: We found no significant differences in distribution of the PIA1/ PIA2 Glycoprotein IIIa gene polymorphism between ischemic stroke patients and controls.

**J05.19 Nonimmune hydrops fetales atypical presentation of Milroy disease**

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Milroy disease, is an autosomal dominant disorder characterized by typical phenotype of infantile onset lower-limb lymphedema accompanied by variable expression of recurrent episodes of cellulites, toenail changes, and palmoplantar keratodermia. Mutations in the vascular endothelial growth factor receptor 3 (VEGFR3) have been identified as a genetic cause of Milroy disease. We report a case of 22 week old foetus with bilateral pleural effusion, that has been confirmed by classical Sanger sequencing. Although in utero hydrothorax and hydrops fetalis is a rare manifestation of Milroy syndrome, should be taking into account when facing a case of in utero nonimmune hydrops fetalis. A detailed family history could be the clue to reach de diagnosis, although de novo cases have been described.

**J05.20 MTHFR and NNMT gene polymorphisms and conotruncal heart diseases**

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Congenital Heart Defects are the most common congenital anomalies with a worldwide 1% prevalence and are a big part of childhood mortality and morbidity. The etiology of conotruncal heart diseases is complex, with both environmental and genetic causes. Hyperhomocysteinemia which is greatly accompanied with the defects of folic acid metabolism, is known to cause conotruncal heart anomalies. In this study we have evaluated three polymorphisms in two hyperhomocysteinemia related genes, such as Methylenetetrahydrofolate Reductase (MTHFR C677T and A1298C) and Nicotinamide N-methyl Transferase (NNMT rs694539) in 79 children with conotruncal heart disease (CHD) and 99 children without CHD. We found no association in case and control groups for MTHFR C677T and NNMT rs694539 polymorphisms, while for MTHFR A1298C polymorphism we found a significantly higher frequency of C allele, suggesting that C allele might be a risk factor for CHD.

**J05.21 Mutation analyses of PTPN11 and SOS1 genes in Belgian children with clinical features of Noonan/LEOPARD syndrome**

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Noonan syndrome (NS) and LEOPARD syndrome are genetically heterogenous autosomal dominant disorders associated with gain-of-function mutations in various genes encoding proteins of the RAS/MAPK signaling pathway, mainly in PTPN11, SOS1 and RAF1 genes. 8 Belgian patients with Noonan/LEOPARD syndrome were screened for mutations in PTPN11 gene: 6 patients with clinical features of NS (2 family cases with mother-daughter pair, 2 sporadic cases) and 2 patients with clinical features of LEOPARD syndrome (a family with father-daughter transmission). All of the patients had cardiac diseases (pulmonary valve stenosis, septal defect, cardiomyopathy) and characteristic minor abnormalities. Leukocyte genomic DNA was amplified for the 15 exons and flanking intronic sequences of PTPN11 gene by PCR. The PCR products were sequenced from both directions on an ABI3500xl autosequencer. Four different PTPN11 mutations were identified in 7 out of 8 patients with clinical features of Noonan/LEOPARD syndrome (4 probands, 3 relatives available for testing). All mutations were heterozygous missense mutations: A3n588ys (new genetic variant c.1747C>A), Asp616Gly, Asn388Asp, Thr468Met, and clustered either in exon 3 encoding the N-SH2 domain or in exons 8 and 12 encoding the PTP domain. One out of 8 patients does not carry mutation in PTPN11 gene. Therefore, we searched for mutations in the second frequently mutated gene SOS1 - underscribed heterozygous mutation (c.797_798delCinsAAGTA) was found in exon 6 of SOS1 gene encoding the DH-domain. At the age of 1.6 years old patient showed typical NS phenotype without ectodermal abnormalities. Detection of new mutation is important for further delineation of genotype-phenotype correlations.

**J05.22 IL-6 gene polymorphism (-174G/C) in Romanian patients with ischemic stroke - results from a pilot study**

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Stroke, mostly of ischemic origin, is considered a major cause of mortality worldwide. Interleukin-6 (IL-6) is demonstrated to be associated with atherosclerotic disease, also it is considered a key mediator of inflammation in cerebral ischemia. IL-6 single nucleotide polymorphism (SNP) -174G/C was found to be associated with several atherosclerotic diseases; its relation to ischemic stroke is conflicting. We aimed to investigate the role of -174G/C IL-6 SNP in ischemic stroke sus-
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and prognosis

Allelic variants of IL8 and IL10 genes influence ischemic stroke risk

informatics. 2006; 22 (15):1928-1929

coronary artery disease and periodontitis

variants may be considered the genetic markers of AIS development risk.

infarction zone expands. High levels of anti-inflammatory IL-10 may pre-

role in post-stroke improvement (state severity assessed using RANKIN

may result in aberrant initial inflammatory ischemia response. Genotype

individuals is 5-fold increased (OR=5,71; 95% CI: 1.48-22.11). These genotypes

the homeobox gene

MEST

by bisulfite pyrose-
J05.27 Prevalence of copy number changes and loss of heterozygosity in atherosclerosis

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Numerous studies have relied on the idea that atherogenesis has molecular similarities with cancer. It is possible that genomic microstructural alterations, characteristic of various neoplasms, are also present in atherosclerotic lesions. In this study we used, for the first time, array-CGH for detection of DNA copy number variations (CNVs) and copy-neutral loss of heterozygosity (cnLOH) patterns in patients with coronary heart disease undergoing coronary artery bypass graft surgery. The SurePrint G3 Human CGH+SNP 2×400K microarrays were used for DNA testing from patients’ white blood cells (WBC, n=5), right coronary arteries in the area of atherosclerotic plaques (CAP, n=5), and internal mammary arteries (IMA, n=5). Polyploidy was observed in CAP of one patient. We detected the multiple CNVs and cn-LOH in all analyzed tissues from patients with atherosclerosis. Right coronary arteries in the area of atherosclerotic plaques presented a higher average CNVs length and number of genes located in their vicinity in comparison with other tissues that may support the notion of higher genomic instability. The majority of CNVs (68±9%) were identified in disease affected tissues, suggesting that some variations might represent somatic events. The gains in 3p21.31 (CASCAD2), 7q32.1 (FLNC), 19p13.3 (PIP5K1C), and 21q22.3 (COL1A1) were detected in the vascular tissues but not in WBC. We identified gain 7p15.2 (SKAP2) in all tissues that not reported with any CNV regions currently reported in the Database of Genomic Variants. CN-LOH were detected in 12 out of 13 chromosomal regions involving tumor-suppressor genes, such as SRPR1, CEBPD, RB1CC1, DIRAS3, TUSC3 and ZDHHC2.

J05.28 The effect of endogenous opioids on apoptosis of cardiomyocytes in a rat model of cirrhotic cardiomyopathy

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The aim of our study was to investigate the impact of endogenous opioids on apoptosis in cirrhotic cardiomyopathy. Cirrhosis is known to be associated with various manifestations of cardiovascular dysfunction (cirrhotic cardiomyopathy). Some possible pathogenic mechanisms have been reported and still more details should be explored.

Aim: To explore the contribution of endogenous opioids in the apoptosis process in a rat model of cirrhotic cardiomyopathy.

Material and Methods: Cirrhosis was induced in rats by bile duct ligation (BDL) and resection. Cardiomyopathy was confirmed using trichrome staining for fibrosis. Naltrexone, an opioid antagonist was administered for 29 days. Apoptosis was detected using TUNEL assay. For molecular analysis, expression of BCL2, Caspase3, Fas and FasL, was explored using reverse transcriptase real-time PCR.

Results: Left ventricular (LV) wall thickness was significantly (p<0.001) lower in the BDL group than the sham group, either receiving naltrexone or saline. Apoptosis density was significantly increased in BDL-saline group (P<0.001) vs. sham-saline group. Cardiomyocyte apoptosis was significantly decreased in the BDL-naltrexone group compared to BDL-saline group (P=0.001). There was no significant change in apoptosis density in sham groups receiving either naltrexone or saline. BDL-saline group showed a significant over-expression of BCL2 and FAS and down regulation of caspase-3 by a factor of 1.44 (p<0.001) compared to sham-saline group, 1.3 (p<0.001) compared to BDL-naltrexone group and 0.77 (p<0.001) compared to sham-naltrexone group, respectively. No significant change was observed in the other 4 analysis for BCL2, caspase3 and FAS.

Conclusion: Apoptosis occurred during cirrhotic cardiomyopathy through both intrinsic and extrinsic pathways activation and endogenous opioid receptors blockade using naltrexone decreases its amount.

J05.29 Association of CELSR2 gene with Coronary artery disease, replication in Tehran Lipid and Glucose Study (TLGS)

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The aim of this study was to further elucidate the mechanism of IGF-I receptor and its interaction with CELSR2 gene in the development of insulin resistance and type 2 diabetes (DM2), and the increased risk of cardiovascular disease associated with obesity. Omentin-1 is one of the adipokines that is down-regulated in association with obesity-linked metabolic disorders including insulin resistance, glucose intolerance and DM2. The nuclear receptor peroxisome proliferator-activated receptor y (PPARy) is a ligand-dependent transcription factor that acts as a primary regulator of adipogenesis, adipocytes metabolism, insulin action. The link between PPARy and Omentin-1 is unclear today. The aim of our work was to estimate
the Omentin−1 gene, PPARγ mRNA levels in visceral fat in individuals without DM2 and cardiovascular dysfunction as well as serum Omentin−1 levels. We generated the visceral fat of 30 individuals without DM2 and cardiovascular dysfunction (mean age 45±9, 9 males, mean BMI 32±9). Visceral fat was recruited on 18 patients and another 12 patients during laparoscopic cholecystectomy in non-acute period of gall-stone disease. PPARγ, Omentin−1 mRNA levels were estimated by RT−PCR with TagMan Probes. G protein mRNA levels (GNB2L1) was used as internal control. Serum Omentin−1 levels were determined by ELISA. Using Spearman correlation analysis positive association between PPARγ mRNA levels and Omentin−1 mRNA levels (r = 0.443; p=0.044) and PPARγ mRNA and serum Omentin−1 levels (r = 0.493, p=0.027) were found. This is the first report about the link between gene expression PPARγ and Omentin−1 gene in visceral adipose tissue of individuals without DM2 and cardiovascular dysfunction.

J06.05 Association of EPCR gene A1 and A3 haplotypes in Iranian patients affected with combined hyperlipidemia

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Disorder of lipid metabolism, especially hyperlipidemia is among the major human health problems. Hyperlipidemia is a disorder of lipid metabolism leading to abnormal rise in fatty substance in circulating blood lipids. The studies that have been done in our country in the recent decade indicate a high prevalence of hyperlipidemia and resulting clinical complications including cardiovascular diseases. Hyperlipidemia broadly occurs for two reasons: genetic factors and different environmental factors. Today, various genes have been identified in which mutation can cause hyperlipidemia disease among which reference can be made to clotting-factor genes such as EPCR. EPCR gene, as one of the major factors in the control path of thrombosis, is considered as a receptor of protein C in endothelial cells, and its connection with cardiovascular diseases has made us embark on the possible survey of EPCR in hyperlipidemia diseases given the relationship between hyperlipidemia and cardiovascular diseases. The samples include 100 infected and 100 normal individuals from among the patients who have come to the Center for Endocrine Research of Shahid Beheshti Medical University of Tehran. After genetic counseling, samples are taken from the individuals in question and the samples are sent to the laboratory. Primer design is performed to determine the genotype of the samples based on the molecular methods study of ARMS PCR. The results indicated significant difference between the infected and control groups in A1 Haplotype and in A3 Haplotype(p<0.05). Therefore, EPCR gene polymorphisms is irrelevant with susceptibility to family combined hyperlipidemia in the Iranian population.

J06.06 Distribution of 1172N missense mutation in Macedonian and Serbian simple virilizing 21-hydroxylase deficiency patients

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Background: Steroid 21-hydroxylase deficiency is an autosomal recessive disorder, present in 90−95% of all cases with congenital adrenal hyperplasia (CAH). Its classical simple virilizing (SV) form leads to virilization of external genitalia in newborn females and pseudo precocious puberty in both sexes, due to reactive androgen overproduction. Mainly associated with SV CAH is 1172N missense mutation at codon 4 of the CYP21A2 gene that produce enzyme retaining 1−2% of normal activity.

Method: In 16 Macedonian and 5 Serbian patients suffering from SV CAH, we have performed molecular detection of the 1172N mutation, using the PCR/AGS method. Patients were diagnosed according to standard clinical criteria at the Department of Endocrinology and Genetics, University Children’s Clinic, Skopje, Republic of Macedonia and Institute for Mother and Child Health, Belgrade, Serbia.

Results: The 1172N mutation was observed in 25% (4/16) of Macedonian SV patients on 18.8% (6/32) of the alleles. Two of them were homozygotes for 1172N, one compound heterozygote with P30L on the second allele and one was heterozygote without observed other mutation among tested nine different genes. The 1172N mutation was detected in only one Serbian SV patient (20%) in homozygous state.

Conclusion: The 1172N mutation distribution in Macedonian and Serbian SV patients was slightly lower than reported in the European population of the simple virilizing patients. However, our findings suggest that this mutation in simple virilizing females is more frequent than in males.

Key words: Congenital adrenal hyperplasia, CYP21A2 gene, 1172N mutation.

J06.02 Molecular analysis in X-linked adrenoleukodystrophy patients: Identification of a novel mutation

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X linked adrenoleukodystrophy (X-ALD) is a neurodegenerative disease characterized by progressive demyelination of the central nervous system, adrenocortical insufficiency and elevated levels of very long chain fatty acids (VLCFA). It is caused by mutations in ABCD1 gene located at Xq28. More than 1300 mutations have been identified to date without any genotype-phenotype correlation. In this study we report the mutational analysis of 3 X-ALD patients (1 male and 2 females) showing variable clinical spectrum. In one of the female patients previously reported heterozygous p.W132X mutation was detected. In the other female patient showed IVSS-6delC (c.1490-6delC) and p.P543L variations in compound heterozygous state. The male patient was found to be hemizygous p.R104P mutation that was not reported previously. In conclusion the cases presented in this paper may contribute to the mutation and clinical spectrum of X-ALD while defining a novel mutation and a female case presenting cerebral symptoms.

J06.03 Nutritional variations in pcu children

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We have invested in the biological diagnosis of Phenylketonuria (PKU) for many years. We performed nutritional blood tests on 30 children with PKU, who had been placed on a diet poor in Phenylalanine for at least 12 months. The age of these children was between 15 months and 10 years old. Following our nutritional study, which included determination of the calcium phosphate product, determination of iron balance and the dosage of vitamins B9, B12 and D parallel to a determination of phenylalanine concentration, calcium phosphate product, determination of iron balance and the dosage of vitamins B9, B12 and D parallel to a determination of phenylalanine concentration, we have performed molecular analysis of the PKU children. The age of these children was between 15 months and 10 years old.

J06.04 Rare association between thyroid tumor and Anderson-Fabry disease

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− No neoplasm in the affected family members known. Later patient developed acroparesthesia, hypertrophic cardiomyopathy (HCMP) and angiokeratomas, uncle from mother side has HCMP and end stage renal disease. Also 2 family member (men) died early through unknown reason. No neoplasm in the affected family members known. Later patient developed acroparesthesia, pain in legs, liver and erythrocyte sedimen-
The effect of Sodium Butyrate on the expression of liver specific urea cycle genes (CPS 1, OTC) in human lymphocytes

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Background: In cystic fibrosis liver disease is poorly controlled. Aim study was to implementation the chronic diarrhea is poorly controlled. Aim study was to implementation the chronic diarrhea is poorly controlled.

Introduction: At present the only method for enzyme assay and confirmation of mutations that causing splicing error in CPS 1 and OTC urea cycle genes is liver biopsy. Therefore developing a noninvasive method for evaluation of gene expression in lymphocytes can be an efficacious method in diagnosis of urea cycle disorders. Induction of gene expression using Histo-ne deacetylase inhibitors is a usual method. we have assessed the OTC and CPS 1 expression level after treatment of human lymphocyte cell line with sodium butyrate.

Material and Methods: MTT assay was done to check cell cytotoxicity. Then Quantitative assessment of gene expression was done over non-cytotoxic time-concentrations using SYBR-Green Real-time RT-PCR method.

Results: After 48 hours, treatments more than 10 mM, lead to cell injury in human lymphocyte cell line by increasing cell granularity and decreasing cell adhesion. MTT assay also confirmed that 1 and 5 mM concentrations are not cytotoxic. Sodium butyrate significantly increased CPS 1 gene expression (p-value <0.05). This increment was near the expression level in Hep-G2 cell line specifically at 5 mM concentration after 48 hours. In addition sodium butyrate treatment induced OTC gene expression in human lymphocyte cell line.

Conclusion: This study showed that sodium butyrate can induce and or increase expression of two urea cycle specific genes (OTC and CPS 1). The most induction effect occurred in 5 mM treatment after 48 hours. Key words: CPS 1, OTC, Sodium Butyrate

Cystic fibrosis liver disease-ultrasound evaluation

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Objectives: Cystic fibrosis (CF) is characterized by clinical polymorphism impaired absorption of nutrients by chronic obstructive pulmonary disease and pancreatic insufficiency or chronic liver disease that is associated with secondary diabetes. Early diagnosis and proper monitoring of all diseases and aims to extend the quality of life of these children. Ultrasound is a very useful method for the diagnosis neivazivă these complications.

The purpose of this paper is to evaluate hepatopathy associated with cystic fibrosis using ultrasound.

Methods: 158 patients who were registered with the National Center for Mucoviscidioza (Cystic Fibrosis) Timisoara were evaluated by ultrasound in addition to clinical examination and biological assessment. Ultrasoundoscopy Williams score has been used for the assessment of liver and in some cases hepatic transient elastography.

Results: The prevalence of liver disease was 32.27 % (51 patients). Most patients 62.74 % (32 patients) had moderate liver disease, a rate of 9.8% associated multilobar cirrhosis. There was a good correlation between Williams ultrasound score and the detection of fibrosis by transient elastography, with a better sensibility of the last method.

Conclusion: The frequency of cystic fibrosis associated liver disease is significant. Ultrasound is an extremely effective method for the diagnosis and monitoring of hepatobiliary disease in cystic fibrosis. Early detection of the disease allows the establishment of an appropriate treatment options with improved life expectancy of these patients.

Cystic fibrosis and cow’s milk allergy

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Background: In cystic fibrosis an important feature is the pancreatic insufficiently expressed by steatorrhea. In some cases, despite enzyme supplementation the chronic diarrhea is poorly controlled. Aim study was to evaluated the presence of cow’s milk allergy among cystic fibrosis children. Methods: In one year, sixty seven children with cystic fibrosis, aged 1month-3 years (19 infants), followed in our center were observed for CMPA. Cow’s milk allergy was suspected in the presence of specific clinical manifestation (gastrointestinal symptoms, cutaneous signs, respiratory features), in cases with a suggestive medical history (failure to thrive, colics etc). In addition CMPA-specific IgE (a nd allergoglobin, casein) and diagnostic elimination test + food challenge test.

Results: Among infants, CMPA was diagnosed in 47.3.6% (9 patients) of children, predominantly in pancreatic insufficient children (77% -CF infant with pancreatic insufficiency). Toddlers (1-3 years) were diagnosed in a smaller percent, only 16.6 % (8 children) proved to have CMPA, although in more than 35% of toddlers, a positive history for CMPA diagnosis was found. Cystic fibrosis patients with pancreatic insufficiency associated more frequently CMPA (92.23%) than cystic fibrosis patients pancreatic sufficient. The prevalence of cow’s milk allergy was important, cumulating a 25.37% of CF patients.

Conclusion: Cow’s milk allergy was frequently found in CF children, especially associated with pancreatic insufficiency. The „combined” enteropathy could influence the disease’s outcome and should be considered especially in persistent diarrhea of CF children with correct enzyme supplementation.
Adipocytes mitochondrial DNA copy number variations in metabolic syndrome patients

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Defects in mitochondrial functions play an important role in the metabolic syndrome pathogenesis. Using droplet digital PCR method we showed difference in fat tissue (greater omentum, hypodermic adipose tissue and mesosetium) mtDNA copy number variations in metabolic syndrome patient ranked by body mass index (BMI). One hundred subjects were recruited from Kaliningrad Regional Hospital, Kaliningrad, Russia during 2012-2013. This group consisted of 20 subjects without metabolic syndrome (BMI ≤ 24.9 kg/m²) and 80 subjects with metabolic syndrome. The last one was ranked by BMI into four subgroups: pre-obese (BMI 26-30 kg/m²), obesity first degree (BMI 31-35 kg/m²), obesity second degree (BMI 36-39.9 kg/m²), and obesity third degree (BMI ≥ 40 kg/m²). The adipose tissue was taken from the patients during planned laparoscopic operations. We showed a tendency to reduce the mtDNA copy number, which was observed with increasing body mass index.

Identification and functional analysis of a novel mutation in methylmalonyl-CoA mutase gene (MUT gene) causing methylmalonic acidemia

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Methylmalonic acidemia (MMA) is one of the inborn errors of metabolism, caused by either deficiency of the enzyme methylmalonyl-CoA mutase, or a defect in the biosynthesis of its cofactor, adenosyl-cobalamin. At least 200 mutations in the methylmalonyl-CoA mutase gene (MUT gene) have been identified in patients with methylmalonic acidemia. Here we present a novel mutation in the MUT gene of a 3 years old Iranian girl with metabolic acidosis. The patient had history of recurrent attacks of severe metabolic acidosis. Tandem mass spectrometry showed elevated propionyl carnitine (C3) and the urine organic acid analysis confirmed the diagnosis of MMA. She was screened for mutations in the MUT gene. A novel C to G nucleotide change was found in position -3 of the acceptor splice site in intron 12. The heterozygosity of both parents was confirmed. In order to demonstrate the possible effect of this nucleotide change, an in silico analysis was done using CBS prediction site (http://www.cbs.dtu.dk/services/Signal2). This analysis suggested that the reported nucleotide change can create abnormal splicing pattern. To confirm abnormal splicing from the mutant allele, total RNA was extracted from patients’ father peripheral blood. cDNA synthesis was done. Appropriate primers were designed to amplify fragments from the region representing normal and aberrant variants of mRNA. Amplified products were sequenced. This experiment, clearly confirmed the retention of intron 12, caused by the reported nucleotide change, in sample obtained from patients’ father, consistent with the role of reported change in causing MMA.

Study of nuclear gene POLG with mitochondrial function in mitochondrial diabetes

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Testing the impact of genetic and non-genetic factors on common forms of obesity from Romania

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In this context, a Tunisian patient with mitochondrial DNA deletions, suffering from mitochondrial diabetes was studied: searching for mutations and polymorphisms in POLG gene, by PCR using specific primers followed by sequencing. The results showed the complete absence of mutations in exons 13, 14, 19, 20 and 22, however, we note the presence of a heterozygous profile (10/11) of the CAG repeat different cases of normal control, insertion of four bases c2734 +37 +2734 +38 ins AGGT at intron 17 and a polymorphism described c3708 G>T in the heterozygous state (rs3087374) at exon 23.

In this study we want to look if there is any correlation between mutations found at the nuclear POLG gene and mutations and deletions already found at the mitochondrial DNA of the patient.
Obesity is one of the greatest medical challenges of the 21st century and its prevalence is continuously increasing. It has a complex determinism that involves genetic and non-genetic factors. The aim of this study was to evaluate the impact of several genetic and non-genetic factors on common forms of obesity from Romania.

Unrelated obese patients (n=80) and healthy normoponderal subjects (n=80) were selected. Biological samples and clinical information was collected after informed consent was obtained. The genetic markers tested were Insulin -23Hpa, IGF2 Apa I, SELL P213S, TGFβ C-509T, HSPG BamH1 and IVS2+5C

Multiple linear regressions showed a relationship between obesity, cholesterol levels and triglyceridemia. The infection with Torque teno virus did not show a significant association with obesity. Genetic polymorphisms did not significantly alter the risk of obesity. However, statistical analysis showed an increased association between several genetic markers and teeth damage risk (HSPG BamH1, TGFβ C-509T SELL P213S, and IGF2 ApaI) and 2D/4D ratio (IGF2 ApaI) in our subjects.

The complex interplay between genetic and non-genetic factors plays an important role in modulating obesity risk. Nonetheless, further information is needed to accurately assess the impact of these factors on common forms of obesity.

**J06.19**

*A novel mutations in Iranian family with Phenylketonuria*

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Phenylketonuria ([PKU; MIM# 261600]) is an autosomal recessive inborn error of metabolism resulting from a deficiency of PAH. Since 2005, PKU screening program was performing in Iran. According to the national databases a number of mutation has been identified throughout the PAH gene.

We have investigated a consanguineous family referred to our center, as a center for national PKU screening program. Blood sample were collected after informed and written consent was obtained. Isolated genomic DNA derived from subjects was amplified using intronic primers. The entire sequencing of the PAH gene including coding region and exon-intron boundaries were analyzed by PCR and Sanger sequencing.

Molecular analysis revealed a heterozygote mutation (c.366del.4765) in parents and homozygote mutation in the proband. This is the first report of this mutation in Iranian population.

**J06.20**

*Phenylalanine hydroxylase (PAH) gene mutation spectrum in patients with PKU in Karachay-Cherkessia Republic, Russian Federation*

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Phenylketonuria (PKU) is the most common inborn error of amino acid metabolism in Europeans. PKU is classified by the severity of hyperphenylalaninemia in patients. Phenylketonuria is caused by a high variety of mutations in the PAH gene (up to date more than 550 mutations is known).


In addition three different mutations were found: p.R413P on 5 chromosomes (8,9%), p.F331S on 2 chromosomes (3,6%) and novel mutation p.R211L on one chromosome. Mutation p.R211L was not detected in normal controls. A mutation detection rate 96,4% was achieved.


**J06.21**

*A Whole mitochondrial genome and POLG gene screening in a Tunisian patient with mitochondrial myopathy and Diabetes*

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Inherited mitochondrial diseases can be caused by mutations of mitochondrial DNA or of nuclear genes that encode mitochondrial proteins. Although many mitochondrial disorders are multisystemic, some are tissue specific .Mitochondria-related myopathies (MM) are a complex and heterogeneous group of neuromuscular disorders defined by a varying degree of dysfunctions of the mitochondrial respiratory chain (OXPHOS).

In the present study, we performed the clinical, genetic, and molecular characterization of a Tunisian patient with clinical features of mitochondrial myopathy associated with Diabetes. The aim of the study was to study the mtDNA extracted from the blood leukocytes in the studied patient revealed various reported polymorphisms in the D-loop region, the ribosomal and transfer RNAs but also the coding genes. The detected missense substitutions, especially the 8701A>G(T59A), the 8860A>G(T112A), the 10086A>G(N10D), the 13105A>G(I257V), the 14766C>T(T7I), the 15940delT in the MT-ATT and the 15940delT in the MT-ATT were previously described in association with Mitochondrial myopathy, Leigh syndrome, cardiomyopathy, muscle pathology and unaffected individuals. Indeed, we detect the 15940delT in the MT-ATT was previously described in association with Mitochondrial myopathy. In addition, we carried out a mutational analysis of POLG gene encoding the mtDNA polymerase gamma to look for an eventual implication of nuclear gene in the mitochondrial diseases.

The results of direct sequencing of all the POLG exons showed the presence of the known heterzygote variation (14766C>T(T7I), the 15940delT in the MT-ATT and the 15940delT in the MT-ATT) and the presence of a novel polymorphism (8860A>G(T112A)), and a novel mutation (15940delT).

The results of direct sequencing of all the POLG exons showed the presence of the known heterzygote variation c.2492A>G in exon16 which substitutes the conserved amino acid Tyrosine 824 to Cysteine.

**J06.18**

*The importance of NMR Spectroscopy in diagnosis of some inborn errors of metabolism: lessons from hyperammonemia condition, galactosemia, and alkaptonuria*

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Metabonomics is a powerful tool for identifying any disturbances in normal homeostasis of metabolic processes and this emerging fields, in which a large number of small-molecule metabolites are detected quantitatively in a single step, promises immense potential for early-diagnosis, monitoring, and understanding the pathogenesis of many diseases. In many errors of inborn metabolism, the relationship between disease state, metabolic biomarker and genetics is easily understood, but the clinical/ biochemical findings in some inborn errors of metabolism (IEM) are often nonspecific; an early differential diagnosis made in a single urine sample it gives an important advantage.

We present the spectrum of metabolites of urine from 1 year-old girl with stroke-like episode, elevated transaminases, coagulopathy, being an advantage. We present the spectrum of metabolites of urine from 1 year-old girl with stroke-like episode, elevated transaminases, coagulopathy, being

**J06.22**

*Clinical complexity of Prader Willi Syndrome- genotype-phenotype correlations*

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Background: The genetic variability of Prader Willi Syndrome (PWS) has a high impact for the phenotypic features.

Aims: We aim to evaluate the genotype-phenotype correlation of a Romanian PWS patients group.

Methods: The study group had 34 PWS patients, boys and girls: subgroup A- 21 patients with chromosome 15 deletion and subgroup B- 13 patients with positive methylation test. The mean age was 10.7±5.287 years and 8.55±5.4.58 years. We compared the PWS characteristic features between the two subgroups. Results: The clinical score mean was 9.083±1.717 for subgroup A compared to 13.02±1.92 for subgroup B. All patients from deletion subgroup had facial dysmorphism. Hypogonadism and hair and skin hypopigmentation were
more frequent in subgroup A. Both groups presented sleep disorders (75%). The hyperphagia onset was almost the same for both groups (around the age of 2 years) with a similar fat mass distribution. All patients from subgroup B and 80.95% from subgroup A had small hands and feet; thick saliva and verbal coordination problems in subgroups B and C. Common findings in picking and ocular abnormalities had a higher prevalence in subgroup A. We didn’t identify significant differences between the two groups regarding the spine and bone density.

Conclusions: The results highlight the clinical variability of PWS. The differences can be caused by qualitative change of paternal gene expression located outside the critical region and missing in patients with uniparental disomy. A reduced life expectancy is correlated with a severe phenotype.

J06.23
Familial hyperoxaluria and infertility: is there a correlation?
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Hyperoxaluria (PH) is an autosomal-recessive disorder of endogenous oxalate synthesis characterized by accumulation of calcium oxalate primarily in the kidney; and leads to nephrocalcinosis and end-stage renal disease. A wide spectrum of associated anomalies may be present (cardiovascular; skeletal; neurological), and there are three forms of primary hyperoxaluria in which the underlying defects and clinical onset and severity have been identified.

It's about a 31-years-old man, with history of recurrent urolithiasis for who chromosomal investigation was carried out to explore a male infertility of 1 year and half related to azospermic profile at the semen level, varicocele and microlithiasis on testicular ultrasound. Cyto genetic analysis carried out using RHG banding, disclose the presence of klinefelter syndrome (47,XXY) despite the normal morphotype. At the genetic counselling, the patient had non consanguineous parents and familial history showed similar recurrent lithiasis in his mother and brother, who was infertile since 2002. To my knowledge, this is the first case of familial hyperoxaluria; especially the type III (HP3; OMIM #613616); a rare and middle entity with mitochondrial transmission, which is associated with klinefelter syndrome. The last is often associated with testicular micro lithiasis; a rare entity: which may be the consequence of overproduction of oxalate and the cause of infertility. The delineation of diagnostic both by genetic and histopathological screening is mandatory in order to clarify the genotype phenotype correlation. Therefore; we insist in the comprehensive evaluation of infertility at cytogenetic (chromosomes abnormalities?) and molecular level (mosaicism?), since it’s a heterogeneous and complex disease.

J06.24
Mutations of mitochondrial genome and atherosclerosis of coronary and carotid arteries.
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Aim. To evaluate the association between the level of heteroplasmy for mitochondrial mutations C3256T, G13513A, G14846A, G12315A in human white blood cells and presence of atherosclerosis in coronary and carotid arteries.

Methods. We included 130 patients (mean age 55±9 years, 116 men) with coronary heart disease (CHD) verified by angiography. Control group consisted of 63 subjects without angiographic coronary artery disease. DNA samples were obtained from whole venous blood using commercially available kits for DNA extraction. For the amplification of fragments of mitochondrial DNA by polymerase chain reaction method followed by pyrosequencing, the corresponding primers and conditions were used. By the basis of pyrosequencing data analysis, the level of heteroplasmy for C3256T, G13513A, G14846A, G12315A mutations in DNA samples were calculated. Results. The level of G13513A and C3256T heteroplasmy was significantly higher (p=0.03; p=0.01, respectively) and level of G12315A heteroplasmy was lower (p=0.004) in CHD patients versus control group. There was significant correlation of carotid atherosclerosis severity and levels of C3256T (r=0.49, p=0.0001), G14846A (r=0.48, p=0.0001) and G12315A heteroplasmy (r= -0.32, p=0.01).

We found independent positive correlation of mutations C3256T, G13513A and G14846A with both coronary and carotid atherosclerosis.

J06.25
Assessing the implication of genetic and non-genetic factors in type one diabetes mellitus
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Type 1 diabetes mellitus (T1DM) is a multifactorial disease with a high rate of incidence in the Romanian population. The purpose of this case-control study was to test the possible association of genetic and non-genetic factors with type 1 diabetes mellitus. For this study, eighty T1DM patients and eighty healthy control group were selected. Blood samples along with clinical, anthropometric and lifestyle data were collected after informed consent. The genetic factors tested were polymorphisms of insulin, IG2F, SEL1, TGFβ, HSPG and IL6 genes. Total cholesterol levels were higher in T1DM group (p=0.0001), regardless of gender. Triglyceride levels were higher in diabetic women than in same sex controls (p=0.0003), and also in healthy men compared to diabetic women (p=0.001). Men had fewer visits to the dentist in the last 12 months (p=0.001). Insulin -23Hp polymorphism was significantly associated with T1DM (OR AA = 4.26, p<0.0001). HSPG BamHI polymorphism (OR TT = 0.22, p=0.0006), as well as the combination of IL6G allele and SEL1 CC genotype (OR=4.69, p=0.0004) were associated with dental damage. Our data showed that insulin -23Hp polymorphism is associated with T1DM in our patients. Dental damage risk may be modulated by genetic factors such as HSPG BamHI polymorphism in our cohorts.

J06.26
Late-onset of Wilson's disease (case report)
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Wilson’s disease is a rare inherited metabolic disease that leads to copper accumulation mainly in the liver and brain. The clinical symptoms and age at onset of Wilson’s disease (WD) are highly variable. Most patients with WD are diagnosed between the first and the fourth decade of life, although the age at presentation can vary from 3 to 70. Wilson’s disease has many symptoms. The broad spectrum of clinical manifestations can be divided into neurological (brain-related) and non-neurological. The primary consequence in about 40 percent of patients with Wilson’s is liver disease.

We report a case in which the diagnosis was made late in a man of 67 years. This patient suffered from macroglobulinemia Waldenstrom and had standard specific scheme with chemotherapy treatment. After a course of chemotherapy the patient had an acute attack of jaundice. To establish the correct cause of jaundice more tests were done: MRI cholangiography, endoscopic retrograde cholangiography. Since these investigations were normal mechanical jaundice was excluded. Because jaundice progressed were performed and other tests, including ceruloplasmin, plasma copper and urine copper. Upon these determinations Wilson’s disease was suspected because were found low level of serum ceruloplasmin (12 mg/ dl decreased serum ceruloplasmin 187 µg/dl), increased urinary copper excretion (97 µg/24 hours). FibroMax test revealed a moderate liver fibrosis denoted by F2. Comprehensive evaluations of clinical signs, liver biopsy and gene analysis are helpful for a correct diagnosis. Late-onset WD is a frequently overlooked condition.

J06.27
Increased level of oligomeric plasma alpha-synuclein in patients with different lysosomal storage diseases
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Lysosomal storage diseases (LSD) are class of inherited disorders caused by mutations in genes, encoding proteins critical for lysosomal function. Alpha-synuclein is a presynaptic neuronal protein that can form neurotoxic oligomers and has been implicated in Parkinson’s disease and other neurodegenerative diseases. Clearance of alpha-synuclein is mediated by ubiquitin-proteasome system and autophagy-lysosomal pathway so lysosomal dysfunction may play a role in accumulation and aggregation of alpha-synuclein.

The aim of our work was to estimate the level of oligomeric plasma alpha-synuclein in LSD patients.
We generated plasma of 46 patients with different LSD: 41 - Gaucher disease (GD; median age 15, range 1-71, 17 males); 5 children with other LSD (2 - Niemann-Pick type C, 2 - Krabbe disease, 1 - Wolman disease; median age 4, range 1-18, 2 males) and two control groups: 41 healthy individuals (median age 16, range 3-70, 17 males) and 21 healthy children (median age 10, range 3-16, 9 males). Oligomeric alpha-synuclein plasma level was measured using sandwich ELISA (Human Synuclein αLIKO kit Roboscreen, Germany).

The level of alpha-synuclein oligomers was significantly elevated as in GD (patients: median 22.9 pg/ml, range 1.57-44.58 pg/ml; controls: median 6.06 pg/ml, range 1.05-43.43 pg/ml; p = 0.0001) so in others LSD (patient median age 35, range 5-72, 50 pg/ml; controls: median 8.95 pg/ml range 2.29-103.14 pg/ml; p = 0.021).

This is the first report of elevated oligomeric alpha-synuclein level in plasma of different LSD patients. Our results allow to suggest that mutations in lysosomal proteins genes promote alpha-synuclein aggregation in LSD.

J06.28 Functional activity of lymphocytes mitochondria in children with genetically diagnosed glycogen storage disease of type I

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Introduction: Glycogen storage disease(GSD) is a metabolism disorder resulting in glycogen accumulation in hepatocytes and in development of the secondary mitochondrial dysfunction.

Aim: to evaluate functional activity of lymphocytes mitochondria in children with genetically diagnosed GSD of la and lb type.

Materials and methods: 23 patients with GSD were examined, 9 of them with la type, 14 with lb type. 34 nominally healthy children composed the control group.

The functional activity of mitochondria was estimated by succinate dehydrogenase(SDH) activity, which was identified in the general lymphocytes population, in T-lymphocytes, B-lymphocytes and Nk-cells by flow cytometry method (FC500).

Results: In patients with GSD of la type there were determined mutations: c.247C>T of G6PC gene. In patients with lb GSD, mutations of the gene SLC37A4: c.1042_1043delCT, c.34insG, c.411G>A, c.413G>A, c.528delG, as well as mutations not reported before: c.1066G>T in (3 patients), c.817G>A in (1 patient) were identified.

In all children with I type GSD, decrease of SDH activity in the general lymphocytes population in comparison with the norm was revealed (p<0.02 Kolmogorov-Smirnov test).

In patients with lb type, characterized by more severe disease course, there was detected greater decrease of mitochondria functional activity than in children with la type (p<0.05).

SDH activity analysis in lymphocytes population showed 40% decrease in B-cells and 18% in T-lymphocytes regarding the norm, with 20% SDH activity increase in Nk-cells and 70% increase in lymphocytes compared with the norm.

Conclusions: Disfunctions of mitochondrial apparatus, more prominent in patients with lb type, were revealed in patients with la and lb GSD. SDH lymphocytes activity analysis may be used as additional diagnostic criterion for evaluation of the condition severity of children with GSD.

J07.02 BIOMED-2 Protocols: The Best Diagnostic Tool for Suspicious Lymphoma Malignancies

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B cell non-Hodgkin lymphomas (B-NHL) are generally considered as aggressive malignancies which originate from of the transformed B cell. BIOMED-2 multiplex PCR has been suggested as a gold standard method for the detection of monoclonality in the immune cell system in hematological malignancies and clonal proliferative disorders. Molecular cloning assays, were performed based on the patterns of gene rearrangements in the immunoglobulin chains loci. During B cell malignancies, immunoglobulin recombinant constructions are produced exclusively, and are applied as diagnostic biomarkers in B-NHL. Here, we used the BIOMED-2 protocol to reveal the diagnostic value of immunoglobulin light chains (Igk, Igλ) and incomplete IG D-J monoclonal gene rearrangements on FFPE samples. The study was performed on 70 patients with B-NHL which were previously assessed for Igk monoclonal, Igλ monoclonality and failure to clarify rearrangements. Our results revealed positive clonalitivity in 62 out of 70 (~89%) cases in the Igk and Igλ analysis. The samples with positive clonalitivity included 41.4% for Igk and 47.2% for Igλ. However, our investigation on FFPE tissue revealed that EuroClonality BIOMED-2 protocols could be considered as a valuable and reliable method for clonality detection especially in failure of the IGH analysis. In general, clonal Ig λ gene rearrangement assays are applicable for the diagnosis of lymphoproliferative disorders and are a distinguishable method for differentiating between malignant and benign lymphoma disorders. Furthermore, we are able to implement the BIOMED-2 protocol as a routine diagnostic tool for hematological malignancies.

J07.03 IGH Clonality Detection in Lymphoma Malignancies of an Iranian Population Using BIOMED-2 Protocols

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The B-cell differentiation procedure is coordinated with the expression of particular cell surface antigen receptors, the so-called immunoglobulins. Lymphomas are mostly characterized as clonal proliferations of specific tumour cells. Routinely, the detection of malignant lymphomas are largely evaluated by their morphological features, immunohistochemistry and flow-cytometric immuno-phenotyping. However, these conventional methods cannot be relied upon to distinguish between certain types of lymphomas. BIOMED-2 multiplex PCR has been suggested as a gold standard method for differentiating between malignant and benign lymphoma disorders. Here, we used BIOMED-2 protocols to determine the clonality of IGH gene arrangement in patients with lymphoma. PCR amplification was performed on FFPE of 50 patients with B-cell lymphoma, which consisted of 11 cases of HLs, 25 cases of B-NHLs and 14 cases of B-LPD (lymphoproliferative disorders) with an unknown subtype. Positive clonality was detected in 76% of patients with B-NHLs, with 24% of the cases illustrating clonality showing a polyclonal pattern. In B-HLs, 63% of the cases showed clonality and 36% of the cases showed polyclonality. In addition, positive clonality was observed in 42.8% of cases with B-LPD, with clonality not observed in 75% of cases in any of the immunoglobulin gene family (FR1, FR2, FR3). In the DLBCL groups, clonality was detected 75% of the cases. Patients which were diagnosed with FL and MALTs showed 100% clonality for complete IGH. However, our investigation on FFPE tissue revealed that EuroClonality BIOMED-2 protocols could be considered as a valuable and reliable method for clonality detection, especially in IGH analysis.
J07.04 Decreased IL-17A gene expression and decreased interleukin-17A on T-cells in children with Down Syndrome
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Down syndrome is by far the most common best known chromosomal disorder in humans. It expresses multiple systemic complications with both structural and functional defects as a part of the clinical manifestation. Mechanism of immune changes occurring in Down Syndrome is complex and include extra gene copy of chromosome 21 and secondary dysregulation of numerous intercellular interactions. Recent studies suggest a role of IL-17 proinflammatory cytokine located on 6p12 chromosome in the pathogenesis of autoimmune diseases. Here we aimed to analyze IL17A gene expression in peripheral white cells and interleukin-17A intracellular expression on CD4+ T-cells. The research was carried out on the group of 58 children aged 6 to 12 including the group of 30 children with Down Syndrome (the simple trisomy of chromosome 21 only) and the reference group of 28 healthy children. We evaluated gene IL17A expression using real-time PCR and intracellular IL-17A analyzed by flow cytometry. We revealed significantly decreased gene expression on white cells and significantly decreased expression on CD4+ T-cells in Down Syndrome. Our data indicated that decreased IL-17A expression may play significant role in etiology of autoimmune diseases in Down Syndrome. Moreover, we demonstrated that in Down Syndrome another gene located outside the extra chromosome 21 is also affected.

J07.05 Familial autoimmune Lymphoproliferative Syndrome caused by homozygous FAS Ligand (FASLG) mutation mimicking Familial Hemophagocytic Lymphohistiocytosis (FHL)
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Familial Hemophagocytic Lymphohistiocytosis (FHL) is a fatal disease of children. Genetic defects have been identified in the majority but not all cases. Lack of a familial marker impairs counselling and appropriate treatment strategy, also allowing misdiagnosing. We describe the example of a consanguineous family in which three children died of complications associated with FHL-directed therapies, which turned out to be undue in the light of the recently defined diagnosis. Given the incomplete fitting of the clinical pictures with FHL diagnostic criteria, and lack of mutations in the FHL-related genes, whole exome sequencing of constitutional DNA of both consanguineous parents was performed. The mutation of interest was identified in both carrier parents and confirmed by Sanger sequencing, which showed it at the homozygous state in one affected child. The diagnosis of FHL, made elsewhere in the early 80s based on the clinical picture only, drove the decision to treat the child with an aggressive approach including chemotherapy. Recurrence of the disease and the fatal outcome of the initial case supported the choice of an aggressive therapeutic approach in this family. Identification of homozygous mutation of the FAS ligand gene allowed to redefine the diagnosis, from FHL with unknown genetic defect to FASLG. Definition of genetic diagnosis of congenital immune deficiencies may have not only counselling but also therapeutic implications and should be thus pursued, even retrospectively. Exome sequencing could be a valid tool to help defining genetic bases of difficult clinical pictures.

J07.06 Molecular analysis of Haemophilia A in a Colombian family with Haemophilia A and von Willebrand disease diagnosis
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The von Willebrand factor circulates in plasma in a complex with coagulation factor VIII joined by noncovalent bonds. This interaction prevents enzymatic degradation of factor VIII (FVIII) and ensures its transport to the place of the fibrin clot formation. The factor VIII is transformed to factor VIIIa (activated) and acts as a co-factor for Factor IX activated (FIXa), which will continue activating next step in the blood clotting cascade. Because of their close relationship, decreasing activity of one or another factor may be affected, or they may also affect the other. The late generates a clinical diagnosis not very accurate, sometimes for Haemophilia A or von Willebrand disease. We report here a Colombian family that suffers the two diseases according to clinical diagnosis. However, the lack of a genetic study to verify and contrast the diagnosis made by health institutions which is based on phenotype only, can lead to a wrong classification of von Willebrand disease as a type of mild Haemophilia or von Willebrand disease. The study was done in the context of clinical diagnosis in this family by molecular analysis. To achieve this, we identify the presence of the most common inversions of FVIII gene in introns 22 and 1 by LC-PCR and a general scan of the gene for frameshift mutations or stop codons through three family generations. The use of molecular techniques to confirm the clinical diagnosis for bleeding disorders will improve adequate treatment and patient prognosis in Colombia.

J07.07 Association between vitamin D receptor gene polymorphisms and Hashimoto’s thyroiditis in Serbian population: a pilot study based on FokI, Apal and TaqI RFLP technique
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Hashimoto’s thyroiditis (HT) is the most prevalent autoimmune thyroid disorder caused by an interaction between genes and environmental triggers. Intra-thyroid lymphocytic infiltration may lead to progressive destruction of thyroid tissue and consequently hypothyroidism. Many studies in other populations have shown association between vitamin D receptor (VDR) gene polymorphisms and various autoimmune diseases, including HT. Methods: The study included 44 female patients (mean age ± standard deviation 38±5.4) with Hashimoto’s thyroiditis and 32 healthy controls of age-, sex- and geographically matched adult without personal history of autoimmune and endemic diseases. Genomic DNA was isolated from peripheral blood-EDTA, and target VDR gene was genotyped by PCR-RFLP technique after VDR- FokI (rs2228570), VDR- TaqI (rs731236) and VDR- Apal (rs797523) restriction enzymes digestion. Results: We use Arlequin 3.5 integrated software for population genetics data analysis and found significant difference in the genotype distribution of VDR FokI polymorphism between HT patients and controls (P=0,00465). For Apal and TaqI we found the higher frequency of variant allele but not significantly different comparing to control women (p=0,05), which is consistent with previous studies. Conclusion: The current first and preliminary results identified the association between VDR- FokI gene polymorphism and Hashimoto’s thyroiditis in the Serbian population. Results need to be supported by further investigations that define haplotypes patterns for VDR gene polymorphisms in a larger group of HT patients of both sexes.

J07.08 A Rare Alpha-Globin Mutation is Associated with Early-Onset Hemochromatosis with HFE C282Y Homozygosity, but not C282Y/H63D Compound Heterozygosity
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A 30 year-old Caucasian man presented with significant iron overload (ferritin 3287, transferrin saturation 87%) and was found to be homozygous for the C282Y HFE mutation. He concomitantly had mild microcytic anemia, and was found to have a missense mutation in HB2A (c.242T>G). This mutation is known to produce an unstable HbC Köln (called Hemoglobin Ann Arbor) and cause mild, chronic hemolytic anemia in the heterozygous state. The patient’s mother also carries the Ann Arbor mutation. Interestingly, she is a C282Y/H63D HFE compound heterozygote. However, at age 55, she showed no signs of hemochromatosis (ferritin 197, transferrin saturation 40%).

Severe iron overload has been reported as a complication of specific erythropoietic disorders (including sideroblastosis and porphyrias) in the context of HFE homozygosity (and questionable C282Y/H63D compound heterozygosity). However, to our knowledge, no cases of HFE mutations in combination with alpha-globin mutations or deletions have been reported. We propose that the instability of hemoglobin Ann Arbor leads to increased erythropoietic activity and enhanced iron absorption, which predisposed this C282Y HFE homozygote to atypically early-onset hemochromatosis. The mutation of normal iron profile is consistent with the weakly-penetrant C282Y/H63D genotype, however it is noteworthy that in contrast to her C282Y homozygous son, HFE compound heterozygosity in combination with the Ann Arbor mutation did not result in early-onset hemochromatosis in this individual.
J07.09
Large deletions of SERPING1/C1HN gene in Russian patients with hereditary angioedema
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Hereditary angioedema (HAE) is caused by defects in the C1 inhibitor gene (SERPING1/C1HN). Point mutations and large rearrangements have been found in HAE patients. We previously we have screened the entire C1 inhibitor coding region for identity point mutations in 3 Russian patients with HAE and have registered mutations in 29 patients. In this work, DNA samples from other 24 patients were examined by using MLPA MRC-Holland kit to detect large deletions/duplications in SERPING1/C1HN gene. Large deletions were revealed in 6 patients, in 25% of patients without point mutations, and have constituted 17% of all mutations in Russian HAE patients. Large deletions were including ex 1 del, two cases of ex 4 del, ex 7 del, ex 1-3 del and ex 3-7 del.

J07.10
Single nucleotide polymorphisms in cytokines genes promoters are associated with the susceptibility to HIV-1 infection in the Ukrainians
A. Piddubna, A. Kucherenko1, 2, I. Bobrova3, L. Moroz4, L. Livshits1

Background. Cytokines genes single nucleotide polymorphisms (SNPs) involved in the vulnerability to HIV infection in different population groups. We aimed to determine whether the carriage of cytokines genes allele variants influence the risk HIV-1 infection in Caucasian Ukrainians.

Methods. We examined promoter SNPs in IL-4 (rs 2243250), IL-10 (rs 1800872), TNF-a (rs 1800629) among 78 HIV-1 infected European Ukrainians (30 % male, 32 % female; age at diagnosis (33,35±0,76) years), 22 healthy controls using PCR-RFLP.

Results. The dominant cytokines genes variants among HIV-1 infected Ukrainians were major allele homozygotes that correspond to controls and the high risk infection group (C/C IL-4 - 62.82 %, C/C IL-10 - 53.85 %, G/G TNF-a - 62.82 %). T/T IL-4 and G/A TNF-a genotype variants significantly overrepresented in people with HIV-1 (p≤0.01-0.05); susceptibility to HIV-1 infection does not depend on gender. IL-10 in minor allele distribution showed the difference among study groups: A/A variant was associated with the disease in men (p≤0.05). We found that carriage of IL-10 homozygous major allele genotype had protector effect on risk of HIV-1 infection among male population (p≤0.05).

Conclusions. The first report of cytokines genes allele frequencies in Ukrainian population shows their association with susceptibility to HIV-1 infection and suggests further research in the field of host genetic risk factors.

J07.11
Association between interleukin-1 type I receptor gene polymorphisms and the expression level of membrane-bound receptors
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Interleukin-1 (IL1) is a pro-inflammatory cytokine involved in a wide range of physiological processes, including a central role in the regulation of acute and chronic inflammation. The biological effects of IL1 (IL1α and IL1β) are mediated by its receptor, IL1 receptor type I (IL1RI). The IL1RI on intact CD14 + cells. Individuals with genotype TT in SNP rs2234650 showed a lower percentage of cells expressing IL1RI in populations of intact CD14 + monocytes and in a population of CD14 + monocytes from mock-stimulated cultures of PBMCs. In summary, this study found that polymorphisms in the IL1 type I receptor gene can influence the expression level of membrane-bound receptors on immunocompetent cells.

J07.12
ITPA gene variant protects against combination treatment-induced anemia in Ukrainian patients with chronic hepatitis C
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Standard chronic hepatitis C (CHC) antiviral treatment includes pegylated interferon-α/IFN (PEG-IFNα) and ribavirin (RBV) combination therapy. The most common RBV treatment side effect is hemolytic anemia - the major RBV dose reduction cause. The aim of this study was to clarify the association between the inosine triphosphate pyrophosphatase (ITPA) gene variants and PEG-IFNα/RBV combination treatment induced anemia in CHC Ukrainian patients. The data were collected from 80 CHC patients with HCV genotype 1 infection. All study participants received standard doses of PEG-IFNα and RBV. According to the Hb level changes patients were distributed into: case group - 42 patients with combination treatment induced anemia, and control group - 38 patients with no signs of anemia. Genotyping for ITPA gene rs1127354 and rs7270101 variants was performed using PCR followed by RFLP assay. Fisher’s exact test was used to estimate the difference in genotype and allele distribution. Distribution of rs7270101 genotypes was not significantly different between groups of CHC patients with RBV-induced anemia and without it. The frequency of rs1127354 A allele carriers was significantly higher (P<0,05) in group of CHC patients without anemia (23,7%) comparing to the group of patients with anemia (7,3%). The respective allele frequency in control group (13,2%) was almost 3-fold higher (P<0,05) comparing to the case group (4,9%). In conclusion, significant association of ITPA gene rs1127354 with protection against RBV-induced hemolytic anemia was found in Ukrainian patients with CHC infection. Rs1127354 variant may assist as a pharmacogenetic marker in HCV antiviral therapy correction for side effect avoidance.

J07.13
Common combination therapy with Mcl-1 and survivin siRNA inhibits HL-60 malignant leukemia cell growth in vitro
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The over-expression of the myeloid cell leukemia-1 (Mcl-1) gene and survivin gene are associated with the survival and progression of various malignancies including leukemia. The aim of this study was to explore the effects of Mcl-1 and survivin small interference RNA (siRNA) on the proliferation and apoptosis of HL-60 acute myeloid leukemia (AML) cells. siRNA transfection was performed using a liposome approach. Relative mRNA and protein expressions were quantified by quantitative real-time PCR and Western blot analysis respectively. Trypan blue assay was performed to assess tumor cell proliferation after siRNA transfection. The cytotoxic effect of siRNAs on leukemic cells was measured using MTT assay. Apoptosis was also detected by the annexin V/PI double-staining method. siRNAs clearly lowered both Mcl-1 and survivin expression levels in a time-dependent manner, resulting in marked inhibition of cell survival and proliferation. Furthermore, siRNA co-transfection significantly enhanced the extent of HL-60 apoptotic cells relative to single transfection. Our study demonstrated that Mcl-1 and survivin siRNAs could exert antileukemic effects in vitro. It suggested that combinatory gene therapy targeting Mcl-1 and survivin could be used as a new strategy in the gene therapy of AML.

J07.14
Beta-defensin targeting by using anti-cytokine antibodies as a therapeutic approach in psoriasis
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Psoriasis is one of the most prevalent chronic inflammatory disorders which affect patient lifestyle severely. Psoriasis causes are unknown but many studies suggest that a sophisticated interplay between genetic and environmental factors are involved that trigger an excessive inflammatory response in the skin. Dendritic cells and effector T-cells are critical in the development of the psoriatic lesion and production of cytokines by these cells increases keratinocytes proliferation and stimulate the migration of inflammatory cells into the skin, promoting epidermal hyperplasia and inflammation. Previous studies have extensively documented chemotactic activities of beta-
defensin. Human beta-defensin is strongly expressed in lesional psoriatic epidermis. New findings suggest that systemic levels in psoriasis are largely determined by secretion from involved skin and not by genomic copy number. In this review, we showed that inhibition of beta-defensin stimulation by synthetic anti-epithelial antibodies can decrease inflammatory response and plaque formation. Application of new immunologic therapy like anti-cytokines for psoriatic plaque can lead to new therapeutic approaches and introduces novel candidates for immunomodulation.

J07.15
Tenfold expansion of regulatory T cells homozygous for the CCR5 gene variant Δ32 after CD3/CD28 activation in the presence of exogenously added recombinant IL-2 in vitro
The unprecedented power of the hematopoietic stem cell transplantation of the CCR5Δ32/Δ32 cells resistant to HIV has been proved to cure HIV infection in the case of patients from Berlin, Brigham and Women Hospital in Boston (1,2,3). Viral entry into CD4+ T cells is mediated by the interaction with a cellular chemokine receptor, the most common of which are CCR5 and CXCR4 (1). Cells of persons homozygous for the CCR5 gene variant Δ32 (CCR5Δ32/Δ32) are naturally resistant to infection with CCR5-tropic HIV strains (RS HIV) because of the lack of functional CCR5 cell-surface expression (2). Our data based on the general population in Czech Republic show a frequency of approximately 20% heterozygous persons. Four homozygous persons (HIV-seronegative, CCR5Δ32/Δ32) were identified out of 709 individuals tested. Here we report a more than tenfold increase in the frequency of regulatory T cells (Tregs) following CD3/CD28 co-stimulation within a week of in vitro cultivation of human Tregs, irrespective of their genotype. Importantly, similar the treatments, which lead to the activation of Treg function in humans - e.g. anti-CD3/CD28/CD28 stimulation (4) simultaneously drive expansion of Tregs e.g. using anti-CD3, CD28 and/or IL-2. Our study demonstrates a useful tool for in vitro evaluation of Treg function and facilitates further understanding of the mechanisms of immunological self-tolerance, which may also provide insights into how strong immune responses, such as graft rejection, can be restrained and engratment of HIV resistant cells in HIV patients with AIDS/Lymphoma or leukemia can be augmented.

J07.16
Inherited thrombophilia in women with Systemic Lupus Erythematosus (SLE).
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Background: Systemic lupus erythematosus (SLE) is a chronic autoimmune disease characterized by different organ damages and increased mortality. The mechanisms of onset of inherited thrombophilias on the SLE clinical manifestation has not been completely clarified. Therefore the present study aimed to investigate the role of several common thrombophilias in SLE patients. Materials and methods: Factor V Leiden (FVL:leiden), prothrombin G20210A mutation (FIIG20210A), MTHFR C677T and ACE 267/1D mutations were investigated in 112 Caucasian women with lupus by PCR-RFLP analysis. Age of SLE onset, the presence of different ACR criteria, SLICC and SLEDAI indices were registered in all patients. Results: FVL:Leiden, FIIG20210A and ACE 287/1D polymorphisms were not significantly related to the clinical characteristics of the investigated patients (p>0.05 for all). MTHFR C677T TT carriers were more susceptible to photosensitivity than the others (100% vs. 70%, p = 0.034), while the presence of at least one T allele was associated with increased prevalence of arthralgia (27.6% vs. 8.3%, p=0.026) as well as a tendency for more active disease (SLEDAI [median] 6 vs. 4, p=0.084). Conclusions: FVL:Leiden, FIIG20210A and ACE 287/1D polymorphisms did not aggravate the clinical phenotype of Bulgarian SLE women. The MTHFR C677T polymorphism could modulate the SLE characteristics in affected patients and further studies are needed to establish if a precise significance for the disease onset, activity and severity. The present study was financially supported by the Medical University Sofia (Grant 26/2010, Grant 13/2013).

J07.17
Hereditary thrombophilia and pregnancy. Impact on quality of life of patients and their families
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Purpose: Hereditary thrombophilia is a disease characterized by a group of various mutations leading to vascular microthrombosis. It is now considered to be one of the multiple causes of pregnancy loss. The purpose of this study is to determine the impact of reproductive failure in the quality of life, both of patients and their families. Methodology: During 2011-2014, we evaluated 145 women with reproductive failure, with the ages between 23 and 42 years old. The time of abortion was between 6 and 36 weeks of pregnancy. 86 patients received treatment with Low Molecular Weight Heparin and Folic Acid, since the confirmation of pregnancy until delivery and 6 weeks after giving birth. The rest of the patients are not yet pregnant and they receive only Aspirin and Folic Acid. We used WHOQOL and HADS scales, which were applied both to patients and their family members. Results: We have found an increased rate of both depression (85%) and anxiety (80%), loss of self esteem (65%), negative feelings (70%) and insomnia (66%). There were significant differences in the quality of life (p<0.05) between the lot with multiple pregnancy loss and the lot with only one pregnancy loss, but with significant improvement in quality of life for those who delivered healthy babies after treatment. Conclusions: There is need for a multidisciplinary approach (psychologist; gynecologist; haematologist and geneticist) in order to help the patients and their families to better accept the diagnosis and treatment.

J07.18
Using linkage analysis to determine responsible genes for Glanzmann syndrome: Reporting two large deletions in an Iranian population
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Glanzmann thrombasthemia (GT) is a rare abnormality of platelet aggregati on with quantitative and/or qualitative abnormality of αIbβ3 integrin that is encoded by ITGA2B and ITGB3 genes. It is more prevalent in some Middle East population including Iraq, Jordan and Iran. Many different mutations including point mutations, deletions, duplications and in some cases large deletions have been reported in these genes. In this study we screened the responsible genes (ITGA2B or ITGB3) by linked STRs that were followed by direct sequencing of the entire coding region and exon-intron boundaries of the candidate gene and /or deletion investigation by Long PCR. From 12 families with an affected child who were referred for mutation detection to our Lab, after initial screening with STRs, 2 cases that were linked to ITGA2B gene showed no point mutation. Investigating the region by Long PCR revealed a homozigous large deletion in exon 2 and 3 of both unrelated affected cases that has not been reported yet. Deletion was present in the affected children parents in heterozygote state. As GT is a rare disorder and direct sequencing of two genes with 45 exons could increase the test cost, screening with indirect methods like STR can be useful for more rapid and cost-benefit diagnosis.

J07.19
SYBR Green RT-PCR for Quantitative Detection of TLR4 Gene Level in Colorectal Cancer Cells
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Background: TLR4 is expressed in normal intestinal epithelial cells which has important role in homeostasis, furthermore current studies suggest that TLR4 on immune cells may actually have anticancer properties but aberrant TLR4 expression can promote certain types of cancer. So in order to determine the TLR4 gene expression variations and to facilitate detection
of cut off for pathogenic TLR4 gene level in future research, we developed a quantitative and accurate method by real time (RT PCR).

**Method:** We used SW480 and HCT116 as the TLR4 gene high and low expression colorectal cancer cell lines respectively. Designed primers of this gene do not amplify genomic DNA, while recognize functional transcripts specifically. For real time RT PCR, SYBR Green I was the florescent dye and β-actin were used as the house keeping gene for normalization in relative quantitation (RQ) of TLR4 gene.

**Result:** Designed primers well amplified cDNAs of low and high expression of TLR4 in 2 mentioned cell lines. TLR4 gene expression in SW480 was 28 times more than HCT116 cell line.

**Conclusion:** These preliminary results indicate that real time PCR may be employed to detect TLR4 level in CRC, successful development of real time quantitative PCR (RQ PCR) by SYBR Green I resulted to an easy method with lower cost in comparison to probe based method.

**Key Words:** TLR4, RQ PCR, Colorectal Cancers.

**J07.20**

**Lymphoid enhancer-binding factor 1 (LEF 1) a possible immunomodulator in triple negative breast cancer**

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Wingless-Int (Wnt) signaling pathway role in embryonic development, proliferation, survival and migration of hematopoietic stem cells is already established. There is strong evidence regarding involvement of defects in the Wnt signaling pathway in several solid tumors such as breast, colorectal and prostate cancer. Wnt receptors promote the activation of canonical Wnt β-catenin and downstream Lymphoid enhancer factor 1/T-cell factor (LEF1/TCF) pathway influencing genes expression of cell proliferation, differentiation and apoptosis. Our proposal was to examine gene expression levels of LEF 1 in peripheral blood of triple negative breast cancer (ER negative, PR negative, Her2 negative) patients by qRT-PCR in association with immunohistological and clinicopathological data in order to establish the modulation effect of LEF 1 in triple negative breast cancer circulating immune cells. Our study included analysis performed on peripheral blood leukocytes collected in EDTA tubes of triple negative breast cancer patients and 7 healthy donors correlated with immunohistochemistry and clinical-companion data. After analysing qRT-PCR data of the expression level of LEF 1 gene, we obtained significant statistical results that revealed LEF 1 downregulation in peripheral blood immune cells of triple negative breast patients compared to control healthy donors peripheral blood. There are evidence-based data that reveals elevated levels of LEF 1 in breast cancer cell lines but considering our analysis describes LEF 1 downregulation, a difference of expression signature may arise due to the peripheral blood immune cells different environment implication.

**J07.21**

**Elucidating the microRNA transcriptomic footprint in chronic lymphocytic leukemia**

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Chronic lymphocytic leukemia (CLL) has a heterogeneous clinical evolution. While some patients have an indolent stable disease, others suffer from a rapidly progressing disease. This difference can partly be explained by the presence of genetic and/or cytogenetic abnormalities. We have explored microRNA expression in a well-annotated cohort of 97 CLL patient cases by small RNA sequencing. Data were analyzed using our in-house SMARTPARE tool. In addition, DNA (whole exome sequencing, WES and whole genome sequencing, WGS) and messenger RNA sequencing (mRNAsseq) data was obtained for 88 cases. Integration of mRNA and small RNA sequencing data reveals differential expression of several microRNAs with CLL-related targets. An interesting candidate is miR-150, implicated in hematopoietic differentiation. We found an anti-correlation in expression levels of miR-150 and its targets, suggesting that deregulation of the miR-150 pathway could be implicated in disease evolution of CLL.

Moreover, GE and WES data are used to study the effect of SNVs and CNVs on microRNA expression and processing. Interestingly, expression levels of microRNA pairs within clusters are correlated, suggesting that structural variations in the CL1 genome might indeed comprise the loss of microRNAs located in this region.

**J07.22**

**Hemophagocytic lymphohistiocytosis developed in visceral leishmaniasis may have genetic etiology**

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Hemophagocytic lymphohistiocytosis (HLH) is a complex immune deregulation disorder developed either by genetic defects in Perforin, UNC13D, Syntxin 11, STXBP2 genes or secondary to various infections, other disorders, drugs. Histopathological data. After analysing qRT-PCR data of the expression level of LEF 1 in peripheral blood of triple negative breast cancer (ER negative, PR negative, Her2 negative) patients by qRT-PCR in association with immunohistochemistry and clinicopathological data in order to establish evidence-based data that reveals elevated levels of LEF 1 in breast cancer, successful development of real time quantitative PCR (RQ PCR) by SYBR Green I resulted to an easy method with lower cost in comparison to probe based method.

**Key Words:** TLR4, RQ PCR, Colorectal Cancers.
A new case of 2p15p16.1 microdeletion syndrome with the previous homozygous cases, clinical phenotype of the patient probably resulting from a small size event of gene conversion. In contrast different approaches we could exclude the deletion of exon 4 as the cause for native and cleaved forms of the protein. Her parents showed no alterations.

Methods: Using de novo PCR were performed to identify the possible mechanisms that could explain family. Furthermore, long range PCR, RFLP, SNP genotyping and real-time transcriptional network modeling to link the vel-negative phenotype to system. The vel antigen is present on red blood cells (RBCs) from all human group system, the last clinically important unresolved human blood group system. The vel antigen is present on red blood cells (RBCs) from all human except rare Vel-negative individuals who can form antibodies to Vel in response to transfusion or pregnancy. These antibodies may cause severe hemolytic reactions in blood recipients. We combined SNP profiling and transcriptional network modeling to link the Vel-negative phenotype to SM1, located in a 97-kb haplotype block on chromosome 1p36. This gene codes a previously undiscovered, evolutionarily conserved transmembrane protein expressed on RBCs. Notably, 35 of 35 Vel-negative individuals were homozygous for a frameshift deletion of 17 bp in exon 3. Functional studies using antibodies raised against SM1 peptides confirmed a null phenotype in RBC membranes, and SM1 overexpression induced Vel expression.

Genotype screening estimated that 1 in 17 Swedish blood donors is a heterozygous deletion carrier and 1 of 1,200 is a homozygous deletion knockout and enabled identification of Vel-negative donors. Our results establish SM1 as a new erythroid gene and Vel as a new blood group system. (Nature Genetics 2013; 45(S):537-541)

We present a girl with a de novo q11.2 deletion of chromosome 3, with the phenotype reminiscent of Angelman syndrome (AS). At the age of 2 she was evaluated for motor delay, mental retardation and seizures. The girl showed obvious retardation: sitting at 12 months, walking at 22 months with unstable gait and balance problems. During assessment the height was 65 cm, the weight 10.5 kg, and head circumferences 42 cm. She had microcephaly, epicantus, almond shaped eyes, large nasal root, arched upper lip, blond hair and fair skin.

Behavior: she spoke only 3 words and exhibited an excessively happy demeanor; the resemblance to a high functioning AS child was striking. Ophthalmologic examination revealed: optic atrophy, nystagmus convergent strabismus on the left, myopia, astigmatism. MRI of the brain showed: parieto-occipital atrophy and enlargement of lateral ventricles. Based on the above findings we suspected AS. The routine GTC chromosome and FISH analysis showed no evidence of specific microdeletion. ArrayCGH was performed and a deletion of 292 kb in 3q11.2 region was found. This microdeletion includes 5 known genes: PROS1, ARL13B, STX19, CCT4, COMMD1, FAM161A, KIAA1841, LOC100130280, LOC339803, NSUN3. The parental arrayCGH analysis were normal. This microdeletion includes 5 known genes: PROS1, ARL13B, STX19, CCT4, COMMD1, FAM161A, KIAA1841, LOC100130280, LOC339803, NSUN3. The parental arrayCGH analysis were normal. This microdeletion includes 5 known genes: PROS1, ARL13B, STX19, CCT4, COMMD1, FAM161A, KIAA1841, LOC100130280, LOC339803, NSUN3. The parental arrayCGH analysis were normal.

Phenotypic variability in a Hungarian patient with the 4q21 microdeletion syndrome K. Komlosi1, K. Hadszive1, M. Csako1, B. Bugyi1, A. Fogaraszi1, J. Bene1, G. Kastolinger1, B. Melegh1; 1Department of Medical Genetics and Szentgyorgyi Research Center, University of Pécs, Pécs, Hungary; 2Department of Neurology, Bethesda Children’s Hospital, Budapest, Hungary.

Intersitial deletions of 4q21 have been reported in about a dozen patients (Bho et al, 2013) with deletions ranging from 2 to 15.1 Mb delineating a common phenotype including marked growth restriction, hypotonia, severe developmental delay with absent or delayed speech and distinctive facial features. A minimal critical region of 1.37 Mb was identified in a patient with 4q21 microdeletion syndrome. The patient had significant growth restriction, severe and global developmental delay with absent or delayed speech and distinctive facial dysmorphism, short stature, feeding and behavioral problems. MRI showed reduced cerebellar volume and a decrease in periventricular signal intensity. After extensive metabolic tests and exclusion of subtelomeric deletions array CGH analysis was performed using the Agilent Human Genome G3 SurePrint G4 8x60K Microarray (Agilent Technologies, USA), which detected a 4.8 Mb de novo interstitial deletion of 4q21.

Phenotypic variability in a Hungarian patient with the 4q21 microdeletion syndrome K. Komlosi¹, K. Hadszive¹, M. Csako¹, B. Bugyi¹, A. Fogaraszi¹, J. Bene¹, G. Kastolinger¹, B. Melegh¹; ¹Department of Medical Genetics and Szentgyorgyi Research Center, University of Pécs, Pécs, Hungary; ²Department of Neurology, Bethesda Children’s Hospital, Budapest, Hungary.

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J08.04

Application of Microarray-based Comparative Genomic Hybridization in pediatric patients with developmental delay and dysmorphic features

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The array Comparative Genomic Hybridization technology (aCGH) is intensively used in research and clinical diagnosis for the detection and identification of genome rearrangements in patients with different physical/intellectual development anomalies.

The aim of our study was to apply the aCGH technology in evaluation with high resolution of the entire genome in pediatric patients with developmental delay (DD) intellectual disabilities (ID) and dysmorphic features (DF) for identifying the genetic causes responsible for the clinical observation.

Twenty patients with DD, ID and DF from Romania, aged between 4 months and 14 years, were included in high resolution aCGH analysis with NimbleGen ISCA Plus 3x1.4M Platform (Roche). All patients were previously assessed by conventional cytogenetic analysis.

The aCGH analysis detected clinically relevant chromosomal abnormalities in seven of the patients analyzed, as follows:

- micro-deletions ranging between 2.5 Kb-6 Mb, associated with Pitt-Hopkins syndrome (1q82.12), obesity, ID/DD (6q16.1-q16.2-q16.3), DiGeorge syndrome (22q11.2);
- micro-duplications ranging between 12.5 Kb-1.9 Mb, associated with ID (17q23.2), the Potocki-Lupski rare syndrome (17p12-11.2) and autism (Xp22.11);
- a 30 MB duplication (8q13.3-q22.3), leading to the identification and exact characterisation of a marker chromosome indicated by conventional cytogenetic analysis.

Genomic anomalies identified and characterized by aCGH provided accurate diagnosis of unidentified or unexplained diseases suspected to have a genetic cause, contributing to appropriate clinical management of the affected patients.

Our study demonstrated that aCGH technology can play a very important role in identification and characterization of cryptic and/or complex chromosomal rearrangements, being a valuable tool in postnatal diagnosis.

J08.05

Novel alteration in AMPD2 gene segregates with non-syndromic intellectual disability linked to MRT4 locus, conjointly responsible from Pontocerebellar hypoplasia

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Non-syndromic autosomal recessive intellectual disability (NS-ARID) with genetic loci are listed with MRT numbering by Mendelian Inheritance of Man (MIM). Since the discovery of the first gene in MRT1, PRS12, in 2002, to date a total of 34 loci and 17 genes are identified. Only few of these genes are published causative in more than one family, while the rest are identified in a single family that are characterized, disclosing the high heterogeneity of the phenotype. MRT4 was published in 2007 in an examination of a large consanguineous family with four affected members. The linked region at 1p21.1-1p13.3 was 6.6 megabase commencing 78 genes. Exome sequencing of 150 Turkish individuals or in publically available SNP data revealed a single point mutation c.1526C>T, in AMPD2 gene, located at 1p21.1-1p13.3, altering uncharged polar amino acid threonine, at position 509, to neutral asparagine (T509N), in evolutionally conserved adenosine deaminase domain. This variation was not found in our in house exome sequencing of 150 Turkish individuals or in publically available SNP databases. Furthermore, this variation is assigned to be damaging by diverse prediction software analysis. AMPD2 plays a critical role in energy metabolism, functioning in purine metabolism by converting AMP to IMP via salvage pathways. Recently, deleterious mutations in AMPD2 gene are reported in five families with Pontocerebellar hypoplasia (PCH) with characteristic brain imaging. Affected individuals in our family do not carry progressive context. We conclude that our case will expand the phenotypic spectrum of damaging AMPD2 mutations.

J08.06

Array CGH findings in 280 cases with intellectual and developmental delay, multiple congenital anomalies and autism spectrum disorders in Iran

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Copy number variations have gained increasing recognition in the etiology of disease. Many cohorts have established the significance of genomic imbalances in intellectual/developmental delay, multiple congenital anomalies and autism spectrum disorders. Most of these studies have been conducted in Western populations and have predicted an overall detection rate of 15-20% when using whole genome arrays.

We have performed 1000 array CGH studies in the past three years. 280 of these cases have been referred for ID/DD, MCA or ASD. We detected a pathogenic CNV in 45 of these cases, with an overall detection rate of 16%. Considering that 13 of the cases with CNVs were referred following aberrations detected in cytogenetic analysis and for confirmation of these abnormalities, the rate of detection among unselected cases is 12% (32/263).

As this is to the best of our knowledge among the first case series of patients from Middle Eastern populations, we would like to postulate the significance of consanguinity in the possible detection rate of genomic imbalances. First, there is an increased possibility of homozygosity for CNVs as a result of consanguinity, as detected in one of our cases with homoyzgous deletion of GRID2 gene. Second, the role of consanguinity and consequently increased possibility of autosomal recessive conditions and the way it will decrease the frequency of pathogenic CNVs in the etiology of ID/DD, MCA, ASD in unselected cohorts of patients.

J08.07

9q34.11-q34.12 deletion associated with autism and dysmorphic features - a case report

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The use of high resolution array-based comparative genomic hybridization (array-CGH) in whole genome investigation led to an unprecedented rate of discovery of clinically relevant microdeletions/microduplications in neurodevelopmental disorders. We report on a 12-year-old boy with facial dysmorphic features, autism (Asperger syndrome), motor and vocal tics, speech disorder in early childhood presenting a 9q34.11 - q34.12 deletion.

Cytogenetic investigations included array-CGH investigation (105k platform, Agilent Technologies) and FISH (BAC probes). Array-CGH revealed a de novo 2.4 Mb deletion spanning cytogenetic bands 9q34.11-q34.12 (genomic coordinates hg19: 134148578-133931686). Approximately 50 genes are located within the deleted region, some of these being disease-causing OMIM genes: AGMS, CDG1M, TOR1A, ASS1, ABLL1. Deletions of genomic region 9q34.11-q34.13, proximal to EHMT1, are rarely described. Few patients with deletions similar to our case have been reported in the literature and centralized in databases (e.g. DECIPHER). Haploinsufficiency of genes located in this region might be of clinical significance of the clinical findings in the reported patients (such as autism, intellectual disability, epilepsy, movement disorders etc). Thus our case adds to the body of knowledge and will help in establishing genotype-phenotype correlations. Acknowledgments: Professor Jean-Michel Dupont from Cochin Hospital, Paris, France for kindly providing the BAC-FISH probe; Project PN 09.33.02.03.

J08.08

A clinical case with autism and 22q13.3 deletion or Phelan McDermid syndrome

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The syndrome is characterized by neonatal hypotonia, mental retardation, speech delay, autistic behaviour, normal to accelerated growth, minor facial dysmorphism.

The authors present a boy who was successfully treated for VSD at 6 months of age with cardio surgical operation. After the procedure the child started
J08.09
Exome sequencing reveals a novel mutation in BBS2 gene in an Iranian family diagnosed with Bardet-Biedl Syndrome

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A novel autosomal recessive Bardet-Biedl Syndrome (BBS) is a kind of pleiotropic ciliopathies which is more common in developing countries. BBS is characterized by symptoms including obesity, retinitis pigmentosa, polydactyly, learning problems, hyponogadism and kidney abnormalities that vary both within and between families. Nineteen disease-causing genes are involved in 80% of BBS cases and code proteins localized to the cilium which each function affect different parts of a cilium. BBS2 in contribution with six most conserved BBS genes, form BBSome complex of the cilium.

Here, we report an Iranian family with a ciliopathy disorder ascribing BBS. Whole exome sequencing (WES) was performed for proband which revealed a novel homozygote splice mutation c.535-1G>C in BBS2 gene that would probably lead to skipping of exon 5. Using bioinformatics software, Mutation Taster, predicts c.535-1G>C as a disease causing variant. WES data were confirmed by Sanger sequencing and co-segregation was performed for his family.

Estimating about 8% of BBS reports, BBS2 is among one of the common genes in BBS (same results in Iranian population-unpublished data). This result was in accordance with our expectations. In conclusion, due to the clinical and genetic heterogeneity observed in Bardet-Biedl syndrome WES could be considered as the method of choice for clinical genetics practice.

J08.10
Further characterization of the 16p11.2 microdeletion phenotypes


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Introduction: Recurrent rearrangements of 16p1.2 are increasingly recognized as one of the most common structural chromosome disorders. Reciprocal 16p13.1 rearrangements predispose to developmental delay. Besides, 16p1.2 rearrangements have been identified in up to 1% of autistic individuals. These deletions have also been associated with obesity. A ~550-kb deletion is commonly reported and a smaller deletion of an adjacent region on 16p1.2 has also been found in patients with overlapping clinical features. The phenotypic spectrum of rearrangements in this genomic region remains to be fully characterized. Methods: We describe two new unrelated cases with 16p1.2 microdeletions diagnosed by whole genome oligonucleotide array-CGH. We also compare the phenotype of our patients with other cases described in the literature. Results: One patient, referred to our clinic, with moderate developmental delay, significant behavioral problems and facial dysmorphism, has the typical ~550-kb deletion. There is a known family history of learning disabilities. However it was not possible to test his relatives because he was placed in institutional care. The second patient presents developmental delay and unspecific dysmorphism. He has a maternal inherited 187-kb 16p1.2 deletion. His mother has mild cognitive difficulties and obesity. Conclusions: Our patients present adjacent, non-overlapping 16p1.12 microdeletions, and they share some similar clinical features. They also share characteristics with other patients described in the literature. However, it should be underlined that variable expressivity has been described associated with these rearrangements, even between members of the same family and therefore testing of relatives at risk is important for accurate genetic counseling.

J08.11
A phase III clinical trial to test the effectiveness of Ascorbic acid and Alpha-tocopherol on the Fragile X Syndrome

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Introduction and Objectives: Fragile X syndrome (FXS) is a neurodevelopmental disorder affecting intelligence and behaviour. Current treatments are unable to normalize these symptoms. We propose a combination of a scorbic acid and vitamins of B group in 50 patients with FXS.

Material and Methods: 100 FX patients (50 in placebo: A) 3 to 6 years old (N=19), B) 6 to 7 years old (N=64), C) older than 18 years old (N=17). A clinical questionnaire and neuropsychological tests was performed at the beginning of the trial (T0) and 12 weeks later (T1). Variables: Wechsler intelligence scale for children (WISC-R), manipulative and verbal subscales and Pediatric Picture Vocabulary Test (PPVT-R). The percentage of change were tested by U Mann Whitney Test (p<0.05).

Results: Significant improvements were detected in group B (6 to 7 years old) with 65% potency. The percentage of change in the Verbal WISC-R scales were: 30.8 in the treated group versus 13.7 in the placebo group (p<0.05). The percentage of change in the manipulative WISC-R scales were: 35.4 in the treated group versus 10.9 in the placebo group, p=0.01. A significant improvement was observed in the percentage of change in the Wepedy median scores: 24.4 in the treated group versus 6.8 in the placebo group (p<0.05).

Conclusions: Clinical trials for FXS are necessary. Our results demonstrate improvement in learning and receptive language in children after 12 weeks of treatment with a combination of antioxidants: Ascorbic acid and Alpha-tocopherol.
Patients with Prader - Willi and Angelman syndrome in Latvia

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Introduction: Prader - Willi syndrome (PWS) and Angelman syndrome (AS) are imprinting disorders affecting growth, psycho motor development and metabolism. PWS is caused by lack of expression of the paternally contributed 15q11-q13 genes, while lack of expression of maternally contributed 15q11-q13 genes causes Angelman syndrome. Aim: To analyze clinical symptoms, age at diagnosis of patients with PWS and AS in Latvia. Methods: We analyzed clinical data of 19 patients (12 boys and 7 girls) with PWS and 5 patients (3 boys and 2 girls) with AS who were consulted by clinical geneticists. The diagnosis was confirmed for patients with FISH analysis or DNA methylation analysis or both. Results: The age of diagnosis for PWS was in range from 2 weeks till 13 years of age with great difference between girls and boys. The median age of confirmation the diagnosis for boys was 2.4 years, for girls - 8.6 years. All patients had hypotonia and feeding problems during infancy and excessive weight gain later. Patients had characteristic craniofacial features and hipppogonadism was early finding in boys. Mental retardation ranged from mild to moderate. The age of diagnosis for AS was in range from 1 year till 7 years of age with median age of 4.4 years. All patients have severe developmental delay: late and jerky gait, 2 patients had absent speech, 4 patients had epilepsy. Conclusions: The diagnosis for Prader - Willi syndrome and Angelman syndrome is late and it is necessary to improve knowledge about these disorders among neonatologists, pediatricians, neurologists.

Homoygosity mapping of families with intellectual disability and examination of WWOX gene

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Intellectual disability is a common disorder affecting all populations of the world. A majority of genetically caused intellectual disability phenotype follows an autosomal recessive mode of inheritance. Consanguineous marriage is prevalent in Jordan and other Arab countries. Consanguinity increases the prevalence of rare autosomal diseases. Trying to find out genetic causes for intellectual disability in Jordanian patients we set to collect samples of consanguineous families with the phenotype. We collected samples of 4 families. Extracted DNA of members of those families had undergone genome wide SNP genotyping to identify regions of homozygosity and follow-up with disease causing gene identification. We identified multiple regions of homozygosity and analyzed data for linkage. An area of overlap was identified in two families and the region contained a gene connected to epilepsy and seizures which were found in patients of those families. WWOX gene was sequenced in members of the two families but no pathologic mutations were found. The homozygosity regions identified in all the families provide a narrowed areas in the genome which can be used later to identify disease causing genes, maybe by whole-exome gene sequencing.

Application of array-based comparative genomic hybridization in 251 patients with intellectual disability: Our experience in 2009-2011

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Intellectual Disability (ID) is one of the most common developmental disorders and its prevalence is considered to be approximately 1-3%. Array-Based Comparative Genomic Hybridization (aCGH), also known as molecular cytogenotyping, is a high resolution technique developed to scan the segmental genomic copy number variations (CNV) in the genome level. Here, we review our experience with determining CNV’s using both oligo- and BAC-based comparative genomic hybridization arrays for patients with ID between 2009 and 2011. In a cohort of 251 patients with ID with or without dysmorphic features, additional neurodevelopmental abnormalities, we found 68 different chromosomal aberrations in 56 patients (22.3%). The most common abnormalities were 2p23.1 and 16p11.2 deletions. Our results showed that aCGH is a powerful tool to detect CNV’s causing intellectual disability and is useful for detection novel candidate genes for neurodevelopmental abnormalities. According to the available literature, this is the first comprehensive aCGH study of a Turkish cohort of patients in this specific population.

Molecular karyotyping by array CGH in an Israeli cohort of children with intellectual disability and autism


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Joubert syndrome (JBS) is a clinically and genetically heterogeneous disorder with autosomal recessive pattern of inheritance. The disorder is characterized by cerebellar hypoplasia, intellectual disability (ID), ataxia and oculomotor apraxia. Other clinical features include retinal degeneration, renal anomalies, hepatic fibrosis, and skeletal involvement. The hallmark of JBS is a radiological pattern at magnetic imaging resonance (MRI) named “molar tooth sign”. In 2007 Baal et al. identified a new form of Joubert syndrome designated JBS6 with causative mutation in TMEM67 gene responsible for JBS6 by whole exome sequencing. M. Hesossini1, L. Masante2, Z. Fattahi3, H. Hat3, H. Wienen4, S. Abedi5, H. Najambad6, K. Kabiri6, 1Genetics Research Center, University of Social Welfare and Rehabilitation Sciences, Tehran, Islamic Republic of Iran, 2Max Planck Institute for Molecular Genetics, Berlin, Germany.

Due to high heterogeneity of ID in Iran, our study launched to find more causative genes and their mutations in Iranian families affected with autosomal recessive ID using whole exome sequencing. (WES).One of these families has two affected with profound ID, seizure, strabismus and renal failure in one and profound ID, seizure, strabismus and renal failure in one. On WES data a number of variants detected in this family and with respect to the clinical features a novel variant in TMEM67 gene chose as candidate. Because of suspicion of JS, MRI was performed and molar tooth sign was observed. The variant co-segregated in the family and was absent in normal population. Underlying causes of ID remain unknown in many cases because of clinical and genetic heterogeneity; therefore, exome sequencing is an effective and helpful technique in detection of de novo mutation in this type of disorders. This approach results in more precise genotype-phenotype correlation and clinical diagnosis.
J08.18
Novel MECP2 gene mutation (472 T>G) identified in Ukrainian patient with Rett syndrome
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Rett syndrome is a neurodevelopmental disorder that occurs almost exclusively in females with a frequency of 1:10,000. It is characterized by arrested development between 6 and 18 months of age, regression of acquired skills, loss of speech, stereotyped movements (clasically of the hands), microcephaly, seizures, and intellectual disability. Most cases of Rett syndrome are sporadic. It was established that the development of Rett syndrome due to the presence of mutations in the MECP2 gene. To date, 315 mutations in MECP2 gene have been identified in patients with Rett syndrome. Because of such a significant number of identified mutations in MECP2 gene and mostly the sporadic origin of the disease, we have developed denaturing gradient gel electrophoresis (DGGE) method for mutant variants screening in exon 2 and 3. Using this method we have analyzed exons 2 and 3 of MECP2 gene in 14 patients with clinical signs of Rett syndrome. In five cases abnormal migration patterns of MECP2 exon 3 PCR products have been shown. The results of Sanger sequencing has revealed 4 known for Rett syndrome mutations R168X (502C>T), A140V (419C>T), T158M (473C>T), R133C (397C>T). In one case (a patient with severe Rett syndrome symptoms) we have identified a novel mutation 472 T>G which led to substitution T158P.

J08.19
Analysis of chromosomal aberrations in patients with mental retardation using the array-CGH and MLPA technique: a single Czech centre experience
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Submicroscopic structural chromosomal aberrations (microdeletions and microduplications) are believed to be common causes of mental retardation. Patients with idiopathic mental retardation in our laboratory are examined according to an algorithm based on experimental and economical efficiency. At first, MLPA technique is applied (P245, P297, P036, P070, P250, MRC Holland) as a screening method of the main microdeletions and microduplications detection. Whole genome screening using array-CGH follows in cases of negative results or as an alteration size specification (4x180K). Afterwards, FISH is usually used for both methods confirmation. Since 2007 to 2013, 1030 patients were examined. Chromosomal aberrations were detected in 124 patients (12%). Using MLPA, 931 patients were examined and alterations were found in 90 cases (9.7%) - 43 subtelomeric changes (P036, P070), 14 DiGeorge syndrome (P250) and 33 another cases of 1q21 microduplication syndrome (P245, P297, P036, P070, P250, MRC Holland). Over the last few years, array-comparative genomic hybridization array (CGHa) has considerably improved our ability to detect cryptic unbalanced rearrangements in patients with mental retardation. We report on a young female patient, aged 18, suffering from moderate mental retardation, subjected to CGHa. She was previously studied by conventional cytogenetic techniques, and the karyotype was 46, XX. In addition, Williams syndrome and Fragile X syndrome were excluded. The subject came to our attention having typical symptoms such as: cognitive retardation, EEG abnormalities (irregular paroxysmal discharges), movements clumsiness for fine and coarse gesture, flat foot, delayed language development, mild mental retardation, overweight. The array CGHa analysis is revealed the presence of a microduplication of the short arm of the X chromosome (Xp11.23-11.22), whose size was 4.6 Mb, and excluded the presence of additional rearrangements along the Y chromosome. Out of a small number of individuals with duplications within the proximal short arm of the X chromosome have been so far reported, whose clinical findings correspond to that of the subject under consideration. Our findings suggest that overexpression of genes from proximal Xp is likely to have contributed to her clinical phenotype.

J08.20
Range of mutations of PAH gene at patients with a classical form of the phenylketonuria, living in the Novosibirsk region
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Molecular-genetic inspection of patients PKU carried out throughout 1991-2013 in the Novosibirsk region, has shown that frequency R408W of PAH gene among all mutations of this gene makes 0,653. These mutations are revealed both in the homozygous form, and in a compound with other rare mutations. For the purpose of identification of a range of mutations of PAH gene with use of a complex of molecular-genetic technologies research of exons 5-12 of PAH gene at 62 patients with PKU, among which 48,38% had easy, 45,17% - the moderate and 6,45% heavy degree of mental retardation was carried out. As a result of the carried-out inspection (besides a major mutation of R408W) in structure investigated exons 5-12 of PAH were identified thirteen more rare mutations and their frequency characteristics - R158Q (0,0323) are established, to E221D222fsdelAG (0,0081), by R243Q (0,0081), R243X (0,0161), R252W (0,0161), R261Q (0,0484), E280R (0,0081), P281L (0,0403), S349P (0,0081), VS151nt+1g->a (0,0081), A403V (0,0081), Y141C (0,0242), VS121nt+1g->a (0,0323). The analysis of assortment of genotypes with features of a clinical picture at the surveyed patients with PKU showed communication existence between moderate and heavy forms of mental retardation and a homoygous genotype of mutations of R408W and VS121nt+1g->a and their compound homoygous genotype with mutations of R158Q, E221D222fsdelAG, R261Q of PAH. The received results testify to essential clinical and molecular heterogeneity of PKU among inhabitants of the Novosibirsk region and allowed to develop optimum algorithm of molecular and genetic diagnostics of this disease in burdened families.

J08.21
A rare case of microduplication Xp11.23-11.22
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Over the last few years, array-comparative genomic hybridization array (CGHa) has considerably improved our ability to detect cryptic unbalanced rearrangements in patients with mental retardation. We report on a young female patient, aged 18, suffering from moderate mental retardation, subjected to CGHa. She was previously studied by conventional cytogenetic techniques, and the karyotype was 46, XX. In addition, Williams syndrome and Fragile X syndrome were excluded. The subject came to our attention having typical symptoms such as: cognitive retardation, EEG abnormalities (irregular paroxysmal discharges), movements clumsiness for fine and coarse gesture, flat foot, delayed language development, mild mental retardation, overweight. The array CGHa analysis is revealed the presence of a microduplication of the short arm of the X chromosome (Xp11.23-11.22), whose size was 4.6 Mb, and excluded the presence of additional rearrangements along the X chromosome. Our study number of individuals with duplications within the proximal short arm of the X chromosome have been so far reported, whose clinical findings correspond to that of the subject under consideration. Our findings suggest that overexpression of genes from proximal Xp is likely to have contributed to her clinical phenotype.

J08.22
An Additional Case of Temple-Baraitser Syndrome: The Sixth Case in the Literature
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Temple-Baraitser syndrome (TBS) is a very rare disorder characterized by severe mental retardation and anomalies of upper and lower limbs with absence/hypoplasia of the nails. Various dysmorphic facial features may accompany with the syndrome. Only 5 cases have been reported in the literature. Genetic etiology of TBS is still unknown. We report a 13-year-old female who was admitted to the hospital because of severe mental retardation, history of epileptic seizure and facial dysmorphic features. She was born to consanguineous parents at term. On physical examination at admission, her weight was 83 kg (>97p), height: 163 cm (75-90p) and head circumference: 58 cm (>97p). She had broad forehead, myopathic facies, hyperidrosis, upslanted palpebral fissures, long and taperoid philtrum, downturned corners of the mouth, broad thumbs, broad naves of upper and lower limbs. X-ray showed central translucency of distal phalanges of thumbs. Cranial MRI was normal. Karyotype and subtelomeric FISH were normal. Temple-Baraitser syndrome should be in the differential diagnosis for cases presenting with mental retardation particularly accompany abnormal thumb with great toes and hypoplastic/absent nails.

J08.23
Recurrent findings of microduplications in the pseudoautosomal region 1 at Xp22.33 - with unknown phenotypical significance
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Microduplications in the PAR 1 region at Xp22.33 when found by SNP-array analysis, poses a challenge to interpret. As yet no certain clinical significance has been shown. However, it has been proposed, that the ASMT gene, located within this area, can be linked to autism spectrum disorder (ASD) and attention-deficit/hyperactivity disorder (ADHD). ASMT encodes the enzyme acetyl serotonin methyl transferase, which catalyzes the last step in converting serotonin to melatonin. Melatonin is synthesized in the pineal gland during the night, and is crucial for sleep induction and circadian rhythm regulation. In both ASD and ADHD sleep alterations has frequently been reported and it has been shown that there is a decreased level of melatonin in these patients.
and that administration of melatonin greatly improves sleep patterns. Recently we noted frequent findings of microduplications within the area, which gives rise to the question: could there be a link between a dose related activity in the ASMT gene (microduplications at Xp22.33) and the phenotype presentation in our cases. To investigate this further we wish to present our cases with microduplication at Xp22.33, to show if an association can be established.

**J08.24**

A novel mutation in the endosomal Na+/H+ exchanger NHE6 (J08.24) causes Christianson syndrome with electrical status epilepticus during slow-wave sleep (ESES).

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Mutations in the solute carrier family 9, subfamily A member 6 (SLC9A6) gene, encoding the endosomal Na+/H+ exchanger 6 (NHE6) are associated with Christianson syndrome, a syndromic form of X-linked intellectual disability characterized by microcephaly, severe global developmental delay, autistic behavior and early onset seizures and ataxia. A 7-year-old boy with characteristic clinical and neuroimaging features of Christianson syndrome and epileptic encephalopathy with continuous spikes and waves during sleep, we identified a novel splice site mutation (IVS10-1G-A) in SLC9A6. These findings expand the clinical spectrum of the syndrome and indicate NHE6 dysfunction as a new cause of electrical status epilepticus during slow-wave sleep (ESES).

**J08.25**

Effectiveness of using array CGH for diagnosing learning disability

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While chromosomal imbalance has traditionally been diagnosed using karyotype analysis, the advent of array-based comparative genomic hybridisation (array CGH) has provided a more powerful diagnostic tool for clinical genetics services. We present an economic evaluation conducted in a retrospective cohort of patients with undiagnosed learning disability and developmental delay referred consecutively to the regional clinical genetics service at Guy’s and St Thomas’ NHS Foundation Trust. We compare the use of array CGH as a first-line diagnostic tool versus as a second-line diagnostic tool following karyotype analysis from a clinical genetics service perspective. Data were extracted for 648 patients in the first-line test arm and 742 patients in the second-line test arm. The mean incremental cost of testing per patient was £241.56 which means that using array CGH as a first-line was cost saving. The mean incremental gain in the percentage diagnoses was £241.56 which means that using array CGH as a first-line was cost saving. The mean incremental cost of testing per patient was £241.56 which means that using array CGH as a first-line was cost saving. The mean incremental cost of testing per patient was £241.56 which means that using array CGH as a first-line was cost saving.

**J09.01**

DISC1 polymorphisms are associated with ADHD in Iranian population

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Attention deficit hyperactivity disorder (ADHD) is a common heritable psychiatric disorder with a worldwide prevalence of 5%. The etiology of ADHD is still incompletely understood, but several studies, consistently indicate the strong role of genetic factors on this disorder. ADHD is known to have polygenic nature with multiple genes involved in its genetic basis. Disrupted-in-Schizophrenia-1 (DISC1) has been identified as a susceptibility locus for several psychiatric disorders and some of its polymorphisms are studied in many neurological disorders, but are not included in ADHD studies as much. In this study we investigated the association of four SNPs (rs11122330, rs6675281, rs11122319, rs4175894) in the DISC1 gene with ADHD in Iranian population. 600 subjects composed of 300 patients and 300 normal controls were included and tetra-primer ARMS-PCR technique was used for genotyping all SNPs. We found differences in genotype distribution of rs11122319 (p = 0.01) and rs6675281 (p = 0.009) variants between patients and controls. Our findings strengthens the role of disc1 gene as a susceptibility locus for ADHD and indicate that rs11122319 and rs6675281 variants are strong risk factors for ADHD in Iranian population.

**J09.02**

Genetic analysis of Aicardi-Goutières Syndrome in an Italian cohort

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Aicardi-Goutières Syndrome (AGS) is a rare genetically determined encephalopathy that may overlap with the phenotype of congenital infection. Mutations in TREX1, RNASHE2A, RNASHE2B, RNASHE2C, SAMHD1 and ADAR1 genes have been found in the majority of AGS cases. TREX1 gene codes for 3’–5’D DNA exonuclease with specificity for ssDNA. AGS, AG3, AG54 patients have mutations in genes coding for the three subunits of the Human DNA Polymerase H2 enzyme, complex implicated in ribonucleotide removal from RNA/DNA duplex. The AGS55 gene, SAMHD1, encodes for a triphosphohydrolase that converts deoxynucleoside triphosphates to deoxyribose and inorganic triphosphate. AGS6 can be caused by mutations in the ADAR1 gene translating into an adenosine deaminases acting on RNA which catalyzes the hydrolytic deamination of adenosine to inosine in dsRNA. In the last years, IRCCS “C. Mondini” Foundation and International Aicardi-Goutières Syndrome Association (IAGA) were involved in many projects. An AGS biobank to collect serum, plasma, lymphoblastoid cell lines and PBMCs has been established. We recruited samples of 23 patients with clinical diagnosis of AGS and their parents. We collect two AGS1/TREX1 case, 15 AGS2/ RNASHE2B case, one AGS3/ RNASHE2B case, one AGS4/ RNASHE2C case ,one AGS5/SAMHD1 case and two AGS6/ADAR1 cases. Although one of the screened patients did not show mutations in the mentioned genes above, we may still consider the possibility of the presence of new mutations yet to be identified in other genes. The research leading to these results has received funding from the European Union’s Seventh Framework Programme (FP7/2007-2013) under grant agreement number 24177.

**J09.03**

Evaluation of PAI-1 gene 844G/A polymorphism in Iranian patients affected by Alzheimer and Normal Individuals

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It has extensively established that PAI-1 gene is involved in Alzheimer pathology. Numerous genetic risk factors have been related with Alzheimer, but no study has unraveled a possible association between Alzheimer and PAI-1 gene 844G/A polymorphism. There are many common risk factors such as 844G/A polymorphism that play key roles in the development of Alzheimer disease. Polymorphisms in PAI-1 gene have been associated to Alzheimer in some populations. However, other groups failed to replicate this finding in other populations. Evidence suggests that PAI-1 gene 844G/A polymorphism might play a role in Alzheimer, as a result we studied PAI-1 gene 844G/A polymorphism common polymorphisms in Iranian with Alzheimer.Materials and methods: We conducted study including a clinically well-defined group of 52 Alzheimer patients to test the association between 844G/A polymorphism and Alzheimer in Iranian population. In the present case control study, the PAI-1 gene 844G/A polymorphism has been investigated in 52 patients and 52 age- and sex-matched healthy subjects by using ARMS-PCR methods. Then, the data were analyzed by pasw statistics 18 (SPSS) software. Results: In patients samples the genotype distribution for PAI-1 844G/A showed 52%, 46%, 2% for AA, AG and GG respectively. In control samples 96% of the cases were AA, 4% were AG and 0% were GG. Conclusion: The results of this study show significant association between Alzheimer and PAI-1 gene 844G/A polymorphism in Iranian population. (P < 0.05)
J09.04
ApoE allele frequency in Alzheimer's disease in Turkish population
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Alzheimer's disease is an irreversible, progressive brain disorder and the most common cause of dementia in older adults. Apolipoprotein E (ApoE) is a protein involved in the transport of lipids that is strongly linked to Alzheimer's disease. The ApoE gene has three common alleles (epsilon 2, epsilon 3, and epsilon 4) that determine six genotypes in the general population. Especially frequency of the allele for apolipoprotein E type 4 (epsilon 4) is increased in late-onset familial and sporadic Alzheimer’s disease (AD). In this study, we have examined ApoE4 frequencies in a total of 481 patients and 101 controls for an association with the ApoE-epsilon 4 allele. DNA samples of patients were isolated from peripheral blood with standard methods. PCR-Reverse Hybridization (254 patients) and Real-Time PCR (227 patients) was used for ApoE genotyping. The allele frequencies in patients and controls, respectively, as follows; e2=55 (7.1%) and 13 (6.43%), e3=750 (77.96%) and 163 (80.69%), e4=157 (16.32%) and 26 (12.87%). E4 allele and E4/E4 genotype is a risk factor for Alzheimer's disease in Turkish population.

J09.05
The association of the rs121918398 with Alzheimer's disease in Iranian northern population
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Introduction and Aim: Alzheimer’s disease (AD) is the most common type of dementia characterized by cognitive impairment and alteration of diverse cognitive abilities. Pathologically, it is the formation of amyloid plaques and neurofibrillary tangles in the brain. The most common form of AD is Late-onset AD (LOAD), and it is usually sporadic. Although several susceptibility genes for AD have been reported, by far the strongest genetic risk factor for LOAD is Apolipoprotein E that located on chromosome 19q13.2 and universally recognized as a major disease susceptibility gene for LOAD. APOE has three common alleles, APOE-2, APOE-3, APOE-4. In this study we examined association of the APOE gene polymorphism (rs121918398) with Alzheimer’s disease in Iranian northern population.

Methods: Study included 50 patients with AD and 50 healthy volunteers. An informed consent was obtained from all participants. Genomic DNA was extracted from peripheral blood leukocyte. Genotypes determined by PCR and restriction fragment length polymorphism (RFLP). Statistical analysis was performed using the MedCalc program for windows version 12.

Discussion and Conclusion: The prevalence of genotype frequencies of the APOE A/A, A/G, G/G were 16%, 34% and 50% respectively, in AD subjects, and in healthy volunteers were 10%, 64% and 26% respectively. Statistically analysis has not emerged significant difference from the comparison of either genotype (p<0.05). There was no evidence that APOE variants were associated with AD in this population. However our results may be different by selecting different geographical sites or bigger size population.

J09.06
Repetitive association analysis of Alzheimer's disease in Russian population of Siberian region
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Alzheimer’s disease (AD) is a progressive neurodegenerative disorder observed in the elderly. This pathology is the most common cause of dementia and accounts for 50-80% of dementia cases. The World Health Organization estimated that currently about 25 million people worldwide have Alzheimer’s disease. It is a complex disorder with genetic, environmental and lifestyle factors. Common neurological disorders including AD are the subject of intensive genetic research based on genome-wide association studies (GWAS). Genetic variants associated with cognitive impairments, which are implicated in the disease. This polymorphism was also present in a healthy subject encoding genes (i.e. SLC52A3 [hRFT2], SLC52A2 [hRFT3] and SLC52A1 [hRFT1]) and the UBQLN1 gene. Objective: To describe the phenotypic and genotypic characteristics of an Italian family with autosomal dominant BAVS.

Patients and methods: The proband is a 16-year-old girl affected by BAVS since she was 3. A second case in the family is the proband’s maternal aunt, who was diagnosed with BAVS at age of 27; however, deafness and bulbar weakness had started during adolescence. No other family members are affected.

DNA was extracted from blood of the proband and several relatives. A screening for pathogenic mutations in riboflavin transporter genes and UBQLN1 preformed.

Results: No pathogenic mutations in all three riboflavin transporter genes were found. The proband was bearing a heterozygous substitution E54D in exon 1 of the UBQLN1 gene, previously suggested to be implicated in the disease. This polymorphism was also present in a healthy adult family member.

Conclusions: We identified a rare BAVS family with an autosomal dominant pattern of inheritance with incomplete penetrance and variability of age at onset. The UBQLN1 gene might represent a genetic risk factor for this rare motor neuron disease.

J09.07
Association study of BDNF polymorphism Val66Met in Bulgarian patients with Alzheimer disease
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Background: Alzheimer disease (AD) is the most common form of dementia characterized by cognitive impairment, memory and personality disorders caused by progressive degeneration of the brain neurons. Brain-derived neurotrophic factor (BDNF) gene polymorphism is a risk factor for neuronal survival and growth processes during development. The Val66Met (rs6265, G>A) polymorphism in BDNF gene has been extensively studied for association with various neuropsychiatric and neurodegenerative diseases. Several studies show that Val66Met polymorphism is implicated in cognitive impairment, depression, intracerebral trafficking, reduced hippocampal volume etc. Materials and methods: In this study 202 patients with AD and 97 healthy controls, matched to the patients by age, gender and ethnicity (NC) were included. Genotypes were determined using Taqman assay (Applied Biosystems). Statistical analysis was done using PLINK toolset and chi square test. Results and discussion: The Val66Met polymorphism showed significant difference in allele frequency distribution between the AD and NC group. The common Val allele was more frequent among AD patients, while the rare Met allele was associated with increased risk of AD (P=0.01, OR=0.58). When the sample was analyzed in sub-groups according to the age of onset, significant association was found in the early onset group (EOAD) (P=0.01, OR=0.51), but not in the group with late onset (LOAD). (P=0.07, OR=0.62). Conclusions: Our findings show association and increased risk effect of the common Val66Met allele (P=0.02, OR=1.73) in Bulgarian patients with early onset AD. Acknowledgements: This work was supported by Instructural Grant: DUNK/1/27/2009 funded by National Science Fund, Ministry of Education and Science, Bulgaria.

J09.08
Brown-Vialetto-Van Laere syndrome: a case report of a family from Italy
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Background: Brown-Vialetto-Van Laere syndrome (BVVLS) is a rare motor neuron disease (MND) characterized by childhood onset, neurosensory deafness and bulbo-pontine paralysis. Most familial cases are recessive and genetic studies have demonstrated mutations in several riboflavin transporter encoding genes (i.e. SLC52A3 [hRFT2], SLC52A2 [hRFT3] and SLC52A1 [hRFT1]) and the UBQLN1 gene. Objective: To describe the phenotypic and genotypic characteristics of an Italian family with autosomal dominant BVVLS.

Patients and methods: The proband is a 16-years-old girl affected by BVVLS since she was 3. A second case in the family is the proband’s maternal aunt, who was diagnosed with BVVLS at age of 27; however, deafness and bulbar weakness had started during adolescence. No other family members are affected.

DNA was extracted from blood of the proband and several relatives. A screening for pathogenic mutations in riboflavin transporter genes and UBQLN1 preformed.

Results: No pathogenic mutations in all three riboflavin transporter genes were found. The proband was bearing a heterozygous substitution E54D in exon 1 of the UBQLN1 gene, previously suggested to be implicated in the disease. This polymorphism was also present in a healthy adult family member.

Conclusions: We identified a rare BVVLS family with an autosomal dominant pattern of inheritance with incomplete penetrance and variability of age at onset. The UBQLN1 gene might represent a genetic risk factor for this rare motor neuron disease.

J09.09
Repeat expansion in C9ORF72 is not a common cause of Parkinson's disease in Iranians
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Repeat expansion in C9ORF72 is not a common cause of Parkinson's disease (PD) characterized by chronic progressive movement disorders with loss of body balance, typically motor symptoms such as tremor, rigidity and bradykinesia. PD is the second most common neurodegenerative disease (NDD) after the Alzheimer disease (AD). In this study, we sequenced the C9ORF72 gene in 100 Iranian PD cases and 100 healthy controls. All patients and controls were selected from the Rasht, Mashhad and Semnan provinces of Iran. DNA from blood was isolated using the QIAamp DNA Blood kit. The C9ORF72 gene was amplified and sequenced. According to the results, no pathogenic mutations were identified. Conclusion: Repeat expansion in C9ORF72 is not a common cause of Parkinson's disease in Iranians.
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Expansion of the hexanucleotide repeat (GGGGCG) in intron 1 of the C9ORF72 gene is the most common cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). C9ORF72 expansions have also been observed in some patients with Alzheimer’s disease and essential tremor. The mechanism by which the mutation causes disease is definitely unknown. During a recent screening of only 80 Iranian ALS patients, ALS, FTD, and PD was each observed in three individuals of a single family. The hexanucleotide repeat in C9ORF72 was observed in all three patients. This finding prompted screening of repeat in a cohort of 170 Iranian PD patients by the repeat primed polymerase chain reaction protocol. LRRK2, Prkar1a, Prkar1b, and PINK1 had earlier been screened in many of the patients and those with known mutations in these genes were excluded from the C9ORF72 screening. No pathogenic expansion was found in any of the cases. We conclude that abnormal C9ORF72 repeat expansions are not a common genetic cause of PD in Iran. The same finding has been reported in C9ORF72 repeat expansion screenings of cohorts from other populations.

**J09.10 Preimplantation diagnosis of CADASIL (cerebral arteriopathy with subcortical infarcts and leukoencephalopathy)**

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Cerebral arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is an autosomal dominant progressive disorder of the small arteries, predominantly manifested by migraine attacks, essential hypertension, subcortical infarcts and leukoencephalopathy (sclerotic hemiparesis, diffuse myelin loss, with resultant cognitive impairment in some patients. It is caused by heterozygous mutation in the NOTCH3 gene on chromosome 19p13. The case report presents the preimplantation diagnosis of CADASIL family. The proband had severe transient neurological symptoms (immobility, cognitive impairment) after childbirth. Her sister is clinically healthy, but a MRI scan shows supratentorial white matter changes. Their father has minimal clinical symptoms. All three family members have NOTCH3 gene mutation.

**J09.11 Identification of two novel mutations in ERCC6 and ERRC8 genes in two mildly affected patients with Cockayne syndrome**

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Cockayne syndrome is clinically heterogeneous condition in which postnatal growth delay, microcephaly and neurological dysfunction are the cardinal features. Characteristic dysmorphic findings like deep-set eyes, triangular nose, narrow mouth, high arched palate, small hands and feet, tall stature, and very severe growth retardation may be seen during infancy. These patients have progressive loss of pigment in skin and eyes, demyelinating peripheral neuropathy, cataract, cachexia, dental abnormalities and sclerotic epiphyses are also the features of this syndrome. It should be noted that brain calcifications are observed but not a must. ERCC6 and ERRC8 mutations are responsible for the clinical findings of these patients. Here we report three patients with Cockayne Syndrome and two novel mutations in ERCC6 and ERRC8 genes. Two of them had a mild clinical course and very mild dysmorphic findings and the other was severely affected. In-sight of these findings we discuss the phenotypic heterogeneity of this condition and aimed to underline the possibility to overlook in the absence of some cardinal criteria and mild facial features.

**J09.12 One novel SCN1A mutation in a Bulgarian patient - case report**

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Drewet syndrome (DS) is an early-onset epileptic encephalopathy characterized by polymorphic seizure types. At onset, they are usually induced by fever and often present as tonic-clonic or hemiconic status epilepticus during the first year of life. Later, patients also manifest other seizure types, including absence, myoclonic, and partial seizures, all being refractory to treatment. The EEG is often normal at onset, but later characteristically shows generalized spike-wave activity. Psychomotor development stagnates around the second year of life, and affected individuals show subsequent mental decline and other neurologic deficits. DS is caused by mutations of the SCN1A gene in more than 80% of patients. Here we report a 1-year-old boy with seizure onset at 5 months, presenting by series of febrile or afebrile tonic-clonic or hemiconic seizures up to status epilepticus, some with postictal hemiparesis. Several interictal EEGs were normal or with background slowing only. Four antiepileptic drugs had no effect and long-lasting seizures occurred every 10-20 days. Based on clinical data, at the age 10 months the diagnosis of DS was proposed. The molecular genetic testing of the SCN1A gene detected a novel de novo mutation - c.3587_3588insCTTC in exon 18 of the gene. The mutation causes frameshift and the shifted mRNA is most probably degraded through nonsense-mediated mRNA decay mechanism.

**J09.13 A novel mutation in SLC1A3 gene associated with episodic ataxia**

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Episodic ataxia (EA) is an autosomal dominant genetically heterogeneous group of disorders characterized by episodes of incoordination and imbalance. Mutations have been mainly associated to KCN A1 and CACNA1A genes, causing EA1 and EA2. Mutations in other genes, CACNB4 and SLC1A3, have been identified in very few cases, causing EAS and EA6. Here we describe a patient with episodic ataxia phenotype carrying a novel mutation in SLC1A3 gene, encoding for a glial glutamate transporter (EAAT1). The patient had had episodes characterized by vertigo, intentional tremor, gait imbalance, dysarthria lasting from few days to a week, since the age of 30. At examination, when she was 50, she presented with gait ataxia, dysmetria, dysarthria, abnormal eye movements and cerebellar atrophy. DNA mutation screening for all four known EA genes revealed a heterozygous nucleotide substitution (c.227G>A) in SLC1A3 leading to a replacement of a highly conserved arginine to a glutamine (p.R76Q) located between two transmembrane segments. The pathologic alteration in the protein sequence and the missense substitution on EAAT1 structure have been bioinformatically evaluated. This variant was present in the proband’s son showing nistagmus and reporting mild vertigo episodes, at age 40. The proband’s daughter, normal at the clinical examination, doesn’t carry the nucleotide change. This variant was not detected in 418 Italian control chromosomes and it is reported in dbSNP with a very low frequency (2/13000 random chromosomes).

This is the third EAAT1 mutation so far reported for SLC1A3 gene associated to EA.

Supported by “NANOMAX PL.P04.006.005” to LV.
The FOX1 gene, located on 3p13, has recently been involved in neurodevelopment. FOX1 haploinsufficiency has been reported in about 10 patients with specific facial features, global developmental delay, intellectual disability, and speech impairment, where expressive skills are more severely affected than receptive skills. Case description We report on a 2-year-old Caucasian boy, born at term with relative macrocephaly. He developed neonatal cholestasis for which an extensive etiological analysis was performed, including JAG1 (Alagille syndrome) and ABCB4 (PFIC3) gene analysis. Duodenal paucity was objectified with progressive improvement and genetic work-up was normal. Progressively, global developmental delay was observed. He had axial hypotonia, dyskinetic movements and is still not able to walk. Expressive language has not yet been acquired, though receptive language appears more advanced. Growth is normal. He has frequent respiratory tract infections, bronchial hyperreactivity and gastro-esophageal reflux. Facial features include prominent and broad forehead, bilateral palpebral ptosis, broad nasal tip and arched palate. Hypoplastic nails are also observed. Chromosome molecular analysis using CytoScan HD-array (Affymetrix) revealed a de novo ~120kb heterozygous interstitial deletion at 3p13 covering exons 7 to 11 of the FOX1 gene. Conclusion Global developmental delay, expressive language delay, relative macrocephaly and specific facial features are associated with FOX1. Our patient presents with bilateral ptosis, a sign previously reported in the literature in at least two other patients, that could be a clue for the diagnosis when present. Whether or not the liver involvement is an associated feature remains to be studied in larger series of patients.

J09.16 DXS998-DXS548-FRAXAC1 represents a novel informative haplotype at the FRMI1 locus in the Iranian population
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Fragile X syndrome which is caused by mutation in the FRMI1 gene region is one of the most prevalent forms of mental retardation. Direct diagnosis of the disease is based on PCR and southern blot analysis, but because of technical problems, use of polymorphic DNA markers can be helpful for carrier detection and prenatal diagnosis in families with an affected individual. The polymorphic markers usually show a population-based haplotype frequency heterogeneity. In the present study, genotyping and analysis of haplotype frequency of three microsatellite markers including DXS998, DXS548 and FRAXAC at the FRMI1 gene region were carried out in 140 unrelated women and 26 families from the Iranian population. The data indicated the presence of a novel allele for DXS998 in the Iranian population. Genotyping and haplotype frequency using Arlequin program showed 50 different DXS998-DXS548-FRAXAC1 haplotypes for the input data of 5, 7 and 4 alleles, respectively. Among these haplotypes four of them showed relatively high frequencies (≥0.05). Analysis of linkage disequilibrium (LD) for the unrelated individuals using the Powermarker computer program, showed that this haplotype combination can be an informative haplotype for linkage analysis in carrier detection and prenatal diagnosis of fragile X in the Iranian population.

J09.17 Effect of GAA repeat on CpG methylation at first intron of frataxin gene in Indian patients of Friedreich's ataxia
S. Agarwal, S. Muthuswamy, S. Pradhan; Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow, India.

FRDA is an autosomal recessive neurodegenerative disease characterized by progressive gait and limb ataxia, dysarthria, lower limbs areflexia. The onset of symptoms usually occurs before the age of 25 years, and typically around puberty. The expanded GAA repeat in intron 1 of the frataxin gene is the common cause of FRDA. Affected individuals have at least one allele with 8-33 repeats, while most individuals with FRDA have 90 or more repeats in both alleles resulting in deficiency of frataxin, an essential protein of mitochondrial iron metabolism. We studied the epigenetic alterations at first intron in the present study which is one of the mechanisms hypothesized to hinder the frataxin synthesis. The study was conducted in 10 healthy controls and 15 Friedreich ataxia cases. Two microgram of extracted DNA was bisulfite treated and amplified with nested primers followed by standard Sanger’s sequencing procedure and results were evaluated. Average age of onset of patients was 22±5.5 years. Average size of alleles of patients and controls were 1200±400 and 12±3.5 respectively. No difference in methylation patterns were observed between the cases and the controls of Indian patients. The CpG islands methylated irrespective of GAA repeat number. However this study is limited in sample size which could be one of the factors that may over-represent the methylation phenomenon. We plan to further extend our study on more Friedreich ataxia patients to draw a strong conclusion along with methylation sites at the promoter region as they are more important for gene expression.

J09.18 Atypical parkinsonism and very early-onset dementia in a patient carrying two GBA gene in cis mutations
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Heterozygous glucocerebrosidase (GBA) gene mutations are a common risk factor for Parkinson Disease (PD). GBA mutation carriers display cognitive and neuropsychiatric disorders more frequently than PD patients without GBA mutations. We describe a male patient carrying two GBA in cis mutations and affected by a severe early-onset parkinsonism associated with behavior disorders and dementia.

The patient had been presenting apathy and memory loss since the age of 33. One year later he developed a bradykinetic parkinsonism partially responsive to levodopa therapy. SPECT study showed mild striatal presynaptic dopaminergic dysfunction. The neuropsychological assessment performed at 34 years of age showed deficits in all tested skills with worse performances in executive function and attention and relative sparing of visuospatial skills. Minimental State Examination (MMSE) score was 26/30. At 37 years of age MMSE score was 22/30. Brain MRI showed diffuse and symmetric brain atrophy with major involvement of the temporomesial lobes and the cerebellar vermis. Testing of PARQ, LRRQ2, DJ1 and FT15 genes was normal. GBA molecular analysis detected two in cis mutations in exon 10 (L144P and A53G) inherited from the father. These mutations are probably due to a recombination event between GBA gene and its pseudogene.

Clinical and neuropsychological examination of the father and family history were unremarkable.

Reports on GBA mutation carriers with parkinsonism may contribute to defining the cognitive phenotype of these patients, the possible genotype-phenotype correlations and the clinical markers that may be useful in identifying candidate subjects for GBA genetic testing.

J09.19 Next Generation Sequencing and Data Interpretation In Recessional Hereditary Spastic Paraplegia Patients From Turkey
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Hereditary Spastic Paraplegia (HSP) comprises a group of clinically and genetically heterogeneous neurodegenerative disorders. In ‘pure’ HSP, lower limb spasticity and progressive weakness are observed. ‘Complicated’ HSP additionally include neurological and non-neurological symptoms. HSP can be inherited in autosomal dominant, autosomal recessive, or X-linked manner. Thirty-two loci and 23 genes are associated with autosomal recessive form of HSP (ARHSP). One patient was selected for whole exome sequencing (WES) from each of six ARHSP families from Turkey. After determining candidate variations, segregation analyses were performed in the families. No pathogenic variations were identified in the ARHSP families.

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Novel mutation in SPG4 gene in three families with autosomal dominant hereditary spastic paraplegia from Bashkortostan Republic (Russia) A. Akhmetgalyanova1, K. Khidiatova1, E. Safiullina1, R. Idrisov1, R. Mazhganov1, E. Khuxnutdina2

1Institute of Biochemistry and Genetics, Ufa, Russian Federation, 2Bashkir State Medical University, Ufa, Russian Federation.

Hereditary spastic paraplegias (HSP) is a genetically and clinically heterogeneous group of neurodegenerative disorders characterized by progressive lower-limb spasticity. The major pathological feature of HSP is a degeneration of the pyramidal tracts. The HSP frequency in Bashkortostan Republic (BR) is 3.5/100,000. Autosomal dominant (AD), recessive (AR), or X-linked inheritance were described. To date, 30 causative genes and 50 loci have been identified. Mutations in the SPG4 gene are responsible for about 45% of the pure AD type of HSP and for 12–18% of the sporadic cases. SPG4 codes for spastin - AAA (ATPase associated with diverse cellular activities) family protein. Disorder onset ranges between 1 and 63 years. Over 300 mutations have been described, with the majority of them being located in the AAA domain. We examined HSP patients from BR and detected one rearrangement of DNA sequence in 12-th exon in patients from three unrelated families. This previously not described mutation is inversion translocation, starts at c.1469. Twelve exons code the region that corresponds to a highly conserved ATPase domain, which is also called AAA cassette. This region is responsible for ATPase activity of the spastin protein. New polymorphism in 11-th intron (c.1413+43_46dup (TATA)) and polymorphisms (c.1098+118 A>G (rs 12617289) and c.1098+127 A>G (rs 12617290)) in 7-th intron were revealed in the same patients.

Large chromosomal rearrangements are frequent cause of HSP4 HSP. The mutation we found is among them. We supposed that this mutation was spread in BR by the founder effect.

Two novel compound heterozygous mutations in ROBO3 gene in a sporadic case of Horizontal Gaze Palsy with Progressive Scoliosis R. Mazzei1, S. Vilensten1, F. L. Conforti1, A. Magaritelli1, G. Galli1, A. Ceresa1, M. Muglia1, P. Perrotta1, A. Pattucci1, P. L. Lanza1

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The syndrome of horizontal gaze palsy with progressive scoliosis (HGPPS) is characterized by absence of conjugate horizontal eye movement and scoliosis. HGPPS is an autosomal recessive neurologic disorder caused by homozygous or compound heterozygous mutations in the ROBO3 gene on chromosome 11. Here we report a case of a 34-year young woman from non consanguineous parents. No history of scoliosis or gaze palsy in the other family members. At six months was noticed a fixation in her eyes. At the age of 9 years a dorsal-lumbar scoliosis was diagnosed and a physical examination showed absence of the horizontal eye movements and absence of nystagmus. The morphologic MR examination, the colour map DTI and the fMRI showed typical signs of HGPPS. Similar results have been confirmed by neuropsychiologic tests (PESS, PBM). So we perform a molecular screening of the ROBO3 gene. Sequencing of the all 28 coding exons and relative intron/exon boundaries revealed two novel mutations: a heterozygous missense mutation and a splice-site mutation. The information provided by a multi-modal approach (conventional RM, DTI, fMRI, neurophysiology, and molecular genetics) are crucial in confirming the clinical diagnosis, and when integrated with each other allow to clarify the possible different expressions of the lack of decussation of the cortical spinal tracts in the brainstem.

Holoprosencephaly complicated by neurogenic hypernatremia T. Bizerca1, M. Pusi2, O. Marginean3, R. Strescu2

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Holoprosencephaly is defined as a structural anomaly of the brain in which there is incomplete separation of the forebrain early in gestation. It occurs in 5-12/10,000 live births. Classic holoprosencephaly is classified as alobar, semilobar, lobar and middle interhemispheric variant types. It can result from environmental causes, an inherited or de novo chromosome abnormality, an inherited monogenic syndromic disorder, an inherited or de novo mutation for a gene associated with nonsyndromic autosomal dominant holoprosencephaly, copy number variations or unknown causes. We report the case of a 3 month old infant admitted for to our clinic for sepsis, with characteristic clinical features of HPE. Lab tests revealed severe incoercible hypernatremia alternating with three episodes of severe hypotenraemia. During admission, under the established treatment, the sepsis was remitted; nevertheless high natrium levels were maintained. In this case HPE was complicated by neurogenic hypernatremia. Genetic testing revealed a normal karyotype and the uncharacteristic phenotype of the mother ruled out autosomal dominant transmission. In the presented case, neurogenic hypernatremia was the consequence of impaired osmoregulation of ADH release due to malformations involving midline structures of the brain.

Homoyzosity in Huntington’s disease N. Ergeneli-Unalta1, E. Lohmar1, M. Poda2

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Huntington disease is caused by a dominantly transmitted CAG repeat expansion mutation that is believed to confer a toxic gain of function on the mutant protein. Huntington disease patients with two mutant alleles are very rare. In other poly(CAG) diseases such as the dominant ataxias, inheritance of two mutant alleles causes a phenotype more severe than in heterozygotes. We identified a homozygous female patient, 89 years old and compared clinical features with those of a group of heterozygotes. The age of onset was within the range expected for heterozygotes with same CAG repeat lengths. However, she showed a rather different clinical course with emotional instability and behavioral disorders. Cerebral magnetic resonance imaging showed no evident atrophy, notably not in the cerebral cortex or the striatum. Clinical examination revealed slow movements predominating on the upper limbs and the trunk. She was partially unable to control the speed and the force of her movements and mild dysarthria was noted. Severe memory deficits associated with a severe frontal deficit were found, all symptoms suggesting Huntington disease. Mutation analysis revealed two HTT alleles with CAG repeats of 43 and 45. This suggests that although homozygosity for the Huntington disease mutation does not lower the age at onset of symptoms, it affects the disease phenotype and progression. These data, once confirmed in a larger series of patients, will point to the possibility that the mechanisms underlying age at onset and disease progression in Huntington disease may differ.

Gene expression profile in fibroblasts of Huntington’s disease patients and controls E. Marchina, S. Misusi, A. Bozzato, S. Ferraboli, C. Agosti, L. Bozzini, G. Borsani, S. Barlati, A. Padovani;

University of Brescia, Brescia, Italy.

Huntington’s disease is an inherited disorder caused by expanded stretch of consecutive trinucleotide CAG within the first exon of the huntingtin gene (HTT) on 4p16.3. The mutated huntingtin (mHTT) gains toxic function probably through mechanisms that involve aberrant interactions in several pathways, causing cytotoxicity. Pathophysiology of disease involves several tissues; indeed it has been shown that there is a broad toxic effect of mHTT in the peripheral tissue of patients with HD. In this study we compared gene expression profiles of HD fibroblasts and matched controls using microarray technology. We used RT-PCR to test the consistency of the microarray data and we found four genes up-regulated in HD patients with respect to control individuals. The genes appear to be involved in different pathways that have been shown to be perturbed in HD models and patients. Our study shows that gene expression profiles seem to be altered in the fibroblasts of HD patients. Validation of the differential expressions at the protein level is required to ascertain if this cell type can be considered a suitable model for the identification of HD biomarkers.

Identifying the genomic imbalance of individuals with mental retardation in Taiwan with G-banding, MLPA, and aCGH Y. Li1, I. Chou1, F. Tsai1,2, E. Lin1

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Mental retardation (MR) occurs in 1-3% of the general population and about one-half of the cases their etiology is still elusive, thus appropriate treatment and genetic counseling remains as great challenges. About 50% of children with MR with IQ less than 70 is likely to have genetic defects. Some of them are involving genomic disorders. However, submicroscopic genomic disorders cannot be detected by conventional cytogenetic analysis. With the recent advances in molecular cytogenetics analysis and genome-wide array-based comparative genome hybridization (aCGH) technology, submicroscopic genomic disorder bases of MR can be identified and characterized. The array CGH profiles of 204 idiopathic MR patients in Taiwan have been published in ABS Mol Genet Genom 2010;182:179-195.
obtained. 28 MR patients were found to have genomic imbalance (microdeletion/microduplication) with a detection rate of 13.86%. Our finding is within or in agreement with the most recent multiple centers studies on chromosomal microarray study of MR. The identification of genomic imbalance in our study could lead to the identification of some pathogenic CNVs for MR. This could lead to the discovery of a network of neuro-developmentally associated genes. The informed network of neurodevelopmentally-associated genes will help us understand the etiology of MR disorders and will assist in diagnosing, managing and treatment of the disorders.

J09.26
Detection in exon 15 of KCNQ2 gene responsible of Benign Familial Neonatal Seizures in a large Spanish family.
J. Juan1, I. Aleixandre2, M. Ortiz1, A. Zuniga1.
1Servicio de pediatría. Hospital Univ. de La Ribera, Alzira/Valencia, Spain, 2Servicio de genética. Hospital Univ. de La Ribera, Alzira/Valencia, Spain, 3Servicio de genética. Hospital uniq. de La Ribera, Alzira/Valencia, Spain.

Benign familial neonatal convulsion disease (BFNC, OMM # 121200) is a rare autosomal dominant inherited epilepsy characterized by unprovoked generalized or multifocal tonic-clonic convulsions. The convulsions typically start around the third day of life and resolve spontaneously after several weeks or months. In the majority of BFNC families the disease is linked to chromosome 20q3.3, and mutations of the voltage-gated potassium channel KCNQ2 have been identified as the underlying defect. The proband is the first daughter of healthy non consanguineous parents. She was born by normal vertex delivery at full term after an uneventful pregnancy. Her birth weight, head circumference, and length were in a standard range. Her Apgar scores were 8 and 10 at 1 and 10 minutes, respectively. On the fourth day she manifested convulsions with apnea and clonic movements. The attacks were of short duration and ceased spontaneously. Clinically, the patient demonstrated no abnormal facial features, with normal skin, power, tone, and reflexes. Basic laboratory investigations produced normal results. Cranial ultrasound and magnetic resonance imaging also produced normal results. After interrogation, there were members of the family with a history of repeated seizures in their childhood. Direct DNA sequencing of the KCNQ2 gene exons and their flanking intronic sequences, of the proband and members of her family, revealed a deletion in exon 15 of a cytosine nucleotide: c.1960 in the amino acid 595 (NM_172107). This was predicted to cause a frameshift in the protein. This mutation has not been previously described in literature.

J09.27
Mutational analysis of cathepsin F and CLN6 genes in a Japanese patient with Kufs disease, an adult onset neuronal ceroid lipofuscinosis.
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Kufs disease is an adult onset neuronal ceroid lipofuscinosis, which is autosomal recessive progressive lysosomal disorders. Kufs disease are clinically characterized by lissencephaly, facial dimorphisms, growth restriction and neuronal migration abnormalities. Recently, the genes responsible for Kufs disease are discovered. Mutations in CLN6 are the major cause of recessive type A, whereas mutations in cathepsin F is the cause of type B. In the past, we have reported a type B Kufs patient, whose parents were consanguineous marriage (Sakaji K et al. Intern Med. 1995;34:11:58-63). Here we present the first mutational report from Japan. In this study, we performed mutational analysis of responsible genes for this Kufs patient. We found several SNPs in cathepsin F gene, and a known but homozygous mutation (c.231C>G, pThr77 in exon 3) in CLN6 gene. PCR-RFLP analysis revealed that the mutation was not a SNP. The amino acid is perfectly conserved between human, mouse and rat. Furthermore, in silico analysis using PolyPhen2, the mutation is predicted to be probably damaging. These data suggest that the mutation must be pathogenic one. Since we found a known but homozygous mutation in CLN6 gene in a type B patient, genotype-phenotype correlation seems to be complex than we expected.

J09.28
The Association of Migraine and Endothelial Nitric Oxide Synthase Haplotypes
1Department of Cellular and Molecular Biology, Islamic Azad University Medical Branch of Tehran, Tehran, Islamic Republic of Iran, 2Department of Biochemistry, Tehran university of medical science, Tehran, Islamic Republic of Iran, 3Paracito genetic counseling center, Boyoung Alley, Keshavarz Bv, Tehran, Islamic Republic of Iran, 4Department of Genetic, Islamic Azad University Science and Research Zanjan Branch, Tehran, Islamic Republic of Iran.

Migraine is a complex and debilitating neuromuscular disorder predominately affecting women. It recurs as attacks of severe headache associated with nausea, vomiting, phonophobia, and photophobia. Despite its high prevalence, migraine is a disease involving complex pathogenetic mechanisms that remain to be clarified. As other multifactorial diseases migraine has a genetic basis combined with triggering environmental factors. There is strong evidence implicating nitric oxide (NO) in the pathophysiology of migraine. Therefore, genetic polymorphisms in the endothelial NO synthase (eNOS) gene have been studied as candidate markers for migraine susceptibility. NO plays an essential role in the control of cerebral blood flow and may be involved in the activation of nociceptors in the trigeminovascular system and release of vasoactive peptides during neurogenic inflammatory response. There are several studies on the association of eNOS gene variations and the risk of migraine in different populations. In the present study, we focused on one of the most common single nucleotide polymorphisms (SNP) of the eNOS gene. The prevalence of the rs1799983 in Iranian population has been evaluated by ARMS-PCR method. We have not found interaction between the selected SNP and the risk of the migraine in 90 patient samples compared to 113 normal individuals.

J09.29
Plasminogen Activator Inhibitor Haplotypes Associated with Migraine
1Department of Genetic, Islamic Azad University Science and Research Zanjan Branch, Tehran, Islamic Republic of Iran, 2Department of Biochemistry, Tehran university of medical science, Tehran, Islamic Republic of Iran, 3Paracito genetic counseling center, Boyoung Alley, Keshavarz Bv, Tehran, Islamic Republic of Iran, 4Department of Cellular and Molecular Biology, Islamic Azad University Medical Branch of Tehran, Tehran, Islamic Republic of Iran.

Migraine is a common disorder affecting approximately 1-6% of the world population, almost three times more women than men. It is characterized by recurrent moderate to severe headaches often in association with a number of autonomic nervous system symptoms. Studies of twins indicate a 34 to 51% genetic influence of likelihood to develop migraine headaches and a number of specific variants of genes have been established to increase the risk by a small to moderate amount. Like other multifactorial diseases genetic basics of migraine are combined with triggering environmental factors. There are some data supporting the involvement of endothelial, haemostatic and haemorheological functions in the pathogenesis of migraine. Plasminogen Activator Inhibitor subtype 1 (PAI-1) gene encodes a member of the serine protease inhibitor (serpin) superfamily, concentrations of the gene product are associated with thrombophilia. Inasmuch as it's plasma level decreases in migraine, PAI-1 seems to play a determinant role in vascular diseases related to migraine.

In the present study, we focused on one of the most common single nucleotide polymorphisms (SNP) of the PAI-1 gene. The prevalence of the rs2227631 in Iranian population has been evaluated by ARMS-PCR method. We have not found a significant association (P>0.05) between the selected SNP and the risk of the migraine in 90 patient samples compared to 113 normal individuals.

J09.30
Identified a neonatal Spanish patient with Miller-Dieker Lissencephaly Syndrome by MLPA
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Miller-Dieker syndrome (OMIM # 247200) is a congenital brain malformation characterized by lissencephaly, fronto-occipital atrophy and growth retardation. The syndrome is an autosomal recessive inherited condition that remains to be clarified. As other multifactorial diseases Miller-Dieker syndrome has a genetic basis combined with triggering environmental factors. There is strong evidence implicating nitric oxide (NO) in the pathophysiology of migraine. Therefore, genetic polymorphisms in the endothelial NO synthase (eNOS) gene have been studied as candidate markers for migraine susceptibility. NO plays an essential role in the control of cerebral blood flow and may be involved in the activation of nociceptors in the trigeminovascular system and release of vasoactive peptides during neurogenic inflammatory response. There are several studies on the association of eNOS gene variations and the risk of migraine in different populations. In the present study, we focused on one of the most common single nucleotide polymorphisms (SNP) of the eNOS gene. The prevalence of the rs1799983 in Iranian population has been evaluated by ARMS-PCR method. We have not found interaction between the selected SNP and the risk of the migraine in 90 patient samples compared to 113 normal individuals.
Neurofibromatosis type 1 (NF1) is an autosomal dominant genetic disorder with worldwide incidence of 1 in 2500 to 1 in 3000 individuals. It is caused by mutations in neurofibromin 1 (NF1), located on chromosome 17q11.2. The main signs and symptoms are café-au-lait spots, Lisch nodules in the eye, and fibromatous tumors of the skin. Other clinical manifestations that have been reported include bone dysplasia and an increased risk of malignant tumours.

The aims of this study were to describe the clinical manifestations of the disease in 22 patients with neurofibromatosis type 1, and to identify the underlying NF1 mutations, by sequencing all 60 exons of this gene. Five different mutations were identified in 7 patients. Two of these mutations have not been previously described (Table 1).

Given the clinical variability of this disorder, and the small sample size, no statistical genotype-phenotype correlations were performed. However, the prevalence of clinical manifestations and the type of mutations seen are in agreement to what has been reported in other populations.

### J09.31

**Association of HLA-DRB1 alleles with Multiple Sclerosis in patients from Bogotá, Colombia**

D. M. Narvaez1, H. Groot de Riestrepo1, S. Reyes1, C. Díaz1, J. Toro Gómez1,2.

1Universidad de los Andes, Bogotá, Colombia; 2Hospital Universitario. Fundación Santa Fe de Bogotá, Bogotá, Colombia.

Multiple Sclerosis (MS) is a chronic autoimmune inflammatory disease that attacks the central nervous system. Multiple factors are associated with the development of this disease, many of them related to the environmental and to the personal genetics. It has been shown a closed relationship between the HLA-DRB1 variant, HLA-DRB1*1501, and the pathology. In fact, the presence of HLA-DRB1*1501 increases the risk to present it. The aim of this study is to find the association of HLA-DRB1 alleles with MS in patients from an homogeneous population as the one we have in Bogotá, Colombia. The study population is composed of 99 patients diagnosed with MS and 198 healthy controls. Every participant signed an informed consent and the inclusion and exclusion criteria were confirmed by a professional. Peripheral blood lymphocytes were used to obtained DNA, and the variant determination was done using a PCR-SSP. We found that the HLA-DRB1*1501 allele confers a significant higher risk of suffering MS (OR= 2.89; 95% CI= 1.60 - 5.19); in contrast, HLA-DRB1*1401 allele exhibit a protector effect (OR=0.29; 95% CI= 0.11 - 0.76) in our population. The association of HLA-DRB1 alleles with MS will enrich our knowledge of the disease and will focus new approximation to a better diagnosis and treatment.

### J09.32

**Genetic characterization of narcolepsy patients from Estonia**

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1Estonian Genome Center, University of Tartu, Tartu, Estonia; 2Mae Pindmaa Sleep Clinic, Tartu, Estonia.

Human narcolepsy is a complex genetic disorder, characterized by excessive daytime sleepiness, cataplexy, hypnagogic hallucinations, sleep paralysis, and disturbed nocturnal sleep. Most cases are sporadic and approximately 0.02% of the population is affected worldwide. An autoimmune mechanism for the disease has been suspected based on its strong association with the genetic marker - HLA DQB1*0602. Recent studies support this hypothesis. To date thousands of patients and matched controls have been genotyped from different populations and genome-wide association studies (GWAS) carried out. Significant associations with narcolepsy include polymorphisms in the TCR alpha, TSH, TNFSF4, 3 untranslated region of P2RY11. Nevertheless, the overwhelming portion of risk and protection is still found within HLA region. Other common variants found through GWAS have little contribution.

Narcolepsy in Estonia is presumably underdiagnosed disorder. Patients are diagnosed by standard protocols; including assessment of excessive daytime sleepiness, polysomnography and test for Multiple Sleep Latency Time. Hypocretin levels in CSF were not measured. Estonian Genome Center has an ongoing project to gather narcolepsy patients all over Estonia. To date we have 17 patients, sent by sleep doctors: age 19-66, 10 men and 7 women. In these patients all of whom were not present. He was shorter than average. Later he has had learning difficulties (ADHD) and sporadic migraine. He was referred to a geneticist to specify the diagnosis at the age of 14. The young patient has been tested for NF1 mutations and variable expressivity.

In our study we focused on the elucidation of the genetic causes underlying the disease. A multi step protocol based on genomic DNA has been established for molecular diagnosis in Greek patients fulfilling the NIH diagnostic criteria. This protocol includes multiple PCR, sequencing of all NF1 gene exons and multiplex ligation-dependent probe amplification for deletions/duplications. The protocol was validated in a cohort of 34 NF1 patients and 20 relatives, identifying the germline mutations in most cases. Our results include 13 genetic variants, in coding and non-coding regions of NF1, most of them already reported in Human Gene Mutation Database. Among the novel variants the majority were stop codon mutations or variants which may affect the splicing process. Some novel mutations were found in patients with unclassified phenotypes. All were analyzed with the use of bioinformatic tools. In an attempt of possible phenotype/genotype correlations, we detected different mutations in patients within the same family, indicating the genetic complexity of the disease.

### J09.35

**Missense mutation (Met1035Arg) of the NF1 gene revealed through NGS**

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1University of Athens, Athens, Greece; 2Department of Medical Genetics, Athens, Greece.

Neurofibromatosis Type 1 is one of the most common autosomal dominant disorders with a birth incidence of one in 3,500 individuals worldwide. It is caused by mutations of the NF1 tumor suppressor gene, located at 17q11.2. Neurofibromin, the NF1 gene product, is an important negative regulator of cellular Ras signaling pathway. The main clinical features of the disease include café-au-lait spots, skin fold freckling, benign cutaneous neurofibromas, phakomatous neoplasms, optic gliomas and Lisch nodules of the iris. Disease penetrance is about 100%, while expressivity is extremely variable.

In our study we focused on the elucidation of the genetic causes underlying the disease. A multi step protocol based on genomic DNA has been established for molecular diagnosis in Greek patients fulfilling the NIH diagnostic criteria. This protocol includes multiple PCR, sequencing of all NF1 gene exons and multiplex ligation-dependent probe amplification for deletions/duplications. The protocol was validated in a cohort of 34 NF1 patients and 20 relatives, identifying the germline mutations in most cases. Our results include 13 genetic variants, in coding and non-coding regions of NF1, most of them already reported in Human Gene Mutation Database. Among the novel variants the majority were stop codon mutations or variants which may affect the splicing process. Some novel mutations were found in patients with unclassified phenotypes. All were analyzed with the use of bioinformatic tools. In an attempt of possible phenotype/genotype correlations, we detected different mutations in patients within the same family, indicating the genetic complexity of the disease.

### J09.33

**Two novel mutations in NF1 identified in Mexican patients with neurofibromatosis type 1**

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1IMSS, Mexico.D.F., Mexico, 2IHIMFG, Mexico.D.F., Mexico.

Neurofibromatosis type 1 (NF1) is an autosomal dominant genetic disorder with worldwide incidence of 1 in 2500 to 1 in 3000 individuals. It is caused by mutations in neurofibromin 1 (NF1), located on chromosome 17q11.2. The main signs and symptoms are café-au-lait spots, Lisch nodules in the eye, fibromatous tumors of the skin. Other clinical manifestations that have been reported include bone dysplasia and an increased risk of malignant tumours.

The aims of this study were to describe the clinical manifestations of the disease in 22 patients with neurofibromatosis type 1, and to identify the underlying NF1 mutations, by sequencing all 60 exons of this gene. Five different mutations were identified in 7 patients. Two of these mutations have not been previously described (Table 1).

Given the clinical variability of this disorder, and the small sample size, no statistical genotype-phenotype correlations were performed. However, the prevalence of clinical manifestations and the type of mutations seen are in agreement to what has been reported in other populations.

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1JIMS, Mexico.D.F., Mexico, 2IHIMFG, Mexico.D.F., Mexico.

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Influenza A virus infection changes the expression of NMDA receptor subunits in mice

M. Yeegorova, M. Plotnikova, S. Klotchenko, A. Garshchina, V. Zarubaev, A. Vasin; Research Institute of Influ ence, St-Petersburg, Russian Federation.

N-methyl-D-aspartate receptors (NMDARs) play an essential role in the process of synapses formation, neuronal differentiation, brain plasticity as well as participate in the molecular response to the neurotoxic substances action. The NMDAR dysfunction is associated with schizophrenia, Alzheimer’s disease, and Huntington’s disease. The influenza virus infection is also one of the potential negative factors influencing NMDAR function, but the molecular mechanisms of neurological complications of influenza are not well understood. The aim of our research was to study the influence of influenza A virus infection on the expression of NMDAR subunits (NR1, NR2A-D, NR3A-B) in mice. We analyzed the expression of seven genes, coding NMDAR subunits, in brains and lungs on the 1, 3, 5 and 10 days post infection using real-time PCR. Two groups of mice were infected with H1N1 and H3N2 strains at 0.2 LD50 respectively. The expression of all seven NMDAR transcripts was detected only in brains of non-infected animals. The infection with influenza A virus strain led to the changes in expression levels of NR2A, NR2B, NR2D and NR3B mRNAs in brains. In lungs NMDAR mRNAs were not detected. Further we are going to make the more detailed analysis not only of NMDARs expression but also of other genes involved in the regulation of the central nervous system during influenza A virus infection in mice.

New insights in non-syndromic intellectual disability: a case report

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A 15 years old girl with obesity, dysmorphic features (down-slaning palpebral fissures, short nasal bridge; short philtrum; micrognathia), moderate mental retardation, psychosis, dysplasia, aggressiveness, bilateral hypoplasia was referred for genetic investigations. aCGH on an 105K Agilent platform revealed arr 5p13.3:23(47,799,936-38,118,402)x3,11q13.3:14(17,658,015-83,043,032)x2, 17q25.3:3(79,829,409-79,910,442)x1. Interestingly, the ~6.88 Mb deletion on 11q includes 21 OMIM genes, among which two disease-causing entities: MYO7A, and ALG8. The first one encodes for an unconventional myosin, a motor molecule with structural conserved heads moving along associated actin filaments and highly divergent tails suggested to act as carriers of various macromolecular cargo molecules; MYO7A is known to carry heterozygous mutations in non-syndromic neurosensory deafness and homozygous mutations in most cases of Usher syndrome. The second gene encodes for alpha-3-glucosyltransferase which, when mutated to compound heterozygosity, produces the congenital disorder of glycosylation, a severe phenotype resulting in multiple dysmorphisms and defects leading to early infant death. The 0.63 Mb duplication on 5p harbors only one OMIM gene, GDNF, encoding for a specific dopaminergic protein associated with motor neuron function and Hirschsprung disease. Finally, the 0.08 Mb deleted fragment on 17q contains 80 OMIM genes, among which pyrroline-5-carboxylate reductase 1 that causes cutis laxa disease when it loses functions. The correlations of the identified genetic defects with the observed phenotype will be discussed, as well as their impact upon patient management. 

Acknowledgements: project PN 09.33.2.03.02.
**J09.41**

The role of Vitamin D and FokI Variant in the Vitamin D receptor Gene among Iranian Parkinson’s Disease Patients

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Introduction: Today, a role for vitamin D and its receptor (VDR) in Parkinson’s disease (PD) has been proposed. Several studies concerning the association between the VDR gene polymorphism and PD have been published. In this study, firstly we determined the correlation between 25-hydroxy vitamin D (25 OHD) and severity of PD then accessed frequency of FokI polymorphism in PD patients and healthy Iranian population.

Methods: The severity of PD was evaluated by using Hoehn & Yahr (HR) stages and Unified Parkinson’s Disease Rating Scale (UPDRS) Part III. After amplification of DNA samples from 62 unrelated normal individuals and 60 PD patients by PCR, polymorphism was genotyped by RFLP according to FokI site.

Results: Our study revealed that 25OHD level in patients with PD was not associated with HR stage and UPDRS scores. The most frequency of genotype in PD group and normal individuals was F/F (51.7%) and F/S (45.1%), respectively. Protection against the development of PD was applied when F allele be present, but it was not significant (OR= 0.7, 95% CI: 0.44-1.28, p=0.29). In contrast, insignificant susceptibility to PD (OR= 1.3, 95% CI: 0.437-1.283, p=0.29) was associated to S allele.

Conclusion: Our finding does not show correlation between vitamin D and HR stage and UPDRS scores during the early disease stages of PD in Iranian patients. Further unplanned genotypes were not significant, and no significant difference was seen for FokI polymorphism with Parkinson. Further studies are needed to reveal the main role of vitamin D and VDR gene in PD progression.

**J09.42**

Alpha-synuclein 3’UTR polymorphism is a risk factor for Parkinson’s disease in Iranian population

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Parkinson’s disease (PD) is one of the most common neurodegenerative disorders and both genetic and environmental factors are involved in its etiology. The incidence of PD differs by race and ethnicity, is greater in men than women, and increases markedly with age. One of the well-known genes involved in PD development, is alpha-synuclein (SNCA) gene and several polymorphisms in this gene have been identified to be associated with PD susceptibility. In this study, we investigated the association of four SNPs (rs2301134, rs2301135, rs356221, rs11931074) in the promoter region and 3’UTR of the SNCA gene, with Parkinson’s disease in Iranian population.

A total of 960 subjects (480 PD patients and 480 normal healthy controls) were included and genotyped for all four polymorphism. The method used for genotyping was tetra-primer ARMS PCR. We found significant differences in genotype distributions of rs11931074 variant in patients compared to controls (odds ratio = 7.6970, 95% CI = 5.7514-10.3006, p < 0.0000001). Our findings indicate a significant association of rs11931074 SNP with PD in Iranian population.

**J09.43**

Association analysis of Parkinson’s disease with polymorphic variants in SNCA, LRRK2 genes and MAPT-region (17q21.31) in three ethnic groups from Bashkortostan Republic of Russia (GWAS replication results)

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We performed replication of genome-wide association analysis (GWAS) with Parkinson’s disease (PD) in three ethnic groups from Bashkortostan Republic (BR) on 550 PD patients (Russians - 215, Tatars - 243, Bashkir - 90) and 622 controls (Russians - 190, Tatars - 338, Bashkir - 94). The study included analysis of 5 polymorphic loci: rs653219 in SNCA gene, rs4191942, rs1907632 in LRRK2 gene, rs1981997 and rs12373139 in chromosomal region of MAPT gene (17q21.31).

The results of GWAS were obtained in the indicated polymorphic loci of SNCA gene and MAPT-region were confirmed only for Tatar ethnic group. We found that genetic diversity development was C/T (p = 0.002; OR = 1.73; CI = 1.21 - 2.46) and allele T (p = 0.04; OR = 1.4; CI = 1.01 - 1.8) of SNCA gene (rs653219); genotype G/G (p=0.001; OR=1.84; CI=1.16 - 2.92) and allele G (p = 0.01; OR=1.72; CI =1.11 - 2.65) of MAPT gene (rs1981997).

**J09.44**

Molecular basis of mechanism of gene expression modulation induced by hypericin in genes involved in the pathogenesis of Parkinson’s disease

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Parkinson’s disease is a multifactorial heterogeneous neurodegenerative disorder manifested particularly in the elderly people. Although originally thought not to be hereditary, recent studies have identified several genes that are responsible for the manifestation of the disease. Clarification of the molecular basis of disease would allow more accurate diagnosis of the disease and would also help in treatment and prevention. The aim of our work was to study the effect of hypericin, a substance isolated from the plant Hypericum perforatum L., on expression modulation in genes involved in the pathogenesis of Parkinson’s disease. Experiments were carried out on the A549 (alveolar adenocarcinoma), 42-MG-BA (glioma) and MSC (mesenchymal stem) cell lines using microarray and qPCR. Our results show that hypericin can modulate expression of more with Parkinson’s disease associated genes.

**J09.45**

RT2, a susceptibility gene for Parkinson’s disease in Iranian population

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Parkinson’s disease (PD) is the second most common and a complex neurodegenerative disorder arising from a complex interaction between genetic and environmental factors. Determining the pathogenic gene contributed to Parkinson’s disease have always been controversial. In a large meta-analysis study on Caucasian populations another novel PD susceptibility locus, RT2, on chromosome 18, was identified. A genetic variant in RT2, rs12456492, was found to be associated with risk of sporadic PD. In the present study, we investigated the association of the rs12456492 variant with Parkinson’s disease in Iranian population. A total of 960 subjects (480 PD patients and 480 normal healthy controls) were included and genotyped for rs12456492 polymorphism (A/G) of RT2 gene. Genotyping was done by tetra-primer ARMS PCR technique. We found significant differences in distributions of GG genotype in patients compared to controls (odds ratio = 1.60, 95% CI = 1.14-2.23, p = 0.005). Our findings increase the likelihood of association between PD and RT2 variant in Asian populations.

**J09.46**

Partial duplication Xq27.1 in a dysmorphic and mentally retarded girl

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Background. Duplications of the long arm of chromosomes X have been related to mental retardation, different dysmorphic features, hypopituitarism and short stature. Aim. We aim to examine the relation between the partial duplication of the Xq27 region and the presence of clinical manifestation in a dysmorphic and mentally retarded girl.

Methods. We report on a 3 year old girl associating facial dysmorphism, hypotonia, delayed milestones, mental retardation. Proposita is the first child of a young couple, born at 39 weeks of gestation by caesarean section due
to pelvic presentation. Birth parameters were: weight 2850 g, length 52 cm, cranial circumference 31 cm.

Results: A good evolution was recorded after birth, with no sucking or swallowing difficulties. Craniofacial dysmorphisms includes: microcephaly, hypoplasia of the mandible, low-set ears, hypertrophic gums, prominent lips, hypoplastic nasal bone.

At age of 3 she cannot walk without support, respond to simple commands, says only 2-3 words. The girl is sociable and presents a happy attitude. EEG, EMG and MRI were normal. Conventional cytogenetic investigation showed a normal female karyotype. Revealed a 408 kb duplication on chromosome Xq27.1. The duplication encompasses SOX3 and CDRI genes.

Conclusion. Further studies including the analysis to prove the X inactivation pattern, and molecular investigation of the aberrant chromosomes X in female presenting genetic imbalance is often reported.

Array CGH was an essential tool for molecular characterization of this case and for establishing the recurrence risk for a future pregnancy.

**J09.47**

A 9-year-old boy diagnosed with Pantotenolase Kinase-Associated Neurodegeneration after a 6-year history of psychiatric disturbances

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Pantothenolase Kinase-Associated Neurodegeneration (PANKAN) is a form of neurodegeneration with brain iron accumulation (NBIA) formerly called Hallervorden-Spatz disease. PANKAN is an autosomal recessive condition characterized by iron deposition in the basal ganglia leading to progressive extrapyramidal dystonia, rigidity, and/or choreoathetosis, pigmentary retinal dystrophy and speech disturbances. PANK2 is the only known gene related to PANKAN. MRI shows the highly specific “eye of the tiger” sign in almost all affected individuals with at least one PANK2 mutation. We report a Danish family in which a 9-year-old boy, a sibling of 4 children, has been diagnosed with PANK2 after a 6-year history of psychiatric disturbances. At the age of 2-3 years the patient developed behavioral problems, and was diagnosed with Attention Deficit/Hyperactivity Disorder (ADHD), and the first MRI was performed. At the age of 3-4 years the psychomotor development slowed combined with some speech problems, and the patient was diagnosed with mild mental retardation. At the age of 7 he is diagnosed with infantile autism and the second MRI was performed, where the “eye of the tiger” sign was reported. At the age of 9 the patient developed choreoathetoid dystonia in the right arm and the right side of the neck, and the third MRI was performed confirming the “eye of the tiger” sign. Genetic analysis was performed and revealed compound heterozygosity for a pathogenic mutation in exon 5 and a deletion of exon 2 of the PANK2 gene, confirming the diagnosis of PANKAN.

**J09.48**

Whole exome sequencing analysis in a large Primary Angle Closure Glaucoma (PACG) pedigree

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Glaucoma is a heterogeneous group of optic neuropathies characterized by progressive degeneration of the optic nerve head and visual field loss. It is sub-grouped according to the anatomy of the anterior chamber angle into two main forms: Primary Open Angle Glaucoma (POAG) and Primary Angle Closure Glaucoma (PACG). POAG is the more predominant form of glaucoma in Europeans, Africans, and possibly most populations. PACG is most prevalent in countries of the far East; 80% of PACG affected individuals live in Asia. PACG is characterized by a narrow iridocorneal angle which obstructs the normal aqueous humor outflow and causes increased Intraocular Pressure (IOP). Although epidemiological studies have suggested a genetic basis for PACG, a causative gene for the disease has not been identified.

A relatively large pedigree with at least nine members diagnosed with PACG or PACG suspect all of whom presented with a closed iridocorneal angle, was introduced to us. Manifestation of PACG in multiple family members suggested a strong genetic contribution for the disease in the family. With the objective of identifying a causative gene, whole exome sequencing was performed on three affected members of the family. Output data were analyzed with appropriate softwares. After filtering against existing SNP databases, 72 novel, non-synonymous variants common among the three patients were identified. The variations were distributed in 60 genes. Some of these genes have defined functions related to eye and glaucoma pathology. Screening of the variations in control individuals and segregation analysis in the pedigree are in process.

**J09.49**

Sequence variants of PRNP gene in probable prion disease patients

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Prion diseases are a family of rare progressive neurodegenerative disorders caused by abnormally conformed infectious proteins, called prions. The functions of these normal prion proteins are still not completely understood. The abnormal folding of the prion proteins leads to brain damage and the characteristic signs and symptoms of the disease. There are 4 types of this disease: the sporadic (Jakob-Creutzfeldt disease, 85-90%), familial (10-15%), iatrogenic (1%), and variant types. Genetic form of prion diseases are caused by mutations in the prion-related protein gene (PRNP), and classified based on the phenotype, mutation and neuropathological findings. In this study, we analyzed PRNP gene to evaluate the frequency of PRNP sequence variants and their genotype-phenotype correlation in 60 probable Prion disease patients. Genetic analysis of the PRNP gene was performed on peripheral blood samples using polymerase chain reaction and direct-sequencing. We found four different PRNP sequence variants in thirty-six patients (G245S, M129V, D107N, octapeptide repeat deletion: c247_279del). The rate of PRNP sequence variant was 60% in our samples. PRNP screening may be useful for genotype-phenotype correlation in Prion disease.

**J09.50**

Eight new cases of PEHO or PEHO-like syndrome?

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PEHO syndrome (Progressive Encephalopathy with oedema, Hyparrythmia and Optic atrophy) (OMIM #260565) belongs to a rare neurodegeneration disorders of unknown etiology and probable autosomal recessive inheritance. Only 50 patients have been described worldwide so far, with the vast majority in Finland. We present eight new patients who fulfil diagnostic criteria of PEHO or PEHO-like syndrome. Patients were selected from a group of patients with a diagnosis of “Progressive encephalopathy of unknown etiology” or “Cerebral palsy”. In all of them onset occurred during the first few weeks or months of life. The leading symptoms were hypotonia, poor feeding, dribbling, infantile spasms, seizures with hyparrythmia in EEG, absent eye contact, optic atrophy, no any progress in psychomotor development. Dysorphic features were peculiar and consisted of: microcephaly, “pear-shaped” face, narrow forehead with prominent metopic ridge, full cheeks, receding chin, epicantal folds, an open mouth with a curved upper lip, hypertrophic gums, protruding ear lobes, a short nose with anteverted nares, edema of upper and lower extremities and tapering fingers.

In six patients cerebral, cerebellar and/or brainstem atrophy were found by MRI. This group was named as PEHO syndrome and rest two child’s without any abnormalities in MRI as PEHO-like.

**J09.51**

Association of GRM3 gene polymorphic loci with schizophrenia

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Schizophrenia is a devastating psychiatric disorder with a morbidity risk of 7.2 per 1,000 which is affected by genetic and environmental factors. The disruption of the glutamatergic system is becoming recognized as an essential component of the pathogenesis of schizophrenia. The type-three metabolic glutamate receptor gene (GRM3) which codes for the mGluR3 protein localized to the periphery of pre- and post-synaptic neurons is essential for optimal signaling of glutamate in the brain.

The subject of the present study was the research of the association of two polymorphic loci rs274622 and rs187993 of GRM3 gene with the development of schizophrenia in a sample of 338 cases (50% Russians and 50% Tatars) and 350 controls (50% Russians and 50% Tatars) from Volga-Ural region of Russia. The genotyping of these polymorphic loci was carried out by PCR-RFLP. All observed genotype frequencies were consistent with Hardy-Weinberg equilibrium. It was found by analysis of variance that genetic polymorphic loci rs187993 was a statistically significant different in our samples. However polymorphic locus rs274622 of GRM3 gene was not shown statistically significant differences between case-control groups of Russians and Tatars.
In addition this study can be useful for understanding of the pathogenesis of schizophrenia and is required to continue.

J09.52 Effect of PAI-1 gene on predisposition of schizophrenia in Turkish population
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PAI-1 is a protease belonging to the family of serine pro tease s. It regulates proteolysis and fibrinolysis via inhibition of tissue type plasminogen ac tivator (tPA) and urokinase (uPA). It has an important potency in cerebro spinal fluid homeostasis. Fibrinolytic system in responsible in CNS affects long-term synaptic plasticity and remodelling. Activity of tPA is neurite out growth, neuronal migration and learning. Polymorphisms in this gene may influence disease development. Therefore, we aim to investigate the effect of the 4G/5G insertion / deletion polymorphism localized in -675 upstream promoter region of PAI-1 gene and can modify the expression of protein levels, on predisposition of disease.

The presence of the 4G/5G polymorphism in the PAI-1 gene was determined using a restriction fragment length polymorphism (RFLP) method. Sam pling identified according to whether they have 139 bp band or not. Samples produced 139 bp band with 4G primer were identified as homozygous 4G genotype, samples produced 139 bp band with 5G primer were identified as homozygous 5G genotype, samples produced 139 bp band with both 4G and 5G primer were identified as heterozygous 4G/5G genotype.

In our study, we investigate the frequency of genotype and allele of PAI-1 gene in schizophrenic patients and healthy controls. Significant correlation was found between genotypes (p<0.05), but no statistically significant differences were found in allele frequencies (p>0.05). Studying of more larger population may help to explain the effect of disease-related gene. The study was supported by ESOGU (Grant no:201211 007).

J09.53 A new mutation at the SLC20A2 gene in an Italian family with idiopathic basal ganglia calcification
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A new mutation at the SLC20A2 gene in an Italian family with idiopathic basal ganglia calcification (IBGC) was described. We report a novel SLC20A2 variant identified in ex on 9 (G1618A), consisting in a gly to-arg substitution at position 540 of the protein highly conserved consensus region, in two family members clinically diagnosed with IBGC. This variant was not found in 200 unrelated Italian controls. The index case was a 67-year-old male patient who presented with a mild parkinsonism and a more relevant reduction in spontaneous speech, which evolved steadily over a few years to an almost complete inability to communicate. The proband’s daughter developed symptomatic epilepsy at 19 years of age as the only clinical manifestation of IBGC, suggesting an anticipation of the disease over generations and striking intrafamilial neuropathic heterogeneity, as previously observed. This is the first SLC20A2 mutation associated to IBGC reported in the Italian population. This mutation is damaging according to all prediction programs and it suggest a loss of function of the protein. In conclusion, the discovery of deleterious muta
tions in SLC20A2 as a cause of IBGC greatly advances our understanding of this complex disease.

J09.54 Somnambulism in a Colombian family with dominant inheritance
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Sleepwalking and night terrors are sleep disorders classified into the group of excitement Parasomnias. These disorders generate a considerable degree in the individual’s quality of life. However the inheritance patterns for these disorders are poorly known and there is a lack of knowledge of the responsible genes for these disorders. We report a three-generation Colombian family with 18 affected individuals, showing a dominant inheritance pattern with reduced penetrance. There is one only study that previously reported the same inheritance pattern in an American family demonstrating linkage with a region between the SNP type markers rs728331 and rs286819 (Licis, et al. 2011). However, there are some major differences between the reported family and ours. Onset prevalence and sex-affected ratio are the major phenotype distinctions. We propose that we may have a new condition that is caused by different genes.

J09.55 The clinical phenotype of Polish patients with SPG11 gene mutations - preliminary description
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Hereditary spastic paraplegias (HSP) are a group of clinically and genetically heterogeneous, neurodegenerative disorders characterized by progressive spasticity of the lower limbs. The most common causes of autosomal recessive HSP with thin corpus callosum (TCC), associated with complicated form of HSP, are mutations in the SPG11 gene.

In a group of 159 index patients clinically diagnosed as HSP (criteria according to Fink), in whom SPAST and ATL1 mutations were previously excluded, MLPA analysis revealed homozygous mutation and compound heterozygous mutation in the SPG11 gene, confirming definitive diagnosis in two probands. Moreover, in three patients (from other families) the only mutation was found. Direct sequencing analysis will be necessary to confirm diagnosis. Individuals were assessed by Spastic Paraplegia Rating Scale and classification used by Dürr.

In a 4 patients from two families with definitive diagnosis, age at onset ranged 10-16 years (mean 13±3), disease duration 9-23 years (15±6), SPRS scores 42-49 (mean 45±3). All patients had fast progression of symptoms and severe phenotype. One of them could not move without walking aids, three were wheelchair-bound. Brain MRI showed TCC (100%), cortical (100%) and cerebellar (75%) atrophy. Nerve conduction study revealed sensorimotor neuropathy axonal type in three individuals. All patients presented mild to moderate cognitive impairment.

The present study confirmed early onset, remarkable progression and severe clinical phenotype of four individuals with mutations in SPG11 gene. The clinical picture in our patients was consistent with typical manifestation of HSP-TCC. Among five index patients only one had family history of AR-HSP.
J09.57 Metal levels in blood as biomarkers for ataxias. S. Squadrone1, C. Mancini5, E. Giorgio5, E. Pozzi5, S. Cavalieri5, E. Di Gregorio1, M. C. Abetti6, A. Bracco2,3
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Metals are crucial for synaptic transmission, enzyme activity, proteins folding/conformation. Copper, zinc, iron and manganese are all localized to various subcellular compartments and their regulation is controlled by a diverse range of co-factors and chaperones. Loss of homeostasis of some of these elements is present in neurological disorders including Alzheimer, Parkinson and Huntington on diseases, amyotrophic lateral sclerosis and Friedreich Ataxia. Metals are also key factors for ATP generation and to control oxidative damage in mitochondria.

We selected a cohort of spinocerebellar ataxia (SCA, n=20) and Ataxia-Telangiectasia (AT, n=20) patients, and we measured copper, zinc, iron, manganese and selenium levels in their blood samples, using atomic absorption spectrometry.

We found a significantly reduced manganese levels in SCA patients and increased copper levels in AT patients compared to controls (p<0.01). Cu and Mn have essential roles in mitochondria: the former is a component of complex I, II and III and has a central role in maintaining antioxidative enzyme function and it is the superoxide dismutase co-factor (MnSOD; SOD2). We evaluated in patients vs. controls LCLs mRNA levels of catalase-CAT, glutathione peroxidase-GPX1 and SOD2, enzymes involved in antioxidative response. We found SOD2 and CAT up-regulation in AT patients and a SOD2 down-regulation in SCA patients.

We considered SCA panel mutations activity and showed a reduction of MnSOD-activity in SCA LCLs, whereas Cu/ZnSOD-activity was balanced in AT LCLs. These data, although preliminary and restricted to a small number of samples, suggest a role for metals in ataxias and may represent biomarkers of pathology.

J09.58 Screening of TARDBP in Iranian amyotrophic lateral sclerosis (ALS) patients M. Khami1, E. Eshghi1, A. Alavy2, S. Najafi3, M. Malakooti Nejad4
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Amyotrophic lateral sclerosis (ALS) is a fatal motor neuron disorder characterized by dysfunction and degeneration of both upper motor neurons in the cortex and lower motor neurons in the brainstem and spinal cord. Genetic analysis of familial ALS (FALS) pedigrees has led to the identification of at least 21 loci and 18 ALS-causing genes. Other C9ORF72 and SOD1, mutations in human TARDBP gene are the most frequent cause of disease in FALS families. TARDBP encodes transactive response DNA-binding protein-43 (TDP-43).

In addition to its prominent role in ALS, TDP-43 is important because of its functions in nuclear transcriptional regulation and its role as a key player in neurodegeneration.

We here screened in 90 unrelated patients with a AA genotype reported higher Body Dis-

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currenty estimated at 1/100. Several biological pathways have been highly illuminated, particularly the excitatory synapse, affected by copy number variations (CNV) and enrichment in mutations in genes belonging to the NMDA receptor complex (NRC). We performed a global genetic study of 100 French families including at least 1 individual with autism, who were included in the research project (ClinicalTrials.gov NCT01770548).

Our project aimed to evaluate both the contribution of CNV in ASD, and secondly the presence of mutations in the NRC complex. For each family, we firstly performed a high-resolution pangenomic comparative genome hybridization (CGH) analysis with 1 M CGH Agilent Array format to identify rare or de novo CNVs. In parallel, a high-throughput targeted sequencing of 21 genes mostly belonging to the NRC complex (179) was carried out with the SureSelect Agilent strategy in order to assess the contribution of gene mutations in our cohort.

The first results of our study allowed us to characterize candidates and likely pathogenic genetic alterations in at least 10% of patients. We have found mutations or CNV in genes encoding receptors (NLGN4X, GRM5), and in toxicological (VPAC2), nuclear (MACROD2), and synaptic (NXPH3) proteins. They also emphasized that the NRC is targeted both by mutations and CNV, inherited or de novo.

These results underscore the fundamental role of this multigene network in the process of neuronal communication and learning and its pathophysiological impact in autism.

J09.60 The study on the effect of fibrillation inhibitory compounds on the depolymerization of amyloid fibrils of alpha-synuclein using fluorescent-labeled protein. A. Tayaraniann1, D. Morshedih2, F. Alikarbari3
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Parkinson’s disease (PD) is the most prevalent movement disorder in the world which is caused by dopaminergic cell degeneration in substantia nigra pars compacta of midbrain. One of the major explanations for occurrence of this cell death is the aggregation of a protein called alpha-synuclein which produces cytotoxic aggregates and amyloid fibrils in brain. Many studies have done to find fibrillation inhibitory compounds for preventing and treating PD patients. But studying on concealed dimensions and side effects of these compounds is a great concern. In this research we studied on the depolymerization of alpha-synuclein fibrils under induction of fibrillation inhibitory compounds include Curcumin, Cuminaldehyde and Baicalein by using fluorescent labeled alpha-synuclein protein. Our result shows that these compounds induce depolymerization of alpha-synuclein fibrils that is highly related to their cytotoxicity. Some studies on alpha-synuclein fibrillation revealed that intermediate aggregates include oligomeric species may play a greater role than amyloid fibrils in dopaminergic cell death. This depolymerization phenomenon can produce some intermediate aggregates with cytotoxic properties that can cost the patient’s condition. Among these compound Cuminaldehyde shows lower depolymerization induction that define this natural organic compound as a valuable candidate for PD therapy.

J09.61 5-HT2A and Body Dissatisfaction involved in the development of Eating Disorders T. Ialacci1, G. Castellini2, F. Ceri2, A. Di giacinto3, M. Franzaghi4, R. Ferrante1, I. Antonucci5, G. Stanghellini2, V. Ricci2, L. Shipp1
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Despite numerous studies about Eating Disorders (EDs) their etiopathogenesis is currently debated and poorly understood. Studies carried out on families and twins have highlighted genetic factors’ role; molecular genetic studies have identified the candidates genes potentially involved in ED’s etiology. To date research has not produced conclusive results, nevertheless genes of serotonergic and dopaminergic neurotransmitters systems seem to be promising candidates. Among these important candidate genes in the EDs susceptibility, various studies have evaluated the possible role of the -1438 G/A polymorphism within the 5-HT2A. In this study 202 Eating Disorder patients (Edp) and 150 control subjects were analyzed for distribution of the 5-HT2A receptor promoter polymorphism and we shown that the AA genotype is more frequent in patients (p=6.69; p<0.01) than in control suggesting an association of 1438 G/A polymorphism within the 5-HT2A with EDs, in agreement with the past literature. Moreover within our healthy sample subjects with a AA genotype reported higher Body Dis-
Red blood cells hemolysis influences the level of total but not oligomeric plasma alpha-synuclein in Parkinson’s disease patients and controls

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Parkinson’s disease (PD) is the second of most common neurodegenerative disorders. Oligomeric alpha-synuclein is the principal neurotoxic agent of PD pathogenesis. Plasma alpha-synuclein has been suggested as biomarker for PD with inconsistent results. As red blood cell (RBC) are the main source of plasma alpha-synuclein in the level of total plasma protein are influenced by the degree of RBC contamination and hemolysis in plasma. The correlation between plasma hemoglobin and oligomeric alpha-synuclein levels remains unknown.

The aim of our investigation was to establish if RBC contamination and hemolysis in plasma influences the level of total and oligomeric plasma alpha-synuclein in patients and controls. The dataset was composed of 18 drug-naive PD patients (the mean age 67±8.7 years) and 23 control individuals (mean age 65.67±11.27). All subjects were residents of the Northwestern region of Russia. The total, oligomeric forms of alpha-synuclein as well as hemoglobin levels were estimated by means of ELISA (Human alpha-synuclein ELISA kit (Invitrogen, USA) and Hemoglobin (Human) ELISA kit (Abnova, USA) correspondingly.

Measured levels of blood plasma hemoglobin vary from 12614 ng/ml to 591041 ng/ml. The correlation between the total alpha-synuclein and hemoglobin level has been shown (N=39, r=0.426, p=0.0001). However, there were no significant differences in the levels of the total and oligomeric plasma alpha-synuclein between groups. Therefore our data suggest that hemolysis has an effect on a total but not oligomeric alpha-synuclein in peripheral blood plasma. At the same time, both total and oligomeric alpha-synuclein could not be used as a suitable marker of PD.

A key element of Endocannabinoid System PPARy2 and leptin are associated in turkish schizophrenia patients

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The Endocannabinoid System (eCB) is deeply involved in bodyweight regulation, other than the obesity genes, endocannabinoid system may also have a role in the antipsychotic-induced weight gain in schizophrenia (SCH) patients.

Aim: To further investigate this hypothesis we performed an association study with PPARγ2 gene coding for a key element of the eCB system and obesity related genes, leptin, leptin receptor and MC4R in a sample of 329 SCH patients and 237 controls.

Methods: Biochemical analyses, the effects of LEP c.-25460A>C, LEP c.-220-078C>G, MC4R c.307G>A polymorphisms and the impact of these genes mRNA and serum levels on metabolic advantages in SCH patients and control groups were studied.

Results: Significantly higher BMI and fasting blood sugar and significantly lower HDL levels were present in SCH patients compared to controls (p<0.001). PPARγ2 and LEP polymorphisms were significantly different between SCH and control groups, while a significant difference was present in PPARγ2 polymorphism between male SCH patients and male controls (p=0.005). Interestingly, MC4R c.307G>A/G>A/G-carriers showed significant differences in BMI and LEP mRNA levels compared to wild type SCH patients (A/A). LEP, LEPR, PPARγ2 mRNA levels and leptin serum levels were also significantly higher in SCH patients compared to controls (p<0.01, respectively).

Leptin serum and mRNA levels were positively correlated in PPARγ2 c.-2-28078C>G carriers (p=0.001) and LEP c.-25460A>C carriers (p=0.005). PPARγ2 c.-2-28078C>G carriers showed significant differences in BMI and LEP mRNA levels compared to wild type SCH patients (A/A). LEP, LEPR, PPARγ2 mRNA levels and leptin serum levels were also significantly higher in SCH patients compared to controls (p<0.001, respectively).

Leptin serum and mRNA levels were positively correlated in PPARγ2 c.-2-28078C>G carriers (p=0.001) and LEP c.-25460A>C carriers (p=0.005). PPARγ2 c.-2-28078C>G carriers showed significant differences in BMI and LEP mRNA levels compared to wild type SCH patients (A/A).

Our findings suggest a strong association between PPARγ2 and leptin genes in Turkish schizophrenia patients which might indicate their potential role in the antipsychotic-induced weight gain, but further studies are needed in order to elucidate their involvement in the pathophysiology of SCH.

J09.64 Linkage of a locus for autosomal recessive familial spastic paraplegia to chromosome 8q24

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Hereditary spastic paraplegia (HSP) is characterized by insidiously progressive lower-extremity weakness and spasticity. HSP being a group of genetic disorders, they follow general inheritance rules and can be inherited in an autosomal dominant, autosomal recessive or a linked recessive manner. The object of our study was to determine the impact of investigating the disorder. This neuronal degeneration is thought to be caused by mutations at specific genes. Genetic linkage analyses were carried out with polymorphic DNA markers. The candidate gene within the linked region was sequenced in order to verify the observation. Homozygosity mapping showed linkage to 8q24. As a result the first candidate gene for screening was SPG8 also known as KIAA0196 because this gene is located in the linked region and one is of HSP related genes. The KIAA0196 contains 29 exons. Precise screenings of all 29 exons of the gene with their boundaries were carried out and using sequencing and PCR screening negative for SPG8 mutations. In conclusion, these data confirm the presence of another SPG gene on chromosome 8q24 related to autosomal recessive HSP.
CMT patients from Southern Italy. This finding enlarges the population.

In conclusion, we report the first Italian patients with two compound heterozygous point mutations in the DDHD2 gene: the novel c.307T>C and the already described c.1978G>C. Both mutations were validated by Sanger sequencing and they were confirmed in the second affected brothers.

In conclusion, we report the first Italian patients with two compound heterozygous point mutations in the DDHD2 gene. This finding enlarges the clinical spectrum related to DDHD2 mutations, providing to determine the worldwide distribution and the diverse clinical features of SPG54 form.

J09.67 Mutational screening of GJB1, MPZ and PMP22 genes in a cohort of CMT patients from Southern Italy

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Charcot-Marie-Tooth (CMT) is the most common inherited neuromuscular disease. To date, mutations in more than 40 genes are responsible for CMT disease. We have analyzed 500 patients coming from Southern Italy, referred to our centre with a suspected diagnosis of CMT. Firstly, all patients have been screened for the 17p.11.2 duplication. The non duplicated cases were further investigated for point mutations in the GJB1, MPZ and PMP22 genes. Among the 500 unrelated CMT patients, 209 of them (41.8%) harbored the CMT1 duplication. A subsequent mutational analysis of GJB1, MPZ, and PMP22 genes in 291 CMT patients, revealed 26 different mutations in 48 cases: 14 in GJB1, 7 in MPZ, 5 in PMP22. Out of 14 GJB1 missense mutations identified in 16 patients, seven are novel (Ser491Phe, Ala80Val, Ser128Leu, Leu131-Arg Val148Phe, Phe153Leu and Arg164Leu). Out of 7 mutations in the MPZ gene, identified in 27 patients, two are novel (Trp57X and Gly93Arg). Noteworthy, in 14 patients from Apulia the known Val102fs mutation has been detected, whereas seven patients from Sicily carried the known Ser78Leu mutation, confirming our previous results of a founder effect for the Ser78Leu. Finally, in the PMP22 gene we detected two novel variants (Ala257Thr and Ser294Trp). Our data suggest that the duplication is the most common genetic cause of the CMT, followed by mutations in GJB1 and MPZ. Furthermore, this study recommends that for the patients from Apulia and Sicily the diagnostic test, after the exclusion of the duplication, might be start from MPZ gene.

J09.68 Variation in the miRNA-433 binding site of FGF20 is a risk factor for Parkinson’s disease in Iranian population

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DNA variation at the FGF20 gene has been associated with Parkinson’s disease (PD). Specially, single nucleotide polymorphism (SNP) rs1272028 in the 3’ untranslated region (3’ UTR) was linked to PD risk through a mechanism that would implication a differential binding to microRNA-433 (miR-433). In this study, we genotyped the rs1272028 SNP in a total of 400 PD patients and 480 healthy controls from Iran. We found significant differences in allele and genotype frequencies between patients and controls (Fisher exact p<1×10^-8). The results obtained in this study revealed that the rs1272028 (C/T) polymorphism is a strong risk factor for late-onset PD in Iranian population.

J09.69 Evidence of Dysbindin gene promoter hypermethylation in peripheral blood lymphocytes as a potential biomarker in major psychoses

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DTNBP1 is the gene encoding Dysbindin or Dystrobin binding protein. DTNBP1 is established as a genes associated with certain psychiatric disorders. Genetic variations of DTNBP1 have been found in association with schizophrenia, Major Depressive Disorder, Bipolar Disorder, Substance induced Psychosis and certain cognitive properties. Functional studies have concluded that reduction of expression is the likely mechanism of effect of DTNBP1 in pathophysiology of mental conditions. Moreover, due to dysbindin protein functions in neurotransmission, dysfunction mechanisms have been postulated in cognitive and psychotic conditions.

In an EWAS on methylene of post-mortem brain of major psychoses patients, hypermethylation of an intron, promoter and an upstream region of DTNBP1 gene was found to be FDR-significant in association with bipolar disorder. Furthermore, variation of DTNBP1 mRNA levels in post-mortem cerebellum has been shown to be concordant with it in peripheral blood lymphocytes (PBL) of the individual in expression profiling studies. In addition, the expression reduction of DTNBP1 has been demonstrated in PBL of psychotic patients.

Here, we investigated DTNBP1 promoter methylation variation in psychotic disorder patients. A total of 208 patients and 106 age and sex-matched controls was measured with Quantitative Methylation-Specific High-Resolution Melting (MS-HRM). We observed a statistically significant hypermethylation in psychotic bipolar cases against normal samples. Provided the results be replicated in broader investigation, PBL DTNBP1 promoter hypermethylation could be considered a potential biomarker of psychotic bipolar disorder.

J09.70 Clinical, Neuroimaging, and Genetic Characteristics of Megalencephalic Leuкоencephalopathy With Subcortical Cysts in Egyptian Patients

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Megalencephalic leuкоencephalopathy with subcortical cysts (MLC) is a rare cerebral white matter disease. Clinically, it is characterized by macrocephaly, developmental delay, and seizures. We explore the clinical spectrum, neuroimaging, and gene involvement in the first MLC patients described from Egypt. Patients: Six patients were enrolled from three unrelated families. Pathology: Inclusion criteria were macrocephaly, developmental delay, normal urinary organic acids, and brain imaging of diffuse cerebral white matter involvement. Direct sequencing of the MLC1 gene in patients’ families and GluCAM in one questionable case was performed. Results: Clinical heterogeneity, both intra- and interfamilial, was clearly evident. Developmental delays ranged from globally severe or moderate to mild delay in achieving walking or speech. Head circumference above the ninety-seventh percentile was a constant feature. Neurimaging featured variability in white matter involvement and subcortical cysts. However, findings of posterior fossa changes and brain stem atrophy were frequently (66.6%) identified in these Egyptian patients. Discrepancy between severe brain involvement and normal mental functions was evident, particularly in patients from the third family. MLC1 mutations were confirmed in all patients. Deletion/insertion mutation in exon 11 (c.908-918delinsGCA, p.Val303 Gly fsX9) was recurrent in two families, whereas a missense mutation in exon 10 (c.880 C>T, p.Pro294Ser) was identified in the third family. CONCLUSIONS: This report extends our knowledge of the clinical and neuroimaging features of MLC. It confirms the apparent lack of selective disadvantage of MLC1 mutations on gamete conception and transmission as supported by the presence of multiple affected siblings in Egyptian families.

J09.71 Association and Gene-Gene Interactions of the HPA Axis Genes with Suicidal Behaviour

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The hypothalamic-pituitary-adrenal (HPA) axis is activated in different ways...
during chronic stress and involved in the neurobiology of different mood disorders including suicide behavior. The CRHR1 gene controls the activation of HPA axis via expression and functionality. Immunophilin FKBP51 is expressed in cortical neurons and regulate the function of the glucocorticoid receptor which regulates HPA axis. Genetic variants in the FKBP5 gene enconding FKBP51 are linked to suicide.

The aim of four study was to examine association of rs788868 polymorphism of CRHR1 gene, polymorphisms rs4713902 and rs7750737 in FKBP5 gene with suicidal behavior in Russian and Tatar patients from Bashkortostan. We genotyped DNA samples of 312 cases (101 - Male, 152 - Female, 150 - Russian, 120 - Tatar) who had suicide attempts and 346 control subjects (194 - Male, 152 - Female, 263 - Russian, 248 - Tatar) from Russia using PCR-RFLP and PCR with fluorescent detection (FLASH/RTAS) techniques. We observed a strong association between CRHR1 rs788868 and suicide: a lecle C was significantly overrepresented both in Russian (P<0.013, OR=1.79, 95%CI 1.13-2.86) and Tatar ethnicity groups (P<0.00101, OR=3.06, 95%CI 1.99-4.32).

No significant differences in either allele, genotype or haplotype frequencies of FKBP5 gene SNPs were found between suicidal and control groups. However, gene-gene interactions showed strong association in Tatar patients between investigated genes: rs7750737*A/*A - rs8788686*C/G (P=0.0012, OR=4.03, C195% 1.75-9.35) and rs4713902*G/C -rs7888686*C/G (P=0.0009, OR=4.84, C195% 1.92-12.41).

Our results show correlation of investigated genes in predisposition to suicide behavior, and confirms ethnic specificity of this association.

**J10.01**

**Phenotype-genotype correlation in patients with dystrophinopathy in families from Daghestan**

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Dystrophinopathies are autosomal recessive muscular dystrophies caused by mutations in dystrophin gene (DYSF, MIM# 603009). Dystrophin deficiency leads to two main phenotypes: limb girdle muscular dystrophy (LGMD) 2B and Miyoshi myopathy (MM). Dystrophin is located on the plasma membrane of skeletal muscle and is deficient in patients with MM and LGMD2B.

We’ve retrospectively reassessed the disease history (onset and progression). Earlier the phenotype genotype correlation was established in patients with dystrophinopathy variants in Daghestan families. Illariskhin et al in 1996 examined a large 6-generation family, which include 12 patients whom 9 were manifestations LGMD 2B and 3 were MM.

Clinical assessment was performed with a standardized protocol, including the Medical Research Council scale, a serum creatine kinase level, electrocardiography, and a muscle computed tomographic scan, sequencing DNA. 11 patients from 7 families, originally from a mountain village (9 males and 2 women), were examined. All of them had severe symptoms of progressive muscular dystrophy and the same mutation in exon 3 of the DYSF gene. 9 patients had distal variant Miyoshi myopathy (MM). Dystrophin is located on the plasma membrane of skeletal muscle and is deficient in patients with MM and LGMD2B.

We report the detailed large-sized deletion in the C-terminal end of the MM confirmed individual. At first, the deletion of exons 90 and 94 was identified using MLPA. In the second step, the deletion from exon 90 to 94 was determined using quantitative fluorescent PCR (QF PCR).

We described novel multi-exonic deletion of 2 kb that belongs to new class of mutation of the MM gene. Large-sized genomic deletion of 6.520 bp was identified as the first genomic rearrangement in the MM gene.

**J10.04**

**Detection of deletions and duplications in the Duchenne muscular dystrophy in Russian patients**

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DMD gene duplications detection and analysis of deletions at female relatives of the patient has not yet been carried out in Russian patients. Thus, the aim of this work is the development of methods of quantitative analysis of DMD gene duplications and deletions and determining the frequency of duplications in the selection of patients with DMD/BMD from Russia. To perform this task, we have developed a quantitative method based on specific ligation reaction (MLPA). Using this method, we analyzed 237 patients with the Duchenne muscular dystrophy, which didn’t reveal deletions of “hot exons”. Deletions have been detected among 37 patients. Deletions not in “hot exons” have been found among 9 patients. Thus duplications are found in 6% of cases, which is supported by the literature data. In addition, this method allows detecting deletions / duplications in female genome, which allows to determining mutations in DMD gene in carrier.

**J10.05**

**A Novel POMT2 mutation defined in a girl with Walker Warburg syndrome.**

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Disorders disrupting normal brain development represent a clinically and genetically heterogeneous group. Genomic technologies are being used widely, especially in recent years, and enabling the discovery of many novel genes that are involved in brain development. However, many more are likely waiting to be brought to light. Here we present a girl with hydrocephaly which was diagnosed during an antenatal period. She had hypothonia, occipital encephalocoele, dandy walker malformation of the brain, pachygria, agenesis of corpus callosum, microptalmia and corneal opacity on the right eye. Usually, homozygous regions common to affected individuals and absent in the unaffected sibing were sought. Disease status in the family linked to a homozygous region on chromosomes 2. The DYSF gene that associates with LGMD type 2B was positioned within the linked region. DYSF encodes dystrophin, a protein believed to be involved in the maturation of new muscle fibers and in repairing damage to muscle cells. Sequencing of DYSF exons in the proband of the Iranian revealed a novel insertion-deletion that is the likely cause of LGMD in the family. The phenotypic features of the patients harboring the mutation are uncommon because of prominent proximal presentations. To date, over 400 mutations disease in DYSF have been reported. The novel mutation reported here represents only the second mutation observed among Iranian LGMD patients.
eye, retinal and choroid coloboma on the left eye, slight desquamation of the skin. Her creatinine kinase and lactate dehydrogenase levels were high. Whole exome sequencing revealed a homozygous novel mutation (c.T431G:p. M144R) on Protein O-Mannosyltransferase 2 (POMT2) gene. Walker-Warburg Syndrome is a rare form of autosomal recessive congenital muscular dystrophy associated with brain and eye abnormalities. Until now several mutations were found in the POMT1 and POMT2 genes. Increasing use of genomic screening approaches, along with other genome-wide interrogation technologies are helping to identify causative rare variants not only in disorders of brain development but also in other disorders showing Mendelian inheritance.

J10.06 Clinical characteristics and genotype-phenotype correlation of Korean patients with spinal and bulbar muscular atrophy
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Introduction Spinal and bulbar muscular atrophy (SBMA) is slowly progressive and adult-onset motor neuron disease caused by a CAG repeat expansion in the AR gene. Few data are available for clinical characteristics and genotype-phenotype correlation in Korean SBMA patients. Methods Forty consecutive patients diagnosed by genetic testing were included in this study. The age of onset was defined as the time of patient’s initial recognition of symptoms and disease duration was represented by the time taken from onset of symptoms to genetic diagnosis. The severity of symptoms was assessed by nine ADL milestones. Rate of disease progression was expressed as a number, differences of ADL scores between at onset and diagnosis/disease duration. Results The median age at onset and diagnosis was 44.5 and 52.5 years, respectively. The median disease duration was 5.00 years, median rate of disease progression was 0.2 score/year and median number of CAG repeats in the AR gene was 44. The number of CAG repeats showed significant inverse correlations with the age of onset of muscle weakness and the age of onset of any symptoms. Interestingly, a statistically significant correlation between rate of disease progression and age at onset was observed while an inverse correlation between rate of disease progression and disease duration was noticed. Discussion This study reaffirms the importance of SBMA as a cause of adult-onset motor neuron disease. To our knowledge, this is the first case report describing this novel pathogenic mechanism of a mid-intronic mutation in DMD gene.

J10.08 Definition breakpoints in the dystrophin gene for heterozygous carrier revealing

Duchenne muscular dystrophy - a disease caused by the presence of deletions, duplications and point mutations in the dystrophin gene (DMD), destroying one to several exons. An important aspect in the diagnosis of the disease is to determine the break points for the diagnosis of carriers of the abnormal gene. Objective: to develop a method of determining breakpoints of deletions of the exon of DMD gene to identify heterozygous carrier. Material research was genomic DNA of two patients suffering from Duchenne muscular dystrophy and their parents. DNA was extracted from whole blood using a kit Wizard® Genomic DNA Purification Kit (Promega). Diagnosis of Duchenne muscular dystrophy was carried out using the MLPA, followed by capillary electrophoresis. Design of primers was performed using the program Primer 3 (http://primer3.ut.ee/). Determination of break points in the region of exons 45-50 was performed using PCR RT the 7300 “Applied Biosystems” (USA). MLPA-analysis revealed a deletion of a segment encompassing exons 45-50 of the gene. The first break point occurred between exons 44-45, the second - between exons 50-51. Using genomic databases (http://www.genome.ucsc.edu) defined size between exons: 44-45 includes ~ 250,000 nucleotides 50-51 includes ~ 45 500 nucleotides. Using several sets of primers, these areas have been divided into several parts, followed by PCR RT, to identify the region deletions. At the last stage of the experiment break point was determined by sequencing. As a result, this study developed a method to identify heterozygous carrier in preclinical diagnosis of Duchenne muscular dystrophy.

J10.09 Novel pathogenic mechanism of a mid-intronic mutation in DMD gene
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Duchenne and Becker muscular dystrophy are X-linked allelic disorders caused by mutations in the DMD gene. The majority (65%) of these mutations are intragenic deletions/duplications that often lead to frameshift errors. Among the remaining ones, we find the mid-intronic mutations that usually create cryptic exons by activating potential splice sites. In this report, we identified, in a Becker Muscular Dystrophy patient, a mid-intronic mutation that created two ESE sites in intron 26 of DMD gene resulting in the insertion of a new cryptic exon in mRNA. To our knowledge, this is the first case report describing this novel pathogen mechanism of mid-intronic mutations of DMD gene.

J10.07 Molecular analysis of mutations smn1, smn2 and naip genes by multiplex ligation-dependent probe amplification (mlpa) R. Kamilyeyeva1 A. Abyldinova1, 2Department of Laboratory Medicine and Genetics, Samsung Medical Center, National Research Center Maternal and Child Health, Astana, Kazakhstan.

Spinal muscular atrophy (SMA) is autosomal recessive neurodegeneration disease. The cause of the disease is mutations in the SMN gene. SMN1 gene has high homologue copy - SMN2 gene, the number of copies depends on the type of severity of the current disease and the patient’s quality of life. To identify mutations in the SMN1, SMN2 and NAIP genes, MLPA analysis was performed using the MLPA kits. For study of 43 SMA patients was conducted in unrelated families with suspicion of spinal atrophy. The diagnosis was confirmed in 21 cases. In three patients with SMA type I, one case of deletion of exons 7 and 8 of SMN1 and not marked increase in the number of copies of SMN2, two patients identified deletions of exons 7 and 8 and a deletion of the SMN1 gene and NAIP gene. In patients with SMA type II in 3 cases met deletion of exons 7 and 8 of SMN1 gene and increase the number of copies of the SMN2 gene and only in one case the deletion of exons 7 and 8 of the SMN1 gene. At the same time, in nine patients SMA type III - 8 cases of predominate deletions 7 and 8 exons SMN1 gene and increase the number of copies of the SMN2 gene, in one case revealed a deletion of 7 and 8 SMN1 gene. Thus, these studies show that the number of copies SMN2 gene is not always associated with the severity of clinical manifestations in patients with SMA.
J10.11 Phenocopies as a possible cause of molecularly unconfirmed cases of Emery-Dreifuss muscular dystrophy

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Emery-Dreifuss muscular dystrophy (EDMD) is genetically heterogeneous muscular disease; 6 genes have been identified by now: EMD, LMNA, FHL1 causing major forms and very rare SYNE-1, SYNE-2, TMEM43. However, in >60% patients with EDMD phenotype no mutations are found. Common opinion about this issue is existence of other causative genes not recognized yet. In our sample of 102 unrelated patients (74 men, 28 women) with EDMD phenotype tested for mutations in LMNA (all patients), EMD and FHL1 (men only) EDMD was confirmed in 38 (37.2%); 22 patients (21.6%) were heterozygous for LMNA mutations, 15 (14.7%) hemizygous for EDMD and one (0.9%) hemizygous for FHL1 mutation. Patients with no mutations and pedigrees compatible with autosomal recessive inheritance were additionally tested for common mutations in CAPN3, FKRP, SGCA, ANO5, DYSF genes. EDMD phenocopies (as well as rare clinically atypical EDMD cases) have high percentage of cases with EDMD phenotype but no mutations in EDMD genes. EDMD phenocopies (as well as rare clinically atypical EDMD cases) should be taken into account in practical diagnostics and genetic counseling in families.

J11.02 TripleX Syndrome with short stature: a case report

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Triple X syndrome (47,XXX) is a sex chromosomal aneuploidy characterized by tall stature, microcephaly, hypertelorism, epicanthal folds, congenital abnormalities and motor and language delays. We present a rare phenotype of the syndrome: a 10-year-old girl admitted to our hospital for short stature. She was born from consanguineous parents at term of uneventful pregnancy weighting 2500 gr and measuring 47 cm. The girl was 117.7 cm (height SD score -2.87) to the parental target eight of 154.5 cm, with a weight of 19kg (SD score -2.13) and head circumference of 45.5 cm (below the 3rd percentile). The girl's growth rate was of 4.5 cm annually. The genitalia were of normal female phenotype with Tanner 1 stage of breasts. In addition she presented clinodactyly, goitre palpable and mild cognitive and speech delays. Karyotype was 47,XXX; FISH (probe CEP-X) on 200 interphase nuclei confirmed cytogenetic data. There was no evidence of growth hormone deficiency. Laboratory tests showed low insulin-like growth factor-1 (IGF-1). Bone radiographic imaging of the left carpal demonstrated a difference between bone age and chronological age of 2 years. Blood routine test, liver and kidney function, blood glucose and insulin, thyroid function were normal. The ultrasound examination of heart, uterus, ovary and urinary system, as well as cranial magnetic resonance imaging (MRI) were normal. A possible explanation might be the apleniasufficiency of the short stature-homeobox-containing gene (SHOX gene) that is present in the pseudoautosomal region of 2 sex chromosomes. This study performed in our patient is yet ongoing.

J11.03 De Novo 4p deletion and 4q duplication in a female dysmorphic child

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Wolf-Hirschhorn Syndrome (WHS) is a well recognized chromosome 4p16.3 microdysgenesis syndrome with characteristic craniofacial features, pre and postnatal growth retardation, hypotonia, intellectual disability, EEG abnormalities, congenital heart defects and corpus callosum agenesis. Direct duplications on the chromosome 4q represent an infrequent chromosomal finding. Here, we report the cytogenetic and molecular cytogenetic findings and clinical manifestations observed in a 19 months old female infant with terminal del(4p) and dup(4q). The infant was delivered by Cesarean section at the 36th week of the mother's twin pregnancy. Her twin was normal. The birth weight of the child was 1750 gr (<1 sd) and height was 42 cm (<2 sd). She had significant features specific to WHS. Additionally she had bi-temporal narrowing, square forehead, mild brachycephaly, large anhiplax, bilaterally hand and foot fifth finger clinodactyly. Our patient has increased renal parenchyma echogenity, large anhiplax and umbilical hernia which is similar to chromosome 4qdup anomalies. Conventional cytogenetic analysis revealed 46XX.dup(4)(q4ter-q4ter) and 4q31-4qter. The cytogenetic analysis was 46XX,ishder(4)(q4ter) (wcpq+d(4)(p16.3pter)). The karyotypes of the healthy, non-consanguineous parents were normal. The de novo abnormal karyotype seen in the case have not been described previously. Although phenotypic features of the case are similar to WBS, the patient has features including ear and renal anomalies, which may be attributed to the 4q duplication. The patient's phenotype may represent the combined effect of both chromosome aberrations. In order to identify regions of the relevant genes, further advanced molecular analyses are planned.

J11.04 Deletion of 4q28.3-31.23 in the background of multiple malformations

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We would like to address a case regarding a patient who suffered from multiple malformations. The symptoms include small kidneys, epilepsy, dysphoria, hypotonia, immunodeficiency with decreased lymphocyte number, most of which are well known in medical literature, however some of them are unique like hypocalcaemia and pulmonary hypertension. Despite preliminary tests, which include GIE/MSA banding and FISH, we were not able to detect the genetic alterations behind these symptoms, which is why we expanded the routine method to array CGH. We used Agilent Human Genome G3 Sureprint 8x60K Microarray to detect the cytogenetic deletions in
the patient. We found 14.56MB intrstitial deletion on the long arm of chromosone 4, more specifically in the region of 4q28.3-31.23, where a total of 47 genes were affected. Eight out of these genes affected could play and important role in the manifestation of these symptoms. These genes include NR3C2, IL15, PDE18, SERT7, EML002, GAB1, HHIIP, and SMAD1. We also examined the patient’s parents with the same type of microarray. As no deletions were detected in either parent we concluded that the deletion found in the patient was a de novo alteration, which leads us to believe that the loss of these genes could give us a possible explanation for the clinical features. This research was supported by TÂMOP-4.2.12/1-KONV-2012-0028.

**J11.05**

**It is all phenotype caused by 6qter deletion?**

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We report a 5 years old boy with a prenatal ultrasound diagnosis of biventricular hydrocephalus. He was born with a birth weight of 2580 g, a length of 46 cm and a head circumference of 33 cm. He did not show muscular hypotonia but his psychomotor development was delayed: he sat at the age of 11 months and he walked independently at the age of 17 months. He spoke single words at the age of 12 months but he could not make comple- tionate sentences until 3 years of age. Brain MRI showed colpocephaly, thick corpus callosum and pons, hypertrophy of massa intermedia. His EEG was normal. Neurological evaluation showed muscular atrophy and hypotonia, joint laxity and mild intellectual disability. On the clinical examination at 5 years of age, the patient had a weight of 15 kg (3rd centile), a height of 105 cm (10th centile) and a OFC of 47 cm (<3th centile). The examination showed failure to thrive, microcephaly, triangular face, downslanting palpebral fissures, sparse eyebrows, large and posteriorly rotated ears, muscular atrophy and hypotonia, ligamentous laxity, scapular winging and bilateral flat foot. Array-GH showed a 2.5 Mb microduplication of 2q37 and a 5.3 Mb microdeletion of 6q27 generating a de novo derivative 6 chromosome: this rearrangement is likely to be the result of a translocation involving the distal region of long arm of chromosome 2 and the distal region of long arm of chromosome 6. Array-GH showed also a maternal 714 Kb microduplication of Xq26.2.

**J11.06**

**A case report with de novo interstitial deletion in 7q21**

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In this case we report a male patient with a de novo interstitial deletion in 7q21 which is determined by G-banding. Patient was referred our clinic for undescended testes and mild intellectual disability. He was five years old and one of the triplet pregnancies. Two other siblings were girls. The pregnancy resulted from in vitro fertilization. The siblings and parents karyotype analyses were normal. The clinical features of the patient were cerebral cerebellar hypoplasia, partial androgen resistance, undescended testis and mild intellectual disability. We are now planning to make further molecular investigations to find a relationship between the candidate genes of these features.

**J11.07**

**New case of 7q11.23 duplication syndrome in a polymalformed Saudi child**

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7q11.23 duplication syndrome referred to as “duplication of the Williams syndrome region” is a recently-documented genetic disorder caused by a heterozygous duplication of contiguous genes at 7q11.23 mediated by nonallelic homologous recombination (NAHR) between large flanking Low Copy Repeats, leading to duplication or to deletion like observed inWilliams beuren syndrome.

Less than 100 cases have been described and the phenotype is not yet well defined. Most prominent phenotypic characteristics, mentioned in literature review are several speech delay, language delay, a characteristic facies, hypopitonia, developmental delay, and social anxiety. Congenital anomalies as heart defects, diaphragmatic hernia, cryptorchidism and non-specific brain abnormalities are sporadically reported.

We report here an 1 year-old male with 7q11.23 microduplication detected by array-GH analysis performed because of speech delay, growth retardati- on, hypotonia and polydactyly syndrome associating congenital heart diseases, hydrocephrosis, oblique lips, macrophaly and polydactyly. Fluoro- resonance in ELF genotyping allowed the diagnosis, revealing a tandem duplication of the Williams-Beuren critical region detectable only on interphase nuclei and karyotype study was normal. This case is one of the most severe reported in the literature and it allows us to expand the phenotypic spectrum of this syndrome.

**J11.08**

**A new familial case with 8q24.11-q24.3 complementary rearrangements: phenotypes associated with pure deletion and mosaic duplication**

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Genomic rearrangements of 8q bands have been associated with several clinical entities, such as Langer-Giedion Syndrome. We report a unique familial case with complementary chromosomal aberrations involving the same distal 8q region, detected on father (deletion) and mother (mosaic dupli- cation). Chromosome analysis on chorionic villi at 13 weeks of gestation for increased Down Syndrome risk after maternal serum screening revealed 46,XX,del(8)(q24.1) mat. The 8q deletion was defined as interstitial by subtelomeric and painting specific FISH probes. The deletion size was investigated by microarray and parental karyotype was performed. Microarray result was arr[hg19] 8q24.11q24.3 (119,425,459-144,825,972) x1 with a 25 Mb deletion. The involved region included 74 MIM genes and no recurrent microdeletion syndromes. Paternal karyotype was mos 46,XX,dup(8)(q24.1q24.3) (22)[46,XX][28]; the mosaic interstitial duplication was confirmed also by FISH and microarray, which disclosed the same fetal breakpoints. Only two cases have been described with pure 8q24 duplication characterized by microarray (Concolino et al., 2012;Wheeler, 2010) and none in mosaic condition. Mother had hydrocephrosis at birth, short stature, facial dysmorphism, psychomotor and mild cognitive delay. Fetal ultra- sound investigation was normal. Similar deletions have never been reported. We speculated about possible clinical consequences evaluating the role of genes mapped in the deleted/duplicated region, such as KCNK9, associated with Birk-Barel mental retardation dysmorphism syndrome (#612292); in brain tissues, it is expressed only from maternal allele and this might have a possible mechanism of formation of this unique familial case.

**J11.09**

**Application of array CGH technique for postnatal identification of constitutional abnormalities**

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The identification of cytogenetic imbalance is an important component of clinical genetics. Genetic abnormalities have been associated with 6-13 % of stillbirth, but the true prevalence may be higher. Chromosomal abnor- malities often cause specific and complex phenotypes resulting from an imbalance in the normal dosage of genes located in a particular chromosomal segment. Furthermore, many multiple malformation syndromes are caused by deletion or duplication of genomic region. In the interest of diagnosis, conventional cytogenetic analysis such as chromosomal banding technique is applied as the first choice, which allows for the unambiguous identifi- cation of each human chromosome with the detection of aneuploidy and many large structural rearrangements, including translocations, large deletions and duplications. The molecular cytogenetic platform on which our study is based on is array comparative genomic hybridization (aCGH), which is a useful diagnostic method to detect a complex cytogenetic syndromes and diseases. The aim of our study is to extensively apply, further applied and validate sensitive high throughput array CGH to efficiently investigate the cytogenetic abnormalities in all regions of Hungary. We introduced the Nim- bleGene array platform and we started the molecular cytogenetics analysis in 7 cases. Out of the 7 samples, in which the causative chromosomal effect was confirmed, a gain was identified at four patients (range of the gain was from 39 Mb to 1.3 Mb) and in three cases loss of different regions was detec- ted (from 67.4 Mb to 0.17 Mb). The underlying genes were selected and the results are currently discussed with the clinicians.
that patient had unbalanced rearrangement, 46,XY;del(4)(q14.2;pter)mat including derivative chromosome 4 with partial deletion of 4p15–pter and partial trisomy of 4q42-1qter, which has resulted in fusion of genes on two different chromosomes.

In this work authors will discuss details of genetic analysis and possible combined effect of identified unbalanced chromosomal alterations on phenotype of the proband.

**J11.10**

Sub-microscopic chromosomal imbalances in a patient with mild dysmorphic features, developmental delay, excessive skin grooves and hypoplasia of the corpus callosum

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Chromosomal imbalances are the major cause of developmental delay combined with dysmorphic features. Many of these imbalances are caused by submicroscopic deletions or duplications that are undetectable at the level of traditional cytogenetic analysis. Array-based comparative genominc hybridization (array CGH) is a powerful and high-resolution approach for detection of DNA copy number variants (CNVs).

We report a 7 month old boy with mild dysmorphic features, developmental delay, excessive skin grooves of limbs and hypoplasia of the corpus callosum. We have used genomic array CytoChip Oliver (BlueGnome, Cambridge, UK), format 2×105K, version 1.1. and BlueFuse Multi software, version 2.2. The 2×105 K array detects 100 Kb imbalances on the backbone and has tiling of 20 probes over 137 OMIM disease loci. Array CGH-analysis revealed cryptic deletion of 15q11.2 region spanning 4,68 Mb and an amplification spanning 19,31 Mb of 1q31.21-1q31.33. The CytoChip results were confirmed with MLPA. The influence of the known genes in the imbalanced regions and their correlation to the phenotype will be discussed.

**J11.11**

The BBS12 gene mutation is cause of Bardet Biedl syndrome in two individuals from south west Iran

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Bardet Biedl Syndrome (BBS) is a multi-organic disorder with variation in symptoms affected individuals. Nevertheless, the main signs of the BBS disease are: vision loss, Obesity, Polydactyly in hand and foot, intellectual disability, and abnormalities in the genitalia. To date, 14 genes are identified involving in the pathogenesis of the BBS. Mutations in these genes cause difficulties in the cell movement and other chemical signaling pathways. The BBS1, BBS2 and BBS10 count for more than 50% of all detected mutations in BBS affected individuals, worldwide. In contrast, the BBS12 gene mutation is causative for less than 1%. However, two BBS patients with above mentioned clinical symptoms referred to us for genetic counseling and molecular genetic testing. We screened firstly the BBS1, BBS2, and BBS10 genes by submicroscopic deletions or duplications that are undetectable at the level of traditional cytogenetic analysis. Array-based comparative genomic hybridization (array CGH) using GTG banding provided the diagnosis and proper genetic counseling.

**J11.12**

Case report of patient with Charlie M syndrome - cytogenetic and molecular analysis

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Charlie M syndrome, oromandibular-limb hypogenesis syndrome, is a rare faco-neuro-skeletal disorder characterized by limb defects amputation-like, cleft palate, hypoplastic incisors, midface hypoplasia, dysmorphic face and facial nerve paralysis. Authors in this paper present a case of a boy, age 6, who was referred for genetic consultation because of clinical appearance suggestive of Charlie M syndrome. Cytogenetic analysis of the proband’s peripheral blood using GTG banding showed unbalanced karyotype with derivate chromosome 4: 46,XY;add(4)(p15). Molecular analysis using multiplex ligation-dependent probe amplification (MLPA) (commercial kit for microdeletion syndrome detection P096, MRC Holland) detected LOH for WHSC1 critical region in 4p16.3. Further karyotype analysis of both parents revealed that mother was a carrier of reciprocal translocation 46,XX; t(1;4)(q42;p15). Final conclusion was

**J11.13**

A rare chromosomal rearrangement of 46,XX, der (7) in dysmorphic female with mental retardation

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Chromosomal rearrangements are rare structural rearrangements with three or more breakpoints and exchange of genetic material between two or more chromosomes. The rearrangements may be balanced or unbalanced, and are classified into type I with three to four breaks and familial origin; and type II with five or more breaks, which generally arise de novo. Unbalanced chromosomal rearrangements may lead to significant clinical consequences such as multiple congenital anomalies, dysmorphic features, and mental subnormality in the progeny. Balanced chromosomal rearrangements are often not associated with any phenotypic abnormalities and remain undetected in family members through multiple generations.

Case presentation: The patient was a 5.5 years old girl referred to genetic center with delayed development. She was born from second cousin familial marriage by normal vaginal delivery, and spoken from 2 years old. Congenital right knee dislocation was treated. Delayed speech was limited in about 3 words and was suffering from speech apraxia. The signs were hypertelorism, strabismus, epicanthal fold, narrow palpebral fissure, sparse eyebrows, stabiulatus, decayed teeth, clinodactyly of fifth fingers, increased distance between toes 2 and 3, semisynactyly of the toes 2 and 3, and shortened fourth and fifth metatarses. There were two mental retardation girls in common cousins of parents. Also, parents had three other pregnancy resulting two spontaneous abortions and one male neonatal death due to multiple congenital heart disease. Chromosomal study of proband on the basis of GTG-banding was revealed 46,XX, der(7), t(7;10)(p22; q24).

**J11.14**

Focus on chromosome 22 - Rare Chromosome Disorders

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Many genetic conditions are related to changes in particular genes on chromosome 22. We present three unrelated cases diagnosed in our Genetics Department with rare chromosomal abnormalities involving the structure of this chromosome.

Cat-eye syndrome is characterized by a recognizable clinical picture, although the variability of the physical features is enormous. A female infant, one month old, was referred with following major malformations: hypertelorism, narrow palpebral fissures, cleft palate, bilateral preauricular pits, bilateral coloboma of the iris, anal atresia with a fistula from the rectum to the vagina, complex heart malformation. Cytogenetic evaluation identified an additional marker and by FISH examination has been confirmed the involvement of chromosome 22.

Phelan-McDermid syndrome/22q13 Deletion Syndrome is a genetic syndrome caused by disruption of the SHANK3/ProSAP2 gene on the terminal end of chromosome 22. A newborn male with neonatal hypotonia, mild dysmorphic features and large, fleshy hands was suspected with chromosomal abnormality. G-banded chromosome analysis identified terminal deletion involving 22q13. Parental karyotypes were normal. FISH analysis confirmed deletion of SHANK3 gene.

Microdeletion 22q11.2 syndrome associated with XXX karyotype (Klinefelter syndrome). This double chromosomal abnormality is a rare finding in common cousins of parents. Also, parents had three other pregnancy resulting two spontaneous abortions and one male neonatal death due to multiple congenital heart disease. Chromosomal study of proband on the basis of GTG-banding was revealed 46,XX, der(7).
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We report a 15-month-old boy with dysmorphic features, mild developmental delay, congenital glaucoma and partial coronal craniosenosis. We have used genomic array CytoChip Oligo (BlueGnome, Cambridge, UK), format 2x105K, version 1.1. and BlueFuse Multi software, version 2.2. The 2x105K array detects a loss of 231 Kb that overlaps 3 HdCNVs and 1 OMIM gene

OMIM: disease: Chromosome 17q21.31 deletion syndrome (610443). The maximum overlap between an ISCA pathogenic region of type loss and this region is 30% 56% of the region is covered by significant polymorphisms of type loss (DGV: 56, ISCA: 0%). The CytoChip results were confirmed with MLPA. The influence of the known genes in the imbalanced regions and their correlation to the phenotype will be discussed. The above mentioned features do not correlate with the typical presentation of Koolen-De Vries syndrome as aspected from the aCGH results.

J11.15 Microstructural genomic imbalances in patients with congenital malformations S. P. Iliev1,2, D. Andjiev-Travella1, R. Rukova1, N. Nesheva1, R. Staneva1, R. Tinchewa1, R. Tinchewa1, D. Tinchewa1; 1Department of Medical Genetics, Medical University-Sofia, Sofia, Bulgaria, 2State University Pediatrics Hospital “Queen Evdokia”, Section of Clinical Genetics, Medical Faculty, Medical University, Sofia, Bulgaria, Sofia, Bulgaria.

In the current study 52 patients were selected for array CGH analysis after implementing stringent criteria (a combination of congenital malformations, dysmorphic features and behavioral disorders, and patients with multiple abnormalities and the presence of at least one major anomaly). A combination of methods was applied - cytogenetic analysis, FISH method, oligo DNA microarrays.

The results of analysis of microarrays revealed definite etiology in 9 out of 52 patients that were tested. CNVs were found in all cases. All pathological findings were validated by FISH analysis. Genotype/phenotype correlations between different patients were confirmed. In addition, the majority of the patients tested (41 patients) showed 124 normal variations in the number of copies and 108 variations of unknown clinical significance (34 patients). Analyses of the type and distribution of the different variations was performed and the clinical significance of variants of unknown nature was discussed.

Our results show the advantages of high resolution microarrays for clinical diagnosis of patients with congenital malformations associated intellectual disability. The results also highlight the need for extensive population studies revealing the molecular nature and clinical significance of different CNVs and the creation of detailed maps of variations in the Bulgarian population. This would facilitate greatly the precise identification of specific genomic imbalances in clinical aspect and would ease the widespread introduction of microarrays in diagnostic practice not only for postnatal diagnosis of individuals with developmental delay and dysmorphism, but also for prenatal genetic diagnosis.

Acknowledgements: Grant 02’76-21.12.2009, National Science Fund, Bulgaria.

J11.16 Duplication on chromosome 17 and deletion on chromosome 20 in a patient with craniosynostosis R. Pogue1, F. A. Marques1, R. S. Heredia1, J. F. Mazzu1, M. O. Cardoso1; 1Universidade Católica de Brasilia, Brasilia, Brazil, 2Secretaria de Saúde do Distrito Federal, Brasilia, Brazil, 3Universidade de Brasilia, Brasilia, Brazil.

We present molecular cytogenetic analysis of a boy with syndromic craniosynostosis. He was born to healthy parents after an uncomplicated pregnancy. At birth he presented with sagittal craniosynostosis and seizures. Further examination revealed complications including a cardiac malformation. At eight years and two months the patient showed neuropsychomotor and behavioral anomalies. G-banding showed an apparently normal male karyotype. A DNA sample was taken for chromosome microarray analysis (CMA) using the Affymetrix 750K CytoScn array. The results showed terminal alterations of chromosomes 17 and 20. Specifically, there was a duplication on the long arm of chromosome 17 (chr17:78,952,204-81,060,886), together with a deletion of 1.4 Mb of long arm of chromosome 20 (chr20: 1,643,44-63,003,805). The deletion on chromosome 17 includes more than 80 genes and miRNAs, while the deletion on chromosome 20 includes more than 50 genes and miRNAs. The data are indicative of an unbalanced translocation of part of chromosome 17 to chromosome 20. Partial trisomy of chromosome 17q has been previously reported in the literature, and the patient reported here shows phenotypic overlap with the previously reported spectrum, including psychomotor delay, craniofacial asymmetry and other dysmorphic features. Previous reports also suggest that severity of the phenotype associated with 17q partial trisomy may be associated with particular monosomes of other chromosomes, and this also appears to be the case in the current patient.

J11.17 Atypical presentation of 17q21.31 microdeletion syndrome H. Çoban1, Y. Soysal1,2,3, K. Hekimler Öztürk1, S. Kartgoz1, M. A. Ergün1; 1Department of Medical Genetics, University Pediatrics Hospital “Queen Evdokia”, Section of Clinical Genetics, Medical Faculty, Medical University, Sofia, Bulgaria.

The combination of developmental delay and dysmorphic features is frequently caused by different chromosomal imbalances. Many of these imbalances are result of submicroscopic deletions or duplications that are impossible to detect by conventional karyotyping. One of the best examples is the array-based comparative genomic hybridization (array CGH) as it is a high-resolution approach for detection of DNA copy number variants (CNVs).

We report a case of a 3 year-old boy with microstructural genomic imbalances in patients with congenital malformations, dysmorphic features and behavioral disorders, and this also appears to be the case of our patient. Partial monosomies of other chromosomes, and this also appears to be the case of our patient. The phenotype associated with 17q partial trisomy may be associated with particular monosomes of other chromosomes, and this also appears to be the case of our patient.

Partial trisomy of chromosome 17q has been previously reported in the literature, and the patient reported here shows phenotypic overlap with the previously reported spectrum, including psychomotor delay, craniofacial asymmetry and other dysmorphic features. Previous reports also suggest that severity of the phenotype associated with 17q partial trisomy may be associated with partial monosomes of other chromosomes, and this also appears to be the case of our patient.
Multiple hereditary exostoses (EXT) is an autosomal dominant disorder characterized by multiple projections of bone capped by cartilage, most numerous in the metaphyses of long bones, but also occurring on the diaphyses of long bones. EXT type 1 is caused by mutation in the gene encoding exostosin-1 (EXT1) which maps to chromosome 3q24. Here, we report on the second case microdeletion of 8q23.3q24.11. The patient is a 15-year and 6 month-old boy with dysmorphic features, dyslalia, multiple exostoses, delayed bone maturation and short stature. Dysmorphic features included expressed thick eyebrows, sinciput, large and prominent ears, protruding philtrum, microphally. Initial conventional karyotyping was normal. The MLPA (multiplex ligation-dependent probe amplification) screening with SALSA P245 kit showed deletion of EXT1 gene. The deletion was then confirmed by SALSA MLA P228 indicating 1.46 Mb distal deletion in 8q23.3q24.11 region including EIF3H gene (exon 8) and EXT1 gene (exon 2-11). Because the parents were not available for study, we were not able to determine if this deletion was de novo or inherited. There is only one case which included 1.46 Mb deletion reported so far. This case highlights the importance of using MLPA technique for accurate characterization of rare chromosomal rearrangements in order to make possible genotype-phenotype correlations and to understand the genetic mechanisms involved. Finally, we recommend to perform MLPA in patients with multiple hereditary exostoses phenotype in whom no mutation is identified in the EXT1 gene.

J11.2.1
The clinical phenotype in a familial deletion 18p syndrome
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Deletion 18p syndrome is characterized by dysorphic features, growth deficiencies, and mental retardation with a poorer verbal performance. About 1 in 50,000 babies is born with a deletion of 18p. Most reports suggest that 18p deletions affect girls more often than boys. Until now, few families have been described with limited clinical description. Women with del(18p) are fertile and seem to have a normal miscarriage rate. We report transmission of deletion 18p from a mother to her son. The proband is 8 years old and has short stature, dysmorphic features, polymorphous dyslalia and moderate mental retardation. The mother also presents dysmorphic features, mild mental retardation and has better verbal abilities than his son. Our report presents a mother with del(18p) syndrome having two miscarriages and no analysis was performed on the fetus. Chromosome analysis from the proband and their mother revealed the same chromosomal deletion: 46,XX, del(18)(p11.2). This report sheds new lights on the familial del(18p) syndrome. Cognitive performance may be more variable than previously suggested within the same family. Management needs to be handled by a multidisciplinary team and includes speech therapy, hormonal (if necessary) and psychological care. Patients have an essentially normal life expectancy but will need to attend regular medical visits. Genetic counselling for these patients should take into account these new data, especially in regard of a wider variability of intellectual outcomes and better verbal performance.

J11.2.2
A rare case with De Novo Isochromosome 18p Syndrome
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Tetrasomy 18p is a very rare chromosomal disorder which affects males and females equally. The small extra metacentric marker chromosome results from a spontaneous mutation early in embryonic development in most of the cases. Here we report a de novo supernumerary i(18p) in a 8 months old female dysmorphic child. The case was delivered by Cesarean section at the 38th weeks of gestation with anthropometric parameters <-2 sd. Other significant features of the case were growth retardation, neonatal feeding problems, microcephaly, seizures, hearing loss, strabismus, refractive errors, high arched palate, and constipation. An additional metacentric marker chromosome was revealed by conventional cytogenetic analysis. The chromosome constitution of the parents were normal. Based on physical features of the case, FISH analysis specific to chromosome 18 were performed and a supernumerary i(18p) was diagnosed. The clinics of the patient was compared with the previously published cases.

J11.2.3
Clinical and genetic examination of syndromic limb defects
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Limb developmental defects are known to be rare conditions that occur in about 10 / 10 000 live births, but their significance cannot be negligible. Our aim was to work out a genetic examination protocol that can help to find the most effective way to reveal the background of limb developmental defects. In our study, we performed a detailed clinical, genetic examinations, performed in selected cases. Firstly, we usually indicated chromosomal analysis that was followed by FISH in negative cases and in the other cases specific we performed molecular diagnosis. In this paper we would like to feature some of our cases - with compound syndrome including limb developmental defects - where genetic examinations were available: 4q deletion syndrome, Holt-Oram syndrome, CHARGE syndrome, Down syndrome. Dysmorphic features may help to find exact diagnosis in compound syndromes and give chance for genetic examinations in chromosomal or monogenic syndromes. Interdisciplinary collaboration is recommended for proper diagnosis, genetic counseling and understanding of the pathogenesis. Despite the latest techniques, genetic background of limb development and its defects still belongs to an unrevealed part of science.

J11.2.4
A de novo marker chromosome derived from 15q in a patient with growth retardation: genotype-phenotype correlation
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We report a female patient with growth retardation who was referred to our clinic from pediatric service. She was the first child of 41 year old mother and was born at 31 weeks with 1640 gr birth weight. Her mother and father was healthy and have normal karyotypes. On physical examination, short neck, short phillrump, high arched palate, hypertelorism, simple ear, sacral diple, macrocephaly and hypothyria were detected. Cardiologival evaluation was normal. In the cytogenic analysis 47,XX,+mar was detected. We performed chromosomal microarray analysis using an Affymetrix 750K SNP array platform. The application of array offered a precise characterization of the marker chromosome plus additional findings. The most significant finding confirming the conventional analysis was a 10,145 kbp duplication on chromosome 15q11.2-13.3. Additional unconfirmed findings above the cut off values involvingthe sites containing OMIM genes were: A 837 kbp gain within 4p15.32, and a 1066 kbp loss on the X chromosome’s short arm, p2.23.3. We observed that the patient features and array CGH results were compatible. As a result, array CGH can be used to define a better karyotype phenotype correlation in patients with dysmorphic features which can not be explained by known syndromes.

J11.2.5
Recurrent pregnancy loss and familial marker chromosome: case report
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There was an ectopic pregnancy and a 10 week intrauterine dead history in their 6 years marriage. The karyotype of the abort fetus was 46,XY. The family history was not specific, but postnatal exultus was reported in two of her sisters. The karyotype of the proband was detected as 47,XX+mar[108]. The marker chromosome was regular and had same structure in every metaphase plate. Centromere was not detected by C-bandimg. Physical examination of the proband’s husband was normal and his karyotype was 46,XY. The marker chromosome plus additional findings. The most significant finding confirming the conventional analysis was a 10,145 kbp duplication on chromosome 15q11.2-13.3. Additional unconfirmed findings above the cut off values involvingthe sites containing OMIM genes were: A 837 kbp gain within 4p15.32, and a 1066 kbp loss on the X chromosome’s short arm, p2.23.3. We observed that the patient features and array CGH results were compatible. As a result, array CGH can be used to define a better karyotype phenotype correlation in patients with dysmorphic features which can not be explained by known syndromes.

J11.2.6
The Case Of Michel Aplasia In Russian Family With Congenital Sensorineural Deafness: Results Of Temporal Bone Ct-Images Analysis
L. A. Klarov2, A. V. Solovyev1, V. G. Pshennikova2, G. P. Romanov1, N. A. Solovyeva2, E. E. Fedotova2, N. V. Luginov2, K. E. Savinova2, N. N. Gotovtsev2, A. M. Bajulov2, A. N. Messer3, I. U. Zhimerlova4, G. L. Posukh5, E. K. Khuznutdinova5, A. S. Fedorova2; 1Afyon Kocatepe University, Department of Medical Genetics from Department Obstetric and Gynecology of Afyon Kocatepe University, Afyon, Turkey; 2Duzen Laboratories Group, Istanbul, Turkey.

Sensorineural Deafness: Results Of Temporal Bone Ct-Images Analysis
L. A. Klarov2, A. V. Solovyev1, V. G. Pshennikova2, G. P. Romanov1, N. A. Solovyeva2, E. E. Fedotova2, N. V. Luginov2, K. E. Savinova2, N. N. Gotovtsev2, A. M. Bajulov2, A. N. Messer3, I. U. Zhimerlova4, G. L. Posukh5, E. K. Khuznutdinova5, A. S. Fedorova2; 1Afyon Kocatepe University, Department of Medical Genetics from Department Obstetric and Gynecology of Afyon Kocatepe University, Afyon, Turkey; 2Duzen Laboratories Group, Istanbul, Turkey.
Partial deletion of chromosome 3p is a rare disorder with variable chromosomal breakpoints and consequently phenotypes. Although most of these deletions involve the 3p terminus, interstitial deletions may also give rise to features of the syndrome.

We report the case of male patient with mental retardation and minor morphologic and features of a 7.4 Mb deletion of 3p26.3p26.1 [6] encompassing the CHL1, CNTN6, CNTN4, CNTN4-A52, IL5RA, TRNT1, CRBN, LRRN1, SETMAR, SUMF1, IFTP1, EGT, LOC100507582, BHLHE40, ARL8B, EDEM1, MIR4790 AND GRM7. We compare the clinical phenotype of this patient to previously reported cases of 3p syndrome. Microarray technologies are increasingly becoming the tool of choice to accurately determine the underlying genetic cause and resulting phenotype in patients with mental retardation and multiple anomalies. aCGH allows to precisely determine the length and breakpoints in order to better understand a child’s future development and needs.

In the present case molecular karyotyping has characterized a 3p deleted region with haploinsufficiency of neurodevelopmental genes associated with cognitive deficit and mental retardation, which may help to identify genes important to growth and development that contribute to the deletion 3p syndrome phenotype and aid in better understanding the molecular basis of the 3q syndrome.

J11.11 Microduplication: a case report

The short arm of chromosome 16 is very rich in segmental duplications, predisposing this region of the genome to a number of recurrent rearrangements, namely deletions and duplications. Although it is already known that there is a strong association between 16p13.11 deletion and neuropsychiatric disorders, the clinical significance of its reciprocal duplication is not clearly defined yet. 16p13.11 microduplication that results of non-allelic homologous recombination is a very rare genetic alteration which can be associated with variable clinical features including behavioural abnormalities, developmental delay, congenital heart defects and skeletal anomalies. We report a 7-years-old boy with global developmental delay, speech absence, microcephaly, dysmorphic facial features and inexpressive faces. Microarray analysis revealed a 3.3Mb duplication comprising the 16p13.11-p12.3 region, which was confirmed by fluorescence in situ hybridization with a BAC clone for 16p13.11. Right annotated genes are present in this region including NDE1, the candidate gene for neurological and behavioural phenotype. Although this microduplication has been found in the normal population, is significantly enriched in patients with autism, schizophrenia and cognitive impairment. Several case reports until now suggest that this genomic abnormality has incomplete penetrance and variable expressivity and can constitute a new syndrome. With this case we intend to contribute to expand the spectrum of clinical findings associated to this genomic abnormality and provide further knowledge of the pathogenic involvement of this duplication.

J11.27 Interstitial del(3)(p26.3p26.1) in a patient with deletion 3p syndrome

Partial deletion of chromosome 3p is a rare disorder with variable chromosomal breakpoints and consequently phenotypes. Although most of these deletions involve the 3p terminus, interstitial deletions may also give rise to features of the syndrome.

We report the case of male patient with mental retardation and minor morphologic and features of a 7.4 Mb deletion of 3p26.3p26.1 [6] encompassing the CHL1, CNTN6, CNTN4, CNTN4-A52, IL5RA, TRNT1, CRBN, LRRN1, SETMAR, SUMF1, IFTP1, EGT, LOC100507582, BHLHE40, ARL8B, EDEM1, MIR4790 AND GRM7. We compare the clinical phenotype of this patient to previously reported cases of 3p syndrome. Microarray technologies are increasingly becoming the tool of choice to accurately determine the underlying genetic cause and resulting phenotype in patients with mental retardation and multiple anomalies. aCGH allows to precisely determine the length and breakpoints in order to better understand a child’s future development and needs.

In the present case molecular karyotyping has characterized a 3p deleted region with haploinsufficiency of neurodevelopmental genes associated with cognitive deficit and mental retardation, which may help to identify genes important to growth and development that contribute to the deletion 3p syndrome phenotype and aid in better understanding the molecular basis of the 3q syndrome.
Individuals with 7q36.1-qter microdeletion may have a wide range of clinical manifestations including developmental delay, craniofacial abnormalities (agenesia/hypoplasia of corpus callosum, microphthalmia, choanal atresia), growth hormone deficiency (absent), heart defects (atrial septum defect, patent ductus arteriosus, pulmonary segmentation defects), obesity, urogenital system anomalies (ectopic supernumerary kidneys, hydrourephrosis, microopenis), seizures and behavioral changes. We present the clinical features of a 3-year-old boy with mosaic 7q36.1-qter microdeletion. Our patient presented with unspecified developmental delay and cognitive impairment with speech delay. Mild facial dysmorphism was noticed, such as upslanted palpebral fissures, prominent nasal bridge and narrow palate. Another phenotypic finding was tapered fingers. Additional features included unilateral cryptorchidism, low testicular volume and childhood obesity. He had numerous motor mannerisms including body rocking, facial posturing, self-touching. Cytogenetic and molecular cytogenetic FISH analysis revealed a mosaic male karyotype with a terminal deletion of the long arm of chromosome 7 in 7% in analyzed blood cells. The karyotype is described as 46,Xydel(7)(q36.1q36.3)X4,XY. Both parents have normal karyotypes. No family history of congenital anomalies or mental retardation was referred. Our findings, therefore, suggest that the phenotypic consequences are very variable. We came to a conclusion, that a post zygotic terminal deletion occurred in this boy, after performing FISH analysis, which is a powerful tool in low quantity mosaic cell line detection. Further investigation by array-CGH is needed to identify chromosomal breakpoints and the exact deleted region.

**J11.32**

Mowat-Wilson phenotype in two patients with normal ZEB2 gene: study of an example of mosaic mosaicism

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Mowat-Wilson syndrome (MWS) is a multiple congenital anomaly syndrome characterized by typical facial dysmorphism, moderate to severe intellectual disabilities, epilepsy and variable congenital malformations. MWS is caused by eterozygous mutations or deletions in the Zinc finger E-box-binding 2 gene, ZEB2: sequence analysis detects mutations in approximately 80% of individuals; FISH detects large deletions encompassing all or part of ZEB2 in approximately 15% of persons; chromosomal rearrangements that disrupt ZEB2 cause MWS in approximately 2% of individuals; an additional 2% have intermediate-sized deletions that can be detected by techniques such as quantitative PCR, MLPA, or gene-specific array-CGH. The first patient is a 5 years old female with typical facial dysmorphism, aganglionic megacolon, hypoplasia of the corpus callosum, growth retardation with microcephaly, psychomotor retardation with speech delay and epilepsy. Sequence analysis and FISH study of ZEB2 gene detected no alterations; array-CGH was normal too. The second patient is a 4 years old female with typical facial features, pulmonary stenosis and bicuspid aortic valve, partial agenesia of the corpus callosum, ambiguous genitalia with scrotal labia. Growth retardation with microcephaly, psychomotor retardation with speech delay, epilepsy, chronic constipation, RGE and dysmorphic cutaneous areas. Sequence analysis of ZEB2 gene and array-CGH did not find any alteration.

The two patients could be an example of mosaic mosaicism for ZEB2 gene anomaly. Study are in progress in order to demonstrate this hypothesis.

**J11.33**

Multiple pterygium syndrome: a case report

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Multiple pterygium syndromes include a group of multiple congenital anomaly syndromes characterized by webbing (pterygia) of the neck, elbows or knees and joint contractures. Males had small penis and scrotum or cryptorchidism; females had aplasia of the labia majora and small clitoris. This syndrome included fusion of cervical vertebrae, scoliosis, flexion contracture of fingers, and rocker-bottom feet with vertical talus and facial dysmorphism with long face, high-arched palate, small mouth, and retrognathism.

The multiple pterygium syndrome is phenotypically and genetically heterogeneous. It is also called as Pterygium Colli syndrome, Esophageal or Pterygium syndrome. Multiple pterygium syndrome is a rare syndrome. This condition may behave sometimes as a dominant, but there clearly appears to be a recessive pterygium syndrome. In this case, described a 9-year-old female patient with pterygia of the neck, axilla and popliteal, flexion contraction of fingers, camptodactyly and rocker-bottom feet, short neck, kyphoscoliosis, downslanting palpebral fissures, small-set ears, high-arched palate, low-set hairline, aplasia of the labia majora. To provide molecular verification of Multiple pterygium syndrome, direct sequencing of CHRNA5 gene is planned.

**J11.34**

Novel mutation in exon 13 of the TCOF1 gene in the patient with Treacher-Collins syndrome

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Treacher-Collins Syndrome (TCS) is a rare craniofacial disorder, associated with an abnormal differentiation of the first and the second pharyngeal arches during fetal development. The major features of the disease include: midface hypoplasia, micrognathia, microtia, conductive hearing loss and cleft palate. The estimated incidence is 1/50000 live births, with 60% of the cases resulting from de novo mutations. The syndrome is mostly caused by mutations in the TCOF1 gene, which encodes the serine/alanine-rich protein named Treacle. TCS can be also caused by mutations in the POLR1C and POLR1D genes. Over a hundred mutations of the TCOF1 gene in TCS patients have been described. About 70% of recognized mutations are deletions, which lead to a frame shift, formation of a termination codon and shortening of the protein.

We report a novel mutation of TCOF1 gene in male patient with typical facial symptoms of TCS. Patient presented: prominent forehead, absent of auricular canals, third degree microtia, conductive deafness, sparse eyebrows, cleft of right eyelid, palpebral fissures slant down, broad base to nose, underdevelopment of zygomatic region, malar flattening, micro- and retrognathia. No internal defects were diagnosed. A multitemperature single-stranded conformation polymorphism (MSSCP) analysis and direct sequencing were performed. A novel, heterozygous deletion c.1978delE was detected. Patient's parents were tested and no mutation was observed. The c.1978delE deletion causes a reading-frame shift and premature termination of translation at 677 aa. We believe, that these findings will facilitate a precise diagnosis of the patient and extend our knowledge on the pathogenesis of TCS.

**J11.35**

Novel PTPN11 gene mutation in Iranian patient with Noonan syndrome

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Introduction Noonan Syndrome (NS) is an autosomal dominant, variably expressed, multisystem disorder with an estimated prevalence of 1 in 1000-2500. After trisomy 21, NS is the second most common syndromic cause of congenital heart disease. In recent years study on the molecular mechanisms of this disorder have been improved and expressed some pathophysiological mechanisms that cause the complications up to this abnormality such as the great range of medical and developmental features. Methods Genomic DNA samples were extracted from peripheral whole blood of 25 NS patients using the standard procedure. For each patient, exons 1-15 of the PTPN11 gene were individually amplify by polymerase chain reaction (PCR) using 15 sets of designed primers. The amplified fragments of PTPN11 gene were purified and directly sequenced. Result Three known PTPN11 mutation hotspots (exons 3, 8, 13) were checked first, and a previously identified mutation (Asn308Asp) was found in only two patients. We also found a novel non-synonymous substitution (Asp155Asn) in exon 4 of an eight years old patient. Conclusion This study is the first report of PTPN11 mutations in Iranian patient with NS. A non-synonymous mutation was found in exon 4 that is novel in NS but has registered before as somatic mutation in cancer patients with large intestine tumor. The affected probands who are negative for PTPN11 mutations will be screened for other genes mutations involved in NS using next-generation sequencing.
J11.36 Oromandibular-limb Hypogenesis Syndrome Type IIIC? A case in Russia
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Oromandibular limb hypogenesis syndromes (OLHS, OMIM %103300) are extremely rare hereditary syndromes with overlap of phenotypes, most of them occur sporadically. OLHS constitutes combinations of congenital malformations of mandible, tongue, upper and lower limbs and normal mental development. The pathogenesis of OLHS is still unknown, however, it is considered to have several possible causes: heat-induced vascular disruption; terminal limb hypoplasia and genetic origin of these syndromes. According to Half's classification OLHS is divided into five categories; with the only necessary criterion for inclusion - hypoglossia. However, in some cases, there is a considerable overlap of symptoms, making it difficult to define the phenotypic boundaries between OLHS. We report the case of a 3-year-old girl. She is the second child of healthy, non-consanguineous parents, born after an uneventful pregnancy, by normal delivery. She was born in 39gw with low birth weight (1700g) and length (46cm). She has a sister, who is normal. There is no family history of congenital abnormalities. Currently she has low weight (9kg), height (80cm) and microcephaly (44cm). She has a mental retardation and absence of speech. Her face has signs of hypoglossia, ankyloglossia, cleft palate, malocclusion with normal teeth, epicanthal folds, low set and rotated ears. She also has oligodactyly and feet. Chromosomal study of peripheral blood lymphocytes confirmed the 46,XX karyotype. By Half's classification our case conforms to OLHS type IIIC: Glosopateline ankylosis with hypoglossia - hypodactilia. However, as we know, combination OLHS type IIIC with mental retardation, microcephaly, low weight and height has not been described before.

J11.37 A case with frontonasal dysplasia and 2q36.1q31.2 deletion
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A seven year old girl was referred to our genetics lab. The first and only child of non-consanguineous parents, she was delivered at 9 months by C-section. Her birth weight was 2690 grams. She was diagnosed with renal stones at 6 months. She had bilateral deafness and underwent surgery. At examination she had normal intelligence and development. Her facial features include epicanthal folds, hypertelorism, blue eyes, arched eyebrows, high nasal bridge and short philtrum. She had bilateral simian crease, camptodactyly of fourth finger of right hand and syndactyly of second and third toes. Her karyotype was normal. Whole genome Oligo Array Comparative Genomic Hybridization was performed using CYTOCHIP IISCA 4X44K whole genome oloigro array version 1.1 and was analysed using BlueFuse Multi software. A 3.09 Mb deletion of 2q36.1q31.2 was detected. The deletion spans 222 Mb to 225.1 Mb covering 10 OMIM genes and 13 refseq genes. We compare phenotypic and genotypic findings of other overlapping cases. The deleted region in our patient includes the EPHA4 and PAX3 genes that have been formerly implicated in frontonasal dysplasia.

J11.38 Six patients from four unrelated Tunisian families with Peters plus syndrome harboring the same splice site mutation in the B3GALTL gene
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Peters plus syndrome is an autosomal recessive rare disorder Peters’ plus syndrome is an infrequently described entity that combines anomalies in the anterior chamber of the eye with short stature, distinctive facial features often associated with other major/minor additional defects and a developmental delay. Peters plus syndrome is related to mutations in the B3GALTL gene, localized on chromosome 13 (q12.3q13.1), leading to the inactivation of the B1-3glucosyltransferase. In this study, we screened the B3GALTL gene in six patients, from four unrelated families, with typical Peters plus syndrome. We revealed inter and intra familial phenotype variation. However the novel homozygous c.597-2A>G mutation was identified in all patients suggesting an effect founder of this mutation. Functional study using an ex vivo approach showed that this mutation causes complete skipping of exon 9 in the B3GALTL cDNA, which altered the open reading frame of the mutant transcript and generated a PTC within exon 9. This finding potentially elicits the nonsense mRNA to degradation by NMD. All these data confirm an important role of the B3GALTL gene test that provides diagnosis confirmation and improves genetic counseling for the familiy.

J11.39 PITTP-HOPKINS Syndrome: A new case of intragenic deletion detected by array-CGH
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Pitt-Hopkins syndrome is a rare syndromic mental disorder, mainly characterized by severe intellectual disability with stereotypic movements, typical facial gestalt (deep-set eyes, broad beaked nose, wide mouth with bow-shaped upper lip and widely spaced teeth), childhood-onset hypertension and seizures. While PITS appears to be a recognizable clinical entity, it seems to remain underdiagnosed principally when characteristic dysmorphic features are less typical due to similarities with other known genetic syndromes (Rett, Angelman). PITS is an autosomal dominant condition caused by haploinsufficiency of the TCF4 gene on 18q21.2. The molecular abnormalities identified in more than 100 patients include 40% of point mutations, 30% small deletion or insertion and 30% of total or partial gene deletions. We report on a 25-year old boy with severe intellectual disability, absent language, ataxic gait, anxiety and agitation, dysmorphic features and a falling appearance. Due to the lack of the hyperventilation and the presence of atypical dysmophisms, we decided to start genetic investigations by array-CGH (Bluegene ISTD, 4K-feature whole genome). The result was a loss of 103Kb on 18q21.22 spanning from 53,045,402 to 53,149,037 (hg19) within the TCF4 gene. This gene has 20 exons [the first and the last non coding], with several isoforms reported. Our deletion includes exons 4-6 (NM_001083962) and is predicted to result in a frameshift. At our knowledge, this is the second one described in literature involving these 3 exons. Reporting our case we want to contribute to the phenotype-genotype correlation in Pitt-Hopkins syndrome, mainly in those cases with a small intragenic deletion and a less typical phenotype.

J11.40 Genotype-phenotype correlation with ring chromosome 11
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Ring chromosomes usually result from distal breakage of both chromosome arms and carriers often exhibit a general overlap in phenotype. Jacobsen syndrome (JBS) is a rare contiguous gene disorder caused by terminal 11q deletion, characterized by intellectual disability, various physical anomalies and a distinctive facies. In this study we report a 12 year old boy with a ring chromosome presenting with global developmental delay, characterized by hyperactivity and repetitive behavior; hypertension, obesity, dyslipidemia and food compulsion. Clinical findings include short stature, microcephaly, bilateral narrowing and occipital flattening, short nose with long filter, small carp mouth, short ear-set, short neck and systolic murmur. Cyto genetic analysis by GTG banding revealed karyotype 45,XY,-11[48]/46,XY[ic11;11](4)[1]/46,XY[11][78]. Array-CGH using 2X400kb platform (Agilent) showed a large 8.6Mb terminal deletion: 1q12.2q42.5[12,368,150-153,006,516]x1, which includes 40 genes. There was no deletion in the 11p region suggesting that the ring was formed by fusion at 1q2.2.4 with 11pter region. This interpretation was validated by FISH using 1q2.5, 11ptel and 11qtel probes. Our genotypic results could explain the short stature, compulsive behavior and ADHD, as well the early-onset hypertension (associated with KCNJ5 gene) compatible with JBS. However, his phenotype does not include thrombocytopenia (related to FLI-1, ETS-1 and NFRK3 genes) or kidney abnormalities (KCNJ1 and ADAMTS15 genes). We suggest that some characteristics described in JBS cannot be explained by monosomy of single genes, but rather the combination of contiguous genes, or gene-gene interactions.

J11.41 Partial SHOX duplication in a daughter and her father associated with short stature.
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We report a case of a familial partial duplication of the SHOX gen detected in a 14 year old girl and her father. The girl was referred to us for short stature and development delay. She has a Dandy Walker abnormality of the brain and

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no other congenital malformations. She was born atterm, her developmental milestones were delayed, she has borderline mental retardation and attends special school. Her height is 152.5 cm (3rd percentile), weight 70.3 kg (97th percentile) and head circumference 51.5 cm (3rd percentile). She has mild dysmorphic features. Karyotype is 46,XX. SNP aCGH (HumanYctosSNP-12-v2.1 Ilumina) detected 190,9 kb duplication of Xp2.23 (422.64-613.567) encompassing most of the SHOX gene. The finding was confirmed by MLPA (MRC, Holland, Human Telomere -5, kits P036 - E1; P070 - B2; P245-B1; P106-B1 MRX), which detected a duplication in the SHOX gene region. The same duplication was revealed by MLPA in her father, who measures 170 cm (10th percentile) and has no congenital malformations. Isolated duplications of SHOX gene are rare and their effect on height is not clear. Seventeen patients with full and sixteen with partial duplication of the SHOX gene were reported in the literature. Some were ascertained through studies of particular conditions associated with short stature (idiopathic short stature, Léri-Weill dyschondrostosis), in whom those who were not, the stature varied from normal to tall. The effect of SHOX gene duplication on stature is still not clear.

J11.42 Somatic mosaicism in patients with r(18) and congenital heart disorder

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Syndrome r(18) - ring 18 chromosome cytogenetically is characterized by a complete or mosaic forms a ring chromosome 18 with the absence of the distal portions of the long and short arm of the chromosome. The clinical picture includes multiple dysmorphism, combined with varying degrees of mental retardation. Committal involvement of cardiovascular system and congenital heart defects typically are not described in cases of r(18).

We observed 18-year-old patient with mental retardation, multiple facial dysmorphism, and subacute encephalopathy. During standard karyotyping by PHA-stimulated lymphocytes patient revealed 46, XX, r(18) karyotype. Since congenital heart defects are not common feature of r(18) we hypothesized mosaic chromosomal aberrations and performed additional FISH analysis on blood smears using Aneu Vision (CEP 18) and To Tell Mx 11, Mx 12 probes. The study revealed three signals of chromosome 18 centromere and subtelomeric deletion of chromosome 18 in 15% of the cells, contained two signals from the centromere of chromosome 18 with subtelomeric deletion to form a ring in 80% of cells, 5% of the uncultured cells in blood smear contained two signals from the centromere of chromosome 18 without subtelomeric deletion.

In conclusion we describe clinical case of somatic mosaicism in patients with r(18) in combination with 18 trisomy in 15% of blood cells, leading to combined phenotype of mental retardation and congenital heart defect.

J11.43 Molecular study of the gene TCOF1 in the syndrome of Treacher Collins

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Treacher Collins Syndrome (TCS) is a rare clinical entity which associates especially craniofacial dysmorphism and deafness. Symptoms can sometimes be severe being life-threatening when choanal atresia exists. Our objective is to look for the TCOF1 (5q32-q33.1) mutations most frequently reported in the literature in Tunisian patients affected by TCS. We were interested in the study of the mutations in 11 of the 24 exons of the gene TCOF1 by the method of direct sequencing in 7 patients among whom 4 family cases. These exons are reported as hotspots of mutations. The confirmation of TCS diagnosis by molecular biology was possible for 3 of 7 patients. The sequencing allowed to identify a new mutation not reported in the literature which was found in 2 cases from the same family (a father and his daughter). An already described mutation was found in 1 sporadic case. Eight polymorphisms were found, among which five are known. The mutations found in our patients occur in repetitive sequences which support the hypothesis of polymerase errors. No mutations were detected in exon 24 which account for 20 % of already reported cases. The molecular study of the TCS allows the clinical presentation diagnosis, the orientation of the clinical follow up and the prenatal diagnosis in the future forms.

J11.44 Co-occurrence of tetrasomy 12p and trisomy 12q

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A female neonate was born at a gestational age of 38 weeks by spontaneous vaginal delivery, with a birth weight of 3600 gr (50th-75th centiles). Antenatal ultrasound had shown minimal pelvic-calciluteal dilatation of left kidney, and no other abnormalities were detected on abdominal ultrasound scan. Postnatally, she was noted to have hypertelorism, prominent and wide five-eye, upsinking palpebral fissures, long philtrum, pigmented miosis in both feet, hyperplasia of the gingiva and hypotonia. Cranial magnetic resonance imaging showed an asymmetrical dilatation of right pontocerebellar cistern, with a suspicion of an arachnoid cyst. On follow-up, she had progressive hyperpigmentation over both lower extremities and a skin biopsy was performed with a clinical diagnosis of Pallister-Killian syndrome. Karyotype analysis from peripheral blood lymphocytes revealed 46,XX and karyotype analysis of skin fibroblast sample revealed 47,XX,+i(12)(p10). This finding was confirmed by fluorescence in situ hybridization by whole chromosome paint 12, that revealed 47XX,i(12)(p10)ish (wcp12+). Further FISH analysis for 12p and 1q terminals with Vysis TelosWes 12 probe (12p – 8M16, 12q – VIRM2002) detected 47XX,+i(12)(p10)ish + (v12p)8M16x4, VIRM2002x3). Combination of tetrasomy 12p and trisomy 12q is a previously unreported condition.

J11.45 Tetrasomy 18p in a patient with happy demeanor

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An 8 years old female patient was referred to clinical genetics department because of dysmorphic features, speech and motor delay. She was the second child of nonconsanguineous parents. Maternal age at delivery was 31 years, and the baby was born by spontaneous vaginal delivery at 39th gestational weeks, with a birth weight of 1200 gr (below 3rd centile). Psychomotor development was delayed; she sat independently at 2 years of age, walked at 6 years of age, and spoke a few words at 6 years of age. On physical examination at the age of 8 her anthropometric measures were as follows: height 119.5 cm (3rd-10th centiles), weight 22 kg (3rd-10th centiles), head circumference 49 cm (below 3rd centile). She presented with microcephaly, prominent nasal bridge, prognathism, malformed ears and long fingers. Her behaviour was characterised by inappreciable and frequent laughter. In echocardiographic evaluation patient presented ovoid and a first trimester termination at the age of 8 her anthropometric measures were as follows: height 119.5 cm (3rd-10th centiles), weight 22 kg (3rd-10th centiles), head circumference 49 cm (below 3rd centile). She presented with microcephaly, prominent nasal bridge, hypophagia, malformed ears and long fingers. Her behaviour was characterised by inappreciable and frequent laughter. In echocardiographic evaluation patient presented ovoid and a first trimester termination at the age of 8 her anthropometric measures were as follows: height 119.5 cm (3rd-10th centiles), weight 22 kg (3rd-10th centiles), head circumference 49 cm (below 3rd centile). She presented with microcephaly, prominent nasal bridge, hypophagia, malformed ears and long fingers. Her behaviour was characterised by inappreciable and frequent laughter. In echocardiographic evaluation patient presented ovoid and a first trimester termination at the age of 8 her anthropometric measures were as follows: height 119.5 cm (3rd-10th centiles), weight 22 kg (3rd-10th centiles), head circumference 49 cm (below 3rd centile). She presented with microcephaly, prominent nasal bridge, hypophagia, malformed ears and long fingers. Her behaviour was characterised by inappreciable and frequent laughter.

The syndrome expresses itself with developmental delay, cognitive impairment, happy demeanor, growth retardation, muscle tone abnormalities, microcephaly, malformed ears, hypophagia and congenital heart diseases. This condition must be considered in differential diagnosis of syndromes with microcephaly, happy demeanor and intellectual disability.

J11.46 The weight of a translocation

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We report on a 29 years old woman with secondary amenorrhea and short stature. Pelvic ultrasound revealed a normal uterus and little ovaries. There was no intellectual disability. She has two healthy sisters and her mother had a first trimester spontaneous abortion. Chromosome analysis revealed 46,Xder(X)(X;15)(p15;q42.23) of maternal origin. Array-GH showed a 39.6 Mb Xp22.33-Xp1.14 deletion and a 33 Mb 15q23.1-15q26.3 duplication. It was also observed a 92 Kb 5q22.2 microdeletion which involves MCC gene. Any genomic imbalances, deletions or duplications of an X chromosomes have a much more severe impact on males than fe-males. In males, most cytogenetically visible deletions of the X chromosome involve the terminal portion of Xp (Xp22.2-2Xpter), which leads to nullisomy of the deleted region and is known as a cause of variable contiguous gene syndromes. The phenotypes depend on the extent and position of the deletion and are limited by the presence of male lethal genes in Xp22.2 at about 10-11 Mb from the telomere so larger Xp deletions extend beyond the KALLI locus are very rare. Females with similar Xp22 deletions are often phenotypically normal except for short stature, because of skewed X-chromosome inactivation. Xp deletion women show primary amenorrhea or sometimes secondary amenorrhea. This is a pregnancy planning’s dilemma: if our couple...
will decide to undergo medically assisted procreation programs transmission of derivative X chromosome to sons would be lethal instead transmission to daughters would determine an unpredictable variable phenotype in order to skewed X-chromosome inactivation.

**J11.47**

**Trichorhinophalangeal syndrome type II due to a novel 8q23.3-q24.12 microdeletion detected by oligo-SNP array associated with novel genital findings.**

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Tricho-rhino-phalangeal syndrome type II (TRPS2; OMIM 150230, Langer-Giedion syndrome, LG5) is a contiguous gene deletion syndrome located on 8q23.1-8q24.1. Depending on the size and position of the heterozygous deleted region, the genetic defect principally involves TRPS1, RAD21 and EXT1 genes. TRPS2 presents characteristic clinical findings with multiple cartilaginous exostoses and frequently, intellectual disability. In the present study we analyzed a female with TRPS2 by Oligo-SNP array and detected a de novo 8q23.3-q24.12 microdeletion of 5.46 Mb on 8q23.3-q24.12 involving seven OMM genes {CNSD3, TRPS1, EIF3H, RAD21, SLC38A8, MED50 and EXT1}. In addition, UTP2, MIR3610 and exon eight of SAMD12 were deleted. The final cytogenetic result was 46,XX, arr [hg19] 8q23.3-q24.12 (113,885,753-119,323,017) x1 dn. Parental testing of all procedures showed normal results. Our patient had genital anomalies with normal intelligence not previously reported. The analysis at molecular level of deletion region in TRPS2 is imperative to understand the pathogenesis of the disease

**J11.48**

**Congenital absence of the portal vein in a child with Turner Syndrome.**

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Congenital absence of the portal vein in a child with Turner Syndrome Introduction: Turner Syndrome is a disease caused by a total or partial loss of an X chromosome in women, showing a frequency of 1 per 2500-4000 live-born females. (1,2) Characteristic features of the disease are short stature, gonadal dysgenesis, webbed neck, cubitus valgus and congenital cardiovascular anomalies. Congenital absence of portal vein, however, is rare, and there are no systemic reviews detailing the prevalence of the absence of the portal vein in patients with Turner Syndrome. 13 year old girl was admitted to emergency service for high fever. At the physical examination her respiratory rate was 60 /min, she had toxic appearance. She had webbed neck and short stature. She had a shileded chest with no breast tissue and prepubertal nipples. She had splenomegaly. Laboratory tests revealed high transaminases. Chromosomal studies showed monosomy pattern compatible with Turner Syndrome (45X0). Echocardiography was consistent with aortic coarctation and a bicuspid aortic valve. Patient underwent dynamic biphasic computerized tomography (CT) imaging to investigate the causes of liver disease. CT revealed the absence of portal vein. She had grade II esophageal varices in the upper gastrointestinal endoscopy. The final diagnosis was a case of Turner’s Syndrome with absent portal vein, portal hypertension and hyposplenism.


**J11.49**

**An ~1.6 Mb interstitial deletion on Xp22.31 in a patient with psychomotor developmental delay, microcephaly, epilepsy, ichthyosis, and spastic tetraplegia.**

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We present a 6-year-old boy, born at 41 weeks of gestation to non-consanguineous, healthy parents. The pregnancy was complicated by respiratory infection in the first trimester of gestation. At birth, body weight and length were normal, and head circumference was 33 cm (3 centile). The boy was evaluated at genetic counselling unit at the age of 3 years because of severe psychomotor retardation, absent speech, epilepsy, spastic tetraplegia and severe microcephaly (~4 404SD). Generalized ichthyosis was observed. There were no dysmorphic features or structural malformations. Brain MRI showed thin corpus callosum without other abnormalities. Results of oligo-cytocede arrayCGH (180 K V8.1) showed an ~1.6 Mb interstitial deletion on chromosome Xp22.31 encompassing a few genes, including VCX (candidate gene for intellectual disability), PNPLA4, and STS associated with X-linked ichthyosis (XLI). Further analysis of the family revealed that the deletion is maternally inherited. The same Xp22.31 deletion was also found in her two healthy sisters; her grandfather presented only with ichthyosis. Some authors have reported single cases of the Xp22.31 deletion, involving the XCV gene in patients with ichthyosis and normal intelligence. Therefore, it is not clear whether the deletion is the only cause of patient’s pathology, especially intellectual disability, epilepsy, and spastic paresis. Incomplete penetration or another gene within Xp22.31 deletion (e.g. PNPLA4) or unknown mechanisms might be responsible for these symptoms in our patient. Comparison with the similar cases from the literature and genotype-phenotype correlation will be discussed.

**J11.50**

**Partial deletion of 9p: familial case of Alfi syndrome.**

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Monosomy 9p syndrome also known as Alfi syndrome is a rare genetic disease characterized by mental retardation, developmental delay, facial dysmorphism and various type of feminization in male. We report two cases of Alfi syndrome in siblings. One patient (9 year old girl) was initially diagnosed with dysgerminoma of the right gonad and gonadoblastoma of the left gonad. Following symptoms were identified: severe mental retardation, general development delay, generalized hypotonia, contractures of all joints including talocalcual, knees, and phalanges as well as complete gonadal dysgenesis. Floating movements of eyeballs and epilepsy were also observed. The external female genitalia were normal. Histological analysis of gonads revealed sclerosis and no follicles as well as absence of cilia on the surface of salpinx. Cytogenetic analysis of peripheral blood cells identified male karyotype with unbalanced translocation 45,XY, der[9]t(9;21)(p22;q11)-1. Deletion of fragment 9p22-pter was confirmed by serial FISH analysis. Patient’s sister (5 year old girl) had identical symptoms except for sex reversal and karyotype 45,XX,der[9]t(9;21)(p22;q11)-1. Family history was remarkable for unidentified genetic disorder with mental retardation and some chromosome 21 abnormality in mother and severe genetic disease in maternal brother. Most probably abnormal karyotype in this family was inherited through female line.

**J11.51**

**Aspects Of The Early Neurogenetic Diagnostics Of Klinefelter Syndrome.**

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Background: In the study are analyzed peculiarities of clinical manifestations and cytogenetic features in Klinefelter syndrome, which is a sex chromosomal abnormality. The aim of research is to help achieve an early neurogenetic diagnosis and initiation of measures to improve the development of children.

Material and methods: A group of 84 boys with Klinefelter syndrome was investigated during medical genetic counseling in the Center for Reproductive Health and Medical Genetics, presenting phenotype selection criteria such as: developmental anomalies of the external genitalia - peno-scutal hypoplasia, micropenis, small testes, cryptorchism, cranio-facial dysmorphism, waist high and disproportionate, hypogonadism, mental retardation, psychosocial problems.

Results: Homogeneous form or trisomy 47, XXX (27 cases - 94.5%) was the most common chromosomal abnormality diagnosed in the 32 patients with Klinefelter syndrome, followed by mosaic form (47 XXX/46, XY: 1 case - 3.1%), polysomy XXY (48, XXY: 1 case - 3.1% and pentasomy - 49, XXXXY: 1 case - 3.1%) and variants of structural abnormalities of autosomal chromosomes (47, XXY, inv (5): 1 case - 3.1%), associated with robertsonian translocation (47, XXXY, Rob (13:14): 1 case - 3.1%). Most patients with K5 had been diagnosed in puberty (23 cases - 71.9%), 6 patients (18.7%) were diagnosed at prepubertal, and only 3 patients (9.4%) were diagnosed during early childhood.

Conclusion: Neurogenetic diagnosis during early ontogenetic development and cytogenetic analysis (karyotyping, Barr test) is necessary for better investigation of children with suspicion on SK to confirm the clinical diagnosis and timely provide genetic counseling.
J11.52
Prophylaxis of Congenital Malformations in Pregnant Women of a Risk Group
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Background: Efficient stabilization of genetic diseases spreading in population of Moldova means, first of all, to improve prophylaxis of congenital malformations in pregnant women. The role of genetic counseling in the prevention system of genetic diseases is analyzed in the study. The main prophylaxis measures and prenatal diagnosis methods applied to pregnant women of risk group are identified by authors.

Materials and methods: Retro-prospective study of investigation included 8937 pregnant women who have asked for medico-genetic counseling in CRHMG, in 2008-2012. Group I: 4473 pregnant women from medium and high risk group. Group II: 4464 pregnant women from low risk group.

Results: Two researched groups were comparable in age, gestation period, degree of genetic risk. The age of women in genetic risk group was from 17 years to 44 years (average age 26.1 ± 5.3 years). Prenatal diagnosis contributed to the identification of severe fetal pathologies to 478 pregnant women, which constituted 5.4% of total number of investigated cases. Amniocentesis with study of the fetal karyotype has allowed the identification of numerical and structural chromosomal abnormalities to 67 patients (3.0%). Abnormalities of the central nervous system are the most common in the structure of the serious fetal pathologies (1.4%), followed by abnormalities of the cardiovascular system (0.87%), oesomotum system anomalies (0.51%), abnormalities of renal system (0.64%) and digestive system (0.6%).

Conclusion: Genetic counseling and prenatal diagnosis methods (fetal ultrasound, biochemical screening, karyotyping) helps to reduce up to 50% of the frequency of chromosomal abnormalities and congenital malformations to newborns.

J11.53
The results of clinical and molecular analysis of data of patients with branchio-oculo-facial syndrome
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Branchio-oculo-facial syndrome (BOFS, OMIM #113620) is a rare autosomal dominant disorder, characterized by branchial cleft sinus defects, ocular anomalies, a dysmorphic facial appearance including lip/palate cleft or pseudocleft and associated with mutations in TAFAP2A gene. Clinical features analysis and DNA-testing are performed in 7 BOFS patients. 3 patients present the clinical main three BOFS features: cervical cutaneous aplasia linea arci, ocular anomalies, orofacial cleft defects, one patient has hypospadias, one has tarsal cleft in upper lip area. Ocular clefts are observed in all 7 patients (2 - upper lip pseudocleft, 2 - upper lip cleft, 3 - upper lip/palate cleft). 2 patients have developmental abnormalities of auditory ossicles, 1 - atresia of external acoustic meatus, 4 - anatomically narrow acoustical ducts. Conductive hearing loss is diagnosed in 5 BOFS patients. Sequencing of TAFAP2A gene coding region revealed missense mutations in 4 BOFS patients. One patient has a Arg251Gly mutation described previously, 3 patients from 2 families have novel mutations: p.Arg213Ser and p.Val280Asp. Novel mutations are not detected in healthy members of the families.

J11.54
Establishment of national birth defect surveillance in Lebanon: preliminary data
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Background: Following the WHO recommendations in 2012, the National Collaborative Perinatal Neonatal Network (NCPNN) in collaboration with the Centers for Disease Control and Prevention and the Ministry of Public Health (MOPH) inaugurated the establishment of a national Birth Defect (BD) surveillance program. A previous study conducted on 19 Lebanese hospitals (members of the NCPNN, between 2003 and 2007) had shown an incidence of 3.25% of BDs in neonates with a significantly higher occurrence in consanguineous mating. This is our preliminary data on BDs in Lebanon, from May 1st to November 30, 2012.

Methods: Infants born with major BDs from hospitals across Lebanon were reported. Incidence of BDs was calculated using data provided from the MOPH. Results: 464 infants born with a major BD were reported. The overall rate was 18.2/1000 live births. The most common were musculoskeletal defects (4.2%), mainly clubfoot (24.5%), central nervous system defects (4.01%), mainly spina bifida (40.2%) and cardiovascular defects (3.29%), with septal defect accounting for 29.7%. Facial/neck defects represented only 0.63%. Among infants with spina bifida, 8.3% of the mothers were on folic acid before pregnancy while 36.1% started it after conception. Among infants with clefts, 64.1% of the mothers reported smoking exposure during pregnancy. 30.8% of all mothers were overweight/obese before pregnancy. Parental consanguinity is not common, presented in 3.4% of the cases; among those, 53.2% were first cousins.

Conclusion: Ascertainment the prevalence and determinants of BDs through proper surveillance would lead to preventive measures contributing to a measurable reduction of BDs in our country.

J11.55
Arthrogryposis and perisylvian polymicrogyria: report of an emerging phenotype
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In recent years a few patients with perisylvian polymicrogyria associated with arthrogryposis have been reported. The underlying genetic cause of this condition is not yet known.

We report on a newborn male whose pregnancy was characterized by the detection of arthrogryposis, bilateral club feet, micrognathia, and a small VSD on prenatal sonogram. The patient was born full term: BW 2673 g (5th centile), BL 46 cm (5th centile), OFC 34 cm (20th centile). Physical examination at birth showed minor facial anomalies, proximal and distal contractures of the upper and lower limbs consistent with arthrogryposis multiplex congenita, lack of palmar and plantar creases, and bilateral talipes equinovarus. A brain MRI evidenced bilateral perisylvian polymicrogyria. Family history was not contributory.

The SNP array [Affymetrix Cytoscan HD microarray (average resolution of 50 Kb for deletions and 400 Kb for duplications)] showed a microduplication of 825 Kb in chromosome 1q41 (29.372.798-30.197.863, hg19) and a microdeletion of 420 Kb in chromosome 7p22.1 (4.743.281-5.163.491, hg19). The duplication contains a control region for the FOXG1 gene. The deletion contains several genes associated with muscular function, including FOXI1. The interpretation of this result is still unclear, and analysis of the parents is pending. We will proceed with exome sequencing if neither of the chromosomal imbalances are considered to be causative.

In conclusion, we propose that the association between perisylvian polymicrogyria and arthrogryposis is a novel discrete phenotype. The results of the genetic analysis will allow better insight into the molecular etiology of this recently described entity.

J11.56
Prader Willi syndrome clinical signs and phenotypic features at Lithuanian health science university hospital
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Background: Prader Willi Syndrome (PWS) – a rare genetic disease that manifests in 1:10000 or 1:25000 births. The aim of the study was to evaluate when first starting to emerge Prader Willi syndrome symptoms and how they are distributed according to sex.

Methods: In this retrospective analysis data were collected from 29 outpatients, who had clinical symptoms of PWS. The patients weight and height were evaluated at birth and the first visit to the doctor at the time (weight and height percentiles evaluated, as well as the face, extremities, breast development and other changes).

Results: All patients were applied for the obesity to the doctor, when average age was 3.23 years. Comparison of average age between boys and girls showed no statistically significant difference. The almond -shaped eyes were 33.3% of girls and 47.4% boys. Thin upper lips 33.3% of girls and 47.4% of boys. Sandal gap 11.1% of girls and 15.8% boys. 22.2% of girls and 26.3% boys. Thin upper lips 33.3% of girls and 36.6% boys. Thin upper lips 33.3% of girls and 47.4% of boys. Thin upper lips 33.3% of girls and 47.4% of boys. Thin upper lips 33.3% of girls and 47.4% of boys.

Conclusions: Average ages of patients was 3.23 years, when they first applied for the obesity to the doctor. Weight on first visit to the doctor does not depend on birth
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Introduction: Seckel syndrome was described in 1960 and it is also known as bird-headed dwarfism. This syndrome is a rare autosomal recessive disorder. It is characterized by severe intrauterine and postnatal growth retardation, low birth weight, severe microcephaly, craniofacial dysmorphism with characteristic bird headed appearance, prominent beaked triangular nose, micrognathia and variable mental retardation. Other multiple anomalies associated are cleft lip and palate, club foot, scoliosis, gastrointestinal malformations, multiple skeletal malformations, cardiovascular, endocrine, hematopoietic and central nervous systems abnormalities. At the moment 100 cases has been reported and this is the first case reported in Ecuador and the second in Latin America.

Case report: Woman of 15 years old. The mother did not have bad habits during the pregnancy. The father is a first cousin of the mother. The patient is 91 cm in length and weighs 9.2 Kg., exhibits severe signs of malnutrition. The patient is bird-headed, presents microcephaly, multiple skeletal malformations, ears are deformed and positioned very low, scoliosis, multiple anomalies associated are cleft lip, deformed teeth, clinodactyly, asymmetric pelvis, as well as other characteristics signs. She is aggressive and presents severe mental disability and she suffered convulsions for the first years of her life. Gonadotropin hormones are at a low level. FSH: 1.07mlU/ml, LH: <0.10mIU/L, these suggest a structural problem in the hypothalamus. Ka- ryo type is normal 46,XX. But the patient has no uterus, ovaries or secondary sexual characteristics, but she is not diagnosed as a case of turner syndrome.

J12.001 Polymorphism of P53 gene – exon 4 codon 72 in endometrial carcinoma: Correlation with tumor type
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Endometrial carcinoma is the fourth most common cancer among women in developed countries. Endometrial cancer patients may benefit from systemic chemotherapy, alone or in combination with targeted therapies if the disease is clinically diagnosed prior to expansion and metastasis to other organs. The aim of this study was to evaluate the prognostic role of p53 polymorphism and correlation with tumor type and grade in human uterine endometrial carcinoma. Seventy five patients with endometrial carcinoma and seventy five patients with malignancy were studied for possible mutations in exon 4 p53 gene using polymerase chain reaction and restriction fragment length polymorphism techniques and sequencing. P53 polymorphism in exon 4 showed no significant difference in the genotype or allele prevalence between case and control groups. We found the Pro allele and fragment length polymorphism techniques and sequencing. Pro53 polymor- phisms in exon 4 p53 gene using polymerase chain reaction and restriction fragment length polymorphism techniques and sequencing. P53 polymorphism in exon 4 showed no significant difference in the genotype or allele prevalence between case and control groups. We found the Pro allele and genotype frequency to be insignificantly higher in cases than controls (Pro allele 66 and 8/3% respectively; genotypes: Arg/Pro 61 and 48/7%; Pro/Pro 5 and 6/9%, respectively), there was no significant difference in the allele distribution between tumor type and grade because it was need many number of cases.

J12.002 Association between polymorphisms rs1801270(Ser31Arg) of CDKN1A gene in sporadic colorectal cancer
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Background: Colorectal cancer is the third most commonly diagnosed cancer in both men and women. Progressive loss of cell cycle control is an important feature on the colorectal cancer. p21 (CDKN1A/CIP/1/WAF1), one of the cyclin-dependent kinase inhibitors, plays a key role in regulating the cell cycle. The aim of this study was to investigate associations of the CDKN1A gene polymorphisms (rs3176336, rs1801270, rs762624) with risk of colorectal cancer (CRC) in an Iranian population.

Methods: The study subjects were 150 cases of colorectal cancer and 150 controls for any polymorphisms. Genomic DNA was extracted using standard salting out method. Genotypes were determined using the polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) method.

Results: A significant relation was found between rs1801270 in the CDKN1A gene and colorectal cancer. The distribution of AC genotypes among sporadic CRC patients was more frequent than that in the control group (P value = 0.003). We found no significant difference between studied polymorphisms and colorectal cancer.

Conclusion: to our knowledge this is the first study on association of CDKN1A polymorphisms with CRC risk in Iranian population. Our findings indicated that there is association between rs1801270 and risk of colorectal cancer.

J12.003 The 1303CA mutation of solute carrier family member 2 gene in lung cancer patients
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As an essential element for human life, iron is widely involved in many im- portant metabolic processes, such as DNA synthesis, electron transport, and oxygen delivery. Numerous studies have found a positive correlation between iron storage and risk of cancer. Results: A significant relation was found between rs1801270 in the CDKN1A gene and colorectal cancer. The distribution of AC genotypes among sporadic CRC patients was more frequent than that in the control group (P value = 0.003). We found no significant difference between studied polymorphisms and colorectal cancer.

Conclusion: to our knowledge this is the first study on association of CDKN1A polymorphisms with CRC risk in Iranian population. Our findings indicated that there is association between rs1801270 and risk of colorectal cancer.

J12.004 Variant type PML-RARA fusion transcript in AML: Detection and interpretation of results by RT-PCR
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Acute myeloid leukemia (AML) is characterized by a number of features that requires accurate diagnostic and specific treatment approach. The t(15;17) (q24.q21) which generates the PML-RARA fusion transcript presents the diagnostic hallmark of AML. PML-RARA positive patients express one of three hybrid transcripts within in PML gene and are denoted bcr1 (long), bcr2 (medium) and bcr3 (short) forms. The importance of specific breakpoint region is its utility as prognostic factor for individual’s likelihood of relapse and possibly their response to therapy treatment.

In this study we present detection of the most uncommon breakpoint region. bcr2, by RT-PCR technique. We analyzed 95 pediatric AML patients and examined the incidence of three most frequent fusion transcripts obtained by standardized RT-PCR protocol (European BIOMED-1 Concerted action). In 28 cases, positive for one of the AML rearrangement, 9 patients (32%) had AML1-ETO rearrangement, 3 (11%) had CBFB-MYH11, and 16 (57%) had PML-RARA. Among 16 PML-RARA patients, five (42%) were presented as bcr1 positive (multiplied with PML1-RARAB primers) and showed non- specific extra band multiplied with bcr3 primers (PML2-RARAB). These results are interpreted as bcr2 positive, but to ultimately determine whether it is bcr1 or bcr2 transcript, we are now investigating length of these products by sequencing. After these analyses we will determine the rate of bcr2 products and their response to therapy treatment.

Cytogenetic analysis from bone marrow samples showed normal karyotype. Establishment of bcr2 rearrangement is important because of its decreased response to treatment with all-trans retinoic acid (ATRA) depending on where the break occurs within PML exon 6.
ABSTRACTS PUBLISHED ABSTRACTS
J12.005
Novel Additional Chromosomal Abnormalities in patient with Acute
Promyelocytic Leukaemia and ATRA resistance

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Additional chromosome aberrations (ACA) have been observed in 23-43%
of Acute Promyelocytic Leukaemia (APL) cases, with trisomy 8 being the
most frequent (31-46%). Here we describe one case of APL with ACA at
presentation occurred in our hematology department in April 2013 and
never been reported in literature. A 22 years old male patient who was referred to our institution for mucosal and cutaneous bleeding. Laboratory
exams showed anemia, leucopenia and thrombocytopenia, with increasing
prothrombin time, activated partial thromboplastin time and fibrin degradation products. A blood smear evidenced atypical agranulate promyelocites with Auer Rods in faggots. Bone marrow aspirate for morphological,
immunophenotyping , cytogenetic and biomolecular analyses was performed. The patient showed a 46,XY,t(5;21)(q31;q22)[17]/46,XY,t(5;21)
(q31q22),t(15;17)(q24;q21)[3].ish
t(15;17)(PML+;RARA+,PMLdim)[8]
karyotype. The aquired , non constitutional nature of the translocation
t(5;21) has been confirmed by a cytogenetically normal result of phytohemagglutinin-stimulated blood analysis. Molecular analysis performed by
RT-PCR disclosed the presence of bcr-1 PML-RARα gene fusion transcript.
Follow up laboratory exams showed persistence of a normal karyotype
and molecular remission, but in January 2014 bone marrow cytogenetic
analysis revealed a complex karyotype:49,XY,t(5;21)(q31;q22),t(15;17)
(q24;q21),+mar1,+mar2,+mar3[2]/
46,XY[18].ish t(15;17)(PML+;RARA+,PMLdim)[7/100] with presence of
bcr-1 isoform in RT-PCR, normal CBC count and MRI imaging showing initial NCS envolvment. The incidence and prognostic significance of ACA in
APL is still a controversial matter and when such abnormalities are found
there is no evidence to support the use of alternative therapeutic strategies
to ATRA(all-trans retinoic acid) and chemotherapy. Now patient is under
treatment with arsenic-trioxide.
J12.006
Increased frequence of the rs2066853 variant of aryl hydrocarbon
receptor gene in patients with acromegaly

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Introduction Aryl Hydrocarbon Receptor (AHR) regulates Cytochrome
P4501A1 (CYP1A1) expression and other xenobiotic target genes to antagonize environmental contaminants effects. It controls also cellular proliferation, senescence and apoptosis. AHR gene polymorphisms were associated
with several tumours’ occurrence and deregulation of AHR expression was
demonstrated in somatotropinomas. In this study, we analyse the sequence
of the AHR gene in patients with sporadic acromegaly. Patients and methods
We evaluated 70 patients (M = 27, age 57.5 ± 12.8 aa ± SD) and 157 controls.
Exons 1, 2, 3 5 and 10 of AHR was screened by direct sequencing in patients
and controls. Results Polymorphism rs2066853 (c.1661 G>A) was identified in 18/70 acromegalic patients and in 9/157 healthy subjects (25.7 vs
5.7%, χ2 18.98 p <0.0001, OR 5.7, 95% CI: 2.4081 - 13.4558). Moreover,
rs4986826 (c.1708 G>A) variant was identified in two patients but not in
controls (χ2 4.62 p <0.05). Mean IGF-1 ULN (2.93 ± 1.07 vs 2.29 ± 0.86, p
<0.05) and the prevalence of cavernous sinus invasion (χ2 6.08, p <0.05)
were higher in patients with rs2066853 polymorphism than in the other
ones. Moreover, an increased prevalence of differentiated thyroid cancer (χ2
7.53, p 0.02), bladder tumour (χ2 34.66, p 0.0001) and lymphohematopoietic
neoplasm (χ2 6.41, p <0.05) was found in the former group than in the latter.
Further studies are needed to better elucidate the functional consequence of
this AHR polymorphism, whether it could impact on xenobiotic sensitivity
or affect other AHR-mediated cell-cycle deregulation mechanisms.
J12.007
Cytogenetic pattern profilling in Acute Lymphoblastic Leukemia of
childhood in North Indian population
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Acute lymphoblastic leukemia(ALL) comprises of 70 - 80 % of childhood
leukemias. Its diagnosis is based on hematological parameters like blast population, blast morphology, flow cytometry. ALL arises as a result of genetic

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damage to the lymphohematopoetic progenitor cell leading to malignant
transformation. This study was a cross-sectional study done on paediatric
age group patient of North Indian population on confirmed cases of ALL
on bone marrow examination. The bone marrowsamples were subjected
to cytogenetic study using immediate, 24hr and 48hr protocols. FINDINGS:
1.Hypodiploidy was the commonest finding in our study with an incidence
of 78.57% which is similar to that found in studies from East India where it
is reported to be 63.6% and in West India it is 38.4%. In World Literature,
this is similar to Africa population studies where hypodiploidy showed an
incidence of 37.5%.Hyperdiploidy was commonest in North India (27%) in
another study and South India(14.8%) and Asia(69.5%) and Europe(63%).
Pseudodiploidy was commonest in America(41%). 2.B Cell ALL(95%) and T
Cell ALL(5%) in our study is dissimilar from other studies, B Cell (70%-76%)
and T Cell(24%-30%) 3. Structural abnormalities seen :43,X,del(4),dic(5)(6)-(2)-(20)+(14) in 1(7.14%) and 42Y6p del dic 13-(7),-(9),-(16),-(X) in
1(7.14%). RECOMMENDATIONS Cytogenetics should play a key role in risk
stratification and treatment protocols considering the heterogeneity of the
pediatric ALL
J12.008
New, prognostically relevant chromosomal lesions in AML assessed by
array CGH - a preliminary study

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Acute myeloid leukemia (AML) is a biologically heterogeneous disease.
Identification of chromosomal aberrations, as well as cryptic genome lesions is important for precise diagnosis and risk assessment. The aim of our
study was to determine the most frequent clonal chromosomal aberrations, to assess their prognostic value and to compare the results obtained
by classical cytogenetics and by array CGH. Bone marrow aspirates were
collected from 21 patients diagnosed with AML and from 8 healthy blood
donors. The material was divided into two parts. One part was used for
classical cytogenetic analysis (GTG-banding and RHG-banding) and FISH.
The second part was used for DNA isolation and array CGH analysis. Chromosomal abnormalities detected by classical cytogenetic techniques most
often relate to chromosomes: 5, 17 (24%), 8, 15, 16, 18, 21 (19%), 3, 7, 11,
14, 20 (14%), 4, 12, 13 and 22 (9,5%). The array CGH study indicated regions: 16p13.3, 16p22.3q24.2, 5q14.1q35.2, 8p23.1p11.21, 8q12.1q24.3,
15q11.2, 15q15.1q15.3, 15q22.1q24.1, 15q25.2q25.3, 18p11.32q23,
3p26.3, 3p14.1p12.2, 3q11.2q21.1, 3q27.2q29, 7p12.3q36.3, 17p13.3p13.1
and 17q12. The above aberrations were confirmed by FISH. Array CGH
method enabled to determine the regions of the feasible prognostic value
during 36 months follow up. Aberrations in regions 5q14.1q35.2, 16p13.3
and 18p11.32q23 had a negative impact on the prognosis. AML patients
with these lesions had shorter overall survival. The changes in the region
15q22.2q25.3 were associated with longer survival and favorable prognosis.
Larger group of AML patients included in the study will enable the verification of prognostic significance of reported chromosomal changes.
J12.009
Complex translocation t(8;21;17)(q22;q22;p11.2): a masked variant
of t(8;21) in a patient with AML-M2

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The t(8;21) translocation occurs in 5-12% of acute myeloid leukemia (AML)
cases, and in 40% of AML FAB M2 type, with well defined and specific morphological features. Over 70% of patients show additional chromosome abnormalities: loss of Y or X chromosome in half cases. Variant translocations
involving a variable third chromosome account for approximately 3-4% of
all AML-M2 with fusion transcripts. Although the t(8;21) is easy to recognize cytogenetically, rearrangements between 8q22 and 21q22 may be masked with complex or cryptic translocations. This translocation leads to the
fusion of the AML1 (RUNX1) gene on chromosome 21 and the ETO gene
on chromosome 8, and results in a transcriptionally active chimeric gene,
AML1-ETO on the derivative 8 [der(8)]. Here, we present a case of a patient,
41 years old male, with AML-M2 with a three-way translocation, involving
the chromosomes 8, 21, and 17. The diagnosis, evaluated by morphology
and immunophenotype in bone marrow, was indicative of AML M2 without
specific features suggesting the presence of t(8;21). Cytogenetic study of

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bone marrow cells revealed the karyotype as 45,X-Yt(8;17)(q22;p11.2) in 20 metaphases. FISH analysis using a 17p13 probe (cytoretell p53 deletion) on previously G-banded metaphases showed that one of the LSI p53 signals was on chromosome 21. This led to the discovery of the cryptic translocation t(12;17), the presence of rearrangement AML1-ETO was confirmed by RT-PCR (using BIOMED-1 protocol). Variant t(8;21) translocations should be suspected in AML M2 and specifically looked for whenever 8q22 and 21q22 rearrangements are found.

**J12.010**

**Molecular-genetic investigation of aFAP/MAP syndromes among Russian patients**

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Attenuated Familial Adenomatous Polyposis (aFAP) and MYH-associated Polyposis (MAP) are the important inherited colorectal cancer syndromes. Germline mutations in APC gene provide aFAP, bacilelac (or heterozygous in some populations) mutations in MYH gene cause MAP. We investigate APC and MYH genes among 25 patients with adenomatous polyposis (4-95). Heterozygous missense mutations in MYH gene were analyzed in control group including 106 healthy probands. As a result 2 mutations p.I1307K in APC and 4 missense variants in MYH (p.G169D, p.G382D and p.D382D) genes were found. All 6 patients with mutations had more than 20 polyps (p<0.0026). Only 1 missense variant (p.G169D) in MYH gene was found among 106 healthy probands (p=0.016). Frequency of germline mutations in APC is 8% (2/25) and 16% (4/25) in MYH. The value of the heterozygous mutations for MYH was demonstrated for the first time among Russian patients.

**J11.011**

**Expression of genes for drug resistance and metabolism in muscle-invasive bladder tumors**

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**Background:**

The primary endpoint of this study was evaluation of the expression of genes related to the resistance to the most common anticancer drugs in muscle invasive bladder carcinomas.

**Materials & Methods:**

- Gene expression analysis of the 168 genes from two panels for Cancer drug resistance and metabolism (PAH004) and Cancer Drug Targets (PAHSS07C) was performed. A total of 47 transitorial cell bladder cancer samples of stage pTa, pT1, pT2, pT2b and lymphoepitheloma-like pT2a were investigated.
- Gene expression analyses of the individual samples as well as the pool samples (pTa, pT1 and pT2) in comparison to negative and positive (non-malignant lesions) controls were carried out.

**Results:**

The pool analysis revealed statistically significant difference (p<0.0001) in the expression level between muscle invasive and non-invasive bladder tumors. More than 10 times higher expression is observed for AKT1, AURK1, CA6, EGFR, ErbB2, ErbB4, HADC and MDM2 genes which are targets for current and trial anticancer drugs. More than 5 times up-regulation was established for ABCG2, ABCB1, ARNT, CYP1A1, CYP3A5, EPHX1, MVP, PPARα and TNN genes. These are involved in the multi-drug resistance and metabolism of cyclosporine, steroid hormones, PAHs as well as anticancer drugs Vincristine, Thiopurine and Taxol.

In the fourteen tested individual samples we found an up-regulation for AHR, AR, CEN1, CLPTM1L, CYP1A1, CYP3A5, MVP and TOP2B genes. In the 78.5% from them the expression of the PPARγ gene, which activity is influenced by retinoid, was elevated.

**Acknowledgements:**

Contribution of O. S. and R. G. to the J11.011 project was supported by RBF 13-06-00101. RBF 13-06-00101.

**J12.012**

**Matrix Metalloproteinase and Tissue inhibitor of metalloproteinase gene polymorphisms involved in bladder cancer development**

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The aim was to investigate MMP1 (rs494379, -519 A>G), MMP9 (rs3918242, -1562C>T; rs17576, 2660A>G), MMP12 (rs2276109, -82 A>G), MMP2 (rs2285053, -735C>T), TIMP3 (rs 9619311, -1267T>C) polymorphisms and bladder cancer susceptibility in population of The Republic Bashkortostan (Russian Federation). A total of 307 bladder cancer patients and 271 controls were genotyped by PCR-RFLP. It was found 2660A>G SNP as association with bladder cancer development in the dominant model (p=0.044, OR=1.45, 95% CI (1.01-2.07) and MMP9 2660C>G haplotype significantly more common in patients (3.52% vs. 21.11%, OR=1.90, 95% CI (1.22-2.97)) compared with control. It was defined MMP2 -735C>T significant association with non-invasive forms (OR = 2.17) Analysis of gene - environment interactions showed a statistically significant interaction between MMP2 and MMP9 polymorphisms with the status and smoking index (p=0.014 and p=0.017, respectively) in dominant model. Thus we can assume MMP9, MMP2, TIMP3 polymorphisms under investigation make a definite contribution to the bladder cancer development.

The investigation was partially supported by RFB 14-04-97006, 1-poivosva.a, 14-06-97003 r_poivosva.a, 13-04-00287 A; RFH 13-06-00101.

**J12.013**

**Bladder cancer risk associated with XPD polymorphism in the Belarusian population**

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Bladder cancer (BC) is the fourth most common cancer among men in Europe, and the annual incidence rate is 1000 in Belarus. Among risk factors, cigarette smoking and occupational chemical exposure lead to accumulation of DNA damage contributing to carcinogenesis. In this context, polymorphism of error-free excision repair genes seems to affect the cancer risk. Polymorphism of XPD Asp312Asn, XRCCL Arg399Gln, OGG1 Ser326Cys, ERCC6 Met1097Val was studied in the group of BC patients (336 individuals) as compared to the control group (370 volunteers). The allelic variants were determined in DNA samples using the PCR-RFLP method. Minor allele frequencies in Belarus were close to those in Caucasians and significantly differed from their frequencies in Asians. Analysis of polymorphisms in a single gene revealed increased BC risk in carriers of heterozygous genotype Asp/Asn of XPD gene that was pronounced in the elderly (OR95%CI=3.34 [1.35-8.28] p=0.009). The combination of this genotype with homozygous genotypes Ser/Ser or OGG1 Met/Met of ERCC6 also increased cancer risk. Analysis of interaction between four genes showed that carriers of combinations Asp/Asn, Arg/Arg, Ser/Ser, Met/Val and Asn/Asn, Arg/Arg, Cys/Cys, Met/Val possessed more predisposition to cancer (OR95%CI=3.20 [1.47-6.95] p=0.003 and OR95%CI=9.97; p=0.1 respectively). Thus, XPD312 polymorphism was predominantly associated with BC risk in Belarus.

**J12.014**

**Cell free DNA quantification in urine of patients with bladder urothelial carcinoma**

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Aim of the study Cell free DNA (cfDNA) represents one of the new bladder cancer markers. Comparision of total urine cfDNA values of the control group and of the patient one was the aim of our study. Patient urine samples were collected from the Department of Urology, University Hospital Motol Prague.

In the fourteen tested individual samples we found an up-regulation for AHR, AR, CEN1, CLPTM1L, CYP1A1, CYP3A5, MVP and TOP2B genes. In the 78.5% from them the expression of the PPARγ gene, which activity is influenced by retinoid, was elevated.

**Acknowledgements:**

Contribution of O. S. and R. G. to the J11.011 project was supported by RBF 13-06-00101. RBF 13-06-00101.
J12.015

BRAF Gene Polymorphisms and chromosomal aberrations as a risk of borderline ovarian carcinoma patients in Tamil Nadu population, South India

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Ovarian cancer is the leading cause of death due to gynaecological malignancies among women. The aim of the study was to analyse the BRAF gene polymorphisms and major chromosomal aberration in the borderline ovarian cancer (BOC) patients. A population-based, case control study of ovarian cancer was performed in Tamil Nadu. Inferred consent forms were obtained from 34 BOC patients individually (Case & Control). We have examined for BRAF, p53, Q56R amino acid changing polymorphisms. Cytogenetic analyses were carried out using 3 ml of blood (100 metaphase). The major chromosomal aberrations were gains from chromosome arms and deletions of 1p, 12q, 14q, 15q, 16p, 17p, 17q, 19p and 19q sites in BOC patients. The correlation between family history, age, infertility, and diet - body weight were selectively evaluated and analysed. The most significant chromosomal abnormality in BOC patients compared to the control subjects. Findings reveal that the patients above 60 years of age were at high risk. Thus the study concludes that the BRAF1 gene polymorphisms and chromosomal aberrations play a key genetic role along with the risk factors for BOC patients.

J12.016

The role of BRAF V600E mutation as a potential marker for prognostic stratification of papillary thyroid carcinoma: A long-term follow-up study

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Papillary carcinoma is the most prevalent malignancy of thyroid gland, and its incidence has been recently increased. The BRAFV600E mutation is the most frequent genetic alteration in papillary thyroid carcinoma (PTC). The role of BRAFV600E mutation as a potential prognostic factor has been controverted in different studies, with short-term follow-up. In this study we evaluated the role of BRAFV600E mutation as a potential marker for prognostic stratification of patients with papillary thyroid carcinoma in long-term follow-up. We studied 69 PTC patients with a mean follow-up period of 45.9 months (median: 60 mo). The BRAFV600E mutation was analyzed with associated age and advanced tumor stage but was not correlated with incomplete response during follow up.

J12.017

Study of genes BRCA1 and 2 in clinical cases of breast and/or ovarian cancer in the Molecular Genetics Unit of Ferrara

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Germlinal mutations in two high susceptibility genes BRCA1 and BRCA2 explain around 25% of familial breast cancers. Patients with a probability value above 10% in the risk assessment calculated by different prediction tools (CaGene, BOADICEA and Gaudzick-Tyrel)) or belonging to the profile 3 of Modena’s criteria were selected for the molecular analysis of BRCA1 and 2 genes by direct sequencing and MLPA. Molecular analysis was completed on 80 patients and for both genes we identified 8 pathogenic mutations (frameshift, missense, nonsense) recurrent in unrelated patients or private. According to the literature, in about 10% of the identified variations the pathogenic significance was not known. The mutations were distributed along the entire gene sequence, therefore the search for specific mutations hot spot is not adoptable as a procedure for the implementation of genetic testing. To date, the Breast Cancer Information Core Database (BIC) contains more than 3000 different sequence variations in BRCA1 and BRCA2 genes. We have found the same mutation in different patients associated to the occurrence of different cancer at a different age, therefore to date it is not possible to define a specific genotype-phenotype correlation. The data obtained in our laboratory so far suggest that the genetic risk of breast cancer could be caused by rare variants and being able to distinguish as deleterious or neutral the large number of new variations with unknown pathogenic significance is a difficult task. Early identification of individuals at risk allow to implement protocols and periodic clinical surveillance for certain risk profiles.

J12.018

Analysis of a novel BRCA1 splicing mutation in hereditary breast and ovarian cancer woman

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Hereditary breast and ovarian cancer (HBOC) is the commonest malignancy in women. HBOC is a familial disease accounting for about 10% of all breast cancers, BRCA1 and BRCA2 being the most prevalent genes involved in this pathology [1]. They play a role in the tumor suppressor pathway, particularly in the homologous recombination (HR) pathway for double-strand DNA repair (2). BRCA1/BRCA2 germline mutations are related to an increased risk of developing HBOCs, therefore the carrier identification is crucial especially before the onset of the disease. Furthermore, testing for BRCA gene mutations allow to improve the clinical management of high-risk patients and of their mutation carriers family members. However, the correct interpretation of variants with uncertain significance or of novel variants still remains a problem for genetic counseling. Here we describe a novel intronic variant identified in a HBOC patient and involved in the BRCA1 gene splicing regulation. This variant is predicted to be deleterious according to Human Splice Finder and NetGene2, causing the loss of a canonic donor splice site at position +2 in the intron 21 of BRCA1 gene. Variant effects were experimentally verified on patient CDNA by PCR amplifications using different primers pairs ad hoc designed. Our results indicate that intron 21 is completely retained in the transcript RNA. Despite other studies are needed to confirm the role of this splice variant, our preliminary results strongly support the pathogenicity of the novel found mutation.


J12.019

Polymorphisms in the FGFR2 and miR146 genes are associated to increased risk in familial breast cancer in young women

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Breast cancer is the most common cancer diagnosed in women worldwide. Only 5-10% of all breast cancer occur in women under 40 On the other hand, of all breast cancer have genetic predisposition and the number of women in the general population with breast cancer attributable to mutations in high penetrance genes BRCA1 And BRCA2 is very low. These observations have led to the proposal that breast cancer susceptibility is largely polygenic and recent GWAS studies have identified some regions in the genome which contains SNPs that can involved risk of developing breast cancer including FGFR2 (rs8211582) and miR146 (rs2910164). Moreover, several SNPs at the BRCA1 gene such as c.2731C>T (rs7999917), c.3232A>G (rs16941), c.3667A>G (rs16942), could modify the risk. We studied 124 women under 40 with breast cancer divided in two main groups: patients who carried pathogenic mutation in BRCA1/2 genes (BRCA + n=22) and noncarriers (BRCA = n=102). DNA was extracted from peripheral blood and allelic discrimination was performed using TaqMan SNP Genotyping Assay. We found a significant association between genotype TT in the FGFR2 polymorphism and BRCA1 group (OR=0.133, CI95%=0.026-0.667, p=0.024 ). Furthermore, we found that the G allele in the miR146 polymorphism is related with
Inclusion of 5382insC mutation test in screening programs for breast cancer

The present study aimed to investigate the frequency of five common deleterious mutations (5382insC in BRCA1) with frequency 1.2%; two polymorphic variants in BRCA2 gene - rs4987117 in exon11N and rs9534262 in exon17 with frequency 2.7% and 36% of the patients, respectively. None LGR was detected.

In conclusion, the insufficient results of our study suggest that the strategy for genetic screening in Bulgarian patients requires complete analysis of the BRCA1/2 genes and stringent compliance to the internationally accepted BGC/NCN criteria.

Mutation Analysis of ESM-1 Gene in Breast Cancer Patient Tissues

Breast cancer is the second common type of cancer in the world. In developed countries, breast cancer is one of the leading causes of women deaths. In recent years, many genes have been identified related to breast cancer. But underlying molecular mechanisms have not been clarified yet. Endocrine, ESM-1: endothelial cell-specific molecule-1) soluble proteoglycans, is secreted by endothelial cells. Expression of ESM-1 gene is regulated by the cytokines. The expression increases with the stimuli of VEGF, TGNF-α, LPS and IL-1β in endothelial and epithelial cells. The ESM-1 gene function has an important role in tumor angiogenesis and growth. It has been shown to be involved in pathogenesis of lung, liver, gastric and breast cancer. ESM-1 gene is reported to have an effective role in cell proliferation, cell migration, invasion, and metastasis, and is involved in the development of breast cancer. Expression of ESM-1 gene has been evaluated in breast cancer tissue samples.

In this study, we analyzed ESM-1 gene mutation in breast cancer from paraffin-embedded tissue samples of breast cancer patients. All three exons for ESM-1 gene were sequenced by Sanger Sequencing. Our results showed a homozygous silent mutation (CA>GAA) in the second exon at codon 118. Another case displayed the same mutation heterozygously. In nine cases, the polymorphism TTG>CTG was evaluated as being significant. These results suggest that ESM-1 gene may play a role in the progression of breast cancer.

Association of estrogen receptor-α A908G (K303R) mutation with breast cancer risk

The association between estrogen receptor-α (ER-α) A908G (Lys303→Arg) polymorphism and breast cancer risk was evaluated in a case-control study. The study included 175 breast cancer patients and 200 matched control individuals. The results showed a significant association between the A908G mutation and an increased risk of breast cancer. The odds ratio for the A908G mutation was 1.5 (95% CI: 1.1-2.0). The study suggests that the A908G mutation may be a risk factor for breast cancer.

Preliminary study of BRCA1/BRCA2 mutations in Bulgarian women with breast cancer

The study aimed to investigate the frequency of common deleterious mutations (5382insC in BRCA1) in a group of Bulgarian women with breast cancer. The results showed a frequency of 1.2% for the 5382insC mutation in BRCA1. This study provides valuable information for genetic counseling and screening programs in Bulgaria.
J12.025
The association between G-2548A polymorphism of leptin gene and breast cancer risk
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Introduction: The breast cancer is the most common cancer in woman and the second cause of death in women between 35 to 55 years. Risk creation the breast cancer is in time life woman 12.5% (one of the eight cases) and risk of death from breast cancer 3/6% (one case of twenty eight case). Leptin hormone is secreted from adipocytes which is involved in the regulation of body weight and serum levels are correlated with breast cancer risk. Genetic polymorphisms G-2548A promoter region of leptin gene expression and hormone secretion rates of fatty tissue will be affected. The purpose of this study is to investigate the relationship between G-2548A polymorphism in the leptin gene and breast cancer risk.

Material and methods: We here carried out a case-control study that included 50 patients and 50 healthy subjects. We examined the genotype distribution of the leptin promoter G-2548A polymorphism, using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) approach, to investigate the possible role of this SNP as a risk factor in breast cancer.

Results: Statistical analysis showed that the AG genotype frequencies in the patient group 26% and in the control group 48%,AG genotype the region -2548A , a SNP that has a protective effect on breast cancer risk and this genotype than other genotypes reduces the breast cancer risk (OR:0.94; 95%CI: 0.16-0.97; P: 0.04). There is a significant association between G-2548A polymorphism of leptin gene and breast cancer risk. This polymorphism can be used as a diagnosis marker for breast cancer.

J12.026
Evaluation of immunostaining by hormone receptors and the receptor HER-2 in breast cancer
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Breast cancer is the most common cancer in women worldwide. The evaluation of the expression of estrogen receptors and progesterone and HER-2 has become essential for the treatment of patients bearing breast cancer since it provides information of prognostic and therapeutic order. The purpose of this study was to evaluate the expression of hormone receptors and Her-2 protein in breast cancer, the study correlates the results between them and with other prognostic factors, compare our results with those in the literature and evaluate our immunohistochemical technique. This is a prospective study of 130 cases of breast cancer diagnosed at the Laboratory of Pathology of the University Military Hospital and Regional Oran (HMIUR) since the year 2006 until 2009. The evaluation of the expression of hormone receptors and HER-2 by immunohistochemistry was performed. ERs are expressed in 64% of cases, PR in 71% of cases and HER2 in 31% of cases. The investigation of 14 microRNA (miR-1, miR-10b, miR-21, miR-31, miR-155, miR-29a, miR-195, miR-192, miR-182, miR-125b, miR-145, miR-221, miR-222, miR-34a, miR-335) expression levels in a sample of breast cancer tumors in relation to the normal breast tissue was performed. All patients of the sample were not subject to radiation, neither to chemotherapy. The expression level of miR-182 most often was increased - in 81% of cases. In 59% of cases there was over expression of miR-182 - more than 10 times. The expression of miR-31, potential metastasis suppressor, was decreased in 54% of cases. There was identified cluster co-expression 5 microRNA: with increased expression miR-10b, miR-21, miR-155, miR-34a and miR-335. The cluster includes 33% of studied tumors. Frequency overexpression of these RNAs among tumors in the cluster was 4 times higher compared to other tumors (p < 0.00001). The transcriptional regulation analysis shows that 4 microRNAs of cluster are transcriptional targets of p53 and RelA-NF-kB. All the tumors in the cluster were characterized by infiltrating lobular and mixed variants (infiltrating lobular combined with infiltrating ductal) breast cancer and were positive in the expression of estrogen and progesterone receptors. The cluster includes 60% of all tumors with local metastases in lymph nodes (i.e., metastases occur approximately 3 times more often than in the other tumors of the sample). It allows assuming existence of the association co-expressed in this cluster microRNA with pathomorphological features of the tumors, in particular, with a predominant presence of metastases.
not observed among SBC patients. In conclusion the TOP2A gene amplification occurs both in FBCs and SBCs but more frequently among FBCs independent of Her-2/neu amplification. This study highlights the importance of TOP2A gene amplification in diagnosis of FBC. In addition, a combined approach using immunohistoechemical analysis and FISH can optimize Her-2/neu testing for breast cancer patients.

J12.030
Burkitt lymphoma: Unfamiliar presentation of a familiar disease
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Recently, Hematology has been witness to great strides in genetic advance-ment. lymphoma is now regarded as heterogeneous genetic disease. The genetic aberrations not only aid diagnosis, but also dictate choice of therapy, therapeutic response and portend prognosis. This is such a case exemplifying application of cytogenetics. A 4 year frail child presented with abdominal pain and distension of 15 days duration. Ultrasonography and Magnetic Resonance Imaging revealed bilateral renal mass. Provisional clinical diagnosis of Bilateral Nephromatosis / Neuroblastoma was offered. Preliminary guided Fine Needle Aspiration showed monomorphic population of vacuolated lymphoid cells suggestive of malignant lymphoma - Burkitt type. Peripheral smear and bone marrow study on aspirate material revealed 46,XY(2.8), add(11)(q24) confirming cytogenetic diagnosis. The patient succumbed to organ failure with short hospital stay of 20 days. We present our case of Burkitt lymphoma with unusual presentation, variant translocation and poor performance. Documentation of such cases can direct complete new prognostic factors, advent of new therapeutic regimens in the future.

J12.031
Expression analysis of four cancer-testis antigens in breast cancer tissues and cell lines
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Introduction: Testis-specific genes which has normal expression in restrict- ted tissues, contains a subgroup demonstrating expression in various malignancies known as Cancer-Testis Antigen (CTAs). Considering testis as an immune privileged organ, aberrant expression in cancers could cause spontaneous humoral and cell-mediated immune responses. Breast cancer is a promising target for vaccination and immunotherapy using CTAs. Methods and material: We analyzed the expression of 4 CTAs, PEPP-2, ODF4, ACRBP and SPATA19 in 40 samples of invasive ductal carcinoma (IDC), adjacent normal tissue and 10 fibroadenomas, as well as MCF-7 and MDA-MB-231 cell lines using RT & Real Time KT-PCR. Results: ACRBP was expressed in normal tissue and SPATA19 expression was not significantly different in normal and cancer tissues. ODF4 and PEPP-2 were expressed in 62.5% and 22.5% of IDC samples respectively. Real Time results showed increased expression of ODF4 and PEPP-2, 2.96 (p<0.001) and 3.31 (p<0.01) times in IDC samples, respectively in comparison with testis tissue. Both genes were up-regulated in MCF-7 and MDA-MB-231 cell lines compared with normal testis sample. Comparing the expression of ODF4 & PEPP-2 in MDA-231 with MCF-7 1.75 & 1.77 (p<0.001) times up-regulated in MDA-MB-231, respectively. Conclusion: ODF4 and PEPP-2 could be potential cancer biomarkers. Therefore, they can be used for active immunotherapeutic interventions. Expression of ODF4 and PEPP-2 in malignant tissues and their absence in benign and normal tissues make them putative cancer biomarkers. We showed ACRBP expression in normal breast tissue, so its application as cancer biomarker or in immunotherapeutic approaches is under question.

J12.032
CAT C262T, GSTM1, GSTT1 and CML risk
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Background: Polymorphisms of oxidative stress related genes enzymes are known to influence the metabolism of different carcinogens and have been associated with incidence and progression of various types of cancer. Material and meth-od: The aim of this study was to evaluate the influence of catalase (CAT) and glutathione S-transferase (GSTM1/GSTT1) as risk factors for Chronic Myelogenous Leukemia (CML) development. We performed a case-control study which included 75 CML patients and 150 healthy volunteers, with no history of malignancy. The genetic polymorphism of CAT C262T was assessed by RFLP-PCR while for GSTM1 and GSTT1 Multiplex PCR assay was used, both followed by gel electrophoresis. Results: For CAT gene polymorphism the molecular analysis identified homozygous mutant allele TT in 13.33% of CML patients and 5.34% in the control group (p = 0.3072), while heterozygo-us CT was found in 21.33% of study population and in 46.66% control cases (p = 0.0008). The prevalence of GSTM1 null genotype in the patients group was 60% and in the control group 53.33% (p = 0.3939). GSTT1 null genotype frequency in CML patients was 17.33% while in the control population it was 16% (p = 0.8494). We found no correlation between GSTM1 and GSTT1 null genotypes in CML development. Our results show that CAT C262T variant genotype is significantly associated with CML risk (p = 0.016, OR = 2.94; 95% CI 1.15-3.62). Conclusion: These preliminary results show that CAT C262T genetic polymorphism might be related with CML development. Acknowledgement: This work was supported by Research Grant number 19/11.12.2013 from University of Medicine and Pharmacy Turgır Mures, Romania.

J12.033
Expression analysis of CD44 isoforms in Esophageal Squamous Cell Carcinoma patients
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Background: CD44 is a member of the cell adhesion molecules family. In normal cells, CD44S, along with CD44V3 and CD44V6 play roles in cell motil-ity, migration, and adhesion, while in tumor cells they are involved in tumor invasion, progression, and metastasis. The aim of this study was to evaluate the expression of CD44S, V3 and V6 in esophageal squamous cell carcino-ma (ESCC) and their correlations with clinicopathological features of the patients.

Methods: The expression of CD44S, V3 and V6 was compared in tumoral and distant tumor-free tissues of the esophagus in fifty ESCC patients using comparative real-time PCR.

Results: A significant overexpression of CD44S, V3 and V6 mRNA was ob-served in 13, 11 and 9 tumor specimens, respectively. The co-expression of the genes (S/V3) were significantly associated with grade of tumor differen-tiation, stage of tumor progression, and depth of tumor invasion and (S/V6) with grade of tumor differentiation and (V3/V6) with grade of tumor differ-entiation, stage of tumor progression and depth of tumor invasion.

Conclusion: Our finding suggested that the reduced expression of CD44 v6 is associated with invasion and stage of tumor and the level of CD44V3 mRNA expression was associated with tumor invasion. In addition, Simulta-neous expression of these genes has an important role in tumor prognosis. Investigating the potential role of alternative splicing in cancer progression may therefore lead to the development of novel therapeutic interventions.

J12.034
Is the chromosome Y loss in oncohematological male patients a disease-related marker or an age-associated phenomenon?
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Loss of the Y-chromosome (Y) is observed in peripheral blood of healthy el-derly men, however it may be found also in bone marrow cells of males with different subtypes of hematological malignancies. Therefore it is unclear whether Y-loss occurs as normal age-related event or it is a marker of neoplastic clone. The aim of our study was to review molecular cytogenetic findings in bone marrow cells of patients with oncohematological diseases and to assess the role of the Y-loss during malignant process.

We found loss of the Y-chromosome in bone marrow samples of 142 males (median age 72 years, range 21-89 years) with myeloid or lymphoid disor-der. The most frequent diagnosis was myelodysplastic syndromes and acute myeloid leukemia (54 patients). All samples were analyzed by conventional G-banding and by interphase FISH. Cut-off level for FISH was established at 10% and the extent of cell clones with Y-loss varied from 12 to 90%. Most of the patients (131) showed a clone with Y at the time of diagnosis. In 114 cases Y-loss was the sole abnormality and in 17 patients it was associated with autosomal structural and/or numerical aberrations. In 11 patients Y clone was detected during the progression of the disease. As quoted in the literature (Van Dyk, 2001) the presence of >75% cells with Y-loss is associated with the malignant disease. In our cohort, this was found in 25 patients (18%) and therefore such findings may be considered as related to malignity.

Supported by MKCR 00023736, RVO-VFN461,645, GACR P302/12/G175/1. Back to index
J12.035
Polymorphism of XRC1, XRC3 and XPD genes and risk of chronic myeloid leukemia in a Romanian population
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The genetic polymorphisms of X-ray repair cross-complementing group 1 (XRC1), X-ray repair cross-complementing group 3 (XRC3) and xeroderma pigmentosum complementation group D (XPD) repair genes may lead to gene expression instability and leukemogenesis. The purpose of the study was to evaluate the association between XRC1 gene Arg399Gln, Arg280His and Arg94Trp, XRC3 Thr241Met and XPD Lys751Gln repair gene polymorphisms and the risk of development of CML in Romanian patients. A total of 156 patients aged 20 to 78 years diagnosed with CML (mean age 51.5 ± 1.1 years) and 190 control individuals (mean age 49.8 ± 2.1 years) were included in this study. The XRC1, XRC3 and XPD genotypes were determined by the polymerase chain reaction-restriction fragment length polymorphism assay. We found no association between chronic myeloid leukemia and XRC1 and XRC3 variant in any of investigated cases. A significant differences were observed in the variant genotype frequencies of the XPD Lys751Gln polymorphism between the with CML and control group (for variant homozygous genotypes, OR = 2.37; 95% CI = 1.20-4.67; p-value = 0.016) and for combined heterozygous and variant homozygous genotypes, OR = 1.72; 95% CI = 1.10-2.69; p-value = 0.019). This was also observed when analyzing the variant 751Gln allele (OR = 1.54; 95% CI = 1.13-2.31; p-value = 0.008). Our result suggest that the XPD Lys751Gln variant genotype increases the risk of chronic myeloid leukemia.

Acknowledgement: This work was supported by internal Research Grants of the University of Medicine and Pharmacy Tirgu Mures, Romania. Project No. 19/11.12.2013

J12.036
Investigation of the role of VHL and SETD2 gene mutations in human clear cell renal cell carcinoma development in patients from Russia
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Renal cell carcinoma is the most common neoplasm affecting the adult kidney. One of the most frequent events in clear cell renal cell carcinoma (cc-RCC) is inactivation of Von Hippel-Lindau gene (VHL). A series of studies recently demonstrated that a number histone modifying and chromatin remodeling genes, including SETD2, have mutation in ccRCC. Furthermore, SETD2 gene is within a 50-Mb region on chromosome 3 that encompasses VHL and is deleted in ~90% of ccRCC.

The goal of the study was to investigate inactivation of VHL and SETD2 genes by mutations. We studied 105 DNA samples of tumor tissues and normal renal parenchyma in ccRCC patients from Bashkortostan Republic of Russia, using PCR, SSCP and direct sequencing. Mutations in VHL gene were found in tumor tissues with the frequency of 21.9% (23/105). We detected 22 mutations in 23 ccRCC patients, including 8 point mutations (35%), 13 deletions (60%) and 1 insertion (5%). Ten somatic mutations hadn’t been described in literature previously. VHL inactivation through sequence alterations in tumor DNA didn’t differ by histopathologic characteristics or occupational exposure. Analysis of SETD2 gene was performed in 50 ccRCCs DNA samples and matched normal tissues. According to literature, mutations in SETD2 gene are detected in 4-15% cases of ccRCC. In our research SETD2 gene mutations were not found. This may be due to an insufficient number of samples or the lack of specific alterations.

The aim of the study was to evaluate the contribution of inversion 751Gln allele (OR = 1.54; 95% CI = 1.13-2.31; p-value = 0.008). Our result suggest that the XPD Lys751Gln variant genotype increases the risk of chronic myeloid leukemia.

Here we reported a 43 years old female patient with Ph chromosome and additive chromosomal abnormalities which has been diagnosed in CML. In the analyses performed using BCR-ABL1 FISH probe into the bone marrow interphase cells, %12 of the cells were normal, %20 had classical fusion, in %60-70% of cells increased ABL signals were present giving rise to complex rearrangements. In the molecular genetic analysis the BCR-ABL1 was positive (%50.75924).

The karyotype of bone marrow cells was 46,XX,[add(1)[q24]]inv(18),add(21)[q2]Ph(+).C and G-banded metaphases of peripheral blood had 16qh+ and inv(18), however add(21)[q22]Ph(+) were observed in the bone marrow cultures were not encountered. The patient had 7 pregnancies but she had only 2 healthy children and 1 child also had 16qh+ and inv(18). It was concluded that only Ph+ and add(21)[q22] was present in the leukemic clones of the patient and that 16qh+ and inv(18) was inherited.

As a result, it was concluded that evaluation of the contribution of inversions identified in hematologic malignancies to unbalanced karyotypes and carcinogenesis should be made wisely.

BACKGROUND: Chronic myeloid leukemia (CML) is a myeloproliferative disease. The cytogenetic hallmark of CML is Philadelphia chromosome (Ph). The aim of this study was to diagnose assumed CML patients, to monitor CML patients under imatinib therapy on the bone marrow and peripheral blood samples by cytogenetic and fluorescence in situ hybridization (FISH), and to also compare the results on both specimens. MATERIALS & METHODS: Chromosome banding and FISH analysis was performed on 30 suspected CML patients on the bone marrow (BM) and peripheral blood (PB) specimens. RESULTS: The comparison of FISH and karyotyping in 30 patients on BM and PB specimens, respectively showed that 9 (30%) and 8 (26.66%) of them were Ph+, and only (18.18%) of Ph positive patients showed atypical patterns. In comparison between BM-cytogenetic and PB-1-FISH, BM-cytogenetic was more reliable than PB-1-FISH to detect Ph. CONCLUSION: Our data demonstrate FISH analysis is a rapid, reliable and sensitive technique. Our study in comparison between BM and PB, showed BM can't replace by PB, even in detecting by FISH.

J12.039
Translocation t(7;11)(p15;p15) in a patient with chronic myelogenous leukemia (CML) Ph-positive
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Translocation t(7;11)(p15;p15) is observed in 2% de novo AML and, much rarely, in Ph-positive CML in blastic phase (BP). The translocation results in a fusion between NUP98 and HOXA9 with a pathogenic role in leukemogenesis. A 49-year old caucasian male was diagnosed with CML-BP. He came to our attention for a marked leukocytosis (190 x 10³/μl) and anemia (Hb 7.6 g/dl), with a normal platelet count and mild splenomegaly. Conventional cytogenetic and fluorescent in situ hybridization (FISH) analyses detected a t(9;22)[q34;q11] combined with a translocation t(7;11)[p15;p15], and BCR-ABL1 together with NUP98-HOXA9 rearrangements, respectively. The patient was at first treated with hydroxyurea briefly followed by high dose imatinib (400-600 mg daily). Then, based on the absence of complete response (CR), sequential therapy with dasatinib (140mg daily) was administered which resulted in primary resistance. Sequencing analysis of ABL-KD domains revealed mutation in E255K, within the P-loop site. Two months later a new induction protocol (AML1310) was administered without response. The patient is alive in relapse six months from diagnosis. To date, only seven CML-BP Ph positive cases have been reported associated with translocation t(7;11) and the present case is the first likely subsequent to both t(7;11) and E255K, which shows a moderate resistance to dasatinib, combined with the presence of t(7;11) as an additional chromosomal abnormality. Furthermore, unsuccessful chemothero therapy still represent a possible effect of t(7;11) / NUP98-HOXA9 fusion gene which is known to be associated with...
severe prognosis in AML cases inhibiting hematopoietic precursor differen-
tiation and increasing self-renewal of hematopoietic stem or progenitor
cells.

J12.040 Study of C-MYC amplification and expression in gastric cancer samples using CISH and IHC methods
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Cancer is a leading cause of death worldwide and accounted for 7.6 milli-
on deaths (around 13% of all deaths). Gastric cancer is the fourth and fifth
most common cancer in men and women, respectively. The northern and
western regions of Iran are high risk areas for gastric cancer.
Gastric cancer carcinogenesis refers to accumulation of genetic alteration of
multiple genes such as oncogenes, tumor suppressor and mismatch repair
genes. C-MYC proto-oncogene is one of the most frequently activated onco-
genues, and is estimated to be involved in 20% of all human cancers.
To evaluate MYC copy number and its protein expression, CISH and IHC ana-
lyses were performed in 50 gastric adenocarcinomas among Iranian indi-
viduals. 29 samples showed low amplification, 9 and 12 samples showed
moderately and high amplification, respectively. MYC immuno-reactivity
was observed in 27 samples. In 31 samples either MYC amplification or MYC im-
uno-reactivity were observed. 19 samples showed low amplification and
were negative IHC. Among 31 samples, 9 samples showed low amplificati-
on, 8 and 14 samples were moderately and high amplification, respectively.
Among 31 samples, 9 samples had strong signal, 16 samples moderate
signal, 2 samples poor signal and 4 samples negative signal for IHC. The ma-
jority of patients with IHC negative had low amplification. Therefore,
C-MYC amplification was correlated with the C-MYC protein overexpression.
This study showed that there was amplification in early gastric cancer and
could be used as a therapeutic target. Therefore, this will contribute to early
diagnosis, therapeutic and prognosis of gastric adenocarcinoma.

J12.041 Extended genetic analysis of Swedish patients with suspected hereditary colorectal cancer (The SWEN study)
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Background
The lifetime risk for colorectal cancer (CRC) in the Swedish population is
approximately 5% with a mortality second only to that of lung cancer. It is
estimated that 5% of all CRC develop in individuals with a Mendelian
predispension for the disease, for example Lynch syndrome (LS), familial
adenomatous polyposis (FAP), MUTYH-associated polyposis (MAP), juvenile
polyposis syndrome (JPS), Peutz-Jeghers syndrome (PJS) or HNPCH mar-
tomatomous tumor syndrome (PHTS). However, diagnosis-selective genetic
screening fails to detect a disease-causing mutation in the majority of pati-
ents with suspected hereditary CRC.
Aim
The SWEdish Extended genetic analysis of hereditary colorectal Neoplasia
(SWEN) is a prospective national study involving researchers and clinically
active staff at the cancer genetics clinics in Sweden with the overall aim to
improve genetic testing and the care of patients and families with genetic
susceptibility for CRC.
Material and Methods
Six hundred adult patients with clinically suspected Mendelian CRC of any
type are offered mutation screening of the 11 genes associated with LS, FAP,
MAP, JPS, PJS, CHS and addional selected candidate CRC susceptibility
genes. Genetic and clinical data for each family is registered in a national
database followed by genotype-phenotype correlation studies.
Current Status
The study is ongoing since February 2014 and inclusion of patients will con-
inue for at least three years.
Significance
The results from the SWEN study should improve genetic testing, personalized
risk estimation and effectiveness of surveillance programs for individ-
uals with hereditary predisposition for CRC.

J12.042 Microsatellite instability analysis in sporadic colon cancer patients in Croatia
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Colorectal cancer is a result of accumulation of genetic and epigenetic alter-
ations associated with the transformation of normal colonic epithelium to
colon adenocarcinoma. Two major pathways involved in colorectal carcino-
genesis, the suppressor and the mutator pathway have been identified, with
different clinical behaviors and response to chemotherapy. Approximately
15% of sporadic colorectal carcinomas (CRC) arise from mutator pathway
which is characterized by microsatellite instability (MSI) caused by deficient
mismatch repair system (MMR) and defects in MMR genes.
In this study we have examined the incidence of MSI using the NCI Bethesda
panel of microsatellite markers (Bat-25a, Bat-26, D2S123, D3S1389, D17S250)
in 200 sporadic CRC patients. Analysis was performed using ABI PRISM 310
genetic analyzer. The sample was denoted as MSI high (MSI-H) if two or
more of the markers demonstrated instability. The sample was denoted as
low microsatellite instability (MSI-L) if only in one of the analyzed markers
the MSI was detected.
The MSI was detected in 11/200 (5.5%) analyzed samples and 8 tumors
with MSI-H and 3 tumors with MSI-L were identified, while the remai-
nung tumors showed no instability and were classified as microsatellite
stable (MSS). MSI was detected in the following markers: Bat-25a in 8 pat-
ients, Bat-26 in 7 patients, D2S123 in 10 patients, D5S346 in 6 patients and
D17S250 in 8 patients.
There was no statistically significant correlation between the MSI and clini-
co-pathological characteristics, although MSI was more frequent in larger,
poorly differentiated and advanced stage tumors.

J12.043 Quantitative JAK2V617F mutation and cytogenetic abnormalities in MPNs: Legnano Hospital experience
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The detection of molecular and cytogenetic alterations is important for the
diagnosis, prognosis and classification of myeloproliferative neoplasms
(MPNs). In our institution bone marrow conventional and molecular cyto-
genetic and detection of JAK2V617F in isolated granulocytes from blood sample are
performed at clinical presentation of suspected MPN. Recently, introduction of
JAK2V617F allele burden has been introduced in our diagnostic flow chart.
While the V617F acquired mutation in JAK2 gene has been descri-
based in a high proportion of MPN patients and allele burden (heterozygosity
versus homozygosity) correlates with a higher risk of secondary fibrosis,
there is no specific cytogenetic alteration but some recurrent abnormali-
ities in MPNs. Among 34 JAK2 mutated patients since the introduction of
quantitative JAK2V617F allele measurement, 3 patients has also shown an abnormal
karyotype. One patient (case 1) presented 2 related clones with trisomy of
chromosome 8 and 9, deletion of chromosome 20q has been observed in
case 2 and a t(4:20) translocation involving deletion of TET2 gene has been
observed in the third case. Prognostic significance of cytogenetics findings
at diagnosis and correlation with JAK2V617F allele burden is presented.
A specific discussion of case 9 case will be done due to the difficulty of inter-
pretation of the allele burden.

J12.044 Clinical and genetic features of paediatric acute lymphoblastic leukaemia in Down syndrome in the Nordic countries
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To address clinical and genetic differences between acute lymphoblastic leukaemia in Down syndrome (DS-ALL) and non-DS-ALL and to ascer-
tain prognostic factors in DS-ALL, we reviewed all 128 paediatric DS-ALL diagnosed in the Nordic countries between 1981 and 2010. All had B-cell precursor ALL, comprising 2.7% of such cases. Within the DS-ALL group, platelet counts and the incidence of extramedullary disease were higher in girls. 5-year event-free survival (EFS) and overall survival were significantly poorer for DS-ALL patients with white blood cell (WBC) counts $\geq 50 \times 10^9$L. The DS-ALL and the 4,637 non-DS-ALL patients did not differ as regards sex ratio and WBC counts, but the age distributions varied between the DS and non-DS cases, with age peaks at 2 and 3 years, respectively, and the platelet counts were lower in the DS-ALL group. Abnormal karyotypes were more common in non-DS-ALL, and there was a significant difference in the distribution of modal numbers, with only 2% high hyperdiploid DS-ALL cases. There was no significant difference in 5-year EFS between DS-ALL and non-DS-ALL patients in the recent NOPHO ALL-2000 protocol (0.670 and 0.785, respectively, $P=0.11$). The present study adds further support for increased survival of DS-ALL patients during the last few years.

**J12.045**

Allele-specific real-time PCR detection of EGFR exon 19 and 21 mutations in various clinical non-small cell lung cancer specimens

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The aim of our study was to evaluate the effectiveness of EGFR gene mutation detection in fresh-frozen tissue. NSCLC patients were characterized by tumor cells content and type of fixation [Table]. 62/707 (9%) specimens were positive for EGFR mutations: 32 detected in exon 19, including 4 rare deletions and p.R748K substitution (c.2243G>A); 28 p.L858R (c.2537T>G) mutations, 1 rare variant of p.L858R (c.2572T>del3insAG) and 1 double mutation p.L858M+p.L861Q (c.2572C>A; c.2502T>A) detected in exon 21. There were 7% women in the EGFR+ group. 98% of EGFR+ were adenocarcinoma samples, thus mutation frequency among adenocarcinoma patients was 9.8% [61/617 patients]. Ultra-sensitive PNA-NGP PCR clamp assay and allele-specific PCR CE-IVD test provided high results conformity with 95.3% overall percent agreement and Cohen’s Kappa score of 0.95 (95% CI=0.88-0.92). Both real-time PCR assays provided reliable and robust detection of most common EGFR activating mutations in various clinical NSCLC specimens.

**J12.046**

An investigation of relationships between hypoxia-inducible factor-1a gene polymorphisms and endometrial cancer

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**Background:** Endometrial carcinoma is the most common malignant tumor of the female genital tract and the fourth most common cancer in women after breast, colorectal and lung cancers. Hypoxia-inducible factor-1 (HIF-1) is a key transcription factor that regulates cellular response to hypoxia. HIF-1 plays important roles in the development and progression of cancer through activation of various genes that are involved in crucial aspects of cancer biology, including angiogenesis, energy metabolism, vasomotor function, erythropoiesis, and cell survival. The aim of the present study was to investigate the association between HIF-1 1772 C/T polymorphisms and endometrial cancer.

**Patients and methods:** 75 patients with endometrial carcinoma and 75 patients who underwent hysterectomy for non-tumoral reasons selected for evaluation of HIF-1 1772 C/T polymorphisms by PCR-RFLP and sequencing. **Results:** Our findings showed that the T allele and genotype TT in HIF-1 1772 C/T was significantly associated with endometrial cancer risk in comparison with control group. **Conclusions:** Our results suggest that the C1772T polymorphism of the HIF-1a may be involved in development and progression of endometrial carcinoma.

**J12.047**

The EMT gene expression signature and somatic mutations in colorectal cancer with peritoneal carcinomatosis


Colorectal cancer (CRC) often metastasizes even at early stages that is the main cause of death. Epithelial-mesenchymal transition (EMT) is a complex process that is required for dissemination of tumor cells. EMT leads to the transformation of epithelial cells phenotype to mesenchymal phenotype. EMT is associated with mesenchymal key transcription factor acting as a proliferation stimulus and involved in the suppression of cell cycle inhibitors such as p27 and p21. HES1 mRNA expression in fresh tumoral tissues from 50 esophageal squamous cell carcinoma (ESCC) samples was compared to their margin microsatellite stability (17 MSS and 3 MSI-L tumors) and low grade. We observed a high concordance between tumor and carcinomatosis node for mutations and MSI status, but not for EMT. We suppose the tumors eventually may lose mesenchymal phenotype (mesenchymal-epithelial transition) or intratumoral heterogeneity exits. Our data shows that the most of CRC with peritoneal carcinomatosis undergo EMT progress process.

**J12.048**

Role of Dido1 in the progression and development of esophageal squamous cell carcinoma

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**Background:** Bone morphogenetic proteins (BMPs) are implicated in several processes during embryonic development and adult tissues homeostasis. Many cancers are linked to either the BMPs or the molecules functioning in this signaling pathway. Dido1 is a novel BMP-specific Smad-regulated target gene, which is studied in few cancers. However, its expression level has not been yet elucidated in esophageal squamous cell carcinoma (ESCC). To determine this level and its probable clinicopathological consequences, expression of the gene was analyzed.

**Methods:** Dido1 expression in fresh tumoral and distant tumor-free tissues from 50 esophageal squamous cell carcinoma samples was compared by real-time polymerase chain reaction (PCR).

**Results:** Dido1 mRNA expression level was overexpressed in 26% of tumors, while its underexpression was detected in 18% of ESCC samples. There was a significant correlation between level of Dido1 mRNA expression and increased depth of tumor invasion (P = 0.043). Furthermore, Dido1 mRNA expression was correlated with the progressed stage of tumor cells in ESCC samples (P = 0.04). Dido1 mRNA expression was inversely correlated with the age of patients (P = 0.05).

**Conclusions:** These results indicate a relationship between Dido1 expression and depth of tumor invasion and staging. Along with the promising evidences of its role in regulation of BMP signaling pathway which is one of the main involved pathways in tumorigenesis, Dido1 may have a role in progression and invasiveness of ESCC.

**Keywords:** Esophageal Squamous Cell Carcinoma (ESCC); Dido1 gene; Expression analysis, Real-time PCR; BMP signaling pathway

**J12.049**

Impact of HES1 on depth of tumor invasion in esophageal squamous cell carcinoma

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Notch signaling is one of the main involved pathways in cell differentiation and organogenesis and its deregulation may lead to tumorigenesis. In this pathway, targeted to the CSL (CBF1, Suppressor of Hairless or Lag-1) complex, notch intracellular domain (NICD) releases coressporers and recruits MAML1 as coactivator triggering the activation of notch signaling transcription complex. HES1 (Hairless split of-1) is one of the notch signaling target genes which is a bHLH transcription factor acting as a proliferation stimulus through the suppression of cell cycle inhibitors such as p27 and p21. HES1 mRNA expression in fresh tumoral tissues from 50 esophageal squamous cell carcinoma (ESCC) samples was compared to their margin normal by real-time polymerase chain reaction (RT-PCR). Thirteen out of 50 cases (26%) had HES1 underexpression while HES1 overexpression was observed only in 4 (8%) samples. HES1 underexpression was significantly
correlated with tumor depth of invasion (P = 0.02). Although we have not observed any significant correlation between the HES1 expression and notch activation in ESCC, this study is the first report that elucidated the HES1 underexpression in ESCC and revealed its correlation with indices of poor prognosis.

J12.050
Clonal genomic changes in progression of multiple myeloma to extramedullary relapse and plasmocellular leukemia - case report
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Extramedullary relapse (EM) of multiple myeloma (MM) is defined as infiltration of plasma cells (PC) outside of the bone marrow. EM is an aggressive form of the disease with a adverse outcome.

We present a case study of a 52-year-old female diagnosed with MM in 2008. Initial cytogenetic analysis (G-banding) proved hypotripliod karyotype (63-64 chromosomes) with structural and numerical abnormalities. Using cIL-FISH, we detected disruption of IGH gene, t(14;14), gain 1q21 and trisomy of chromosomes 9 and 15. In 2011, patient relapsed twice and progressed in 2011. Peripheral blood was infiltrated by abnormal PCs and an extramedul- lary lesion in the head was formed. At the time of progression, the same chromosomal abnormalities were present in the bone marrow, peripheral blood and the EM lesion: deletion of R81 and TP53, IGH rearrangement, t(14;14), gain 1q21 and non-hypotripliody. Array-CGH showed that genome profiles before and after progression were different - during disease progression, hypotripliod karyotype turned into non-hypotripliod karyotype together with loss of trisomies of chromosomes 2,3,7,8,9,11,17,18,19,20 and gain of new abnormalities - deletion of 1p, 2p, 9q, 11p, 12p, 13, 1q, 17p and 22p.

Similarly, sequencing of TP53 showed different mutations before and after progression. We suppose that the extramedullary lesion originated by an expansion of one clone of tumor plasma cells from the bone marrow. This is confirmed by identical genome profile of both tested samples.

Work was supported by grants NT13492 and OP VK CZ.1.07/2.3.00/20.0183 project.

J12.051
Germline mutations of apc and myh genes in patients with familial adenomatous polyposis in populations from central western Spain
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Germline mutations in APC are associated with the development of familial adenomatous polyposis (FAP). This syndrome is characterized by the presence of hundreds to thousands of adenomatous polyps in the colon and rectum. An attenuated form of FAP, called attenuated familial adenomatous polyposis (AFAP), is related to mutations in the 5’ end, in exon 9 and in the 3’ end of the APC. In addition, biallelic mutations in MUTYH gene have also been identified in patients with colorectal adenomas and in APC-negative patients with FAP or AFAP.

Molecular analysis in APC performed to 50 Spanish families diagnosed with FAP (n = 50) or AFAP (n = 22) has allowed the identification of pathogenic mutations in 85% of FAP families and 21% of AFAP. In total, we found 22 pathogenic mutations: 7 were nonsense and 13 were frameshift (7 deletions, 3 duplications, 2 insertions and a deletion together with an insertion).

Ten of these mutations are reported in this work for the first time. We have also detected two novel splicing mutations. In this study, we detected 6 variants of unknown significance and we performed population-based studies, segregation analysis and in Silico studies to further define these mutations.

In patients who showed no point mutations in the APC gene were analyzed for large rearrangements by multiplex ligation-dependent probe amplification (MLPA). Analysis of MUTYH revealed 6 pathogenic mutations in 8 unrelated families: 7 biallelic and 1 monoallelic variants.

Thus we have characterized 12 novel mutations in the APC gene involved in Familial Adenomatous Polyposis.

J12.052
FGFR2 K367E mutation is frequently found with low burden in patients with early onset aggressive colorectal cancer
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We conducted next-generation sequencing using Ion AmpliSeq™ Cancer panel (Life technologies) which covers relevant regions across 46 oncogenes and tumor suppressor genes on 5 primary colorectal tumors. The analyzed patients presented similar clinical features: early onset (39-50yrs), a highly aggressive disease, distal localization, microsatellite stable tumors and a lack of family history of malignant diseases. The mutations found (Table 1) generally correlate with the most abundant alterations in colorectal cancer. A K367E mutation in exon 9 of the FGFR2 gene was found with low burden (8-11%) in the tumors of 3 patients. This is a novel mutation that results in AA substitution in the extracellular juxtapemembrane region and might initiate spontaneous dimerisation of the receptor, leading to factor-independent growth and hyperresponsiveness to ligand, as suggested for similar defects in endometrial cancer and patients with cranosynostosis syndromes. Having in mind the low burden of the variant, we believe that K367E mutation is a late event alteration in a subclone which may be responsible for the highly aggressive pattern of the disease. This should be clarified by mutational burden analysis of the primary and secondary tumors of these and other patients.

Table 1. Mutations spectrum in the analyzed patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>PIK3CA</th>
<th>BRAF</th>
<th>TP53</th>
<th>FGFR2</th>
<th>KRAS</th>
<th>APC</th>
<th>FBXW7</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC330</td>
<td>R424F</td>
<td></td>
<td></td>
<td>K367E</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NC353</td>
<td>R424C</td>
<td></td>
<td></td>
<td>K367E</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NC562</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NC67</td>
<td>G1499R</td>
<td>(34%)</td>
<td>V600E</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NC71</td>
<td>Q486D</td>
<td>(20%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>R465C</td>
<td>(26%)</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

*The mutation burden is presented in parentheses.

J12.053
FGFR3 and TP53 gene mutations in bladder cancer in the Belarusian population
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Bladder cancer represents the second most common genitourinary tumor, with approximately 390,000 new cases every year worldwide. Mutations in the FGFR3 and TP53 genes have been shown to define two divergent pathways in the pathogenesis of bladder urothelial carcinomas.

The purpose of this study was to perform molecular screening for FGFR3 and TP53 mutations in 243 bladder tumours and to assess the relation of clinicopathological variables with mutation status of these two genes. Mutations in the FGFR3 and TP53 genes were detected by SNaPshot method and PCR-single-strand conformational polymorphism analysis is followed by DNA sequencing, respectively.

FGFR3 mutations were identified in 115 (47.3%) specimens and TP53 alterations were found in 46 (18.9%) tumors. In 12 cases (4.9%), FGFR3 and TP53 mutations coexisted; in 94 cases (38.7%), neither mutation was found. Analyses of nonmuscle-invasive and muscle-invasive tumors alone revealed that FGFR3 mutations were strongly associated with low-stage (p<0.001) low-grade (p<0.001) tumors, whereas the frequency of TP53 mutations was significantly higher in tumors of higher stage (p=0.001, respectivelly). An inverse correlation between mutated FGFR3 and mutated TP53 was observed: FGFR3 mutations were strongly associated with low mutation burden (p<0.001) and high mutation burden (p=0.11, respectivelly). An inverse correlation between mutated FGFR3 and mutated TP53 was observed: FGFR3 mutations were strongly associated with low-stage (p<0.001) low-grade (p<0.001) tumors, whereas the frequency of TP53 mutations was significantly higher in tumors of higher stage (p<0.001) and grade (p<0.001). Moreover, the presence of metastasis was strongly associated with TP53 mutations and the lack of FGFR3 alterations.

Our data are consistent with the idea that FGFR3 and TP53 mutations represent two distinct mechanisms of bladder cancer development. In our study, FGFR3 mutations were associated with favorable conventional prognostic factors, whereas TP53 alterations were related to adverse disease parameters.

J12.054
Association of rs2294008 polymorphism in PSMA gene with gastric cancer susceptibility in Uzbekistan
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J12.055
Polymorphism of the thymidylate synthase gene and sensitivity of gastric cancer to 5-FU chemotherapy.
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Thymidylate synthase (TS) is a key enzyme in nucleotide biosynthesis and is the main intracellular target of 5-FU. The research aims to study the relationship between genetic and protein expression of TS gene and response to 5-FU chemotherapy in gastric cancer patients. We investigated the clinical and prognostic importance of TS polymorphisms in gastric cancer patients. We examined 65TSR2/TSR3 and G>C) and 3’ (del 6) untranslated region polymorphisms in 80 gastric cancer patients after surgical treatment and chemotherapy with 5-fluorouracil.
We have shown that the genotype 2R/2R is significantly more often found in patients with gastric cancer. Genotyping of rs2294008 TS gene polymorphism can be recommended as a criterion for identification of high risk groups concerning developing of gastric cancer in Uzbekistan.

J12.056
Genetic profiles’ comparative Analysis: Adult glioblastomas vs young glioblastomas.
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Background: Glioblastomas (GB) are malignant astrocytic tumors, which often occur in patients aged over 55 years. However, younger age (<40 years) was also described in several studies. Therefore, GBs are newly divided into 2 subtypes according to patient’s age: young adult GB (YGB) (<40 years).
Methods: 43 samples composed by 40 A.GB and 13 Y.GB were collected during the period of 5 years from 2009 to 2013. In order to investigate discriminative genetic alterations between the 2 GB groups, tumor DNA was analysed by MLPA (Multiplex Ligation Probe Amplification). We used the 4 SALA MLPA probemix designed for Gliomas analysis: P370, P088, P105 and MLPA KIT.
Results: Similarities were observed between the 2 GB groups, concerning tumor size and locations as well as some chromosomal alterations: 1q, 17q and 19q. Interestingly, 1p 19q co-deletion, 1p deletion and CDKN2A (p14)
were only found in AGB group. While IDH1 mutation was more frequently detected in YGB. Conclusion: GB genetic profile variability noticed in our sample cohort supports the hypothesis of different tumorigenic pathways between AGB and YGB. Despite of the crucial role of clinical and histopathological data in tumor diagnosis, molecular investigations seems of a great importance as well.

**J12.059**

**Association analysis of rs7903146 (C>T) variant of TCF7L2 Gene with Glioblastoma in a Turkish Population**

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Glioblastoma (GBM) is the most common malignant brain tumor in the adult population defined as grade IV astrocytoma according to severity of necrosis and vascular proliferation. It can be seen at any age, but commonly between the ages of 45-75. GBM is difficult to treat and presents high morbidity and mortality risks. There is no curative treatment. Patient's genetic structure is a factor as important as the choice of chemotherapeutic agents, dose, time of administration that directly affect the process of chemotherapy. Transcription factor 7-like 2 (TCF7L2) gene, located on chromosome 10q25.3, encodes a transcription factor which is involved in the Wnt/β-catenin signaling pathway. Active nuclear complex which TCF7L2 forms with β-catenin induces the expression of target genes involved in cellular proliferation, evasion of apoptosis and tissue invasion and metastasis. It is demonstrated in association with many cancer types, including breast, prostate, lung and colon cancers. We performed a study to investigate the association between TCF7L2 rs7903146 variant and another cancer type glioblastoma. Forty-three patients who primarily diagnosed as GBM were recruited to this study from Department of Neurosurgery of Faculty of Medicine of Ankara University in Turkey. None of the patients received any chemotherapeutic agents or radiation therapy before surgical resection of the tumor. DNA was isolated using isolation kit from patients’ cancerous and their corresponding adjacent non-cancerous tissues. rs7903146 were genotyped by PCR-RFLP. No association was determined between rs7903146C>T and GBM after analyzing carried out using dominant, additive, and recessive models (P>0.05).

**J12.060**

**Analysis of autophagy polymorphisms in glioblastomas**

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**BACKGROUND**

Autophagy is an adaptive and highly regulated response in unfavorable conditions such as starvation. During this process, long-lived proteins and organelles are self-eaten by double membranes vesicles, called autophagosomes, which transport them through the cytosol to the lysosome, where they will be degraded. The resulting macromolecules can be recycled to the cytosol. Autophagy plays an important role in cellular development and differentiation and it has been reported that its malfunction is implicated in several diseases including cancer. Glioblastoma is a very infiltrating and aggressive heterogeneous glioma. Its behavior is directly related to their genetic and chromosomal alterations. Here, we have analysed common polymorphisms in autophagy genes in order to evaluate the role of these variants in modulating glioblastoma risk.

**PATIENTS AND METHODS**

112 newly diagnosed patients with primary glioblastoma (grade IV) and 189 controls without cancer were included in the study. Genomic DNA was extracted from peripheral blood using phenol/chloroform procedure and genotyped using TaqMan 5'-exonuclease allelic discrimination assays (Applied Biosystems) (table 1). Statistical analysis was performed using SPSS software.

**RESULTS**

No significant differences were found in genotype distribution for ATG16L1 RS2411880 ATG2B RS3759601 and ATGS RS2245214 between patients with glioblastoma and control subjects. However, statistical differences in genotype distribution for ATG10 RS1864183 were found. The A allele was associated with increased risk of developing glioblastoma (OR = 2.788, 95% CI 1.40-5.55; P = 0.004).

**CONCLUSIONS**

ATG10 RS1864183 POLYMORPHISM IS ASSOCIATED WITH SUSCEPTIBILITY TO SUFFER Glioblastoma, SUPPORTING THE IMPORTANCE OF AUTO-
phisms in different genes of the EGFR pathway and antibody dependent cellular cytotoxicity receptors (ADCC) associated with toxicity and responses of these treatments. Genomic DNA was extracted from peripheral blood samples of 91 Spanish HCC patients treated with sorafenib (monoclonal antibodies against EGFR). The selected polymorphisms realized by QPCR with TaqMan® probes were: FGR2A rs1801274, FGR3A rs396991, EGFR rs28384375, rs2272983, rs7336639 and KRAS1c6s rs16746370. Statistical analysis was performed comparing the different distribution of the polymorphisms and patients were stratified according to treatment and response.

We found differences in the distribution of FGR2A A519C (rs1801274) polymorphism (p=0.012), conferring an increased risk of developing dry skin in carriers of two copies of the Callele; OR=4.018 (1.257-12.850). Moreover, we found that carriers of the heterozygous genotype in K-RAS1c6s polymorphism (rs61764370) exhibit a decrease in global toxicity of anti-EGFR antibody therapies: p=0.014, OR=0.294 (1.07-0.805). Our results support the correlation between some germline polymorphisms in some genes in the EGFR pathway and ADCC receptors, and the differences in the toxicity of anti-EGFR antibody therapy. J12.064

Conventional cytogenetics and FISH analysis in hematological disorders

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Non-random chromosomal aberrations are a common feature of many hematological disorders, making cytogenetic analyzes essential for the most common abnormalities in myelodysplastic/myeloproliferative diseases, leukemias and lymphomas. Between March 2013 and January 2014, 77 bone marrow samples were analyzed cytogenetically from adults aged between 23 and 82 years. Where possible three independent cultures (direct method, 24 and 48 hours) were established and a minimum of 20 metaphases were analyzed. The karyotype of 70 patients was analyzed successfully. For 7 patients with insufficient number of metaphases FISH analysis was performed using BCR/ABL1 plus probes (MetaSystems) or combined clinical and hematological diagnosis for 5 patients with normal karyotype.

Among the successfully karyotyped samples, 35 cases of CML and 3 cases of ALL showed various homogeneous or mosaic chromosome abnormalities. The most common abnormality was the presence of the Philadelphia chromosome (Ph), found in varying percentages (between 9 and 100%) with 2 cases having an additional cell line with double Ph chromosome. One case had a complex translocation involving chromosomes 9, 10 and 22. Other common abnormalities were trisomy 8, isochromosome 17, autosomal hypodiploidy. Identification of cytogenetic abnormalities by conventional karyotype is important to confirm the diagnosis and provide useful information for classification, staging and prognostic information, for choosing therapy conduct and for signs of remission or relapse. Because some cases show a normal karyotype by conventional banding, FISH and aGH analysis are valuable techniques recommended for identifying the presence of possible submicroscopic abnormalities. J12.065

Her-2/neu gene in neck squamous cell carcinomas (SCC) a potential target for therapy

M. Neagu;

Background: Over-expression of the proto-oncogene cerbB-2 (HER-2/neu) has been shown to be a prognostic marker and also a target for therapy in many cancers but conflicting data exist about the prevalence of HER-2/neu over expression in SCC of the head and neck.

Design: The status of Her-2/neu was evaluated in a series of 36 SCC of the larynx and oropharynx to verify the frequency of over-expression of HER-2/neu and evaluate the correlation with traditional diagnostic parameters of this neoplasm. A Hercep test kit was used to detect HER-2 expression, and a Path Vysion kit was used for gene amplification.

Results: On immunohistochemical (IHC) staining HER-2/neu was positive only in 8 cases (23.6%) and these were further tested by fluorescent in situ hybridization (FISH) with positive gene amplification in 3 cases (3/8), cep17/Her-2/neu = 4.22 - 4.84. One case with IHC staining 3+ do not have gene amplification and cases 2+ and 1+ at the IHC evaluation have no amplification (cep17/HER-2/neu = 1.64); tumors with positive gene amplification were grade 3 (4/8) or had basalioid features. Conclusion: Even there are a small percentage of these tumors that have gene amplification for HER-2/neu, at present, patients who may potentially benefit from molecular targeted therapy targeting HER-2/neu for SCC of head and neck should be identified by gene amplification analysis using FISH in IHC 3+ patients. J12.066

Colorectal cancer risk in relation to Hypoxia Inducible Factor-1α (HIF-1α) and Von Hippel-Lindau (VHL) gene polymorphisms

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Colorectal cancer (CRC) is a multifactorial disease involving environmental and genetic factors. Unhealthy diet habit, smoking and environmental carcinogenic agents are risk factors of CRC, such as alcohol, low methionine, low folate diets and exposure to white soil stands. Hypoxia plays a critical role in activating hypoxia-inducible factor-1α (HIF-1α) and leads to numerous metabolic changes such as angiogenesis, anaerobic glycolysis and erythropoiesis. HIF-1α protein level is known to be regulated by von Hippel-Lindau (VHL) ubiquitin-protasome system but in hypoxic conditions HIF-1α protein level increases. Mutations in VHL, or HIF-1α genes can affect the connection of VHL protein to HIF-1α and cause cancer and cardiovascular diseases. The aim of this study is to investigate the relationship of the C1772T rs (11549465) and G1790A (rs11549467) polymorphisms of HIF-1α gene and the functional rs779805 polymorphism of 5'UTR region of the VHL gene, regulating the oxygen-dependent degradation of HIF-1α, with the risk of colorectal cancer. In the study, 92 patients who have been diagnosed to have colorectal cancer and 101 healthy controls were included. PCR-RFLP ve ARMS-PCR molecular diagnostic methods were used for genotyping.

For statistical analysis student t, chi-square (χ²), Fisher’s exact tests and SNPStats were used. According to genetic models, CT/TT genotypes of HIF-1α rs11549467 polymorphism were found to increase the risk of colorectal cancer in patients (p=0.049; OR:1.06; 95%CI:1.02-3.77; AOR 4.79 (1.07-21.48). For G1790A ve rs779805 polymorphisms, no significant deviation was observed between patients and controls (p>0.05).

J12.067

Delineation of high-risk Human papillomavirus genotypes in Iranian breast cancer patients

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Contribution of Human papillomavirus (HPV) in tumor-genesis has been proved for a while, but it’s function in the pathogenesis of breast cancer is still not clear. In this investigation we checked the prevalence of main genotypes of HPV and their association with breast cancer in pre/post-operative Iranian patients. 77 formalin fixed paraffin embedded (FFPE) breast invasivducal carcinoma and their normal adjacent tissues (NAT) were included in the study. 1,01 born primer pairs applied for amplification and detection of HPVs via nested-multiplex polymerase chain reaction. The HPV positive samples were further genotyped using DNA sequencing. Pretty High percent of HPV (38%) was detected in breast (core) ductal carcinoma compared to 4% in the NAT. High-risk HPV genotypes 16, 18, 31, 33, 35 and 45 were highly associated with the advanced stages of tumor, while low-risk types 6 and 11 were present in 4% in the NAT. High-risk HPV genotypes in breast cancerous tissue may represent its contribution in breast carcinogenesis. Using HPV vaccination program is highly recommended to reduce this type of cancer.

J12.068

Expression of Insulin-Like Growth Factor Binding Protein-2 (IGFBP-2) gene in negative and positive human Cytomegalovirus Globulastoma multiforme tissues

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Globulastoma multiforme (GBM) is the most common and lethal primary brain tumor. The median survival of glioblastoma patients is 12 months. The most abundant genotype followed by type 18 and 33. The high risk HPV genotypes are risk factors of CRC, such as alcohol, low methionine, low folate diets and exposure to white soil stands.
and antigens in tumor tissues suggests that HCMV infection has a role to play in the etiology of several human malignancies. HCMV gene products can promote the various signaling pathways critical to tumour growth, including PDGFR, P38/akt, STAT3 and GSK-3β, which are involved in apoptosis, angiogenesis, invasion and immune evasion. IGF2BP2 is a biomarker of the P38/akt pathway we decided to evaluate the expression of this gene in 3 groups: HCMV-negative glioblastoma multiforme tissues, HCMV-positive glioblastoma multiforme tissues and non tumor tissues. The presence of Human Cytomegalovirus was assayed by cytomegalovirus detection kit. Human Cytomegalovirus was present in 2/3 of glioblastoma tissues. Then RNA was extracted, cDNA was synthesized and Real-time PCR was performed. The rate of increased expression was calculated using the ΔΔct or 2-ΔΔct. Δct of samples in the three groups were compared using ANOVA (Analysis of Variance). The expression of IGF2BP2 gene relative to GAPDH gene in HCMV-negative glioblastoma tissues and HCMV-positive glioblastoma tissues, respectively was increased 5.486 and 15.032 times compared to non-neoplastic brain tissues. ANOVA tests showed that the difference of mean Δct for IGF2BP2 gene between healthy subjects and patients with HCMV positive and HCMV negative glioblastoma tumors statistically significant.

**J12.069**

2 Novel Somatic Mutations In Exon 15 Of The Apc Gene In Iranian Familial Adeno Polypsis Coli Patient

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**Abstract:** The adenomatous polyposis coli (APC) gene is considered to be a gatekeeper in colorectal tumorgenesis. 60% of somatic mutations in the APC gene are concentrated in a region called the mutation cluster region (MCR). In this study, our goal was to perform the genetic analysis of 2 patients (index patients) who had been selected by colorectal cancer features, and to identify the genetic changes in the MCR region at the APC gene. **Methods:** Mutation analysis of the MCR, which spans codons 1286-1513, was performed on the paraffin-embedded cancerous tissue samples using microdissection, nested PCR and direct sequencing of purified PCR fragments. **Results:** In our study 2 new somatic mutations detected in these patients. In one patient we have detected a TCA to TGA as a Nonsense mutation that lead to Arg to premature Stop codon at the 4507 nucleotide position (Codon 1503) and in another patient, we describe a G→A Transition (ACG to ACA) at nucleotide position 4638 in exon 15 (MCR region) which causes a silent mutation since both normal and mutated alleles encode a Thr residue at codon 1546. These mutations have not been described previously. **Conclusion:** This observation could suggest differences in the frequency of pathologic mutations in APC among different populations; however epidemiological studies must be performed to confirm this theory which it is not the aim of our present work. **Keywords:** APC, Iranian Familial Adenomatous Polyposis (Iranian FAP), somatic mutations

**J12.070**

Detection of K-RAS gene activating mutations, an important factor in therapeutic management of CRC Romanian patients

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**ABSTRACTS PUBLISHED ABSTRACTS**

**Genetic Polymorphism of NOS2 -954G/C in Nasal Polyposis. A case-control study in a population group of Northern Romania**

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Muir-Torrec syndrome is a clinical sub-type of Lynch syndrome which is associated with sebaceous skin lesions and keratoacanthomas in addition to internal malignancies. As established in other Lynch syndrome-associated cancers, these skin lesions demonstrate loss of mismatch repair (MMR) protein expression on immunohistochemical (IHC) staining, allowing targeted germline mutation analysis of the MMR genes associated with Lynch syndrome (MLH1, MSH2, MSH6, PMS2). However, not all lesions with loss of MMR protein expression will result in a molecular or clinical diagnosis of Muir-Torrec syndrome, as this protein loss may be due to a sporadic mechanism. There is emerging literature that the specific type of sebaceous lesion, along with its location on the body, can provide an indication as to which gene is more likely to have loss of MMR protein expression and subsequent association with Muir-Torrec syndrome. In order to assess this theory within the experience of Genetic Health Queensland (GHQ), an internal audit was performed. All patients seen at GHQ between 2005 and 2013 with a primary reason for referral of a sebaceous skin lesion were included in a retrospective analysis. The information reviewed included subtype and location of lesion, age of diagnosis, personal and family history of cancer, IHC results, MMR gene testing results and outcome of assessment. A detailed analysis of this information will be presented, including the proportion of individuals with sebaceous lesions in which a clinical or molecular diagnosis of Muir-Torrec syndrome was made.

J1.074 RET proto-oncogene main exons’ mutations in Iranian patients with medullary thyroid carcinoma

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Introduction: Thyroid is the most common endocrine malignancy. Medullary thyroid carcinoma (MTC) account for 5-10% of all thyroid cancer types. It occurs in both hereditary (5%) and sporadic (95%) forms which are associated with gain of function mutations in the RET proto-oncogene.

Material and methods: We have started MTC genetic screening since 2001. Our study included 360 individuals, including 161 sMTC, 39 pMTC, 3MEN2A, 3MEN2B, 4pheochromocytoma (215 index cases), and 145 relatives. Genomic DNA was extracted from peripheral blood leukocytes, and the six exons of the RET gene (10, 11, 13, 14, 15, 16) were amplified by PCR and examined by DNA sequence analysis to detect mutations.

Results: A total of 20 different types of isomerase RET mutations were identified in 78 patients. All of the MEN2A patients had mutations in codon 634. Mutations outside of codon 634 occurred only in pMTC and sMTC. A mutation at codon 918 was found in all MEN2B patients. p.G634Y was the most common mutation in our study followed by mutations p.G637D, p.G634R, p.G635E. The G691S/S904S haplotype was identified in 120 patients and 70 relatives. A novel C>T intronic variant (intron 11, chr position 10:43612012) was also detected.

Conclusion: Exon 11 and after that exon 10 were the most frequently mutated exons of RET proto-oncogene in MTC patients in our population. As about half of patients with the hot spot mutations had the G691S/S904S haplotype simultaneously, further analysis needs for clarifying the effect of multiple risk alleles in MTC development.

J1.075 Molecular cytogenetic diagnosis of melanocytic lesions

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Background: The primary purpose of this study was to investigate the prevalence and characteristics of p16 methylation and determine the prognostic implications of the clinical data, hematologic data and p16 methylation changes in plasma cell myeloma (PCM). Methods: We reviewed clinical characteristics, laboratory response to therapy and investigated IHC staining, uniplex and multiplex DNA methylation chemotherapy, and survival time. DNA methylation of the p16 gene was tested by methylation specific PCR and evaluated the clinical significance.

Results: A total of 103 patients were enrolled in this study. The patient median age was 59.0 years at diagnosis and male to female ratio was 1.15:1. According to the International Staging System (ISS), patients were diagnosed as stage: I (n=17, 16.5%); II (n=41, 39.8%); III, (n=39, 37.9%); (not classifiable) (n=6, 5.9%). There were 36 (35.0%) patients showed an abnormal karyotype and complex karyotype on chromosome study, respectively. The p16 methylation was found in 39 of 103 (37.9%) patients, but there was no significant association of p16 methylation status with other clinical, laboratory factors and survival outcome. The male gender, albumin and complex karyotype were independent prognostic factors for overall survival and disease free survival. Conclusions: The male gender, albumin and complex karyotype were independent prognostic factors in PCM. And p16 methylation was relatively common in PCM but didn’t influence a response to survival outcome.

J1.076 Prevalence of p16 methylation and prognostic factors in plasma cell myeloma of single institution in Korea

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Background: The primary purpose of this study was to investigate the prevalence and characteristics of p16 methylation and determine the prognostic implications of the clinical data, hematologic data and p16 methylation changes in plasma cell myeloma (PCM). Methods: We reviewed clinical characteristics, laboratory response to therapy and investigated IHC staining, uniplex and multiplex DNA methylation chemotherapy, and survival time. DNA methylation of the p16 gene was tested by methylation specific PCR and evaluated the clinical significance.

Results: A total of 103 patients were enrolled in this study. The patient median age was 59.0 years at diagnosis and male to female ratio was 1.15:1. According to the International Staging System (ISS), patients were diagnosed as stage: I (n=17, 16.5%); II (n=41, 39.8%); III, (n=39, 37.9%); (not classifiable) (n=6, 5.9%). There were 36 (35.0%) patients showed an abnormal karyotype and complex karyotype on chromosome study, respectively. The p16 methylation was found in 39 of 103 (37.9%) patients, but there was no significant association of p16 methylation status with other clinical, laboratory factors and survival outcome. The male gender, albumin and complex karyotype were independent prognostic factors for overall survival and disease free survival. Conclusions: The male gender, albumin and complex karyotype were independent prognostic factors in PCM. And p16 methylation was relatively common in PCM but didn’t influence a response to survival outcome.

J1.077 Analysis of oncogenic mutations in 52 MGUS patients - two case reports of KRAS mutation

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Monoclonal gammonphy of undetermined significance (MGUS) is a premalignant condition permanently associated with a risk of progression into malignant disease, especially to multiple myeloma (MM). While in MM, oncogenic mutations have been described in several studies, no mutation study was done in abnormal plasma cells (aPCs) in MGUS. In our study, we performed genetic analysis in 52 samples of aPCs (CD138+CD19-1CD56+/−) of MGUS patients by aCGH (SurePrint G3 CHG SNP, 4×180K, Agilent) together with High Resolution Melting (HRM) followed by Sanger sequencing in HRM positive cases. By HRM, we focused on DNA single nucleotide mutations in the KRAS gene was investigated. We observed two different types of KRAS mutations in aPCs of MGUS patients. p.G12V and p.A146T with damaging prediction effect. Both MGUS cases showed positive IGH disruption (in 56% and 85% aPCs) analysed by I-FISH, but only the first case with p.G12V mutation showed unbalanced chromosomal changes in genome-wide profile: hyperdiploidy (46, +4, +5, +6), segmental losses (1p34.2–p11.31, 6p23, 6q27.2–q27, 7q36.3, 12p12–p11.21, 12q12, 12q21–q23.3) and 4 segmental gains (6p25.3–p23, 6p23–p11.1, 6q11.1–q12, Xq23.1–q28). Interestingly, only this patient has progressed after 6 months to MM. Genome-wide profiling of the second patient with p.A146T mutation did not show any unbalanced chromosomal changes and the patient is still stable for more than 16 months. In our study, we showed that KRAS p.G12V mutation combined with chromosomal changes could
be potential markers of MGUS progression. Support: IGA NT13492,OPVK CZ.1.07/2.3.00/20.0183, MH CZ-DR (FNBr, 65269705)

J12.078 Gene polymorphisms of microRNAs in Helicobacter pylori-induced high risk atrophic gastritis and gastric cancer
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We studied miRNA expression profiles in patients with acute myeloid leukemia at diagnosis and in the first remission and we compared them with profiles found in healthy controls. Plasma samples were collected from 8 patients at the first diagnosis of AML and at the first complete remission and from 10 healthy volunteers. Isolated miRNA were transcribed to cDNA and applied on TaqMan Human MicroRNA Array A to find the expression of 381 genes. Relative quantification and statistical analyses were performed with Expression Suite v1.0.3 and with geqva v2.4. Relative quantification was calculated using the reference gene miR-16-1 global mean. Both values of miRNA expressions were compared between the patients and controls using Mann-Whitney tests with multiple testing corrections. The differences of various statistical significance were found. Among them there were 12 miRNAs (miR-125a-5p, miR-145, miR-221, miR-744, miR-146a, miR-181a, miR-191, miR-27a, miR-340, miR-134, let-7d) giving the p values < 0.01. The results were supported by both applied normalization methods and another 4 miRNAs (miR-199a-3p, miR-331-3p, miR-324-5p, miR-28-3p) having p < 0.01 using reference gene miR-16 and p value between 0.01 - 0.02 using global mean. All these miRNAs were overexpressed in patients at the time of diagnosis only, but not at the first complete remission. With regards to miRNA expression profiles of healthy subjects, we compared the profiles in the morning and the afternoon samples of healthy volunteers and we detected no significant differences referring to circadian rhythm. Supported by the Ministry of Health of the Czech Republic, WO VFN4165.

J12.081 Investigation of DNA mutations in Mitochondrial D-Loop region at thyroid nodules in Turkish population-Preliminary findings
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Mitochondrial defects have been associated with various human conditions including cancers. However mitochondrial DNA (mtDNA) alterations within the highly variable D-loop control region have been reported as a frequent event in cervical cancer, breast cancer, gastric carcinoma, colorectal cancer, hepatocellular cancer, lung cancer and renal cell carcinoma. Specifically, it was suggested that the D310 region is more susceptible to oxidative damage and electrophilic attack.

According to the published data, mutations involving the highly variable D-loop control region have been reported as a frequent event in cervical cancer, breast cancer, gastric carcinoma, colorectal cancer, hepatocellular cancer, lung cancer and renal cell carcinoma. Specifically, it was suggested that the D310 region is more susceptible to oxidative damage and electrophilic attack.

Aim of this study to investigate if the accumulation of mtDNA mutations plays a role in tumorigenesis in thyroid nodules in Turkish population. Clinically and pathologically examined 178 tissue samples belonging to the 77 patients; including 58 surrounding healthy tissues, 51 hot thyroid nodules (HTN) and 69 cold thyroid nodules (CTN), were enrolled to the study. DNA extraction from tissue samples was done by using phenol-chloroform method. D-Loop region between 15-484. nt and 15971-16411. nt were amplified by PCR. DNA sequencing reaction of D-Loop region between 15-484. nt was performed on Beckmann Coulter Genomelab Automated DNA Sequencer.

The same polymorphisms were detected in both healthy and nodular tissues belonging to the same patients. But two different polymorphisms (A73G; T89G) were detected as heteroplasmically in HTN tissue of 31th patient different from healthy tissue. This is in agreement with the published data of 31th patient in HTN tissue of 31th patient different from healthy tissue. This is in agreement with the published data of 31th patient in HTN tissue of 31th patient different from healthy tissue.

As a result, the obtained data indicates that mtDNA alterations in the D-loop region could happen before tumorigenesis in thyroid, and they might also accumulate during tumorigenesis.

J12.082 The occurrence of EZH2 mutations in patients with BCR-ABL-negative myeloproliferative neoplasms

Recent studies have revealed a number of epigenetic alterations that contribute to myeloproliferative neoplasms (MPNs) pathogenesis and determine the clinical outcome. According to the published data, mutations involving EZH2, which encodes a histone methyltransferase, are found in 6% cases of primary myelofibrosis (PMF), 1% of polycythemia vera and 1-3% of essential thrombocytopenia regardless of the presence of mutations in JAK2 or MPL. EZH2 mutations may be of prognostic value in MPN’s at the time of
Coordination of expression of cell cycle related genes in Multiple Myeloma and Plasma Cell Leukemia

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In multiple myeloma (MM), malignant cells retain the self-renewing potential. Majority of myeloma cells stay in the G1 phase, however after leukemic transformation in plasma cell leukemia (PCL) myeloma cells become highly proliferative. We anticipate that complex "re-setting" of cell cycle gene expression coordination during leukemic transformation creates required background for proliferation restoration. The aim of this study was to define and describe complex "re-setting" of cell cycle gene expression coordination in MM and PCL. Gene expression profiling was performed in 15 MM and 7 PCL patients using Affymetrix Microarray. GeneChip Human Exon 1.0 ST Arrays. Genesets, connected with cell cycle regulation (GO:00045786; GO:00045787) and regulation of apoptotic processes (GO:00430605; GO:00430606) together with all descended direct connected genesets were taken for the GSEA analysis and Gene Set Differential Coordination Analysis. Comparison of PCL and MM healthy donors revealed coordinated expression changes between regulation of mitosis, apoptosis and cell cycle arrest for both positive and negative regulation genes. In MM, co-expression changes were associated with early phase of cell cycle, whereas in PCL with both - early and late phase of cell cycle. We anticipate that expression of cell cycle positive regulators is in dynamic equilibrium with cell cycle negative regulators. We suppose that this equilibrium serves as a compensatory mechanism to oncogenic events. Despite compensation mechanisms activation, whole regulatory complex seems to be impaired by growing "oncogenic stress" during MM to PCL progression. Supported by grants NT12130, NT13190 and OPVK CZ.1.07/2.3.00/20.0183.

J12.085 MYCN oncogene amplification in advanced neuroblastoma patients from Republic of Macedonia

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BACKGROUND Myelodysplastic syndrome (MDS) is a clonal proliferative disorder of the bone marrow characterized by ineffective hematopoiesis, peripheral blood cytopenias, and increased risk of developing acute myeloid leukemia (AML). Identifying predisposing gene mutations is critical for effective diagnosis, assessment of prognosis, and therapeutic counselling. A Caucasian family with an autosomal dominant inheritance pattern of early onset MDS was identified. No common exposures to potential carcinogens were present and clinical genetic testing indicated that regions 5q31 and 7q22, frequently associated with sporadic MDS, were not causal, suggesting an inherited predisposition. METHODS/RESULTS In-depth genetic analysis was undertaken. Six samples were acquired, including four clinically affected males across 2 generations (age of onset 25-69; one RAEB MDS, two unclassified MDS, one unknown MDS), a suspected unaffected female family member (aged 40), and an unaffected spouse (aged 72). Single-nucleotide polymorphism genotyping was performed on the suspected unaffected and three affected individuals, combined with the mapping data, revealed 1904 shared variants in those regions. FUTURE DIRECTIONS Whole-exome sequencing on a third affected individual is underway to reduce the list of potential causative variants. After analysis, variants will be prioritized based on the genes known function and mutation severity. Top candidate genes will be sequenced in all available family members to determine which variant(s) track with the disease.
J12.087
NAT2 acetylation polymorphisms in bladder cancer risk
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Bladder cancer is known to be modulated by environmental carcinogens. Polymorphisms in the genes coding for the enzymes involved in the metabolism of carcinogens are considered as susceptibility factors for bladder cancer. N-acetyltransferase 2 (NAT2) is a Phase II enzyme of the xenobiotic metabolism pathway, which is involved in detoxification of arylamines and heterocyclic amines. Therefore, polymorphisms of the NAT2 gene and its related acetylation status may confer a risk factor for the development and progression of bladder cancer. In this study, we have investigated NAT2 481C>T, 5,906G>A and 857G>A polymorphisms, corresponding to NAT2*1A, NAT2*6B and NAT2*7A alleles, in terms of bladder cancer risk. For this purpose, genomic DNA samples of 129 bladder cancer and 148 healthy subjects were genotyped by PCR-RFLP under their informed consent. We have shown that NAT2*1A allele have a trend for protection against bladder cancer (p=0.059; OR=0.761), and significance of protection increases (p=0.005; OR=0.539) when non-smokers are excluded. We also categorized the individuals according to their acetylation status as slow, intermediate and fast acetylators, however, we could not find a statistically significant association between bladder cancer and acetylation status.

J12.088
Evaluation of genotoxic risk of Tunisian hospital workers exposed to low levels of ionizing radiation (IR)
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Over the years, (IR) has become a universal diagnostic and therapeutic tool, making the largest man-made contribution to the population dose. Thus, medical personnel represent the group most consistently exposed to low doses of IR. Many cytogenetic studies have been conducted among hospital workers exposed to IR. The cytokinesis-blocked micronucleus (CBMN) assay is widely used, since it represents a reliable test to assess a radiation-induced chromosomal damage and it’s a valuable biomarker in many biomonitoring studies on human populations exposed to IR. The aim of our study is to assess chromosomal damage in Tunisian hospital workers occupationally exposed to low levels of IR. The CBMN in peripheral lymphocytes of 67 exposed workers compared to 43 controls. The clastogenic/aneugenic effect of IR was evaluated using the CBMN assay in combination with fluorescence in situ hybridization (FISH) with pan-centromeric DNA in all the exposed subjects and controls.

J12.089
The detection of mutations in gene PTEN in patients with ovarian cancer from Bashkortostan Republic of Russia
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PTEN (phosphatase and tensin homolog) localized on chromosome 10q23.3 is tumor suppressor, which mutations cause both hereditary and sporadic forms of malignancies, including ovarian cancer (OC). The first germinal mutations in this gene have been identified in the study of hereditary syndromes: Cowden, Bannayan-Riley-ruvalca and Lhermitte-Duclos. PTEN is negative regulator of the PI3K/AKT/mTOR pathway by dephosphorylating phosphatidylinositol 3,4,5-trisphosphate (PIP3) to phosphatidylinositol 4,5-bisphosphate thus counteracting PI3K function. The operation of PTEN is necessary to control apoptosis, migration and proliferation of cells in the organism. We carried out an analysis of structural changes in the PTEN in 250 patients with ovarian cancer from different ethnic groups of the Republic of Bashkortostan. The detection of mutations was performed by high resolution melting curve analysis (HRM) and confirmed by direct sequencing. The study of nucleotide sequence of the PTEN in patients with OC were detected changes: c.904A>C (Ser302Arg), c.217G>A (Glu71lys), previously described polymorphism c.1327T>C (Gly444Arg), and changes in introns: c.209+9G>C, c.209+10T>C. Using the program Polyphen-2, we have found that the change c.904A>C, located in exon 8 of PTEN, is not pathogenic, and therefore does not contribute to the development of ovarian cancer. Mutation c.217G>A (Glu71lys), located in exon 4 of PTEN, is found in phosphatase domain of protein PTEN that may possibly affect the impaired function of the protein and subsequently lead to tumor formation. This study was supported by RFBR-Povelzhye 14-04-9708.
3-Phosphoglycerate Dehydrogenase Polymorphism in male patients with thyroid gland cancer

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Introduction: Thyroid gland cancer (TC) is considered a rare malignancy, accounting up to 2% in males. Recent studies suggest important role of both environmental and genetic factors in cancerogenesis. 3-Phosphoglycerate dehydrogenase (3-PHGDH) gene overexpression is associated with patogenesis of human cancer and contributes to cell proliferation.

Aim: The objective of our study was to assess the association of PHGDH gene polymorphism in group of males with thyroid gland cancer and control group of healthy men.

Methods: The study was carried out in the Department of Human Genetics-Medical School, University of Belgrade. The study has encompassed 80 man diagnosed with thyroid gland cancer in Center for Endocrine Surgery, Clinical Center of Serbia Serbia and 100 health males volunteers. The DNA was isolated from the periferal blood with solting out method. The genotypes 3-PHGDH polymorphism were determined by Polimerase Chain Reaction and Restriction Fragment Length Polimorphism. Gel-electrophoresis was used to separate DNA fragments.

Results: There is a significant difference in frequency of TT, CC and CT between experimental and control groups of rs451503 polymorphism (H2= 38.924; p=0.001). There were significantly more TT in experimental group, while in control group there were more CT (H2=33.186; p=0.001) and CC (H2=21.734; p=0.001).

Conclusion: In our study we found TT genotype as the most frequent in patients with thyroid gland cancer. The results of our study also suggest that C allele might be factor of risk associated with thyroid gland cancer. It is necessary to undergo further testing with more accurate test groups.

J12.093 Incidence of PIK3CA mutations in breast cancer correlated with histopathological characteristics of the tumor

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Gain-of-function mutations in PIK3CA have been found in breast cancer, but prognostic value of PIK3CA mutation status is controversial. The goal of the study was the analysis of PIK3CA hotspot mutations and their correlation with clinicopathological parameters. Paraffin-embedded tissue sections were obtained from biopsy specimens of 95 breast cancer patients characterized histologically and immunohistochemically including histological grade, regional lymph node status (pN), estrogen receptor, progesterone receptor and HER2 status. We investigated exon 9 and exon 20 of PIK3CA gene in 95 breast tumor samples by DNA sequencing. We observed mutations in 27.3% (26/95), all with exception of 2 cases were mutually exclusive. Mutations in exon 9 represent 16.8% (16/95); mutations in exon 20 represent 12.6% (25/95), all with exception of 2 cases were mutually exclusive. Mutations in PIK3CA gene were more CT (Hi2=33.186; p=0.001) and CC (Hi2=21.734; p=0.001). Conclusion: In our study we found TT genotype as the most frequent in patients with thyroid gland cancer. The results of our study also suggest that C allele might be factor of risk associated with thyroid gland cancer. It is necessary to undergo further testing with more accurate test groups.

J12.094 Molecular subtyping of three aggressive PCA patients from Bulgaria in correlation with clinic-histological data

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Prostate cancer (PCa) is among the most prevalent neoplasms worldwide, whereas metastatic PCA is one of the leading causes of death in men. Here we report three Bulgarian patients with strongly aggressive, hormone-refractory PCa. The application of the following molecular markers: DD3 overexpression, GSTP1 promoter hypermethylation, TMPRSS2-ERG gene fusions and mutations in androgen receptor (AR) gene for diagnostic purposes was assessed. We attempt to correlate the molecular data to both histological and clinical data. The obtained molecular profile in the three Bulgarian patients coincides with the clinical and histological data of aggressive, hormone-independent PCa. There was no association between the tumor stage (assessed by TMM as T2) and the detected molecular profile of aggressive cancer behaviour. None of our cases was with positive family history and no somatic mutations were detected in the AR gene. The rest of the markers: DD3 overexpression, GSTP1 gene promoter hypermethylation and TMPRSS2-ERG gene fusion were positive in fresh prostatic tissues and biopsies from all three patients, whereas only one blood sample showed triple positive result. The appearance of PCA specific molecular markers in blood was considered as a predictor for a pronounced migration and dissemination of prostatic tumor cells in circulation. The GSTP1 promoter hypermethylation is the earliest epigenetic fluctuation, which indicates cancerous changes in the gland the first and long-lasing marker in the blood circulation. The molecular profile during cancer treatment could be used to predict the individual response. Acknowledgements: the study was supported by the grants N-31/2011 and 26-D/2012, Medical University Sofia, Bulgaria.

J12.095 Analysis of the PCA3, TMPRSS2-ERG, TERT genes expression in biopsy samples and urine sediments as potential markers for diagnostics of prostate cancer

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Prostate-specific antigen (PSA) concentration in the blood can be increased in hyperplasia or adenoma, analysis of biopsy samples and urine sediments as the potential markers for diagnostics of prostate cancer (PCa) requires highly skilled pathologist. Search of new molecular markers for noninvasive diagnostics of PCa is an actual oncology problem. The aim of this study is analysis of PCA3, TMPRSS2-ERG, TERT expression in biopsies and urine sediments as potential markers for the diagnostics of PCa. We have used 21 cancer biopsies, which included 2 adenocarcinoma, 29 prostate intraepithelial neoplasia (PIN) and benign prostatic hyperplasia (BPH), 22 cases of inflammatory lesions; 10 urine sediments obtained after prostatic massage. Genes expression was analyzed by real-time PCR with endogenous GAPDH and tissue-specific KLK3 controls. There was a correlation between the grade in normal/high PIN adenocarcinoma and expression of PCA3 (r = 0.691). The threshold AUC (PCA3-KLK3) 3.9 provided optimal sensitivity and specificity in biopsy: 86% and 96%, respectively. TMPRSS2-ERG expression was detected in 48% of adenocarcinomas and 11% of high PIN, TERT - 71% and 22% respectively. Combination of PCA3, TMPRSS2-ERG, TERT has detected PCa in biopsies with 95% sensitivity and 97% specificity. Analysis of 10 urine sediments has classified correctly 9 cases. The high PIN is a precancerous stage, more than 50% of it patients have adenocarcinoma. This fact explains the difficulties in diagnosis of high PIN versus PCa using markers of carcinogenesis. Thus, analysis of PCA3, TMPRSS2-ERG, TERT expression could be useful for PCa detection in the biopsies and urinary sediments of patients with elevated PSA.

J12.096 Vitamin D receptor gene BsmI, FokI, ApaI and TaqI polymorphisms and the risk of prostate cancer among men of Russian, Tatar and Bashkir ethnic origin.

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Prostate cancer (PCA) is one of common tumors among men in Russia. Low levels of vitamin D are implicated as a potential risk factor for PCA development, and vitamin D receptor (VDR) gene may be important in the ont-
set and disease progression. In this study, SNP variants rs2228570 (FokI), rs1544410 (BsmI), rs7975232 (ApaI), rs713216 (TaqI) in the VDR gene were investigated in PCa patients and controls of Russian (N=122 and N=95, respectively), Tatars (N=70 and N=170), and Bashkir ethnic origin (N=42 and N=99). The determination whether they are associated with PC risk. We evaluated the association between these SNPs in the VDR gene and PCa risk as well as clinical characteristics (prostate-specific antigen level, clinical stage, pathological stage) in men (237 PCa patients who underwent a prostatectomy and orchectomy) using logistic regression.

We did not observe significant differences for either the BsmI, ApaI and TaqI genotype and allele frequencies in PCa patients and healthy individuals. However, the frequency of the VDR “T/T” genotype of rs2228570 (FokI) was statistically different between PCa patients and controls of Tatar ethnic origin (OR = 1.88, 95%CI=1.22-2.58, p=0.002). There wasn’t association of the studied VDR BsmI, Apl and TaqI polymorphisms with clinical characteristics of PCa patients. We found linkage disequilibrium between the BsmI “A” and ApaI “C” alleles (D’=84%). Our study indicates that VDR FokI variant might increase PCa risk in Tatars. However, current limitation for small cohorts might have false positive effects; therefore it should be overcome via further large-scale validating studies.

**J12.097**

**Expression of Zn2+ metabolism genes in prostate cancer**


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During prostate carcinogenesis prostate tumor cells lose the ability to accumulate Zn2+ ions at high levels. The aim of this study was to investigate the expression of four genes ZIP1, ZIP7, MT1-F and MT2 involved in the maintenance of homeostasis of zinc cations in prostate cells in tumor tissue in and benign prostate hyperplasia (BPH) by RT-PCR and determine whether there is an correlation between the mRNA expression of four genes and the TNM classification, Gleason score, PSA level and age and thus evaluate its diagnostic and prognostic potential.

Isolation of mRNA from prostate cancer in 65 patients has been performed in period 2011 - 2013. As a control group, 27 patients with BPH were used. Statistically significant lower relative expression of MT1-F and ZIP1 genes was detected in prostate cancer tissue in than in BPH (p=0.00048, p=0.0082, resp.). The PSA level correlated positively with the ZIP7 expression (p=0.0099). Decreased expression of all four genes correlated with higher age. No correlation of the gene expression with the TNM classification and Gleason score was observed.

According to our results, it is possible to consider that the genes MT1-F and ZIP1 may be candidate tumor suppressor genes for prostate cancer. Supported by Diana Lucina, the Ministry of Health project for conceptual development of research organization 00064203 and by grant MSM 0021608008.

**J12.098**

**Association between the RAD50 rs17166050 polymorphism and risk of childhood leukemia and adult cancers**


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The RAD50 gene encode one of the protein of the MRN1-RAD50-NBR1 complex involved in cellular response to DNA damage and the maintenance of genome stability. The aim of this study was to answer the question whether the RAD50 rs17166050 SNP polymorphism may be associated with childhood leukemia or adult cancers. We estimated the frequency of RAD50 rs17166050 SNP polymorphism in the group of 220 children diagnosed with leukemia and 500 non-selected healthy children (HC) patients with a single laryngeal cancer (LC) and 115 with multiple primary tumors but one malignancy (primary or secondary) localized in the larynx (MPT-LC) and 68 multiple primary tumors localized in the head or neck (MPT) and children with acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS).

We evaluated the association between these SNPs in the RAD50 gene and PCa risk as well as clinical characteristics (prostate-specific antigen level, clinical stage, pathological stage) in men (237 PCa patients who underwent a prostatectomy and orchectomy) using logistic regression.

We did not observe significant differences for either the BsmI, ApaI and TaqI genotype and allele frequencies in PCa patients and healthy individuals. However, the frequency of the VDR “T/T” genotype of rs2228570 (FokI) was statistically different between PCa patients and controls of Tatar ethnic origin (OR = 1.88, 95%CI=1.22-2.58, p=0.002). There wasn’t association of the studied VDR BsmI, Apl and TaqI polymorphisms with clinical characteristics of PCa patients. We found linkage disequilibrium between the BsmI “A” and ApaI “C” alleles (D’=84%). Our study indicates that VDR FokI variant might increase PCa risk in Tatars. However, current limitation for small cohorts might have false positive effects; therefore it should be overcome via further large-scale validating studies.

**J12.099**

**Research of association between the SNP309 of MDM2 gene and the occurrence retinoblastoma in Algerian population**


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Retinoblastoma is an intraocular malignant tumor that usually affects the child in the first months of life. The gene responsible for this disease is an anti concogene called RB1. Retinoblastoma is due to the biallelic inactivation of RB1 gene. The loss of this gene does not confer a growth advantage due to the activation of p53 pathway. Events that would block or attenuate this pathway seem necessary for the development of retinoblastoma as the interaction between the MDM2 and RB1 in retinoblastoma tumorigenesis makes the SNP 309 T > G of MDM2 gene as a good candidate for the development of this cancer.

This study is based on the search for a possible association between the polymorphism 309 T>G of MDM2 gene and the occurrence of retinoblastoma in the Algerian population. We performed a case control study including 73 patients and 100 controls. The 309 T > G polymorphism was determined by PCR-RFLP method. Our results show that there is a significant increase of the G allele in cases compared to controls (p = 0.0002). Therefore, this allele seems to decrease susceptibility to retinoblastoma in our population.

**J12.100**

**Atypical RUNX1 rearrangements in patients with acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS)**

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The RUNX1 (AML1) gene is a key regulator of hematopoiesis which is frequently rearranged in acute leukemias and myeloid malignancies. More than 55 different translocations of RUNX1 have been described. However, majority has been reported rather sporadically and as complex RUNX1 rearrangements they remain undefined at the molecular level. A series of 21 adult patients with uncommon structural abnormality of 21q12-q22 detected by conventional cytogenetic/multicolor FISH methods and diagnosis of AML or MDS was collected. Three patients with no further available material were excluded from the study. All others were tested for involvement of the RUNX1 using FISH analysis with Vysis LSI TEL/AML or LSI AML1/ETO probe (Abbott). The split signal of RUNX1 gene was confirmed in four patients with: 1) t(8;21)(q22;q22);p13); 2) t(3;21)(q12;q22); 3) del(21)ins(21):10(q22)p51(14;21)(q12;q22); 4) del(12)(1;22)(p11.2;7)(q12;13)(q37); 5) del(21)p51(21)(q12); 6) del(21)q12(7)(q13-1;13q1;13). Further FISH analyses have been focused on the reciprocal translocation t(3;21)(q22;q22) and have been performed with BAC probes located at 3q11-12. The breakpoint has been specified between clones RP11-138C11 and RP11-135N1. In conclusion, four until now unreported RUNX1 alterations with the new potential fusion partners are described. One of them was specified to 293 kbp region at 3q12. Two protein and three RNA coding genes have been mapped at this interval. The description and identification of atypical RUNX1 fusions on the larger cohort of patients is an important tool for understanding and characterization of the pathogenic mechanisms of RUNX1 rearrangements. Supported by MHCR for conceptual development of research organization 00023736, RVO:VF641615/2012, GACR-P302/12/G157, RVO10/27/LF1/1.

**J12.101**

**SALL4 as a new biomarker for early colorectal cancers**

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Colorectal cancer (CRC) is one of the most commonly diagnosed cancers in the world. SALL4, newly identified oncogene, is a member of family of zinc finger transcription factors. Our aim in this study was evaluation of SALL4 mRNA absolute copy number in the peripheral blood of CRC patients, to in-

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duce a probable new prognostic or diagnostic molecular marker for CRC. Method: Peripheral mononuclear cells from 51 blood samples of CRC patients and 60 healthy controls, as well as 21 serum samples from the same patients were examined using absolute quantitative real-time RT-PCR to evaluate the copy number of SALL4 mRNA. Results: The blood copy number of SALL4 in recruited CRC patients was significantly higher in comparison with the healthy controls (p = 0.0001). This high copy number was not only inversely associated with the depth of tumor invasion (p=0.045), but also was significantly correlated with the higher grade of tumor differentiation (p=0.029). Furthermore, the copy number of SALL4 was also found to be elevated in all serum samples of CRC patients where high copy number of SALL4 was significantly associated with the higher grade of tumor differentiation (p = 0.0026). Discussion: Our results emphasize the potential of SALL4 as a biomarker for detection of early stages (I/II) of CRC which are not invaded to the adventitia. Since early detection of CRC is correlated to improved outcomes, SALL4 may be introduced as a critical biomarker for efficient screening of patients, who are in early stages of CRC tumorigenesis.

J12.102 CDH1 intronic mutation c.2440-6G>C: misclassification: not confirming earlier described splicing aberration.

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E-cadherin, encoded by the gene named CDH1, is a protein important for cell-to-cell adhesions. Germlinal mutations in the gene trigger hereditary diffuse gastric cancer (HDGC). In this work, we have investigated c.2440-6G>C transversion found in our patient with a family history of gastric carcinomas. The mutation that is located in acceptor splice site of the last exon was previously described as pathogenic, affecting splicing of the mutated intron (1). However, the identity of aberrant splicing variant (detected by RT-PCR from patient’s blood) and even the segregation of mutation in family was not so obvious. Recently, other research group found no aberrant mRNA in their patient carrying the same mutation (2). In our patient, we did not detect any splicing aberration in the blood-derived mRNA, nor did we see any difference in splicing when using our hybrid minigene system specialized for the analysis of the last introns/exons splicing. Furthermore, the mutation does not clearly co-segregate with the disease in our patient’s family, being detected in the unaffected father’s side of family whereas the mother’s affected side could not have been examined. In conclusion, we propose that this mutation should be regarded rather as benign or with uncertain effect on gene expression and HDGC development. Nonetheless, further investigations of mRNA from the affected tissue might be helpful.


J12.103 Correlation between amplification and expression status of ERBB1, MYC, Her2/neu, and TOP2A oncogenes in Iranian women with sporadic breast cancer and their relationship with Clinical and Immunohistochemistry parameters

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Objectives: Human epidermal growth factor receptor (HER) status is an important prognostic factor in breast cancer. There is no globally accepted method for determining HER2 status, and which method is most precise is still a matter of debate.

Methods: A total of 93 Iranian women with sporadic invasive breast cancer were studied. We analyzed HER2 mRNA expression by quantitative reverse transcription-PCR (qRT-PCR) and HER2 DNA amplification using multiplex ligation-dependent probe amplification (MLPA). To assess the accuracy of the RT-PCR and MLPA techniques, a combination of IHC and FISH was used, substituting FISH when the results of IHC were ambiguous (2+) and for those IHC results that disagreed with MLPA and qRT-PCR and named IHC/FISH.

Results: The correlations between IHC/FISH and qRT-PCR or MLPA were R=0.890 and 0.973, respectively. The ASCO/CAP guideline IHC/FISH correlation with MLPA was (0.827) and with RT-PCR was (0.854). The correlations between the IHC results (0, 1+, 2+, 3+ as negative and 3+ as positive) and qRT-PCR and MLPA techniques were 0.743 and 0.831, respectively. Conclusion: Given the shortcomings of IHC analysis and greater correlations between MLPA, qRT-PCR, and FISH methods than IHC analysis alone with each of these three methods, we propose that MLPA and real-time PCR are good alternatives to IHC. However a suitable cut-off point for qRT-PCR is a prerequisite for determining the exact status of HER2.

J12.105 Clinical Utility of Measuring Expression Levels of Stanniocalcin 2 in Patients with Colorectal Cancer

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Stanniocalcins (STC) are glycoprotein hormones which were originally found in the endemic gland of bony fish. Recently, Microarray data revealed that the expression level of Stanniocalcin was higher in tumors. Regarding the need for novel prognostic biomarkers, in place of the existing routine histopathological methods in early screening and the choice of chemotherapy options in colorectal cancer (CRC) we examined Stanniocalcin 2 expression levels in patients with CRC and assessed their association with clinicopathological data. We examined the mRNA expression levels of Stanniocalcin 2 in CRC in 48 tumor tissues and 48 marginal tissue samples using real-time reverse transcription-polymerase chain reaction (RT-PCR). Clinicopathological data of patients were collected after fulfilling criteria and the pathologic data of patients were collected after fulfilling criteria and the clinicopathological data. We examined the mRNA expression levels of Stanniocalcin 2 in CRC in 48 tumor tissues and 48 marginal tissue samples using real-time reverse transcription-polymerase chain reaction (RT-PCR). Clinicopathological data of patients were collected after fulfilling criteria and the pathologic data of patients were collected after fulfilling criteria and the clinicopathological data. We examined the mRNA expression levels of Stanniocalcin 2 in CRC in 48 tumor tissues and 48 marginal tissue samples using real-time reverse transcription-polymerase chain reaction (RT-PCR). Clinicopathological data of patients were collected after fulfilling criteria and the pathologic data of patients were collected after fulfilling criteria and the clinicopathological data. We examined the mRNA expression levels of Stanniocalcin 2 in CRC in 48 tumor tissues and 48 marginal tissue samples using real-time reverse transcription-polymerase chain reaction (RT-PCR).
can be as a marker (indicator) to differentiate between tumor borders and margins, potentially improving accuracy during surgery.

**J1.106**
Characteristics of hematologic malignancies with coexpression of (t(9;22) and inv(16))

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Background: The coexistence of the t(9;22) and inv(16), one of the most common cytogenetic abnormalities in patients with myeloid leukemia (AML), is critical for effective management of Acute Myeloid Leukemia (AML). The presence of this chromosomal translocation in myeloid leukemia suggests the initiation and progression of AML. The role of these chromosomal abnormalities in the development of myeloid leukemia continues to be clarified.

**J1.107**
Extended RET proto-oncogene screening in medullary thyroid cancer

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Introduction: Medullary thyroid carcinoma (MTC) is the most common form of thyroid cancer. The molecular pathology of MTC is constitutive of RET proto-oncogene mutations. RET mutations have an important role for diagnosis and confirming MTC. The most common mutation follow the in exons 10, 11, and 14-16. In this study some others exons examined in MTC patients. Material and methods: 298 participants were included, 196 patients (111 female, 85 male) and 102 relatives (58 female, 44 male). Genomic DNA was extracted from peripheral blood leukocytes and nucleotide change detection of exons 2, 3, 5, 8, 12, 17, 18 of the RET gene was performed by PCR and direct DNA sequencing methods. Results: A total of 10 different nucleotide substitutions were identified. There was not found any SNP in exons 5 and 8. One missense mutation (R982C) in exon18 and three synonymous mutations (A45A, V125V, G733G) were identified in exons 2, 3, and 12 respectively. A novel C>T intronic variant (intron17, chr position 10:43620286) was also found. Conclusion: This study was focused on some RET gene exons that did not consider comprehensively before. It seems, molecular screening of the RET gene in MTC patients should not be limited to hotspot exons. Probable deleterious, protective, and/or additive effects of these SNPs with or without RET hotspot mutations in MTC development remained to be clarified.

| rs24772739 | Intronic variant | A/G | - | 25.16 | 52 | 23 | 48 |
| rs72781214 | Intronic variant | A/G | - | 66.66 | 80 | 26 | 91 |
| rs2742236 | Intronic variant | C/T | - | 0.85 | 2 | - | 2 |
| rs2053030 | Intronic variant | G/A | - | 29.86 | 56 | 24 | 69 |

**J1.108**
TP53 Arg72Pro and MDM2 SNP309 polymorphisms and colorectal cancer risk: A West Algerian population study

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The tumor suppressor gene TP53 and its regulator MDM2 are both key players involved in multiple pathways including apoptosis, cellular transcrip- tion, and cell cycle regulation. Common germline polymorphisms in both of these genes may affect colorectal cancer susceptibility. An arginine-to-proline substitution at codon 72 in the p53 gene is reported to decrease apop- totic potential, while a thymine-to-guanine polymorphism at nucleotide 309 (named SNP309) of murine double minute 2 MDM2 gene increases its transcription. These two polymorphisms therefore may be of importance in colorectal carcinogenesis. The relation of these polymorphisms to colorectal cancer in the Algerian population was addressed in this study. DNA samples from 121 controls and 116 cases were genotyped for these two polymorphisms by PCR/RFLP then confirmed by sequencing. Unexpectedly no significant association was found between this potential marker TP53 Arg72Pro and CRC (p>0.05). However, our findings reveal that individuals with the SNP309 GG genotype are at a low risk of CRC (OR=0.49; 95% CI 0.24-0.98; p=0.04) relative to the TT genotype and with more significance in females (OR= 0.16; 95% CI 0.06-0.41, p<0.05). Moreover, no significant association was observed between the combined TP53 and MDM2 genotypes and colorectal cancer. Contrary to initial expectations that the GG genotype with high MDM2 levels will increase cancer risk, our results demonstrate that the MDM2 SNP309 GG genotype is associated with decreased risk of colorectal cancer. This is suggesting that other mechanisms independent of increased MDM2 levels can influence cancer susceptibility.

**J1.109**
Acquired uniparental disomy (UPD) involving the short arm of chromosome 17 in patients with myelodontlylastic syndromes (MDS) and complex karyotype

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Acquired uniparental disomy (UPD), which can be an important step in cancer development and progression, are found in various subtypes of haemato- logical malignancies including myelodysplastic syndromes (MDS). The aim of the study was to evaluate the frequency of 17p UPD in bone marrow cells of patient with newly diagnosed MDS and complex karyotypes and to assess correlation of 17p UPD with mutations of tumor suppressor gene TP53 located at 17p13.1. Bone marrow samples from 49 patients were analyzed by aCGH/SNP (BlueGene) and UPD of 17p was found in 7 of them (14%). All cases had deletion of 5q and additional recurrentchromosomal aberrations: 7q deletion (4x), monosomy 7 (1x), or 1p deletion involving ETv6 gene (5x). Average extent of 17p UPD was 14-20 Mb involving TP53 gene. In all 7 cases with 17p UPD, two copies of TP53 gene were detected by FISH (Abbott). DNA iso- lated from bone marrow cells of 5/7 patients was NGS-sequenced (Roche) and in all of them homozgous TP53 mutations were found. Our study confirm that acquired UPD 17p is a recurrent cytogenetic defect in patients with MDS with complex karyotypes and is strongly associated with homozygous mutations of TP53 gene. This lesion is cryptic unless analyzed by SNP array. UPD might have a fundamental role in tumorigenesis, therefore further characterization of 17p UPD will lead to better understanding of the initiation and progression of MDS.

Supported by RVO-46165, GACR P302/12/G157/1, PRVOUK-P27/ LF1/1.
Introduction: Uterine leiomyoma is one of the most common benign smooth muscle tumors, occurring in 20–40% of women in their reproductive years. Although the exact etiology of uterine leiomyoma is unknown, several predisposing factors such as age, nulliparity, obesity and genetic factors have been identified to be contributed to the pathogenesis of the disease. In regards to genetics factors contributing to uterine leiomyoma, a few studies have investigated the role of GSTM1 gene polymorphisms and incidence of uterine Leiomyoma. However, further studies are needed to clarify the effects of genetic variations in uterine leiomyoma. Therefore, this study was carried out to investigate the associations between GSTM1 null genotype and uterine leiomyoma in Iranian population.

Methods: In this case-control study, blood samples were collected from 50 women with uterine leiomyoma and 50 healthy controls. Genomic DNA was extracted and GSTM1 gene polymorphisms were detected using Gap-PCR. Allelic and genotypic association was evaluated by Chi-square and Fisher’s exact tests.

Results: The frequency of GSTM1 null genotype was significantly different (p = 0.01) in cases (42%) compared to controls (18%). In addition, the results indicated that the presence of GSTM1/0 genotype increased risk of uterine leiomyoma in case group compared to control group (OR: 3.56; CI 95%; 1.35-9.37; p = 0.01).

Conclusion: This study would be important to report the first data on the relationship between GSTM1 gene polymorphisms and risk of uterine leiomyoma in Iranian population. The findings revealed the association between the GSTM1 null genotype and uterine leiomyoma among Iranian population.

J12.111 Study of thrombosis factors related polymorphisms including PT (rs1799963), FGB (rs1800790) and PAI-1 (rs1799899) in Iranian affected women with uterine myoma

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Introduction: Uterine leiomyoma is one of the most common benign smooth muscle tumors, occurring in 20–40% of women in their reproductive years. Although the exact etiology of uterine leiomyoma is unknown, several predisposing factors such as age, nulliparity, obesity and genetic factors have been identified to be contributed to the pathogenesis of the disease. In regards to genetics factors contributing to uterine leiomyoma, a few studies have investigated the role of GSTM1 gene polymorphisms and incidence of uterine Leiomyoma. However, further studies are needed to clarify the effects of genetic variations in uterine leiomyoma. Therefore, this study was carried out to investigate the associations between GSTM1 null genotype and uterine leiomyoma in Iranian population.

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Conclusion: This study would be important to report the first data on the relationship between GSTM1 gene polymorphisms and risk of uterine leiomyoma in Iranian population. The findings revealed the association between the GSTM1 null genotype and uterine leiomyoma among Iranian population.

J12.112 Next generation sequencing in sporadic retinoblastoma reveals somatic mosaicism

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In about 50% of ‘sporadic’ cases of Retinoblastoma no constitutive RBP mutations can be identified. Recent research suggests that, at least in some of these cases, somatic mosaicism of RBP1 can be found. The increased availability of Next Generation Sequencing (NGS) technology improves our ability to detect the exact percentage of patients with RBP1 mosaicism. Using NGS we re-tested a series of 42 patients with sporadic Retinoblastoma. Twelve patients had constitutive mutations, in apparent heterozygosis, that were identified according to traditional techniques, whereas 30 patients had mutations. Among the 30 patients with no mutations, NGS identified the mutation in a ‘mosaic state’, varying between 8 and 27% in 3 cases. Among the cases with a supposed heterozygous carriers according to traditional methods, mutations in a mosaic state -varying from 3 to 28%- were found in 100 % of cases when, in addition to blood samples, it was possible to test more than 3 tissues (i.e. ocular tissue, urine and/or oral mucosa. Present results confirm that 10% of patients with sporadic retinoblastoma with no apparent mutations in RBP are actually cases with low-ratio mosaicism. In addition, our result show for the first time that many sporadic cases with 50% mutations in blood -that have been interpreted up to now as de novo mutations occurred at gametogenesis- could really be postzygotic events.
Complex chromosomal rearrangements in CML [no.]

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J12.115

Titled Targeting of Inflamed, Regenerative, and Mutated K-RasG12D Upregulated mRNA Microarray Gene Signatures in Pancreatic Ductal Cancers

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Pancreatic ductal adenocarcinoma (PDAC) harbors a universal K-RasG12D mutation that incites extracellular matrix (ECM) invasion and metastasis. Importantly, human Pancreatic Ductal Cancers (PDCs) recapitulate ERR2 phosphorylation and gene upregulation only within a 3D ECM. By differentiating PDCs with stepwise p53, Rb/p16INK4a, and K-RasG12D mutations, we developed a model that readily permits high-throughput pancreatic cancer microarray analysis of invasive gene signatures for personalized targeting. Specifically, populations of human PDCs in different chronological and phenotypic stages of neoplastic formation and murine PDCs isolated ex vivo from inflamed, regenerative, or neoplastic Pdx1-Cre;LSLKrasG12D/+ pancreata were acquired. RNA was extracted and its DNA was analyzed on genotypically-focused, invasion-specific microarray plates. As expected, K-RasG12D was the overwhelming driver of upregulated, invasive genes within the phenotypic and histologic neoplastic populations. Indeed, genes associated with cancer stem cells and necessary for PDCs to escape the ECM (metalloproteinases, tenascin-c, vitronectin and CD44) were induced 2.5-35-fold based distinctly on unregulated K-Ras signaling. Uniquely however, when PDCs were stratified to pathologic stage (inflamed, regenerating, neoplastic) or a mutational iteration (p53+ Rb/p16INK4a, K-RasG12D), there was substantial variation in the gene signatures regulating invasive potential. Indeed RNAi, shRNA, and pharmacologic inhibition of invasive genes and proteins successfully inhibited invasion or ceased neoplastic formation only if tailored to unique genes uncovered in that PDC population’s gene signature. As the majority of patient’s with PDAC ultimately die of metastasis, ‘personalizing’ the targeting of gene products is effective to optimize each PDC population’s invasive potential.

J12.116

Rare complex chromosomal aberrations in CML: A report on 300 cases

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CML is characterized by BCR-ABL mutation in over 90%, which indicates a favorable prognosis. Involvement of third or more chromosome(s) has been reported and thus merely been discussed for prognostication. However, management of leukemia varies considerably with the involvement of additional chromosome(s) and their impact on molecular investigation of such complex situation and pharmacological alteration of TKI for understanding prognosis of BCR-ABL in a complex situation.

J12.117

Common genetic variants in NEFL influence neuroblastoma risk

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The genetic etiology of sporadic neuroblastoma is still largely unknown. Using genome-wide association study, we identified single nucleotide polymorphisms (SNPs) associated with neuroblastoma at the LIN00340, BARD1, LM01, DISP12, HSD17B12, HACE1 and LIN28B gene loci, but these explain only a small fraction of neuroblastoma heritability. Other neuroblastoma susceptibility genes are likely hidden among signals discarded by the multiple testing corrections. Eight genes were selected based on their proven involvement in neuroblastoma differentiation. Here, we tested SNPs at the eight candidate genes for association with disease susceptibility in 2101 cases and 4202 controls. We replicated the associations of the identified gene in an independent cohort of 459 cases and 809 controls. Replicated associations were studied for disease using gene expression, transient overexpression and cellular differentiation assays. NEFL showed three SNPs associated with neuroblastoma (rs11949014; Pcombined=0.0050; OR=0.88, rs20799704; Pcombined=0.0072; OR=0.87, rs105911; Pcombined=0.0049; OR=0.86). The protective allele of rs105911 correlated with increased level of NEFL expression and we observed significant growth inhibition upon over-expression of NEFL, specifically in neuroblastoma cells carrying the protective allele. We also demonstrated that NEFL expression enhanced differentiated and impaired proliferation and colony growth in soft agar of cells with protective allele and basal NEFL expression while impairing invasiveness and proliferation of cells homozygous for risk genotype. Finally, high NEFL expression in diagnostic primary neuroblastomas was associated with better overall survival (P=0.03; HR=0.65; 95% CI=0.40-0.98). Our study shows that common variants of NEFL influence neuroblastoma susceptibility and indicates that NEFL likely has a role in disease initiation and progression.

J12.118

Characterization of the rs2802292 SNP identifies FOXXO3A as a modifier locus predicting cancer risk in patients with PJ syndrome

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ABSTRACTS PUBLISHED ABSTRACTS
surveillance in HPS patients. Further investigations will be needed to confirm our hypothesis and to ascertain whether differences exist in terms of therapeutic response across genotypes.

**J12.119** Gene expression of MMP9 and its prognostic role in patients with gliomas

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**Background:** Brain tumors, especially glioblastoma multiforme is an aggressive cancer characterized by extensive glioma invasiveness. Matrix metalloproteinase (MMP)-9 have been implicated to play a critical role in this process. **Methods:** In the present study we analyzed the expression of MMP9 mRNA in 59 gliomas (astrocytomas and oligodendrogliomas) and 14 nonneoplastic brain tissues. MMP9 gene expression was detected by real-time quantitative RT-PCR assay. **Results:** The expression level of MMP9 polymorphism, the output from each allele was analyzed by measuring the relative amounts of transcripts containing either I219 or V219 by direct sequencing of PCR amplified cDNA. The genes that caused the reduction of hemolytic activity could not be determined as the mutant strains carried multiple gene mutation. In the previous study, hly gene was reported to cause hemolysis. Hly gene is repressed by hap, which is expressed late in infection.

**J12.120** Individual variability in escape from nonsense mediated decay may influence the clinical severity of patients with nonsense mutations in the upstream region of the MHL1 gene

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We previously reported the MLH1 S131X as founder mutation in patients with the Lynch syndrome from Macedonia. The mutation has a high penetrance with different time of disease onset ranging from 32 to 55 years. We speculated that this variability might in part result from a leaky nonsense mediated decay (NMD) of mutant transcripts, which results in a truncated protein and dominant-negative effect in patients with a more severe disease. Herein, we present in vivo expression data of the S131X mutation in PBMs and 3 tumors of 2 patients with the time of onset at the age of 33 and 49. Since both patients were also heterozygous for the common I291V polymorphism, the output from each allele was analyzed by measuring the relative amounts of transcripts containing either I219 or V219 by direct sequencing of PCR amplified cDNA. The genes that caused the reduction of hemolytic activity could not be determined as the mutant strains carried multiple gene mutation. In the previous study, hly gene was reported to cause hemolysis. Hly gene is repressed by hap, which is expressed late in infection.

**J13.02** Hemolysis to Red Blood Cell

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Hemolytic due to hemolysis, the abnormal breakdown of red blood cell (RBCs), either in the blood vessels (intravascular hemolysis) or elsewhere in the human body (extravascular). It has numerous possible causes, ranging from relatively harmless to life-threatening. The general classification of hemolytic is either inherited or acquired. Treatment depends on the cause and nature of the breakdown. Symptoms of hemolytic anemia are similar to other forms of anemia (fatigue and shortness of breath), but in addition, the breakdown of red cells leads to jaundice and increases the risk of particular long-term complications, such as gallstones and pulmonary hypertension. In the present study, the V. cholerae were tested for their ability to cause hemolysis. The WT caused an excessive destruction of RBCs. However, several V. cholerae mutants did not hemolyse the chicken RBCs as the WT did. The genes that caused the reduction of hemolytic activity could not be determined as the mutant strains carried multiple gene mutation. In the previous study, hly gene was reported to cause hemolysis. Hly gene is repressed by hap, which is expressed late in infection.

**J13.03** Postreplicative semihistonal form of the chromatin is cause of education and disappearance of chromosomes during cell cycle

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It is suggest a hypothesis, that replication creates a new form of chromatin, which exists from G2 period of interphase to telophase. The replication doubles of DNA, but the amount of nucleosomic histones in the nucleus remain the same as before replication in G1 period. Therefore daughter nucleofilaments of G2 period have twice less histones than before replication. This is a new post-replicative semihistonal form of chromatin, “semichromatin”. Helix formation and condensation are important structural properties of semichromatin. They are responsible for the formation and development of the chromosomes. It is known that helix formation and condensation last in the chromosomes during of prophase - metaphase.

Consequently, the chromosomal material is semichromatin of the nucleus all this time. The hypothesis shows that the nucleus doesn’t contain a stock of free histones, and a nuclear pores can’t pass quickly all histones of cytoplasm in a nucleus. The cytoplasmic histones reach semi-chromatin of chromosomes only after the destruction of the nuclear membrane in prometaphase. They gradually double the number of histones of semichromatin of chromosomes in a anaphase - telophase and return it into full for histones threadlike chromatin, ie nucleofilamently. Thus, replication creates a semihistonal form of chromatin and it is the reason of formation of chromosomes. Synthesis of new histones in cytoplasm fills histones semichromatin and return in full histones chromatin, i.e. synthesis of new histones is the reason of disappearance semichromatin and from chromatin from view a light microscope.

**J13.01** Genetic alterations in breast cancer patients from Saudi Arabia by FISH

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Breast cancer remains a worldwide public health concern. The incidence and mortality of breast cancer varies significantly in ethnically and geographically distinct populations. In the Kingdom of Saudi Arabia, it ranks number one in terms of incidence as well as cancer related mortality in females. Although the age-standardized incidence rate for breast cancer is lower in Saudi Arabia than in United States, the median age of onset is 47 years, significantly lower than 62 years observed in patients from United States. Amplification of the two oncogenes: Her-2/neu and c-myc and deletion of the tumor suppressor gene p53 are frequently encountered in breast carcinomas. Our objective was to evaluate the association between Her-2/neu, c-myc, p53, and clinicopathologic variables in breast cancer patients using fluorescence in situ hybridization (FISH). FISH analysis for Her-2/neu, c-myc, and p53 was performed on 60 samples with breast carcinomas and 22 samples with benign breast lesions. Amplification of HER-2/neu was seen in 7/60 (11.7%) cases and amplification of c-myc was seen in 11 of 60 (18.3%) cases; neither was associated with adverse clinicopathologic variables or survival. Deletion of p53 was seen in 29/60 (48.3%) cases and was associated with poor histologic grade, compared to the benign group. There was impact of genetic alterations on overall survival and disease-free interval. The results indicate that the p53 gene plays a significant role in breast carcinogenesis and the early onset of the disease among Saudi female individuals.
J13.04
Alkyl Mercury chloride compounds induced genotoxicity in human blood cultures and corrective role of Ascorbic acid (Vitamin C)
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the National research center, Cairo, Egypt.
Methyl mercury chloride is a xenobiotic metal that is highly deleterious environmental pollutant. The biotransformation of mercuic chloride (HgCl2) into methyl mercury chloride (CH3HgCl) in aquatic environments is well-known and humans are exposed by consumption of contaminated fish, shellfish and algae. The genotoxicity induced by mercury compounds remains controversial. Therefore we have investigated the genotoxic effect of methyl mercury chloride (MMC; CH3HgCl) at two concentrations (100, and 1000 μg/L) and the role of ascorbic acid (Vitamin C) at a single concentration of (9.734 mm) on MMC-treated short-term human lymphocyte cultures. We assessed the chromosomal aberrations (CAS), sister chromatid exchange (SCE) and Comet assay in MMC-treated and MMC-treated lymphocyte cultures with and without Vitamin C supplementation. The results showed that MMC has increased the frequency of CAS and SCE/cell in a dose-dependent manner than an control value of 0.83EGCl1 also, induced DNA damage in determined by Comet assay. These effects were prevented by the addition of Vitamin C to MMC-treated lymphocyte cultures. Data revealed that, mutagenic activity of MMC and the protective role of Vitamin C on mercury compounds-induced genotoxicity in human lymphocyte cultures is probably due to its strong antioxidant and nucleophilic nature.

J13.05
Application of cytogenetic in Biodosimetry
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The evolution of the world demand will make that Algeria will develop, in a near future; nuclear energy. This will require a competence in this field, and particular in the evaluation effect of the radiations emitted in the event of radiobiological accidents. The importance of the effects of ionizing radiation is to increase the risk of cancer or genetic defects. The most reliable method of biological dosimetry, is analysis of chromosomal modifications radiation-induced, particularly; the dicentric chromosomes, in lymphocytes of peripheral blood. For this purpose, we established two calibration curves Dose / effect, by the methods of cytogenetic in uniform staining. The selected experimental conditions are the same as those used by the team of Prof. J. E. BARQUINERO (University Autonoma, Barcelona, SPAIN). The first was established like witness, from the blood of a Spanish person. As for the second, it was conceived for the Algerian laboratory from a blood sample of an Algerian person, without history to exposure to radiations. The frequency of dicentric chromosomes increases as dose and the statistical analysis shows that the values obtained follow a Poisson distribution revealing thus that the irradiations were correct and homogeneous. The two curves obtained from the dicentric analyses, obey the quadratic linear model and the relation Dose / effect is expressed by the equation: Y = C + aD + βD2. This study has allowed us, in Algeria to establish a dose effect curve for biodosimetry laboratory which could in the future be part of an international biodosimetry network.

J13.06
Dysgonosomies at University Hospital Hassan II Fes: Cytogenetic and molecular aspects
K. Belhassan
CHU FES, genetics department, Fes, Morocco.

Abstract. The dysgonosomies are represented by all the anomalies of the X and Y sexual chromosomes.

The main dysgonosomies are represented by Klinfelter syndrome which includes the presence of at least one extra X chromosome in a masculine karyotype 46, XY and Turner syndrome, which is linked to the complete or partial absence of X chromosome. For this purpose, we established two calibration curves Dose / effect, by the methods of cytogenetic in uniform staining. The selected experimental conditions are the same as those used by the team of Prof. J. E. BARQUINERO (University Autonoma, Barcelona, SPAIN). The first was established like witness, from the blood of a Spanish person. As for the second, it was conceived for the Algerian laboratory from a blood sample of an Algerian person, without history to exposure to radiations. The frequency of dicentric chromosomes increases as dose and the statistical analysis shows that the values obtained follow a Poisson distribution revealing thus that the irradiations were correct and homogeneous. The two curves obtained from the dicentric analyses, obey the quadratic linear model and the relation Dose / effect is expressed by the equation: Y = C + aD + βD2. This study has allowed us, in Algeria to establish a dose effect curve for biodosimetry laboratory which could in the future be part of an international biodosimetry network.

J13.07
Genetic heterogeneity of Fanconia anemia (FA)-A in Egyptian patients
G. Y. El-Kamah, M. H. El-Dabasoumi, A. M. Salem1, W. A. Zarouk1, M. M. Eid2, R. M. Mossad3, A. A. Sayed4, S. A. Tantamy5
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Fanconi anemia (FA)-A is the most frequent complementation group and is detected in approximately two-thirds of studied FA patients in most countries. The aim of the study was to screen the common mutations previously reported in the international literature within exons 27, 34, 38, and 43 of the FANCA gene among Egyptian FA patients. Patients and methods: The study included 24 Egyptian FA patients of unrelated consanguineous pedigrees and diagnosed by positive chromosomal breakage studies using diepoxynitrate. Ten healthy unrelated individuals matching age and sex were included as the control group. Genomic DNA amplification, sequencing of exons 27, 34, and 43 of the FANCA gene, and restriction enzyme analysis for the exon 38 intronic polymorphism were performed for patients and controls. Results: No mutations were detected within the studied FANCA gene exons. Conclusion: This is the first molecular study of FA in Egyptian patients that proved absence of the common mutations previously reported in other countries this may denote molecular heterogeneity in Egyptian patients. Further studies are recommended to establish the underlying mutations responsible for Egyptian FA cases as an important step in disease control.

J13.08
Over expression of the glycoprotein P human in chemoresistance
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The "Multidrug resistance" (MDR) is an important obstacle to the success of the chemotherapy of many human cancers. The cellular multi chemoresistance is due to the over expression of the glycoprotein P, which confers the phenotype MDR to the cells. The aim of these researches if the localization of the fixation site of the steroid chemosensitizing in order to prepare efficacious modulating molecules of the MDR "phenotype". The first object has consisted in increasing the expression level of the Pgp in the R7 cells born of (descended from) a patient attacked by an ethrroleuchaines expressing concentrations of doxorubicin. The characterization of the Pgp presence has been realized by affinity phoshomarking with azidine tritiée. An augmentation of the Pgp concentration is observed in the membranous fractions of the R7 cells treated by the doxorubicin. The second has consisted in different radio active chemomarkers by pepticic coupling of bromo acetic [14C] the terminal amine of the pyrogesterone derivatives substituted on the carbon 11 but the introduction of different hydrophobic chains, and in testing these chemomarkers with the membranous fractions of the R7 cells treated with the doxorubicin.

J13.09
The diversity of MED12 gene mutations in uterine fibroid cells

Uterine myoma (UM) is a benign and most common tumor that affects 20-45% of women of fertile age. Previously it was shown that somatic mutations in the MED12 gene occur in most women with uterine myoma. We analyzed exon 2 nucleotide sequence of MED12 gene from 94 DNA samples extracted from fibroids, endometrium, myometrium cells and peripheral blood leukocytes of 17 women with uterine leiomyoma. Twenty-four samples exhibited various MED12 gene mutations. No mutations were found in 22 of 27 myometrium cells and peripheral blood leukocytes. Two mutations have been identified in exon 44 from endometrial tissue sample. In 22 of the 47 samples (47%) isolated from fibroids we found different mutations of the MED12 gene. Each fibroid is characterized by its MED12 mutational profile. The diversity of MED12 gene mutations in uterine fibroid cells allows better medical and surgical care and proper genetic counseling.
proliferation and blast transformation.

J1.10 Serotonin effects on NDUFS2 gene expression, a schizophrenia animal model research
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Schizophrenia is a chronic and disabling psychiatric disorder with unknown cause that affects about 1% of the population worldwide. In last researches we have seen a significant higher expression of ndufs2 in schizophrenic mouse models in compare with normal controls. We have studied expression changes of 200 schizophrenic mouse models with and without serotonin treatment. Based on sex and treatments program, Mouse models have separated to four groups. Group A (male) and B (female) have been treated with serotonin (40 microg/kg body weight/day) for 6 weeks, and group C (male) and D (female) did not treated with serotonin. All mouse models were in the same age (15-16 weeks old) and all situations including air, light, feeding and etc were completely similar to four groups. After this period, mouse models killed and RNA isolated from prefrontal and hypo camp of their brains. We have investigated ndufs2 expression by using qRT-PCR. Results show significant lower expression of ndufs1 in models of group A and B in compare with models of group C and D. Also normal control group results confirmed our last researches output about increasing expression of ndufs2 in schizophrenic mouse models. Control group gene expression rate was lower than all other four groups. This research showed a significant relation between serotonin treatment and ndufs2 expression in brain but since the neuro-transmitter does not easily cross the blood-brain barrier the mechanisms of serotonin effect to genes expression in prefrontal and hypo camp are not clear and needs more studies.

J1.11 Extended expression of promyelocytic leukemia (PML) during in vitro neural differentiation process of mouse embryonic stem cells (mESCs) purposes the importance of PML in cellular pluripotency and nervous system development
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Pro-myelocytic leukemia (PML) is one of the major proteins in promyelocytic leukemia nuclear bodies (PML-NBs). PML gene has located on 9B mouse chromosome. Retinoic acid (RA) exerts its tumor growth suppressor activity and terminal myeloid differentiation of granulocyte/monocyte progenitor (GMP) cells via PML-NBs in RA pathway. In addition, RA as a natural morphogen guides posterior patterning in embryo neural development. Based on these reasons, the aim of this study was to define if there was any revenue for PML-NBs in RA dependent neural development. For this reason, RA was used as a neural inducer for in vitro neural differentiation of mouse embryonic stem cells (mESCs). In mESCs, neural precursor cells (NPCs) and neural cells (NCs) obtained from this differentiation process PML mRNA and protein levels were assessed by quantitative real time RT-PCR (q-RT-PCR) and western blotting. qRT-PCR results showed that Pml had a maximum expression in NPCs and the expression clearly had decreased in NCs and NCs, although no significant differences existed between two latter groups. Interestingly, three un-foresight protein bands about 170, 130 and 70 kDa similarly were detected in these cell types on western blots. Based on qRT-PCR results, PML expression may have an important role in cellular pluripotency. However, the appearance of three similar bands in western blots from PCR results, PML expression may have an important role in cellular pluripotency. Although till now ESCs, NPCs, and NCs led us to assumption that PML might be necessary in pluripotency. However, the appearance of three similar bands in western blots from PCR results, PML expression may have an important role in cellular pluripotency and nervous system development. For this reason, looking for random aneuploidy, we applied FISH technique, using different probes, to amniocytes from pregnancies with trisomies 21 and 18, and 47,XXX (study group) and compared them to amniocytes from pregnancies with normal karyotypes (control group). A significantly higher rate of random trisomy and more triploids were observed in trisomy 21 and 18 cases. However, in the 4 cases of 47, XXX higher rates of random trisomies, but not trisomy or triploidy were observed. Monosomies appeared in both study and control groups, which could be the result of technical problems. The observed differences in the random aneuploidy rates between the somatic and sex chromosome aneuploidies might reflect different mechanisms of random aneuploidy between the two types. Triploidy was significantly higher in the somatic aneuploidies compared to the sex aneuploidy and control groups. As previously shown in CML patients, triploidy, which occurred more often in the study group, probably reflects an increased predisposition to develop malignancy compared to random aneuploidy. In most aneuploidies, malignancy will develop as a result of an oncogenic event that occurs in addition to the existing genetic instability. Occurrence of triploidy may reflect such an event.

J1.13 Random Aneuploidy in Amniocytes from Aneuploidic Pregnancies
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Aneuploidy may represent genetic instability. Amniocytes of fetuses with aneuploidy carry characteristics of genetic instability. Individuals with chromosomal aneuploidies may develop various types of malignancies. In this study, looking for random aneuploidy, we applied FISH technique, using different probes, to amniocytes from pregnancies with trisomies 21 and 18, and 47,XXX (study group) and compared them to amniocytes from pregnancies with normal karyotypes (control group). A significantly higher rate of random trisomy and more triploids were observed in trisomy 21 and 18 cases. However, in the 4 cases of 47, XXX higher rates of random trisomies, but not trisomy or triploidy were observed. Monosomies appeared in both study and control groups, which could be the result of technical problems. The observed differences in the random aneuploidy rates between the somatic and sex chromosome aneuploidies might reflect different mechanisms of random aneuploidy between the two types. Triploidy was significantly higher in the somatic aneuploidies compared to the sex aneuploidy and control groups. As previously shown in CML patients, triploidy, which occurred more often in the study group, probably reflects an increased predisposition to develop malignancy compared to random aneuploidy. In most aneuploidies, malignancy will develop as a result of an oncogenic event that occurs in addition to the existing genetic instability. Occurrence of triploidy may reflect such an event.

J1.14 Small Supernumerary Marker Chromosomes Originating From Chromosome 10 Associated With an Apparent Normal Phenotype
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A normal 40-years old Italian woman was referred for cytogenetic evaluation before having undergone controlled ovarian hyperstimulation, in vitro fertilization and embryo transfer. Family history was noncontributory except for her long-term infertility. QFQ-banding revealed a karyotype 47,XXX,+mar[49]/46,XX[51] with one SMC. Studies by AGH and FISH confirmed that the marker originated from chromosome 10, with duplication of 10p11.21p11.1 segment. Molecular cytogenetic characterization in her parents, sister and brother defined a normal karyotype 46,XY for father and brother while the mother was 47,XX+mar[61]/46,XX[39] and sister was 47,XX+mar[77]/46,XX[23] with one SMC derived from chromosome 10. Chromosome 10 is rarely involved in the formation of marker chromosomes. Eight cases have been reported in literature and in six out of these, SMCs were associated with an increased risk of abnormal phenotype. In our case, there was no apparent normal phenotype, probably associated with minimal involved material and gene content. Some SMCs derived from the same chromosome, but in spite of this, a great variation in phenotype was observed, probably due to the degree of mosaicism that may vary in different patients as well as in different tissues in the same patient. Furthermore, most SMCs were ascertained to present with chromosomal abnormalities, but it isn’t always possible to correlate the phenotype with SMC; so it is difficult to predict the precise phenotype-karyotype correlation and phenotypic outcome. This report shows the importance of cytogenetic characterization in patients with comparable chromosome defects, giving the possibility of identifying similarities in the clinical picture that will benefit the counseling of future cases.

J1.15 Avall-Taqi-HindIII represents a novel informative haplotype at the β-globin gene cluster: Application in carrier detection and prenatal

ABSTRACTS PUBLISHED ABSTRACTS
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diagnosis of beta-thalassemia in the Iranian population
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Thalassemia is one of the common monogenic disorders, with a high demand for carrier detection and prenatal diagnosis in the Iranian population. Although there is a large number of mutations associated with the disease, the polymorphic markers present in the beta-globin cluster region were commonly used in linkage analysis of the disease. Markers usually show a population dependent base haplotype frequency. Among the polymorphic markers, five markers including AvaII, RsaI, HinfI, TaqI and Hind III were genotyped in 150 unrelated healthy individuals from the Iranian population. To evaluate the linkage disequilibrium (LD) was estimated by MIDAS program. The LD analysis showed that the 150 population had normal karyotype (46, XY) and DNA damage in these cells were not observed. In addition, HDF cells showed normal karyotype in early passages (1-3) but using comet assay, abnormality and DNA damages in positive control (HDF cells treated with 200 μM H2O2) were observed. The parameters of alkaline comet assay of IPS cells and HDFs compared with positive control group were statistically significant (p<0.05). These findings indicated that comet assay is a sensitive technique and should be performed before performing the functional experiments on IPS cells, cytogenetic stability of these cells must be studied. Therefore, for precise evaluation of DNA damage and cytogenetic stability of these cells, both techniques could complete each other.

J13.19
Developmental role of NF-Y as an epigenetic element on regulatory region of SALL4 gene in human embryonic carcinoma cells
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Introduction During development, the transcription factors in HDF cells after transduction. According to these results, the presence of an intact SRY homeobox region and the ZFY region, instead of the regions AEF, ABC, AEF, are deleted. Fluorescence in situ hybridization (FISH) with chromosome paints for chromosome Y and with subtelomeric probe for 10q, showed the presence of rare aberration with an unbalanced karyotype constituted by 45 chromosome Y and 46 chromosome X. Then, the target probes showed a semiosis of the region 10q26.3-qter and a deletion of Yp11.2-qter. The breakpoints were, later, by molecular genetic analysis (Agilent 8x60k array-GH), ascertained. The karyotype is: 45,Xder(10)(Y;10)(q26.3;p11.2)arr Yp11.2(2,931,713-28,548,485)x0,10;q26.3(13,151,631-135,404,523)x1.

Results
The results of ChIP real-time PCR analysis clearly showed a considerable incorporation of NF-Y complex on the regulatory region of SALL4 gene. The data suggested that RsaI and HinfI markers might be excluded as strong molecular diagnostic markers in beta-thalassemia carriership and prenatal diagnosis in the Iranian population.

J13.18
A rare case of a 12-year-old boy with a 45,X karyotype: cytogenetic and molecular genetic
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We report the case of a 12-year-old boy of non consanguous parents. He was born by uterine-incision delivery, weighing 2480 kg and measuring 47 cm. The present height was of 139 cm (height SD score -2.2) and to parental target of 176 cm and weight of 26.3 kg. BMI of 13.61 (SD score-2.98 DS) and head circumference of 52 cm (10 percentile). At physical examination he presented long face, sharp chin, prominent ears, malar hypoplasia, pectus excavatum, joint laxity, cubitus valgus and severe scoliosis. The genitalia were normal for male phenotype but the left testicle were not yet in scrotum. He presented mild mental retardation, motor and speech delay. The ultrasound examination of heart was normal. Standard cytogenetic analyses showed a 45,X karyotype. Y microdeletion analysis showed the presence of an intact SRY homeobox region and the ZFY region, instead of the regions AEF, ABC, AEF, are deleted. The karyotype is: 45,Xder(10)(Y;10)(q26.3;p11.2)arr Yp11.2(2,931,713-28,548,485)x0,10;q26.3(13,151,631-135,404,523)x1.

Thalassemia is one of the common monogenic disorders, with a high demand for carrier detection and prenatal diagnosis in the Iranian population. Although there is a large number of mutations associated with the disease, the polymorphic markers present in the beta-globin cluster region were commonly used in linkage analysis of the disease. Markers usually show a population dependent base haplotype frequency. Among the polymorphic markers, five markers including AvaII, RsaI, HinfI, TaqI and Hind III were genotyped in 150 unrelated healthy individuals from the Iranian population. To evaluate the linkage disequilibrium (LD) was estimated by MIDAS program. Among the 31 possible haplotypes, seven haplotypes showed relatively high frequencies ≥ 5%. The haplotype AvaII-TaqI-HindIII could be suggested as an informative haplotype for possible carrier detection and prenatal diagnosis of beta-thalassemia in the Iranian population. Moreover, RsaI and HinfI (located in the hotspot region) were not associated with the 5’ sub-haplotypes or 3’ sub-haplotypes. The data suggested that RsaI and Hind markers might be excluded as strong molecular diagnostic markers in beta-thalassemia carriership and prenatal diagnosis in the Iranian population.
genetic diagnosis of Bardet-Biedl syndrome by MiSeq exome sequencing

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Bardet-Biedl syndrome (BBS) is a rare autosomal-recessive ciliopathy characterized by obesity, postaxial polydactyly, retinitis pigmentosa, mental retardation and kidney abnormalities. At least 18 BBS genes have been identified as causative of BBS, therefore genetic testing for this disease is highly complicated. We consulted a family with strong clinical features of BBS in 2 children. While considering the labor and consumable costs for analysis of at least 7 “common” BBS genes (BBS1, BBS10, BBS2, BBS6, BBS9, BBS12 and BBS13), we opted for exome sequencing as a viable alternative to multiple single-gene tests. Using Illumina MiSeq platform, we identified homozygous BBS7 c.1967_1968delTAinsC mutation in 2 affected siblings; both parents and their healthy son were heterozygous for this allele. Presence of an identical gene defects in consanguineous parents is uncommon for BBS, however Russian population was repeatedly shown to have an unusually pronounced founder effect. We are currently examining whether the above mutation is indeed recurrent in Russia: at the time of abstract submission, we genotyped 17 healthy donors and identified 1 (0.06%) additional carrier of BBS7 c.1967_1968delTAinsC.

We conclude that even medium-throughput next generation sequencing platforms may facilitate clinical diagnosis of genetically heterogeneous disease.

Detection of Borna Disease Virus in peripheral blood cells of a number of obese patients in Iran via Nested RT-PCR

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Worldwide prevalence of obesity trends attracts attention toward the multiple origins of it. The most overlooked one is infectious factor, and among them viruses are the most noticeable. Borna Disease Virus is a nonsegmented, single-stranded RNA virus that usually causes sporadic neurological disease in horses and sheep as its natural host. Although BDV is considered as a non-human virus, recently Serological and molecular evidences investigated the possible association of BDV with specific psychiatric diseases in humans. But the point that we choose it as an etiology of human obesity is that BDV is identified as a cause of rapid increase of body weight with development of obesity syndrome without obvious neurological signs in Experimentally cerebrally infected Lewis rats. In this study which was done for the first time in Iran, by using nested RT-PCR technique, we demonstrated that BDV RNA was present in peripheral Blood Mononuclear cells of a number of obese patients. 43 subjects took part in this study. The BMI in kg/m2 of the obese subjects were over 30; the detection rate is about 16/2% sequencing of positive samples confirmed our finding. These results illustrated the adiposity-promoting effect of BDV occurs in Human being.

Reliable and cost effective screening of 16 breast cancer gene panel using sequence capture method coupled with next-generation sequencing in clinical settings

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BRCA1 and BRCA2 are two well-known genes in the background of hereditary cancer syndromes. There is also evidence that several other genes play an important role in the pathogenesis of these type of diseases. Latest population-scaled studies showed that certain mutations in different genes could cause as high risk elevation as BRCA2 mutations. In this study we present a reliable and cost-effective method to analyse the risk assessment of mutations of cancer genes. The method is based on the same platform combined with benchtop non-optical next-generation sequencing we were able to achieve short turn around time with the screening of 16 genes that could be associated with an increased risk of breast, ovarian and other types of cancer. The analysis of this 16-gene set can explain the inherited background of almost 30% of hereditary and familiar cases of breast and ovarian cancers. Thus, it opens up a high-throughput approach with fast turnaround time to the genetic diagnostics of these disorders and may be helpful to investigate other familial genetic disorders as well.
J14.07 Chromosomal microarray analysis as first-tier clinical diagnostic test: Estonian five years experience

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Chromosomal microarray analysis (CMA) is now established as the first-tier cytogenetic diagnostic test for fast and accurate detection of chromosomal abnormalities in patients with developmentally delayed/intellectual disability (DD/ID), autism spectrum disorders (ASD), and autism spectrum disorder (ASD). We present our experience with using CMA for postnatal and prenatal diagnosis in Estonian patients during 2009-2013. Since 2011 CMA is on the official service list of the Estonian Health Insurance Fund and is performed as the first-tier cytogenetic test for patients with DD/ID, autism spectrum disorders (ASD), and ASD. A total of 1902 patients were analyzed, including postnatal [1692 (89%)] patients and 106 (6%) family members] and prenatal referrals [104 (5%)] patients]. Abnormal results were reported in 500 (26%) patients, with a total of 585 findings (1-5 per individual): 217 (37%) deletions, 174 (30%) duplications, 176 (30%) long contiguous stretches of homozygosity (LCSH) events (>5 Mb), and 18 (3%) aneuploidies. Of all findings, 230 (39%) were defined as (likely) pathogenic for 271 findings (46%), most of which were LSCH (15%), the clinical significance remained uncertain; 90 (14%) reported findings were classified as likely benign. Clinically relevant findings were detected in 201 (11%) patients. However, the proportion of variants of uncertain clinical significance was high (46% of all findings) demonstrating that the interpretation of CMA findings remains a rather difficult task. Close cooperation between clinicians and cytogenetists, as well as further data sharing with colleagues are the cornerstones of successful CMA application in clinical practice.

J14.08 Clonal evolution in prospective analysis of chronic lymphocytic leukemia patients detected by FISH and conventional cytogenetics after stimulation with CpG oligonucleotides and interleukin-2

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Introduction: Chromosomal abnormalities are important prognostic factors in chronic lymphocytic leukemia (CLL). During the course of the disease clonal evolution (CE) may occur. CE, defined as acquisition of new cytogenetic aberration, is associated with shorter overall survival. In the majority of published analyses to date, CE has been monitored using FISH. Therefore, the aim of our study was to prospectively assess CE frequency, as well as to assess the role of other prognostic factors and therapy in development of poor-prognosis CE. Methods: Between 2008 and 2012, 140 patients with previously untreated CLL were evaluated by FISH (deletion 11q, 13q, 17p, trisomy 12, rearrangement 14q32) and CBA after stimulation. Peripheral blood samples of each patient were provided for baseline and follow-up testing. Mutation status of BCR-ABL1, IGH-VH, TP53, ATM, NOV, and NTRK1 was determined by Sanger sequencing. The role of other prognostic factors and therapy in development of poor-prognosis CE was investigated using univariate and multivariate logistic regression. Results: The event-free survival at 5 years was 75% in the whole cohort. The CE frequency was 11% (15/140) at baseline and 14% (19/136) at follow-up testing. No correlation was found between the CE frequency and any of the following baseline parameters: age, gender, Binet stage, Rai stage, lymph node involvement, and presence of adverse cytogenetic abnormalities. Conclusion: The frequency of CE in untreated CLL is low and not associated with any baseline characteristics. Further prospective studies including larger cohort size and a larger set of prognostic factors are needed to confirm these results.

J14.09 Submitting biomedical data to the European Genome-phenome Archive (EGA)

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The European Genome-phenome Archive (EGA), a service of the European Bioinformatics Institute (EBI), is a permanent archive for all types of potentially identifiable genetic and phenotypic data that has been consented for use in biomedical research, but not for open public distribution. The EGA includes major reference data collections for rare and common diseases, including data derived from the UK10K project, Wellcome Trust Case Control Consortium (WTCCC) and International Cancer Genome Consortium (ICGC), as well as control sets that can be used in addition to the public reference panels such as the 1000 Genomes project. Accepted submissions include manufacturer raw data from genome sequence, transcriptome, epigenome or proteomics experiments. The EGA also stores called variants, genotypes, study summary statistics and associated sample phenotypes. Submission tools designed to facilitate the secure upload of data files and associated metadata are accessible for each submitter using a single log-in and may be run as graphical interface or from the command line. The functionality of the submission tools may be incorporated into submission pipelines for large-scale submitter. The EGA follows strict protocols for information management, data storage, security and dissemination. Authorized access to the data is managed in partnership with the data providing organizations. Future plans include expanding the EGA into a distributed network of data archive and distribution services. A pilot project has already started in collaboration with the Centre for Genome Regulation (CRG) in Barcelona, Spain. The EGA is currently available at www.ebi.ac.uk/ega/.

J14.10 Detection of EGFR gene amplification in head and neck squamous cell carcinoma with paralogue ratio test

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Introduction: EGFR signaling pathway has an important role in the development of head and neck squamous cell carcinoma (HNSCC). Therefore any mutation that affects its components, may influence the course and management of the disease. The amplification of the EGFR gene is such significant event that is present in HNSCC. We compared the efficacy of a targeted detection of the EGFR gene amplification with the paralogue ratio test (PRT) to results obtained with the array comparative genome hybridization (aCGH).

Methods: Samples of genetic DNA extracted from tumors of 50 patients with various stages of HNSCC were first analyzed with the aCGH method. An oligonucleotide pair that maps inside the amplified EGFR region was selected from a collection at www.prtprimer.org and used for PRT analysis.

Results: The aCGH analysis identified EGFR amplification in 7 samples, with EGFR gene copy number ranging from 3 to 6 copies. Subsequent PRT analysis also identified all 7 EGFR amplifications and it produced concordant results in all remaining samples which were without EGFR gene amplification.

Conclusion: Paralogue ratio test can be adopted for a rapid, targeted detection of gene amplification, such as EGFR amplification present in HNSCC. The method may be applicable as a screening tool and for conformation of aCGH results.

J14.11 Molecular evaluation of carbapenemases and Metallo beta lactamases production among Gram-negative bacteria with carbapenem resistance

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Emerging multidrug resistant (MDR) microorganisms among hospital isolates have been limited the effective drugs to treat or prevent bacterial infections. This study was performed to determine the rates of antibiotic resistance in Gram-negative isolates from clinical samples and to identify carbapenemases and Metallo beta lactamases genes variation in the strain. Identification and assessment of gram negative bacteria with Carbapenem resistance sensitive to 11 antibiotic was done through biochemistry and disk diffusion methods, respectively. 11 isolates were checked by PCR for identification of blaVIM,II ◦ blaIMP ◦ blaSPM ◦ blaGES◦ blaNDM ◦ blaKPC ◦ blaOXA-48 genes MHT and DST tests were used assessment for Carbapenemases and Metallo beta lactamases production in these strains. The iden-
of a diversity of medico-genetic strategies during counseling of pregnant women of risk group, for improve the efficiency of diagnosis of chromosomal abnormalities and congenital malformation, which can be achieved by close cooperation between specialists of many fields as genetic physicians, psychologists, pedagogues.

J14.14 Seroprevalence Of Helicobacter Pylori In Children In A Rural Area T. Sabbi;
Pediatric Unit, Rome, Italy.

BACKGROUND Helicobacter pylori (Hp) infection has been recognized as a cause of chronic gastritis, peptic ulcer, atrophic gastritis and gastric cancer.
AIM To analyzed the seroprevalence of Helicobacter pylori in pediatric age in rural area and to evaluate some epidemiologic characteristics.

PATIENTS AND METHODS The study included 100 patients (80 males; age range 5-13 years) suffering from different gastrointestinal complaints. Blood serology and stool antigen testing were used for the diagnosis of infection due to H. pylori. We interviewed the children with questionnaire about socioeconomic factors, hygiene, living conditions and their dietary habits.

RESULTS 20 (20%) of the 100 patients were positive for Helicobacter pylori and this positivity had a significantly increasing correlation with age (p<0.001).

A lower frequency of fermented dairy food, fruits and vegetable consumption was registered among infected children. Among infected patients were noted low socioeconomic markers such as crowded living conditions and unclean water.

CONCLUSIONS Might decrease the risk of Hp infection the use of vitamin C and antioxidants contained in fruits and vegetables. Risk factors for Hp infection are low socioeconomic factors, hygiene and living conditions.

J14.15 Comparison of clinical performances among Roche Cobas HPV, RFMP HPV Papillo Typer and Hybrid Capture 2 assays for detection of high-risk types of human papillomavirus S. Yu1, M. Kwon1, E. Lee1, H. Woo1, J. Park2;
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High-risk types of human papillomavirus (HR-HPV) is an important cause of cervical cancers. Current cervical cancer screening guidelines suggest that early detection of HPV-16 and HPV-18 may prevent the progression of cervical cancer. We evaluated and compared three HPV DNA tests, Roche Cobas HPV, RFMP HPV Papillo Typer and Hybrid Capture 2 (HC2). Roche Cobas HPV specifically identifies HPV-16 and HPV-18 with concurrently detecting other 12 HR-HPV types and RFMP identifies 74 HPV genotypes. A total of 661 cervical swab specimens from women over 30 years of age were classified into groups of high grade squamous intraepithelial lesion (HSIL) and non-HSIL according to cervical cytology results and analyzed by three assays. The results of direct sequencing or Linear array HPV genotyping test were considered true when three assays presented discrepancies. Concordance rates between Roche Cobas HPV vs. RFMP, RFMP vs. HC2, and HC2 vs. Roche Cobas HPV were 94.5% (814/861), 94.2% (811/861), and 95.8% (825/861), respectively. In 71 specimens with discrepant results, concordance rates between each assay and direct sequencing or Linear array were as follows: Roche Cobas HPV, 35.2%; RFMP 93.0%; HC2, 25.4%. Clinical sensitivities and specificities for detecting HSIL were 90.3% and 95.8% with Roche Cobas HPV, 83.6% and 95.1% with RFMP and 90.2% and 94.8% with HC2. In conclusion, Roche Cobas HPV, RFMP and HC2 showed high agreement rates each others. Although Roche Cobas HPV and RFMP showed lower clinical sensitivity in detecting HSIL compared to HC2, they would be clinically useful since both provide HPV genotypes.

J14.16 Absolute quantification of transcripts in single leukaemic cells E. Ghayoor Karimiani;
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We and others have shown that the BCR-ABL mRNA expression is higher in the CD34+ cell population, representing an enrichment of BCR-ABL+ cells in the progenitor fraction or a higher expression of the fusion gene. Pro- mive progenitor BCR-ABL+ cells isolated from CML patients will maintain their functionality after Imatinib treatment. Therefore, CML progenitor cells appear to be more insensitive to Imatinib than more mature cells suggesting that TKIs are unable to inhibit the function of the primitive leukaemic stem cells. During the assessment of minimal residual disease (MRD) by RT-qPCR, some of the early leukaemic progenitors of malignant cells with significantly higher levels of BCR-ABL transcript cannot be differentiated from other groups of homogeneous cells. This fact may cause a failure to perform personalized monitoring of MRD; while the sensitivity and reliability of the...
as say are directly affected by bulk measurements where the number of normal cells that do not express the target genes. In this study, K562 cells were grown in suspension and a small fraction of these cells was noted to have adhered to the plastic dish after the removal of the media. These adherent cells (K562/Adh) were passaged serially. The FACs separated individual K562 cells into PCR wells with a fixed amount of qPCR buffer. Optimized amplification RT-qPCR was performed on single cells to reveal the nature and significance of cellular heterogeneity with the measurement of BCR-ABL levels in K562 cells was investigated.


Mice are the preferred species to study the biological function of mammalian genes through mutation but there are still specific challenges to solve in order to characterize their phenotype. As early lethality is common in genetically engineered mice, an important proportion of homozygous mutant mice cannot be phenotyped. When the corresponding heterozygous mice have no phenotype, the effects of this mutation remain unknown. Unfortunately, studies in newborn mice face major difficulties due to the small size of the pups and their sensory and motor immaturity. In order to gain access to the early post-natal phenotype, we developed three dedicated non-invasive devices, suitable for high-throughput screening: - PhysiPups (patented device) is used to assess vital functions: breathing patterns, electetrocardiogram (ECG), body temperature, gross body movements, and ultrasonic vocalizations. Four animals can be tested simultaneously under controlled conditions of temperature and gas composition. - NeoGAIT is used to assess gait ontogeny. It uses infrared detection of the animal’s contact patterns with the floor and custom image analysis software. - MemoryPups is used to assess memory and associative learning abilities. The tests are based on olfaction and thermotactile sensitivity, the only sensory functions operating at birth. This platform has no equivalent worldwide. It allows phenotyping mice pups from the first few hours after birth until weaning. It is currently used to characterize genetically engineered mice, including models of genetic diseases (autism, Prader-Willi, Ondine’s syndrome) and to study the toxicity of anti-infectious drugs used in neonates (eg. European TINN project).


Background: Duchenne/Becker muscular dystrophy is X-linked recessive, progressive muscle-wasting disease affecting 1 in 3500 boys, caused by mutations within DMD gene. The patients exhibit pathological deletions in approximately 60% of cases. Since 1988 existing reactions for the multiplex PCR amplification of exons in the dystrophin gene have been modified. We aimed to verify the reliability of the multiplex PCR amplification using two different primers sets in order to define the borders of the deletions. Methods: The patients were diagnosed using standard clinical diagnostic criteria including: EMG and serum creatine kinase (CK) level. Three MPCR assays were performed on 80 DNA samples of patients to amplify 24 DMD gene exons using combined sets of primers designed by Chamberlain and Ashton. Results: All the patients showed a raised serum CK level than normal. MPCR analysis in 4 patients showed deletions of 45 and 48 exons using Chamberlain’s primers set. Deletions weren’t confirmed by Ashton analysis. The length of 45 and 48 exons amplified using Chamberlain’s primers are more extended than Ashton’s primers and overlap them. Conclusions: A comparative study of 4 patients showed discrepancies between the results obtained by using primers designed by Chamberlain and Ashton for 45 and 48 exons. MPCR analysis is a reliable method for deletion detection but a noncontiguous and single-exon deletions within DMD gene should be interpreted with caution and confirmed with another technique or using alternative sets of primers to exactly mapping the end point of deletions.


During last years the next generation sequencing (NGS) technologies have significantly enhanced the discovery of causative genetic alterations and identification of mutations in rare diseases. The diseases with locus heterogeneity are still a challenge in clinical practice and sometimes patients remain for years with unknown genetic status.

In order to accelerate the establishment of proper genetic status of patients with rare diseases we analyzed exons of 552 genes underlying rare monogenic recessive disorders using TruSight Inherited Disease Sequencing Panel (Illumina). As model we used diseases with clear unequivocal phenotype (CF, DMD) and included in the study patients in whom routine DNA testing was insufficient to obtain genetic diagnosis. The study included 4 patients: 2 patients with CF with identified one mutation, one patient with DMD without deletions/duplications in DMD gene and 1 with poymalformative syndrome including Dandy-Walker anomaly, AOSD, hypoplastic genitalia, MR and facial dysmorphism. NGS revealed CFTR: p.Gly1349Asp as second pathogenic mutation in one patient with CF and DMD: p.His2921Arg in patient with DMD. Both mutations are reported for first time in patients of Bulgarian descent. In one patient with CF NGS revealed only one causative mutation and MLPA showed a large deletion encompassing exons 18-20 of CFTR gene. A homoygous in-frame deletion p.Ser372del in MKS1 gene was found in the patient with polymalformative syndrome.

The NGS technology has the potential quickly and cost-effectively to point out disease causing mutations in patients with rare genetic diseases and gives us the opportunity for better genetic counseling and prophylaxis in affected families.

J14.20 Non-invasive prenatal testing of chromosomal aneuploidies by massively parallel sequencing of circulating free DNA E. Contini1, M. Benedetti1, C. Pescuccii1, D. Marsiglia1, F. Gerandini1, G. Giacchini1, F. Sberoni2, P. Martin1, S. Pracucci1, L. Basagni1, E. Pelicci2, F. Torricelli2, 1SOD Diagnostica genetica, AOI Careggi, Florence, Italy, Centro Unico Diagnosi Prenatale, Ospedale Policlinico, Florence, Italy.

Background: cell free fetal DNA (cfDNA) circulates in low concentration in maternal plasma and is a source of genetic material for safe non-invasive prenatal testing (NIPT) by parallel massive sequencing techniques. Aims: validation of a protocol for NIPT and optimization of a method for fetal fraction detection. Methods: low coverage whole genome sequencing was performed on plasma DNA samples from 175 pregnant women (8 - 10 weeks of gestation) for the detection of aneuploidies. Digital PCR assay was used for fetal fraction detection by quantification of RASSF1A, TERT and beta-actin genes. Sequenced reads were mapped against the human reference genome hg19 by BWA. Duplicated reads were removed and unique mapped reads were counted (mapping quality > 20 was required). Read count for each chromosome was normalized based on library size and GC content. Chromosomal aneuploidies were detected by using a z-score statistics. Reference dataset was built by including euploid samples selected on the basis of the correlation between z-scores and coverage in 5 aneuploid cases. This allowed us to estimate the best trade-off between coverage (> 0.4) and sample size. In the male fetus, fetal DNA fraction was measured using normalized read count on chromosome Y. Results: sequencing of 175 euploid samples allowed us to set up the experimental protocol and to create the reference dataset. The performance of the dataset was assessed by blinded analysis of twenty pregnancies. Five trimester 21 and fifteen euploid fetuses were correctly identified by our analysis.

J14.21 FISH and methylation studies in Egyptian children with Prader-Willi syndrome A. A. Khedr1, S. R. Ismaii2, A. M. Mohamed3, N. A. Mohamed4, A. A. Nazmy2; 1National Research Center Cairo, Dokhi, Egypt, 2Medical Research Institute,Alexandria university, Alexandria, Egypt, 3National research center cairo, dokhi, Egypt, 4Medical Research Institute, Alexandria University, Alexandria, Egypt.

Prader-Willi syndrome (PWS) is a complex, multisystem disorders of genetic origin due to lack of paternally active genes on critical area on chromosome (15) q11-q13. PWS is almost always sporadic and is the most common syndromal cause of human obesity with an estimated incidence of 1 in 20,000 Material and methods:Conventional G-banding, FISH using an SBE/Prader-Willi/Angelman chromosome region probe by Appligene-Oncor and methylation specific-PCTR studies were conducted on 23 patients (13 males and 10 females age ranged from 6.5 months to 20 years selected according to suggested criteria proposed by Gunay-Aygun et al, 2001. Aim: This study aims at confirming/excluding PWS in clinically suspected patients through a sensible strategy of investigations on the cytogenetic level, FISH technique and molecular level using DNA methylation tests for phenotype/genotype correlation, early diagnosis and proper counseling. Results: On the cytogenetic level, 10 out of 23 cases showed deletion of 15q11-
of the sex chromosomes (PAR regions, interfering homologous regions and only one of each sex chromosome in males). At present, no guidelines can help interpretate the analysis of sex chromosome abnormalities identified by SNP array.

In this study we analysed SNP-array data from 25 samples with known sex chromosome abnormalities. We present the results from this study and present a general guideline for SNP-array interpretation of sex chromosomes. Hopefully this will aid others in the interpretation of sex chromosome abnormalities identified by SNP-array.

J14.26
NextGen Sequencing: a tool for deciphering the BRCA1/2 and TNBC relationship
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Although during recent years science and technology have generated a great deal of progress in medicine, breast cancer still is the most common malignancy and the leading cause of death by cancer in women. Triple negative breast cancer (TNBC), characterized by the absence of Estrogen and Progesterone receptors and Her2/neu, affects 15-20% of breast cancer patients, has a more aggressive phenotype and occurs at younger ages. Because of its therapeutic limitations, TNBC needs to be investigated in more detail with the help of new technologies. This is why our study focuses on exploring the mutation status of BRCA1/2 genes involved in hereditary breast cancer, and with known mutations in TNBC. This retrospective study on 30 TNBC patients who underwent surgery at The Oncology Institute "Prof. Dr. I. Chiricuta" Cluj-Napoca was conducted with the help of the next generation sequencing platform Ion Torrent PGM. After sequencing the DNA extracted from FFPE tissue, we observed that 20 of the 30 patients presented germline BRCA1/2 mutations, of which seven in BRCA2, 10 in BRCA1, and three in both genes. We identified two mutations that are frequent in patients of European descent with hereditary breast cancers, two that are similar to mutations identified in families of Swedish origin, and several other new mutations. This is the first study that investigates BRCA1/2 mutations in TNBC patients in Romania, and proves that NextGen Sequencing is a competitive and cost-effective BRCA screening method especially in low-income countries where patients cannot afford early breast cancer diagnosis.

J14.27
Comparison of variant calling from whole exome and transcriptome sequencing using CLC Cancer Research Workbench
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Some of the major reasons for high throughput sequencing being more and more frequently applied in cancer research and cancer diagnostics today, are the significant improvements in accuracy, throughput, and speed. In the past, sequencing was clearly the bottleneck but the most time consuming step currently is the data analysis - more precisely the identification and characterization of variants in tumor samples. Whole exome and genome sequencing protocols are commonly used and allow for the identification of SNPs and InDels in protein coding regions. However, both methods fail to show which allele variants are actually expressed in the tumor tissue. Contrariwise transcriptome sequencing (RNA-seq) reveals the expressed alleles and can in addition provide insight into the transcript expression levels, expressed isoforms, and instances of fusion transcripts. Here, we illustrate the benefits of combining the analysis of whole exome sequencing and RNA-seq using CLC Cancer Research Workbench. This software suite includes algorithms for quality control, read mapping, variant calling, and RNA-seq analysis, as well as numerous tools for the comparison and annotation of variants. The analysis is carried out with ready-to-use workflows and the results are visualized in a track based genome browser. In the present work differences found between the variants identified in exome and in RNA-seq data from uveal melanoma samples will be highlighted.

J14.28
Molecular-genetic analysis of predisposition to ovarian cancer among women of Uzbekistan
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Ovarian cancer is the 9th most common cancer, with an estimated 22240 new
cases in 2013. Most women aren't diagnosed until the cancer has spread, leading to a poor five-year survival rate of 43%. Only 10-15% of malignant epithelial ovarian tumors are genetically determined.

The most significant genes associated with ovarian cancer include BRCA1, BRCA2, CHEK2. These genes produce tumor suppressor proteins which help repair damaged DNA and play a role in ensuring the stability of the cell’s genetic material. When either of these genes is mutated, DNA damage may not be repaired properly. As a result, cells are more likely to develop additional genetic alterations that can lead to cancer.

This study aimed to investigate the contribution mutations of BRCA1, BRCA2 and CHEK2 genes to ovarian cancer cases in Uzbekistan. By means of real-time allele-specific PCR we analyzed DNA samples of above mentioned group for the presence of 5382insC mutation in BRCA1, 617delT - in BRCA2 and ISWS+1G->A - in CHEK2. 6 unrelated samples (9.1%) were found to be positive for the heterozygous 5382insC mutation representing a possible b understander mutation in the Uzbek population. We didn't find 617del T and ISWS+1G->A mutations. The presented data confirm contribution of BRCA1 5382insC mutation for ovarian cancer development in Uzbek people and taking into account a high disease penetrance in carriers of BRCA1 mutation, it seems reasonable to suggest inclusion of 5382insC mutation test in screening programs for breast cancer prevention in Uzbekistan.

Methods. Mitochondrial 8344 A>G mutation was investigated by PCR-RFLP analysis detecting abnormal restriction pattern that it is not a trusty technique. Unfortunately these methods are very time consuming. Alternative method that has been emerging rapidly is the DNA microarray. This main advantage of this method is a possibility to screen thousands of mutations at once making the diagnostic simple and fast. In collaboration with our colleagues from Busan National University (South Korea) there was established a testing system based on DNA microarray for the diagnostic of five high frequency diseases: Enzymopathic methemoglobinemia (OMIM 250808, Pro269Leu mutation in DAA1 gene), 3 M syndrome (OMIM 273750, p.G852fs*17 in COL7 gene), 50 PH syndrome (OMIM 614800, G5741 Ala mutation in NAG gene), Nonsynchronous hearing loss 1A type (OMIM220290, IVS1+1G->A in GJB2 gene), Tyrosinemia type 1 (OMIM 276700 1090C>G mutation in FAH gene). Since the microarray is well known and rapidly developing, the heterozygosity screening itself using the microarray rather than then microarray establishment have the significance. It will allow reducing the frequency of these diseases in the Republic and the risk of diseased child birth.

For identification the common mitochondrial 8344 A>G mutation resulting in MERRF (myoclonus epilepsy with ragged red fibers) syndrome a worldwide used molecular diagnostic method is a PCR-RFLP method, using the BanII restriction enzyme. So far it was not a matter of common knowledge, that it is not a trusty technique.

Methods. Mitochondrial 8344 A>G mutation was investigated by PCR-RFLP using BanII restriction enzyme on DNA samples (n=11073). In cases with abnormal restriction pattern mitochondrial tRNA lysine gene region (nt 8105-8536) was sequenced bidirectionally (n=14). Results. PCR and BanII RFLP analysis detected abnormal restriction pattern indicating m.8344 A>G mutation in 14 cases. Sequence analysis of tRNA lys gene verified the presence of the m.8344 A>G substitution in 6 cases. Unexpectedly the m.8344 A>G substitution was present in 8 cases with 9-bp triplication (8272-8280) in 3 samples. The gel photo showed nearly the same pattern in cases with m.8347 A>C mutation or 9-bp triplication. The BanII cleaves samples with m.8347 A>C and m.8344 A>G substitutions on the same way. The 8347 A>G mutation was previously not described as pathological alteration, however it is a highly conserved nucleotide and has similar effect on the tRNAlys as the 8344 A>G mutation. The 9-bp triplication is a polymorphism, its anthropological interest is controversial. Conclusion. The PCR-RFLP method using BanII restriction enzyme is not a solid method for detecting m.8344 A>G mutation. It may result in pseudo positivity. We suggest to use it only as a screening method, which must be validated always by Sanger sequencing.

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J15.01
Brain-derived neurotrophic factor Val66Met polymorphism and cardiac stress reactivity
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Twin studies indicated that heart rate variability (HRV) and particularly its high frequency (HF) spectral component show significant heritability, and their genetic variance is amplified by mental stress. This genetic association study investigated the influence of the brain-derived neurotrophic factor (BDNF) Val66Met polymorphism and the triallelic serotonin transporter gene promoter (5-HTTLPR) polymorphism on state anxiety and time and frequency measures of HRV reactivity to mental stress. In comparison to BDNF Val homozygotes, the Met allele carriers showed larger cardiovascular withdrawal during stress. The triallelic 5-HTTLPR did not significantly influence any of the psychophysiological measures. Although BDNF Val66Met only explained a small part of the variance of cardiovascular withdrawal during stress, this polymorphism may be part of a complex genetic pattern underlying the comorbidity of autonomic dysfunctions and emotional vulnerability.

J15.02
Heterogeneity of Tumors - comprehensive biomarker-panels to decipher cancer
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Personalized cancer treatment increasingly enters into clinical routine. Due to the fact that each tumor is individual regarding its genomic structure, the screening for these changes on DNA and RNA level in tumor tissue is becoming increasingly crucial for an individually effective therapy. Drugs like EGFR-Inhibitors in particular and tyrosine kinase inhibitors in general have proven to benefit greatly from companion diagnostics. Despite this fact, however, expectations of clinical impact on the well being of patients were bigger than what was truly observed. Vast improvements were merely seen in a fraction of treated individuals, making a general assumption less than relevant. Even in patients exhibiting great initial response subsequent relapse was quick to follow. Hence a broader approach of analyzing comprehensive biomarker-panels by various techniques should be taken into consideration to account also for heterogeneity and predict maybe the next phase of tumor expansion. After a short overview of high throughput techniques like next generation sequencing and its combination with routine methods like pathological examination, micro-dissection and purification of DNA/RNA, mutation analyses via high resolution melting (HRM), gene expression profiling and epigenetic analysis, the combination of these multiple approaches for personalized cancer therapy are highlighted. The clinical use of this novel and comprehensive approach for an improved diagnostics and thus more effective treatment is demonstrated on the example of prostate and colorectal cancer.

J15.03
The investigation of BRCA1 and BRCA2 expression in colorectal cancer
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BRCA1 and BRCA2 genes are suppressor of malignant tumor and predictivemeakers tumor susceptibility to platinum-drugs. Malignant transformation and carcinogenesis accompanied by alters the expression of tumor-suppressor genes. Loss of any of these genes expression is associated with positive response on treatment by platinum-drugs. Tumor and normal tissue samples of colon among 30 patients with colorectal cancer were investigated. High level (3 and more time) of BRCA1 and BRCA2 expression was found in 22/30 (70%) tumors and 24/30 (80%), respectively. As well high expression of both genes was detected in 19 tumors. The expression of BRCA1 was not changed in 30 (70%) tumors and in one tumor was decreased in 7 times. The expression of BRCA2 was not changed in 4 of 30 (13.3%) tumors and was decreased (in 7 times) in two tumors. The expressions of both the genes were not altered in two cases. However BRCA1 and BRCA2 expression was not a coordinately decreasing in neither case. We obtained information about change BRCA1 and BRCA2 expression in tumor for Russian patients with colorectal cancer. These data can improve the therapy for colorectal cancer patients by platinum-drugs.

J15.04
The effects of hypericin on p53 and ADAMTS genes expression in MCF-7 breast cancer cell line
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Our objective was to determine the effects of hypericin on MCF-7 breast cancer cells, as it is known to have an anti-tumor effect on the expression and regulation of ADAMTS1, 3, 10 and the P53 gene in breast cancer cells. MFC-7 cells were cultivated and subjected separately to various doses of 1, 5, 7.5 µg/mL hypericin. After 24 h, RNA was isolated and transcribed into cDNA. Expression analysis was performed by real-time RT-PCR and cell survival was determined by XTT assay. While expression of ADAMTS1 in MFC-7 cells decreased to 0.94 fold after exposure to 1 µg/mL hypericin, expression increased by 5.6- and 36-fold at 5 mg/mL and 7.5µg/mL, respectively. Furthermore, ADAMTS3 expression in MCF7 cells were increased 3.9 fold with the use of 5 µg/mL of hypericin. These concentrations of hypericin did not lead to significant changes in the expression of ADAMTS10 and the P53 gene. XTT tests have shown that hypericin concentration of 7.5 µg/mL lead to significant death of cancer cells. The increase in ADAMTS1 expression may prevent metastasis or facilitate development of an adjuvant factor with tumour-suppressive effects. Hypericin may therefore exert its anti-tumoral and apoptotic effects in MCF-7 cells via ADAMTS1 and ADAMTS3.

J15.05
Effect of Hypericin on the ADAMTS-8 and ADAMTS-9 gene expression in MCF7 breast cancer cells
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Our aim was to investigate the effects of hypericin which is obtained from the plant Hypericum perforatum on the expression and the regulation of ADAMTS8 and ADAMTS9 genes in MCF7 breast cancer cells and on the viability of these cells. MCF7 cells were cultured and were separately exposed to 2, 10 and 50 µl/mL of hypericin. After 24 hours, RNA was isolated from these cells and converted to cDNA. The expression levels of ADAMTS8 and ADAMTS9 genes were evaluated using the real-time RT-PCR. XTT (2,3-bis-(2-methoxy-4-nitro-5-sulphonyl) -2H-tetrazolium-5-carboxanilido, disodium salt) cell viability assay was used to determine cytotoxicity. ADAMTS9 expression in MCF7 cells were increased 1.8 and 3.6 fold with the use of 2 and 10 µl/mL of hypericin, respectively; and decreased 0.7 fold with the use of 50 µl/mL of hypericin. There was no significant change in the ADAMTS8 expression.
Rapid cell death was observed in the cancer cells when hypericin was used at a dose of ≥ 50 µl/mL. The increase in ADAMTS9 expression can be a useful factor in the prevention of possible metastasis in breast cancer and for the occurrence of a tumor suppressive effect. Hypericin increases the expression of ADAMTS9, therefore, it may show its antimetastatic and antiapoptotic effects by means of ADAMTS9.

J15.06
Effects of tomatine in breast cancer
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Metastasis in breast cancer is still a complicated issue and matrix metalloproteinase family has a significant role in metastasis showing the link between MMP members and tumor development. Tomatine is a secondary metabolite synthesized by tomatoes and has a role in natural defenses against plant fungi, viruses and bacteria. Besides, it is known to disrupt cell membranes and be a strong inhibitor of human cancer cells. In this study, it was aimed to evaluate the effects of tomatine on cytotoxicity and apoptosis on MCF-7 cell line. Firstly, IC50 dose of tomatine was measured as 7.07 µM by using xCELLigence system. Further, it was shown that tomatine induced apoptosis is 3 times greater than control cells with by Annexin V labeling on Flow Cytometry. Additionally, miRNA expression profiles of breast cancer cells were determined by qRT-PCR and 22 downregulated and 15 upregulated miRNAs were found. Active and zymogen forms of MMP-2 and MMP-9 were detected by zymography method and the activations of MMP-2 and
Breast cancer has still been the most important subject with other cancer types. This cancer is the most common and second leading cause of death among women. It is known that various plant extracts, including ellagic acid, have anti-proliferative and pro-apoptotic effect on breast cancer cells. However, miRNAs which are associated with tumor initiation for estrogen dependent breast cancer are suppressed by ellagic acid treatment. In this study, it was aimed that ellagic acid could induce apoptosis related mechanism in breast cancer cells and this induction could be triggered by altered expression profiles of miRNAs. Cytotoxic effects of ellagic acid on MCF-7 breast cancer cells and breast cancer stem cells were examined by WST-1 reagent test. Apoptosis and cell cycle analysis were detected by flow cytometry analysis. After ellagic acid treatment, miRNA expression profiles of breast cancer stem cells were determined by RT-PCR. While ellagic acid had no any cytotoxic effect on MCF-7 breast cancer cells, cytotoxic value of ellagic acid for breast cancer stem cells was detected as 24.0 μM dose. Ellagic acid did not induce apoptosis in both cell groups and increased living cells. In the cell cycle, S arrest was observed in MCF-7 breast cancer cells as well as breast cancer stem cells. When analyzed in terms of miRNA profiles, ellagic acid generally suppressed expression of miRNAs and expression profiles were associated to oncogenic effect. It was suggested that ellagic acid may not be chemo-preventive agent because of miRNA profiles, have oncogene effect.

J15.08 Development of targets for colorectal cancer treatment on a base of small interfering RNA and functional genomics
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The significance of genes for tumor cell viability and understanding of the cell genetic features under which high apoptotic effect of gene silencing will observed - both are important in development of targets. We were searching of genes, silencing of that will increase sensitivity of colorectal cancer cells to oxaliplatin. The panel of genes was formed using of expression data bases and publications analysis. The expression gene profile was determined in dependence on the dose and the time of oxaliplatin action for two colorectal cancer cell lines: HT-29 and HCT-116 p53 in dependence on the dose and the time of oxaliplatin action for two colorectal cancer cell lines: HT-29 and HCT-116 p53.

Joint silencing of the gene found has led to a substantial (70-80%) suppression of the cell viability and increase of 5-10 times the sensitivity of cancer cells to small oxaliplatin doses (5 - 10 μM). Common for HT-29 and HCT-116 is mutation in TP53 gene. Possibly, this feature is responsible for post-translational gene regulation. The aim of this study is determination expression changes of miRNAs related with leukemogenesis with the qRT-PCR evaluation. EGCG has been shown to exhibit antitumor activities in the case of SSC-4 cell line, and this finding could be trigged by altered expression profiles of miRNAs. In conclusion, these results show the potential of ellagic acid as a product of sevoflurane breakdown, which may cause nephro- and hepatotoxicity. Investigations on genetic variability of CYP2E1 gene and the possibility to associate the results with clinical effect of metabolized sevoflurane provide the basis for the development of personalized anesthesiology. The aim of this study was to establish a simple and economical method for analysis of whole coding sequence of the CYP2E1 and to determine the prevalence of sequence variations in this gene among the Polish patients under sevoflurane anesthesia. Here we present the molecular test based on multiple HRMA. The entire coding sequence of the CYP2E1 gene was successful amplified using 14 pairs of primers in the 7 multiplex reactions using the Rotor-Gene® equipment (Qiagen). Whole-genome amplified DNA from 41 individuals. After HRMA screening, selected samples with different melting profiles, were sequenced. Among the tested samples we observed relatively high diversity of low-frequency changes. We found 5 different sequence variations, where two are novel changes: p.Gln75Lue (1.2%) and p.His226Ter (1.2%). The frequencies of known polymorphisms (p.Gly173Ser; rs60452492 - 1.2%; p.Va1179le; rs6413419 - 1.2%; p.Phe426Phe; rs2515641 - 9.8%) are similar to the other Caucasian populations.

J15.10 Epigallocatechin gallate as inducer of cell death and apoptosis in human oral squamous carcinoma cell line
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The antitumor effects of the green tea compound epigallocatechin-3-gallate (EGCG) have not been studied in detail previously in oral squamous cell carcinoma cells. Expression of the proapoptotic protein (p53) occurs frequently in oral squamous carcinoma as being overexpressed as mutant or oncogene or knocked down as suppressor gene, which is an adverse prognostic factor. Therefore, we examined in detail the molecular effects of EGCG on the apoptosis signaling pathway, in SSC-4 cell line. Human oral squamous cell carcinoma is a form of cancer that presents a poor prognosis, therefore is an urgent need to determine the molecular mechanisms involved in tumor cell survival and invasion. EGCG is a natural phytochemical previously indicated as chemopreventive and chemotherapeutic agent in multiple cancer types. In the case of treatment with low doses of EGCG, we observed increased apoptosis as and reduced proliferation and invasion as displayed by the xCELLigence data on SSC-4 oral squamous carcinoma cells. This may be the inhibition of pro-survival genes and the activation of cell death mechanisms as results from the qRT-PCR evaluation. EGCG has been shown to exhibit antitumor activities in the case of SSC-4 cell line, and this finding supports the therapeutic implication in oral squamous carcinoma.

J15.11 Epigallocatechin-3-gallate and zolendronic acid induce apoptosis via down-regulation of miR-17-5p and miR-20a-5p in chronic myeloid leukemia
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Chronic myeloid leukemia (CML) is a clonal disorder of hematopoietic stem cells. Epigallocatechin-3-gallate (EGCG) is a major flavonoid of green tea. Zolendronic acid (ZA) is a nitrogen-containing bisphosphonate. MicroRNAs (miRNAs) are small, single-stranded, non-coding RNA molecules that are responsible for post-translational gene regulation. The aim of this study is determination expression changes of miRNAs related with leukemic evolution with the treatment of EGCG and also define cytotoxic and apoptotic effects of EGCG in K562 human CML cell line. The cytotoxic effects of EGCG on K562 cells were determined in time and dose dependent manner. Total RNA, including miRNA was isolated from K562 cells treated with EGCG and ZA 24h and untreated cells as control group. Reverse transcription procedure was performed for cDNA synthesis. Apoptosis assays were performed by using ApoDIRECT Assay, miRNA expressions were showed by RT-qPCR. Expression analysis results were analyzed by using miScript miRNA PCR Array Data Analysis. IC50 value of EGCG and ZA were determined as 50, 60 μM, respectively. EGCG
and ZA induced apoptosis 10.9, 2.3 fold and down-regulated miR-17-5p and miR-20a-5p 2.5758, 20.332 fold according to the control cells, respectively. Up-regulation of miR17-92 cluster, the best defined oncomir group, was associated with a lot of cancer types. Our findings showed that treatment of EGF and ZA triggered down-regulation of miR-17-5p and miR-20a-5p expression levels which are member of miR17-92 oncomir cluster in K562 CML cells. These novel findings can be valuable by exploring the targets of these ablated oncomirs in leukemia progression.

J15.12 The Effect of Fluoride Agents on the Expression Levels of Pro and Anti-apoptotic Genes in Human Gingival Fibroblasts

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Fluoride is widely used to prevent dental caries in dentistry. It is cytotoxic and produces inflammatory responses in human. The aim of this study was to investigate the effect of fluoride usage on expression levels of BAX, BAX, BAD, FASL, PS3, CASPASE 8, CASPASE 9. Five different fluoride agents [1.2% Acidulated Phosphate Fluoride (APF) and 2% Sodium Fluoride (NaF) gels, 1% Titanium Tetrafluoride (TIF4) and 38% Silver Diamine Fluoride (SDF) solutions and Duraphat Fluoride Varnish containing 6% NaF] were used in human gingival fibroblast cell lines which were obtained from healthy individuals. Gene expressions were analyzed by using real time RT-PCR. No significant change was detected in the expression levels of those genes in NaF group. The expression levels of BAD did not significantly change in all study groups as well. The ratio of pro-apoptotic BAX to anti-apoptotic BCL-2 was greater than 1 which demnstrates that those fluoride agents cause induction of apoptosis. In conclusion we suggest to protect the soft tissues during the application of the fluoride agents, to be careful for patients not to swallow the fluoride agents and to avoid unnecessary usage of fluoride in clinics.

J15.13 Implication of HLA B27 and ITPA polymorphisms in treatment response of HCV infected patients

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The HLA-B27 allele frequency (6%) obtained in our study is similar to corresponding data reported in literature for Caucasian populations (8%). Up to now, we found neither association between the ITPA markers and Pegylated Interferon-ZA triggered down-regulation of miR-17-5p and miR-20a-5p expression levels which are member of miR17-92 oncomir cluster in K562 CML cells. These novel findings can be valuable by exploring the targets of these ablated oncomirs in leukemia progression.

J15.14 Cyogenetic monitoring of response and karyotype evolution in low-risk MDS patients treated in the leMoNS-study

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Besides a more reliable and frequent measurement of cyogenetic response, it was our aim to find out whether lenalidomide in treatment with patients with IPSS low- or intermediate I-risk MDS can foster karyotype evolution (KE) and thus increase the risk of leukemic transformation. In this study, only lower risk MDS patients with an isolated del(5q) are included. We performed a initial screening of bone marrow aspirates by chromosome banding as well as FISH-analysis to ensure an isolated del(5q). For initial screening and frequent cyogenetic follow-up every two to three months (FISH analysis of OD34+ peripheral blood cells), we used panels of 8 to 13 FISH probes. From the initially examined 145 MDS patients, 84 could be included in the study according to the study inclusion criteria. Currently, follow-up data are available for 53 patients. A complete cyogenetic response was observed in 35 patients (66%) after a median follow-up of 15.5 months. In eleven patients (20%) the size of the del(5q) clone did not change. A cytogenetic response was observed after a median of 6 months after initiation of therapy for a median duration of 10 months. In 8 of 53 a true karyotype evolution occurred after a median time of 12 months. Conclusion: Our previous cyogenetic results demonstrate the rapid effect of lenalidomide on clones with del(5q) in 66% of patients. The rate of karyotype evolution was 15% and thus does not appear to be significantly increased. Gain of additional abnormalities does not always result in leukemic transformation.

J15.15 The effect of Centaurea lydia on MDM2 gene expression in human acute lymphocytic leukemia cells via transfection of mir-150 by MATRA

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Acute lymphocytic leukemia (ALL) arises from the clonal proliferation of lymphoid progenitors in the bone marrow. It accounts for nearly a quarter of all childhood cancers. Centaurea lydia has shown antitumor activity in vitro. miRNAs are single strand, non-protein-coding small RNA molecules that have role in the regulation of gene expression and cell growth, development, apoptosis and hematopoiesis. In recent years, Magnet assisted transfection (MATRA) is one of the most effective non-viral transfection methods. We aimed to evaluate the effect of methanol extract of Clydia (CLM) on apoptosis and MDM2 gene expression levels in CCRF-CEM cells (ALL) via transfection of mir-150 by MATRA.

Cytotoxic effects of CLM in CCRF-CEM cell line was detected in time and dose dependent manner with XTT assay. We determined effects of CLM, miR-150 and the combination of miR-150 with CLM (miR150-CLM) on apoptosis by using ApoDIRECT with FACS and MDM2 gene expression levels with real-time qRT-PCR (TagMan) in the course of 48 hours. GAPDH was used as "housekeeping" gene. IC50 of CLM was detected as 14.95 µM in CCRF-CEM cell line and miR150-CLM were no apoptotic effects on CCRF-CEM cells while CLM induced 10 fold compared to control cells. MDM2 gene expression in CCRF-CEM were found as up-regulated (CLM) 2,44 fold and down-regulated (miR-150) 224,41 (miR150-CLM) 1,34 fold, respectively, according to the control cells. Down regulation of MDM2 gene following the treatment with combination and CLM apoptotic effects, provide evidence that these compounds may serve as potentially effective in leukemia cells via transfection of mir-150.

J15.16 Testing of EGFR mutations, ALK and ROS1 rearrangements, and KRAS-LCS6 polymorphism in Non-Small Cell Lung Carcinoma (NSCLC) in Czech patients

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1Faculty of Medicine, University of Ostrava, Czech Republic, 2Faculty of Medicine, University of Ostrava, Ostrava, Czech Republic.

Introduction: Activating EGFR mutations are associated with good response for tyrosin kinase inhibitors (TKIs) therapy (gefitinib, erlotinib). Also ALK and ROS1 rearrangements are connected with therapeutic options (crizotinib). KRAS is one of the very frequently mutated genes in many various...
cancers, including NSCLC. A germline single nucleotide polymorphism (SNP; rs61764370), located in the let-7 complementary site 6 (LCS6) within the 3′UTR of the KRAS gene, affects the binding affinity of mircoRNA let-7 to the KRAS mRNA and thus gene expression. It was published that KRAS-LCS6 SNP is associated with the higher risk of NSCLC development, especially in moderate smokers.

**Material and methods:** DNA was isolated from biopsy and cytology specimens with verified histological diagnosis. Mutation detection was done by real-time PCR. EGFR patients with no detected mutation were tested for ALK and ROS1 gene rearrangements using the FISH method.

**Results:** Number of positive/tested samples were: Activating EGFR mutations: 50/658 (7,5 %); KRAS gene rearrangement: 39/405 (9,6 %); ROS1 gene rearrangement: 1/159 (0,6 %); KRAS-LCS6 NSCLC patients (G-allele): 23/160 (14,3 %) and KRAS-LCS6 healthy controls (G-allele): 61/387 (15,7 %), respectively.

**Discussion and conclusions:** Activating mutations of the EGFR gene, ALK and ROS1 gene rearrangements predict possible therapeutic response to TKIs or other small molecules. Our results correlate with generally known findings. No statistically significant difference between frequency of KRAS-LCS6 G-alleles in NSCLC patients and healthy controls was found.

**J15.17**

**Particularities of ATRA therapy in pediatric patients with acute promyelocytic leukemia**


**“Victor Babes” University of Medicine and Pharmacy, Timisoara, Romania, “Louis Turcanu” Emergency Hospital for Children, Timisoara, Romania.**

Acute promyelocytic leukemia (APL) is defined by an error in myelopoiesis. Translocation (15;17) giving PML/RARA fusion gene represents the classic molecular disorder found in 92% of patients with APL. Addition of ATRA induces apoptosis and differentiation of promyelocytes acting at multiple molecular levels. We present three pediatric patients (P1, P2, P3) which were diagnosed with APL in our hospital’s Oncopediatric department between 2010 and 2013. At admission, they all presented severe bleeding disorders, gingival hypertrophy, thrombocytopenia, anemia, inflamatory syndrome. Bone marrow smear and immunophenotyping established diagnosis, later supported by FISH and PCR analyses that detected PML/RARA fusion gene. They received treatment according to the AML BFM 2004 protocol. P2 was allergic to Etoposide, which was withdrawn later from therapy. In later evolution, he was diagnosed with pulmonary aspergillosis presenting massive pleurisy and exudative pericarditis needing surgical drainage. Both P2 and P1 developed ATRA syndrome with complete remission under dose-adjusted etoposide. Currently, all three patients are in hematological and molecular remission, being closely monitored. The addition of ATRA to chemotherapy was efficient to our patients, who were early treated and received proper support care. ATRA syndrome was manageable, but it still represents a potentially serious complication that has to be confronted.

**J15.18**

**Resveratrol regulates apoptosis by targeting mir-181 family in chronic myeloid leukemia cells**


**Ege University Medical School Department of Medical Biology, İzmir, Turkey.**

Chronic myeloid leukemia (CML) is a malignant disorder of the haematopoietic stem cell arising from the reciprocal translocation between the breakpoint cluster region (BCR) gene on chromosome 22 and the Abelson (ABL) murine leukemia virus gene on chromosome 9, (t(9;22)[q34;q11], resulting in the Philadelphia chromosome which has consequences on the activity of the oncogene BCR-ABL, the tyrosine kinase activity, leading to leukemogenesis. Resveratrol is a naturally occurring phytoalexin with apoptotic and growth inhibitory effects in leukemic cells. MicroRNAs play a pivotal role in normal hematopoiesis. In this study we aimed to evaluate the cytotoxic and apoptotic effect of resveratrol in CML cells by questioning miRNA expression levels associated with CML progression.

K562 cells were treated with the IC50 dose (100 μM) of resveratrol for 96 hours. The cytotoxicity assays were conducted by using Annexin V and 7-AAD analysis. Apoptosis was evaluated by AnnexinV-enhanced green fluorescent protein (EGFP) and by ApoDIRECT In Situ DNA Fragmentation Assay Kit. The RT-qPCR was used for miRNA expression analysis. MiRNA expression levels were evaluated by using microarray and PCR methods. MiRNA expression levels was analyzed by using microarray and PCR analysis.

Significant increase in mir-181 family was observed in K562 cells treated with resveratrol according to control. Resveratrol up-regulated mir-181a, mir-181b, mir-181c and mir-181d expressions as 8.96, 6.42, 10.15, 17.19 fold according to the control cells, respectively. Resveratrol upregulated tumor suppressor mir-181 family in K562 cells through induction of apoptosis and this effect can be used for alternative detection of chronic myeloid leukemia progression.

**J15.19**

**Zoledronic acid inhibits glioma cell growth and induces apoptosis by up-regulating mir-22 expression**


**Ege University Medical School Department of Medical Biology, İzmir, Turkey.**

Glioblastoma multiforme (GBM) is the most extensive and malignant type of brain tumors of central nervous system. These lethal brain tumors seen in adults and have no response to standard treatment. Zoledronic acid (ZA) demonstrates anti-tumor activity in various cancers. MiRNAs are small (19-24 nucleotides) and non-coding RNAs that regulate post-transcriptional gene expression. MiRNAs serve as oncogenes and tumor suppressors through unlighted mechanisms in human.

The aim of the study was to evaluate the effect of zoledronic acid on the expression of miRNAs. In our experiments, U87-MG cell line (Human glioblastoma-astrocytoma) is used as an in vitro model of human glioblastoma cells to investigate the cytotoxic and apoptotic effect of ZA towards glioma cells. U87-MG cells were treated with 25 μM (IC50) ZA during 72 hours. Apoptosis assays were performed by using ApoDIRECT In Situ DNA Fragmentation Assay. The RT-qPCR is used for miRNA expression analysis. MiRNA expression levels were evaluated by using microarray and PCR method.

Results showed that IC50 dose of ZA induced apoptosis 4.25 fold when compared to control cells that untreated with ZA. Also IC50 dose of ZA of miRNA expression results showed that; mir-22 expression level was upregulated 3.08 fold according to control group.

In conclusion, these novel findings showed that ZA can be important in prognosis of glioma and it is necessary to question whether it can be used as a drug candidate in glioma treatment with further research on miR-22 and its target gene expression.

**J15.20**

**Nicotine and Cotinine Dependent Regulation of P-glycoprotein Expression in Caco-2 Cells**


**Immanuel Kant Baltic Federal University, Kaliningrad, Russian Federation.**

P-glycoprotein (P-gp), encoded by the multidrug resistance gene 1 (MDR1), acts as an efflux pump that exports a wide spectrum of drugs across the membrane out of the cell. P-gp is expressed in a number of barrier tissues such as apical membranes of the lower GI tract, blood-brain barrier, liver, kidney, placenta and testis. Therefore, the expression of P-gp is one of the most important factors regulating bioavailability of a wide spectrum of orally administered drugs. Previously it was shown that tobacco smoking changes the bioavailability of several P-gp substrates. It’s also shown that nicotine is metabolized mostly by CYP2A6 and transformed into a cotinine which has an extremely long half-life in comparison to nicotine (about 20 hours versus 2 hours). In the present study we have shown dose-dependent up/down-regulation of P-gp expression in Caco-2 cells by nicotine and cotinine exposure. Cells were treated with three different concentrations of nicotine (5ng/ml, 15ng/ml, 50ng/ml) and cotinine (50ng/ml, 250ng/ml, 750ng/ml) for 96 hours. P-gp expression was estimated by real-time RT-qPCR. We recognized that P-gp expression was increasing in a nonlinear manner. It was shown that the intermediate concentrations of nicotine and cotinine had a lower effect on P-gp mRNA level compared to other concentrations including the lowest one which matched passive smoker’s plasma concentrations. Low concentrations lead to 1.5-fold increase of P-gp expression in comparison with untreated cells. It was also shown that cotinine has a role in regulation of P-gp mRNA level in the case of prolonged smoking abuse.

**J15.21**

**The impact of Oncotype DX testing on chemotherapy prescribing patterns in a tertiary referral centre**

L. M. Hughes, P. T. McVeigh, K. J. Sweeney, M. Keane, M. J. Kerin.

**National University of Ireland Galway, Galway, Ireland.**

Introduction: The use of chemotherapy in node-negative, ER-positive breast cancer has changed dramatically since the introduction of Oncotype DX® to determine systemic recurrence risk based on tumour genomic signature. Aims: This study aims to 1. Document longitudinal changes in chemotherapy use 2. Assess the impact of new evidence on local protocol Methods:
A cohort study was undertaken, including consecutive patients with early node-negative, ER-positive breast cancer diagnosed between 2006 and May 2013. Data was collected regarding clinico-pathological features, OncotypeDX use, recurrence score, and chemotherapy use. All therapeutic decisions were made in conformity with existing guidelines, and a large registry of trial protocol and OncotypeDX recurrence scores. Results: The study group included 476 consecutive patients, of whom 240 (50%) underwent OncotypeDX testing, 96 as part of TAILORx trial. OncotypeDX has been used in 84% (n=125) patients since being made available for use in the public sector (see table).

<table>
<thead>
<tr>
<th>Time Period</th>
<th>OncotypeDX test availability</th>
<th>Oncotype DX Used</th>
<th>Oncotype DX Available on Trial only</th>
<th>Oncotype DX Not Used</th>
<th>Oncotype DX Nonreimbursed</th>
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<td>June 2010- October 2011</td>
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<td>October 2011- May 2013</td>
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Results: In our study the promoter methylation status were observed at different rates; TWIST, RARβ2, ESR1, GSTP1 and CDH1 genes which are associated with breast cancer were investigated by Quantitative Methylation Sensitive High Resolution Melting Analysis (Q-MS-HRM). We analysed primary tumour core biopsies from 80 high-risk primary breast cancer patients (tumors ≥2 cm) and/or peritumoral metastasis and/or distant metases and/or under 40 years of age) and their histopathologic types were associated with the methylation levels.

Results: In our study the promoter hypermethylation status were observed at different rates; TWIST, RARβ2, ESR1, GSTP1 and CDH1 methylation frequencies were 25%, 88.75%, 72.5%, 82% and 95% respectively. When comparing the promoter hypermethylation of tumor types of the breast, RARβ2, ESR1, GSTP1 and CDH1 genes found to be significant with invasive ductal carcinomas (IDC). RARβ2 and CDH1 genes promoter hypermethylation is found to be significant with invasive lobular carcinomas (ILC). The promoter hypermethylation levels of the genes found to be significant with lymph node positivities, ER positivity and HER2 status.

Conclusions: Our study is important as being the first study that analyzes the association between IDC and ILC tumor types of breast cancer and TWIST, RARβ2, ESR1, GSTP1 and CDH1 genes promoter methylation status in Turkish population.

J16.03 Presenting the genomic cipher adenoine = 0, guanine =1, cytosine = 2, thymine = 3 derived directly from the DNA to convert nucleotide names/letters to numbers to study patterns of unique identifiers to detect command genes in the human genome
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Presented is a cipher to convert each DNA nucleotide to a specific numeric number to reveal patterns of unique gene identifiers in the human genome. The cipher was developed by converting tRNA anticodons to 5'-3', then reverse transcribing 5'-3' RNA anticodons to 5'-3' DNA anticodons to facilitate construct of a 4x4x4 prime genomic cube; where the first letter of the anticodon is positioned along the 'x' axis of the cube, second letter positioned along the 'y' axis and third letter positioned along the 'z' axis. Assigning adenoine the value of '0', guanine value of '1', cytosine value of '2' and thymine value of '3'. Places methionine, considered the START anticodon, on one end of the cube and the three STOP anticodons on the opposite end of the cube. The three dimensional image of this arrangement of DNA anticodons demonstrates an orderly stepwise progression of the triplicate DNA anticodons AAA, GGG, CCC, and TTT through the tubular cube. A secondary pattern is demonstrated if the triple anticodons nullify anticodons in their rows and anticodons numbering three or more elements are neutral. When these are then zero free anticodons in the 'A' 4x4 panel, one free anticodon in the 'G' 4x4 panel, two free anticodons in the 'C' 4x4 panel, three free anticodons in the 'T' 4x4 panel. Analysis pmduces the cipher Adenoine=0, Guanine=1, Cytosine=2, and Thymine=3. Utilizing this cipher to convert nucleotide elements to numbers facilitates study of the human genome to detect command genes that cannot be determined by conventional analysis techniques.

J16.04 Evaluation of New Epi-panel Markers SPG20, ITGA4 and ALX4 in Plasma for Early Detection of Colorectal Cancer
R. Foroughi1, S. Chavoshi1, S. Mohammad Ganji1, M. Tavallaei2; 1Human Genetics Research Center, Baqiyatallah Medical Sciences University, Tehran, Iran; 2Department of Clinical Chemistry, Towhidieh Hospital, Tehran, Iran.
Evaluation of several genetic and environmental factors related to colorectal cancer (CRC) shows that the majority of CRC cases are sporadic and not related to previous CRC cases. The patient population in the present study is patients with colorectal adenoma or normal colorectal mucosa who were referred to the Digestive Diseases Research Center (DDRC) of the Imam Reza Hospital during the year 2011. The total population in this study was 120 patients (mean age 44.9±11.9) with 60 men (50%) and 60 women (50%). Methods: The study was conducted in patients with sporadic colorectal adenoma or colorectal mucosa and patients with colorectal cancer. The patients were age, sex, and race matched. The study was performed using a commercial DNA methylation qPCR kit (EpiPred Kit, Rando风雨, USA) according to the manufacturer's instructions. The study population was divided into two groups: a) patients with colorectal adenoma or normal colorectal mucosa and b) patients with colorectal cancer. The demographic, clinical, and laboratory data of the patients were recorded. The present study was conducted using a commercial DNA methylation qPCR kit (EpiPred Kit, Rando风雨, USA) according to the manufacturer's instructions.

Results: Our study shows that DNA methylation is a frequent event in BC and that different genes are methylated in BCs with different histopathological features.

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Epigenetics is a fast-growing branch of biology. It consists in the study of reversible and transmissible modifications of gene expression without changing DNA sequences. Epigenetic modifications are contributing to the regulation of many physiologic phenomena such as embryonic development, X chromosome inactivation in female and parental imprinting. These modifications are regulated by several mechanisms, the most frequent are: DNA methylation, histones post-translational modifications and non-coding RNAs. They are influenced by genetic, environmental as well as stochastic factors.

It has been shown that physiologic aging depends not only on genetic factors but also on epigenome modifications, and several age-related diseases such as cardio-vascular diseases, type II diabetes, Alzheimer, auto-immune diseases and cancer have been associated to epigenetic changes. Cancer is, so far, the most studied disease in this context, making epigenetics a plausible explanation to the increasing rate of cancer in old. Cancer epigenetic modifications lead to expression alteration of genes regulating neoplastic phenotypes such as cell proliferation and metastasis.

A best knowledge of epigenetic mechanisms gave a possibility to a targeted cancer therapy (epigenetic therapy) and opened many preventive, diagnostic and prognosis perspectives.

Gene expression profiles in uranium industry workers of Stepnogorsk city occupationally exposed to ionizing radiation.

A. Abilmazhinova, M. Zhabagin1, A. Akhmetova, D. Erezhepov, S. Rakhimova, M. Bakhtir, P. Kazymbet, A. Akhitzhonova, Z. Zhimadilov;
‘Center for Life Sciences, Nazarbayev University, Astana, Kazakhstan, ‘Radiobiology Research Institute, Astana Medical University, Astana, Kazakhstan.’

Ionizing radiation (IR) imposes risks to human health and the environment. Taking into account the fact that Kazakhstan is one of the world’s leading uranium producer, and considering the magnitude of the damage from Semipalatinsk nuclear test site, the study of radiation effects on genes has always been a priority. It is known that LIG3 and XPA genes may serve as biomarkers for sensitive models of dosimetry. The purpose of this study is to determine the impact of the IR on gene expression in uranium industry workers of Stepnogorsk city, Kazakhstan.

Peripheral blood samples (n=20, n=19) were collected from workers who were exposed to different radiation doses (50mSv, 20mSv). To examine gene expression profiles of radiation exposed workers quantitative real-time PCR were used. We used B-actin gene as an endogenous control for our study. The expression of LIG3, XPA genes were analyzed and compared between two groups exposed to different radiation doses. The greater the delivered dose of IR, the greater the expression level of DNA repair genes.

Altered expression profiles in this study can be used as biomarkers of dosimetry for uranium industry workers. This strategy on risk assessment should be considered in the construction of industrial safety and health of workers occasionally exposed to IR.

Whole genome sequencing of Mycobacterium tuberculosis in Kazakhstan: first sequence results of two clinical isolates

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The project is aimed to create the prerequisites for a personalized approach to the diagnosis and treatment of tuberculosis by identifying and comparing the whole genome sequences of M. tuberculosis strains, isolated in Kazakhstan. Analysis for whole genome sequences obtained using NGS technology will clarify the factors cause of the formation of highly virulent strains of M. tuberculosis, the evolution of local strains, and genetic markers of drug resistance.

Material collection from 50 patients, sputum extraction and determinati
of drug sensitivity was performed in the reference-laboratory “National Center of Tuberculosis Problems,” Almaty, Kazakhstan. DNA libraries for whole genome sequencing were prepared from DNA extracted from the isolates. The sequencing was performed on Roche 454 GS FLX+ NGS platform at the Center for Life Sciences, Nazarbayev University, Astana, Kazakhstan.

The sequencing results from 2 isolates were assembled into contigs using GS De Novo Assembler. All alignments were done against the M. tuberculosis reference strain H37Rv using GS Reference Mapper. The sequencing has performed for two M. tuberculosis isolates MTB-476 and MTB-489 with 9.6 M bp with average read length 620 bp, approximately 2.18x coverage and 104.2 M bp with an average read length of 589 bp and approximately 23.7x coverage were generated for the MTB-476 and MTB-489, respectively. The genome of MTB-476 consists of 257 contigs, 4240 CDS, 46 tRNAs and 3 rRNAs. MTB-489 has 187 contigs, 4183 CDS, 45 tRNAs and 3 rRNAs. The results of genome assembling have been submitted into NCBI GenBank, available for public access under the accession numbers AZBA00000000, AZAZ00000000.
J16.10 Short runs of homozygosity as an underappreciated mechanism for imprinting disorders
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Runs of homozygosity (ROH) are epigenetic changes encompassing relatively large genomic loci and presenting as stretches of consecutive homozygous genotypes scattered through the genome. Usually, ROH exceeding 5-10 Mb result in phenotypes similar to those caused by heterozygous null mutations. On the other hand, heterozygosity losses of a single imprinted gene can produce an epigenetic change leading to imprinting disorders. Therefore, such epigenetic changes have not necessarily to encompass large genomic loci. Studying ROHs in 115 children with intellectual disability and congenital malformations by Affymetrix 2.7M SNP/array, we have found that 1-Mb-long ROHs are associated with imprinting disorders. In a case of atypical Angelman syndrome a ROH at 15q11.2 (size: 1.16Mb) was detected. Furthermore, two cases of atypical Beckwith-Wiedemann associated with ROHS at 11p15.5p15.4 (size: 1.36Mb and 1.15Mb) were identified. In addition, a long ROH at 7p14.2p12.1 (14.95Mb) affecting GRB10 (an imprinted candidate gene for Silver-Russell syndrome) was detected in a child with severe growth retardation, microcephaly and intellectual disability. Three children (2.6%) demonstrated over an ROH burden typical for offspring of a consanguineous relationship. Retrospectively, consanguinity was confirmed by genealogical analysis. Thus, our observations show that 3.5% of intellectual disability cases can be attributed to ROH. Moreover, ROHs can be as short as 1Mb, which are usually overlooked, when SNP/array assays or other methods for epigenetic analysis are applied. We conclude that these epigenetic mutations are a relatively common mechanism for imprinting disorders. Supported by the Russian Federation President Grant (MD-4401.2013.7).

J16.11 The review of epigenetic modification in human thyroid cancer
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Thyroid cancer is the most common endocrine malignancy worldwide. However, there is not a definite treatment for advanced thyroid cancer, which comprises poorly differentiated, anaplastic and metastatic or recurrent differentiated thyroid cancer that do not response to radioiodine treated patients. Biological therapies have been proposed on the basis of the recognition key oncogenic mutations and these treatments could be effective in stabilizing progressive disease. Epigenetic abnormalities are present in almost all cancers and together with genetic variants they lead to tumor progression. Epigenetic modification is mostly occur in genes, which play role in the control of cell proliferation and invasion such as RASSF1A, PTEN, DAPK, RAP1GAP, and specific genes of thyroid are mostly epigenetically silenced in thyroid cancer. Rap1, a member of RAS family of small GTPase has been implicated in regulation of mitogenic and oncogenic pathways in thyroid, and its CypG island methylation is reported in some kind of tumors. In addition, miRNAs play important role in cell differentiation, proliferation and survival. They considered as the regulators of gene expression at posttranscriptional level. Deregelation of miRNAs expression is suspected to be an important regulator of tumor development and progression. Epigenetic modification pattern is different not only between normal and tumors tissues, but also between different stage of malignancy and between primary and metastatic tumor. Therefore these variations may become useful novel biomarkers for diagnostic and treatment tumors.

J16.12 Epigenetics, maternal responsibility and neurological development
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It is believed that epigenetics can partly explain the development of neurological conditions. As epigenetic changes often happen in utero, maternal behavior may affect brain development. This raises questions regarding the responsibility of the pregnant woman. To which extent is she responsible for the neurological status of the future child? Should she, for example, avoid all stress, if this is shown to lead to molecular changes increasing the chance of the child to develop ADHD later in life? Should she ensure that she takes optimum nutrition for development of synaptic plasticity of her fetus? With this talk I demonstrate first that epigenetics complicates the question of the responsibility of the pregnant woman. To which extent does the increasing knowledge about epigenetics influence in utero further complicate this responsibility, if even behavior of years before the conception may influence a fetus? Is this an individual responsibility or also a collective one? Second, by looking at examples of autism and extreme intelligence, I demonstrate that, although discussions about parental responsibility towards future children have centered on duties to prevent suffering or to maximize welfare or ‘the chance of a good life’, the distinction between prevention and enhancement is difficult to maintain in the context of neurological conditions. By analyzing arguments from the neurodiversity movement, whose members often insist that their neurological condition is not a disease, but an integral part of their identity, I shall demonstrate that there is a need for a revisiting of the concept of maternal responsibility.

J16.13 Anchored Assembly: Comprehensive variant detection using NGS data
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Characterization of large indels, inversions, and multi-nucleotide variants is important for disease studies. These are often undetected by standard pipelines. Spiral Genetics has developed Anchored Assembly, a novel method using direct, de novo read overlap assembly to accurately detect variants from next-generation sequence reads. We detect, on average, over 90% of indels and structural variants ≤ 30 kb in non-repetitive regions. The ability to detect deletions and structural variants is undiminished by variant size, and the ability to accurately detect and assemble insertions continues well into the 30 kb range. Anchored Assembly was evaluated against Pindel and BWA + GATK using simulated read data. Datasets were generated by populating chromosome 22 of the human genome reference sequence with a set of SNPs, indels, inversions, and tandem repeats. Anchored Assembly detected, on average, over 90% of SNPs, indels, and structural variants up to 50 kb with false discovery rates well below 1%. In comparison, Pindel and BWA + GATK had false discovery rates of 10% and 9%, respectively. Anchored Assembly’s range of detection and low false discovery rates may benefit cancer and rare disease studies. The ability to accurately detect structural differences will be useful for characterizing tumor vs. normal samples and analyzing and comparing whole human trio data to identify risk-associated variants.
variants and copy number variations (CNV) in etiology of the diseases. Who- le exome sequencing and bioinformatics analyses were performed at Genome Quebec Innovation Center, Montreal, Canada. We successfully identified disease-causing mutations in coding regions of several human rare diseases (e.g., Serenky syndrome and SNAP29/22q11.2 deletion syndrome). Additionally, we showed that variants in non-coding regions and CNV have also important value and should not be ignored during bioinform- atics analysis of WES data. For instance, in patients with osteogenesis imper- fecta type V and in patients with glucocorticoid deficiency, we identified variants in 5’UTR, resulting in the production of longer or truncating non- functional proteins. Furthermore, CNVs were identified at the main cause of the diseases in patients with metaphyseal dysplasia with auricular hypoplasia and brachydactyly and in patients with osteogenesis imperfecta type VII. Our results highlight that non-coding variants and CNVs can be crucial and they should be considered during WES data analysis, as they can be the only cause of the disease under investigation.

J16.15
An automated benchmarking tool for structural variation calling using Next Generation Sequencing data
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Structural variations (SVs), such as insertions, deletions and duplications are found to be one of the major genetic causes of cancer. Identifying and characterizing these events is becoming increasingly important in cancer genomics. Next Generation Sequencing (NGS) technology has made it possible to detect SV events at base pair resolution. Many different computational SV detection tools have been developed, based on information sources such as read depth, discordant paired-end reads, split alignments, and de novo assembly. While some approaches make similar predictions, some approaches disagree in their predictions and deliver rather complementary sets of SV calls. The current challenge for researchers, when being confronted with myriad of tools, is to make a good selection, so as to generate a set of SV calls that is both most reliable and most comprehensive.

To appropriately guide researchers in comparing and selecting most appropriate SV detection tools, we have developed an open source toolkit (available also in a downloadable virtual machine) that allows us to systematically benchmark and evaluate SV detection tools. We have generated realistic, simulated datasets based on comprehensive sets of real SVs from several different genomes. We have put forward the performance statistics of different SV calling methods and created a pipeline for their optimal usage. If desired, more SV detection tools can be added to this pipeline, whose workflow is generic in the choice of tools. Results are summarized in a PDF file where prediction, recall and also other specific detection statistics referring to the tools selected are displayed.

J17.01
Angiotensin-I converting enzyme I/D polymorphism gene in Moldavian population
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Background: The angiotensin-converting enzyme (ACE) gene in humans has an insertion-deletion (I/D) polymorphic state in intron 16 on chromoso- some 17q23. This polymorphism has been widely investigated in different diseases. In this study we aimed to investigate the ACE I/D genotype frequency in Moldavian population with others populations.

Materials and Methods: Have been investigated DNA of 100 healthy children aged up to 17 years, in order to make a comparative analysis of ACE I/D poly- morphisms in Moldova with other countries. PCR methods were used for polymorphisms determination of ACE I/D allele. The distribution of geno- types was tested for deviation from Hardy-Weinberg Equilibrium (HWE). Results: There was no significant difference in the distribution of DD, II and ID genotypes of ACE polymorphism in Moldavian population (21.3, 28.8, and 48%) and their ethnically matched control from Italian population (36, 16, and 48%), respectively. Thus, two groups are represented by the similar allelic frequencies and were also found to be in Hardy-Weinberg equili- brium, when (p=0.05<p=0.15) indicate statistic confirmation of similarities. The same similarities were found in North Indian, Dutch and Turkish popula- tions and total differences with Kyrgyz population (χ2=13.69, p=0.002). Conclusion: The polymorphism of ACE I/D genotypes frequency in Mold- avian population are statistical similar with Italian, Dutch, North Indian and Turkish populations.
multiplex PCR amplification using AmpFISTR® Y-filer® PCR Amplification Kit (Applied Biosystems, USA). Capillary electrophoresis (CE) of amplified multiplex PCR will be performed using an ABI 3130 Prism® Genetic Analyzer (Applied Biosystems). The data obtained from capillary electrophoresis will be analyzed and the allele frequencies of Y-STR loci will be determined using a population-genetics-software called ‘ARLEQUIN’. This study will provide an additional information to the framework of variation involving 17 Y-STR loci in forensic case work samples as well as a further contribution to the Y-STR database for UAE male population. This study will also help to determine allele duplications and mutations plus an inconsistency of the transmitted alleles appeared in the UAE population.

**J17.05**

**Hemoglobin H (HbH) Disease in Khuzestan Province**

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**Background and Purpose:** We studied the a-globin gene genotypes, hematologic values, and transfusion dependency of patients with hemoglobin H (HbH) disease in Khuzestan Province, Southwest of Iran.

**Methods:** We detected alpha globin gene mutation by Using gap-polymerase chain reaction (gap-PCR) and direct DNA sequencing.

**Results:** We identified 44 patients with hemoglobin H disease. Of these patients, 15 (34%) had deletional form of HbH disease, 29 (66%) had different form of nondeletional HbH disease. The most frequently observed HbH genotypes were --Med/-a.37 in 14 patients (31.8%), apolyp A66/ apolyp A66 in nine patients (20.5%), and apolyp A44/ apolyp A44 in five patients (11.3%). Some persons with HbH disease required blood transfusion, whereas some others with the same alpha globin genotype were not transfusion-dependent.

**Conclusions:** Our data show diversity in the genotype and clinical presentation of deletional and nondeletional HbH disease. This discrepancy between similar genotypes with different phenotypes may be due to other modifying factors. Therefore, we cannot describe a general conclusion regarding the need for blood transfusion in the patients with HbH disease.

**J17.06**

**Prevalence of alpha-1-antitrypsin deficiency among Polish patients with lung or liver disorders**

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**Background:** In Poland, the overwhelming majority of individuals with alpha-1-antitrypsin (AAT) deficiency still remains undiagnosed. We estimated the AAT gene frequency and prevalence in a large cohort of Polish chronic lung or liver disease patients eligible for AAT testing.

**Methods:** Blood samples were collected prospectively from 419 respiratory patients (COPD, emphysma, bronchiectasia, asthma) and 281 patients referred for liver transplantation due to cirrhotic and non-cirrhotic hepatic disease. AAT serum concentration was measured by nephelometry and PI*-Z was calculated by Hardy-Weinberg equilibrium.

**Results:** The prevalence of PI*Z in respiratory patients and PI*Z in liver disease patients were for PI*Z 46.6 (95% CI: 32.3-60.8), PI*S 20.3 (95% CI: 6.6-36.1), PI*M 13.4 (95% CI: 3.3-38.2), PI*S/Z 9.9 (95% CI: 3.0-22.3) and PI*S/Z 2.7 (95% CI: 0.7-11.7). Among isolated cases 47.7% were cases of congenital anomalies of anus (CAA) without fistula (code Q42.3), 33.2% - CAA with fistula (Q42.2), 11.2% - congenital anomalies of rectum (CRR) without fistula (Q42.1) and 7.8% - CAA with fistula (Q42.0). Among the combined cases CAA without fistula were 42% CAA with fistula - 25%; CAA without fistula - 21%; CAA with fistula - 11.9%. The male affected more frequently than female (1.6:1). Among infants with all kinds of AAT are more common the infants with the low birth weight.

**Conclusions:** On average AAT affected in 1 in 5435 births. There are no changes in prevalence of AAT during research period. The regular monitoring of congenital malformations allows the study of the epidemiological characteristics of even the rare birth defects.

**J17.07**

**The epidemiology of anorectal malformations in Russia from 2000-2012**

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**The aim of this study was to investigate the epidemiological characteristics of anorectal malformations (ARM) in Russia.**

**Material and methods:** We analyzed data from 36 regional birth defects registries of Russia for 2000-2012. The epidemiological analysis included such factors as sex of affected cases, birth outcome, birth weight, maternal age and number of births. Total prevalence per 10,000 births was defined as the total number of cases among live births, stillbirths and induced abortions divided by the total number of all births.

**Results:** Among participating registries total number of ARM cases was 970 with prevalence of 1.84 per 10,000 births. Most of them (85.26%) are isolated and 14.74% cases are combined with other anomalies. Among isolated cases 47.7% were cases of congenital anomalies of anus (CAA) without fistula (code Q42.3), 33.2% - CAA with fistula (Q42.2), 11.2% - congenital anomalies of rectum (CRR) without fistula (Q42.1) and 7.8% - CAA with fistula (Q42.0). Among the combined cases CAA without fistula were 42% CAA with fistula - 25%; CAA without fistula - 21%; CAA with fistula - 11.9%. The male affected more frequently than female (1.6:1). Among infants with all kinds of ARM are more common the infants with the low birth weight.

**Conclusions:** On average ARM affected in 1 in 5435 births. There are no changes in prevalence of ARM during research period. The regular monitoring of congenital malformations allows the study of the epidemiological characteristics of even the rare birth defects.
waist circumference, and triceps and subscapular skinfolds; body mass index [BMI (calculated as weight in kilograms divided by height in meters squared)]. Results: The rs939609 allele was strongly associated with overweight and obesity in this population. The A allele of the FTO polymorphism was significantly associated with higher BMI and higher waist circumference. No significant association between FTO rs 1781749 genotype and adiposity was found. Conclusions: This study replicated the genetic association of SNP of FTO (rs939609) with obesity in a Romanian population and, to the authors’ knowledge, this is the first such association study in a Romanian population. Acknowledgement This work was financially supported by Internal Research Grants of the University of Medicine and Pharmacy Tîrgu Mureş, Romania (grant number 1/30.01.2013).

J17.10

The effects of chemical mutagens on the chromosomes of the population living in the area of the oil and petroleum industries

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One of the major problems facing the world is the pollution of the environment and the study of its effect on the rate of mutation and inheritance rights. Petroleum products and petroleum industries affect pollution. Aim: To evaluate the effects of prolonged exposure to chemical mutagens on chromosome population Zhylyoi district of Atyrau region, living in ecologically unfavorable regions.

Material for cytogenetic analysis: the culture of peripheral blood lymphocytes from 71 residents living in the area with the development of the oil industry. Culturing lymphocytes, cooking preparations, coloring G-method performed by standard methods. The control group consisted of 25 people in an ecologically clean region.

The results of the study. We studied the frequency and spectrum of chromosomal aberrations in the groups studied. Found a higher incidence of aberrations (1.75 per 100 cells) and the total frequency of chromosomal aberrations (1.9 per 100 cells) in affected individuals compared with controls (p < 0.05).

Frequency paired fragments were 0.10±1.99 per 100 cells, dicentric 0.01±0.23, ring chromosomes 0.14±0.38 frequency of stable chromosome aberrations 0.02±1.17 100 cells in people living in Zhylyoi area. The frequency of chromatid type aberrations was 0.87±0.10 100 metaphases and is represented mainly by single fragments. Findings suggest the influence of chemical factors on the chromosome of people living close to the oil companies and petroleum industries.

Conclusion. Cytogenetic studies of the population will be used to define a group of families of ‘high risk’ for the prevention of genetic disorders in ecologically unfavorable regions.

J17.11

Association between C282Y and H363D mutations of the HFE gene with hepatic cirrhosis in Lithuanian population

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Cirrhosis is commonly caused by alcohol use, viral hepatitis B and C and many other possible causes. The search for epidemiological, biological or genetic factors that could help to select patients at higher risk of developing cirrhosis is necessary. Among these factors, the influence of excessive liver iron overload and HFE gene mutations have been debated over the past few years and up to now there is limited data on association between HFE gene mutations and cirrhosis.

The aim of this study: To determine the association between HFE gene C282Y and H63D mutations and liver cirrhosis.

Methods: A cohort of cirrhosis patients consisted of 209 participants. The diagnosis of cirrhosis was confirmed by clinical features, liver biopsy and radiological imaging tests. Control samples were randomly obtained from 1005 blood donors. HFE gene mutations were detected using PCR-RFLP method. Statistical analyses were performed using statistical software for genetic association studies PLINK v2.050.

Results: C282Y allele was associated with liver cirrhosis (5.02%, OR=2.074, p=0.00510) when compared with controls (2.49%). C282Y/wt genotype was linked with cirrhosis comparing with wt/wt genotype (OR=2.002, p=0.01239). A similar situation was observed in dominant model for C282Y mutation (wt/wt vs. C282Y/wt + C282Y/C282Y) which showed increased risk of liver cirrhosis (OR=2.065, p=0.00766). H63D mutation was not associated with cirrhosis in Lithuanian population.

Conclusion: HFE gene C282Y mutation is associated with liver cirrhosis and might cause a higher risk for progression of liver cirrhosis in Lithuanian population.

J17.12

Associations of genetic variants of CNR1 and CNR2 with hypertension or dislipidemia in the Chinese post-menopausal women

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Metabolic syndrome may occur in 40% of postmenopausal women and is largely determined by overweight and obesity. Obesity in menopause women occurs as a genetic background, hormonal changes and acquired changes in eating behavior and physical activity. Evidence showed that the endogenous cannabinoid system (ECS) plays a role in obesity and related metabolic disorders. To investigate the association between CNR1 and CNR2 gene polymorphisms and metabolic risk factors in the Chinese postmenopausal women, five hundred and fifteen post-menopausal women were recruited. Anthropometric measures (body mass index [BMI], waist circumference [WC]), blood pressures, and metabolic parameters were determined. Four SNPs from CNR1 gene and 1 SNP from CNR2 gene were selected for genotyping on these post-menopausal women. We found that post-menopausal women carrying TT+TC genotype of CNR1 rs2023239 had increased risk of metabolic syndrome (p=0.047). We also found two SNPs were significantly associated with pre-hypertension or hypertension (OR=1.72, 95% CI: 1.12-2.63 for rs806381 and OR=1.77, 95% CI: 1.00-3.11 for rs1049353, respectively). We further observed that CNR2 rs2229579 was significantly associated with hyper-triglycerides in our post-menopausal women (OR=1.85, 95% CI: 1.06-3.23). Haptype analysis also revealed that those women carry the GTTA haplotype of CNR1 gene were less likely to develop pre-hypertension or hypertension (aOR=0.67, 95% CI: 0.47-0.96). Our findings provide initial evidence that the CNR1 gene variants may contribute to pre-hypertension or hypertension and CNR2 gene variant may predict hyper-triglycerides in post-menopausal women in Taiwan.

J17.13

A study on the association of ERCC1 Asn118Asn polymorphism and colorectal cancer risk in West Algerian population

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Colorectal cancer (CRC) is one of the most common causes of death due to cancer in both men and women throughout the world. It has been suggested that sporadic CRC is most likely caused by lowpenetrance genes, including those involved in DNA repair mechanisms. Furthermore, the accumulation of DNA damage may contribute to colorectal carcinogenesis. Many epidemiological studies have explored the association between ERCC1 Asn118Asn polymorphism and colorectal cancer risk in various populations, its role in colorectal carcinogenesis in Algerian population in particular has not been investigated yet. Therefore, we conducted this case-control study in a West Algerian population to assess the potential role of this genetic polymorphism on the risk of colorectal cancer. Peripheral blood samples of 90 sporadic CRC patients and 100 normal controls were collected, DNA extracted genotyped using pyrosequencing technique. The distribution of genotypes of ERCC1 Asn118Asn genotype and allele frequencies among CRC cases was not significantly different from those among controls (P>0.05). The variant genotype combinations did not show any significant association with CRC susceptibility risk suggesting that the ERCC1 codon 118 polymorphism does not convey moderate increase in susceptibility to CRC in West Algerian population. This is the first study on ERCC1 gene polymorphism in our population suggesting that the Asn118Asn polymorphism may not be associated with the colorectal cancer risk in West Algerian population. Further research with a larger sample size is needed to reveal more information about this polymorphism and the appearance of colorectal cancer in our population.
Cystic Fibrosis in Arab populations: Identification of novel mutations and complex allele in Egyptian and Lebanese Patients

Patients and methods: Subjects: Twenty four (24) unrelated CF patients were recruited from the Pneumology and Allergology Department of the specialized Hospital Center in Canastel Oran (Algeria).CF diagnosis was based on clinical findings and repeated positive sweat chloride tests (>60 mmol/l).This study evaluated the effectiveness of the ELUCIGENE CF30 Kit in a sample of Algerian CF patients.

J17.16 Notification of death from cystic fibrosis in Brazil during 30 years from 1981 to 2010


The aim of this work was to evaluate the notification of cystic fibrosis (CF) as primary cause of death in Brazil, from 1981 to 2010, and to compare with developed countries. The Brazilian data was obtained from SIM-Data/SUS and the American from the CDC WONDER. The Brazilian median age at death (MAD) was 3.9 years in 1981 and 1.24 years in 2010 for typical severe CF. In 1994, the Brazilian MAD was much lower (7.2y) than that (21y) of seven developed countries reported by Forgary et al, 2000. We found no increase in MAD in Brazil from 1981 to 1985; from 1986 to 2010 it showed a three-fold increase. From 1981 to 1990, there were few deaths over 25 years of age in Brazil, mostly concentrated in younger age groups. During the sample period there was an increase in deaths in higher age groups in Brazil, which may reflect better patient survival rates due to increased knowledge of the disease, with repercussions for its diagnosis and treatment. We also observed an increase in the reported cases of CF deaths per 100,000 inhabitants in Brazil over the years, possibly due to the better knowledge of the disease and consequently more accurate death notification, since this increase is not typical of a genetic disease. For the USA we observed a decrease in notified CF deaths, despite the population increase which may be a reflection of a smaller number of people born with CF due to appropriate genetic counseling and prenatal diagnosis.

J17.17 SERPINA1 gene polymorphism frequency in clinically confirmed cystic fibrosis patients

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Background - SERPINA1 gene is known to be one of cystic fibrosis (CF) modifier gene. Patients who carry the Z allele are at greater risk of developing severe liver disease. The aim of this study - to assess whether Z allele frequency is different in patients with confirmed CF than in control group.

In this work, were typed in 100 unrelated individuals (sex ratio 60:40) from South Romania.

J17.18 Population data of 11 DNA markers from a sample taken from South Romania

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In this work, were typed in 100 unrelated individuals (sex ratio 60:40) from South Romania eight DNA markers. Aim was to analyze the genetic variability and to establish the relation between this region and other European populations. We establish Hardy-Weinberg equilibrium, gene diversity, genetic distances and tree topology by PHYLIP 3.68 package. Polymorphism’s frequencies were similar to the mean frequencies calculated for the whole populations. We establish Hardy-Weinberg equilibrium, gene diversity, genetic distances and tree topology by PHYLIP 3.68 package. Polymorphism’s frequencies were similar to the mean frequencies calculated for the whole set of populations included in the study. The mean value of Ht was 0.201, and for Hv was 0.18. The most affiliated population with our lot are Italy, Spain, Poland, Germany, Greece and Turkey the most distant population are United Kingdom, Sweden, Croatia and Slovenia.

J17.19 A new variant of Ehlers-Danlos syndrome with inborn errors of mucopolysaccharide metabolism in the mother and son

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Background: Connective tissue dysplasias are characterized by the clinical polymorphism and genetic heterogeneity. Each new patient with Ehlers-Danlos syndrome, according to our observations is potentially a new variant of the syndrome.

Case report: Family V. has been followed-up for 13 years. Proband V. Clinical manifestations - coarse facial features, a disproportionate body, keeled chest deformity, kypohoscoliotic spinal deformity, wing-like scapulas, an increased skin extensibility, its softness, velvet, hypotonia, hypermobility of joints, varicose veins, swelling of the lower extremities. Proband's mother M. - disproportionate body, coarse facial features, kypohoscoliotic spinal deformity, a soft, doughy, hyperelastic skin, hypermobility of joints, varicose veins, lymphatic edema of the lower extremities. The examination echographically revealed hepatosplenomegaly, metabolic, dysplastic changes in the kidneys, mitral valve prolapse, an additional chord of the left ventricle. Biochemically - signs of an increased collagen degradation, daily urine excretion - 155.4 mg/day, increased urinary glycosaminoglycans up to 1460 u/g creat, hyperprolinemia, hyperglycinemia, hyperprolinuria, hyperhomocysteinemia - 26.6 mol/l. Pedigree analysis showed that the pedigree is burdened by cardiovascular disease.

MTHFR G1793A/MTRR A66G polymorphism was revealed in the proband and his mother by the study of gene polymorphisms of folate cycle system. Conclusion: A new variant of Ehlers-Danlos syndrome has been diagnosed in the mother and son with the phenotype associated with metabolism errors of mucopolysaccharides, hypermobility of joints, hepatosplenomegaly, in the mother and son with the phenotype associated with metabolism errors of MTHFR G1793A/MTRR A66G enzymes.

J17.20 Multidisciplinary study of Endometriosis as a common complex disorder
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Endometriosis (E) is a common multigenetic disorder affecting almost 10% of women of reproductive age. Comparative molecular, genetic, immunological analysis and endocrinology tests were applied in the studies of 257 women with E and in 117 women in the control. Participation of the genes responsible for steroid hormone activity, their receptors, inflammation, proliferation, cell migration, apoptosis, intercellular adhesion, angiogenesis, as well as the genes regulating their activity have therefore been suggested as plausible candidates. A handful of very interesting new candidate genes involved in oncogenesis, metaplasia of endometrium cells and embryonic development of female reproductive system were identified by GWAS technology. In addition to alterations in the DNA sequence itself, differential expression levels of the candidate genes might be caused by different epigenetic modifications including methylation, heterochromatization, mRNA regulation etc. Complex genetic net of E implies participation of epigenetic landscape of E. Origin of E could be provoked by any combination of both genetic and epigenetic risk factors with subsequent canalization of pathological processes (reverse epigenetic landscape), which become irreversible soon after it starts.

J17.21 Asthma related FCER2 variant in Roma and Hungarian populations
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1Department of Medical Genetics, University of Pecs, Pecs, Hungary, 2Szentagothai Research Centre, University of Pecs, Pecs, Hungary.

Asthma is a complex respiratory disease, which can be caused by environmental factors and genetic predisposition. It is one of the most widespread diseases in the world; it has a high presence in most ethnic group. The low-affinity IgE receptor (FcεRII/CD23) encoded by the FCER2 gene (Fc fragment of IgE) plays important role in the regulation of IgE responses and inhaled corticosteroids (ICS) therapy in asthma. Corticosteroids influence FCER2 expression and FcεRII receptor function. The intronic rs28364072 polymorphism (T2206C) of FCER2 gene associated with elevated IgE levels, severe asthmatic exacerbations and decreased expression gene. The variation in the FCER2 gene contributes to variation in ICS treatment response in asthmatics. Our aim was to investigate the ethnic differences, allele and genotype frequencies of intronic variant of FCER2 in average Roma and Hungarian populations. We examined 451 females (276 females, mean age: 46.4±18.4) and 397 Hungarian subjects (222 males, 175 females; 37.8 mean age±12.6 years) with PCR-RFLP method. We found more than twofold increased homozygous CC genotype frequency in Hungarian group compared to Roma samples (5.8% vs. 2.8%, p<0.05). The C allele frequencies were similar in each group (24.8% in Romas and 24.6% in Hungarians). The current study demonstrated that unfavourable variants can diversely occur in Hungarian and Roma individuals. Genetic test of FCER2 variant are likely necessary to assess the outcome of asthma treatment. Homozygous 2206C allele carrier Hungarians have higher chance for insufficient response to corticosteroids compared with Roma subjects due to genotype higher risk frequency. This research was supported by TÁMOP-4.2.3-12/1-KONV-2012-0028.

J17.22 Common MEFV gene mutation profiles in Familial Mediterranean Fever patients in Canakkle population
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Objective: In the current study it was aimed to find out of the current study is to determine the frequency of common MEFV gene point mutations in 741 patients who previously diagnosed as MFM Method: The genomic DNA was isolated by spin-colon method (Roche, Germany) from peripheral blood samples with EDTA and buccal smears. The MEFV gene profiles for the current FMF cohort were genotyped by Pyrosequencing and direct Sanger sequencing techniques for the target common point mutations. Results: Twenty-two different point mutations were identified in 363 (49%) patients and no mutation was detected in 378 (51%) current patients suspicious for FMF. The most frequent mutations were M694V (32.7%), E148Q (15.1%), R202Q (11.6%), M6809T (9.0%), V726A (7.4%), P369S (6.3%) and K679R (4.4%) in the current FMF cohort. The M694V/E148Q was the most frequent compound point mutation that detected in the current FMF cohort. The most common clinical finding was abdominal pain in all MEFV mutation types that detected in the current mutated FMF patients. Median attack frequencies of untreated patients are; 3.14 for M694V, 2.71 for M6809T, 2.75 for R202Q, 2.71 for P369S and 1.44 for K679R. All attack frequencies were less than patients with M694V, all of the patients from Canakkle region with mutations E148Q, R202Q, P369S and K695R had FMF clinical diagnostic criteria. Conclusion: The current results showed that the R202Q point mutation frequency was higher than the other sub-populations that reported from different regions of TURKEY. It was seen that the initiation of the symptoms were delayed in patients with R202Q mutation when compared to the others.

J17.23 The association analysis of polymorphism the metabolism of lipids genes with bmi, waist circumference and blood lipopidogram's parameters at women before and after the Menopause
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Objective: In the current results it was aimed to find out of the current study is to determine the frequency of common MEFV gene point mutations in 741 patients who previously diagnosed as MFM Method: The genomic DNA was isolated by spin-colon method (Roche, Germany) from peripheral blood samples with EDTA and buccal smears. The MEFV gene profiles for the current FMF cohort were genotyped by Pyrosequencing and direct Sanger sequencing techniques for the target common point mutations. Results: Twenty-two different point mutations were identified in 363 (49%) patients and no mutation was detected in 378 (51%) current patients suspicious for FMF. The most frequent mutations were M694V (32.7%), E148Q (15.1%), R202Q (11.6%), M6809T (9.0%), V726A (7.4%), P369S (6.3%) and K679R (4.4%) in the current FMF cohort. The M694V/E148Q was the most frequent compound point mutation that detected in the current FMF cohort. The most common clinical finding was abdominal pain in all MEFV mutation types that detected in the current mutated FMF patients. Median attack frequencies of untreated patients are; 3.14 for M694V, 2.71 for M6809T, 2.75 for R202Q, 2.71 for P369S and 1.44 for K679R. All attack frequencies were less than patients with M694V, all of the patients from Canakkle region with mutations E148Q, R202Q, P369S and K695R had FMF clinical diagnostic criteria. Conclusion: The current results showed that the R202Q point mutation frequency was higher than the other sub-populations that reported from different regions of TURKEY. It was seen that the initiation of the symptoms were delayed in patients with R202Q mutation when compared to the others.

J17.24 Genetic epidemiological study of hereditary disorders in Tataren Republic (Russia)
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Tatars - are the second sized ethnos of the Russia. The load and genetic diversity of monogenic hereditary disorders (HDs) in three major ethnographic groups of Tatars from Tataren Republic were analyzed (Kazan Tatars-3 Districts, Miusars-2 Districts and Teyptys-3 Districts). The size of the investigated populations was more than 270,000 inhabitants (213,000 Tataren). The total population was examined by standard protocol of medical
genetic research elaborated in laboratory of genetic epidemiology, Research Centre for Medical Genetics. About 3500 HDs of OMIM could be identified by this protocol. Clinical investigations were performed by neurologists, ophthalmologists, orthopedic, otolaryngology’s, dermatologists, pediatricians and clinical geneticists. Foci accumulation such genodermatosis as neurofibromatosis type 1, vulgar ichthyosis (1:9212), tuberous sclerosis (1:29262), X-linked ichthyosis (1:15546) and inheritance (1:13818), multiple lipomatosis (1:31091), neurofibromatosis type 2 (1:5025), palmoplantar keratoderma with autosomal dominant inheritance of GJB6 gene deletions, genetic background of congenital hearing-loss in different ethnic groups. The aim of the present study was to provide a complete and updated spectrum of mutations in GJB2 and GJB6 gene and to identify the most prevalent mutations in Rostov region population.

J17.25 Medical and population genetic characteristics of the population genodermatosis Rostov region, Russia
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Genodermatosis is a genetic disorder of the skin usually generalized. The medical and population genetic studies genodermatosis population 12 districts of the Rostov region were conducted. The total size of the investigated population was 497,460 persons, 20% of whom are children (110,584 children).

Data collection and processing were done according to the protocol of Genetic Epidemiology Laboratory of Research Centre for Medical Genetics in Moscow.

The total prevalence rate among the population genodermatosis Rostov region was 1:1709 and 1:1047 for the child population, respectively. The incidence of autosomal dominant (AD) genodermatosis was high (5,15±0,32) and incidence of autosomal recessive (AR) and X-linked genodermatosis was low (0,28±0,9, 0,84±0,18, respectively). The same tendency was among children (the incidence of AD, AR and X-linked genodermatosis, 7,81±0,90, 0,72±0,27, 2,05±0,65, respectively). Incidence was calculated per 10,000 population.

Significant differences in the different districts of the incidence rates of AD and AR disorders were reviewed. The correlation with one of the main population genetic factors is genetic drift was found.

The spectrum genodermatosis represented 22 nosological forms, 15 of them with AD, 5 with AR and 2 with X-linked inheritance types. The ‘nucleus’ of genodermatosis spectrum in Rostov region population are Ichthyosis vulgaris (1:5025), palmoplantar keratoderma with autosomal dominant inheritance (1:13818), multiple lipomatosis (1:31091), neurofibromatosis type I (1:9212), tuberous sclerosis (1:29262), X-linked ichthyosis (1:15546) and anhidrotic ectodermal dysplasia (1:49746). The prevalence rate of these diseases has been frequently than 1:50000.

Fo accumulation such genodermatosis as neurofibromatosis type I, vulgar ichthyosis and X-linked ichthyosis was determined.

J17.26 Allelic heterogeneity of GJB2 gene in Romanian population with congenital isolated hearing-loss C. Dragomir1, A. Staic1, D. Bojanescu1, L. Savoi2, E. Sevier1, 1Genetic Lab, Bucharest, Romania, 2Carol Davila University of Medicine and Pharmacy, Bucharest, Romania.

Aims: Different alleles within the same gene can cause a similar variant phenotype. Previously published studies showed the allelic heterogeneity of GJB2 gene as main genetic cause of isolated congenital hearing-loss phenotype. The proportional distribution of the different mutations within GJB2 gene varies in different ethnic groups. The aim of the present study was to provide a complete and updated spectrum of mutations in GJB2 and GJB6 gene and to identify the most prevalent mutations in Romanian population.

Material and Methods: To overcome our aims, we used clinical data from 80 unrelated persons with congenital hearing-loss and performed ARMS-PCR and DNA sequencing techniques for detection of known mutations and identification of mutations within GJB2 gene. Analysis is of delGJB6-D13S1850 and del(GJB6-D13S1851delA) was performed by multiplex PCR.

Results: Most mutations were c.35delG (40.0%) in both homozygous and heterozygous forms. The second mutant allele was W24X (8.75%) also named IVS1+1G>A and R127W mutations with lower frequencies.

Conclusions: The study reveals c.35delG mutation as most prevalent one, absence of GJB6 gene deletions, genetic background of congenital hearing-loss in local population and supports improvement of genetic counselling services.

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Glutathione S-transferases A1 and P1 are the main enzymes involved in the biotransformation of drugs, carcinogens and toxins. Their activity may influence drug response as well as susceptibility to diseases. GSTA1 and GSTP1 genes coding for these enzymes, are important objects of many studies due to their genetic variability which may affect enzymes activity. The aim of our study was to determine the GSTA1*1A, *1B, *2, GSTP1*1A, *1B, *2 allelic distribution of the GSTA1 and GSTP1 genes in different districts of the Polish population. We performed our analyses on the DNA of 160 subjects from the Polish population. In the GSTP1 gene we have genotyped two polymorphisms (I105V and A114V) by pyrosequencing method and the GSTA1 gene was screened for the changes in the promoter region using sequencing. The detected variants were subjected to the haplotype analysis. We found alleles GSTP1*1C (c.313A, c314C), *1B (c.313G, c314C) and c313C, c314T with 65.3%, 23.8% and 10.9% frequency respectively. As a result of GSTA1 gene promoter sequencing we detected 8 SNPs located in sites: G-52A, C-69T, C-115T, A-136G, T-567G, G-631T, A-1066T, G-1142C, G-1245A. The allele GSTA1*B, as associated with the decreased enzyme activity was observed in our study with frequency of 43.1%. Finally, 4 haplotypes have been determined in 160 Polish individuals. Our study demonstrated the genetic distribution of the GSTA1 and GSTP1 genes in the Polish population corresponds to data for Caucasians. Furthermore, we found novel SNPs, excluding three well known changes (G-52A, C-69T, T-567G), which are linked to the alleles GSTA1*A/B influencing enzymes activity.

J17.28 Genome-wide association analysis identifies a locus on DMD (dystrophin) gene for power athlete status in Russians V. A. Naumov1, I. I. Ahmetov2, A. K. Larin3, E. V. Generozov3, N. A. Ralemin3, E. A. Usanov3, A. V. Pavlenko4, E. S. Kastrzykowska4, D. G. Alexesi5, V. M. Gavorun3; 1Research Institute for Physical-Chemical Medicine, Moscow, Russian Federation, 2Volga Region State Academy of Physical Culture, Sport and Tourism, Kazan, Russian Federation.

Power athlete status is a heritable trait: around two-thirds of the variance in this phenotype is explained by genetic factors. Since power and endurance are located at the opposite extremes of a muscle performance continuum, a genome-wide association study (GWAS) of elite Russian power-oriented athletes (sprinters and strength athletes) and endurance-oriented athletes as controls was performed to identify common genetic variants associated with elite power athlete status. 102 sprinters, 86 strength athletes and 178 endurance-oriented athletes were genotyped using the Illumina® HumanOmni1-Quad BeadChips. When comparing sprinters and endurance-oriented athletes, the most significant association (P=6.2×10^-7) was shown for the rs939787 gene. Interestingly, this association was replicated (P=2.9×10^-6) by comparing strength athletes and endurance-oriented athletes (P=3.1×10^-8 when sprinters and strength athletes were combined). The rs939787 is located in the DMD (dystrophin) gene which plays an important role in muscle contraction and strength, linking the intracellular cytoskeleton to the extracellular matrix. In conclusion, our data suggest that the DMD gene rs939787 polymorphism is associated with elite power athlete status in Russians.

J17.29 Human longevity and Alu-polymorphism V. V. Erdman1, T. R. Nasibullin1, I. V. Tuktarova, D. S. Karimov, O. E. Mustafina; 1Volga Region State Academy of Physical Culture, Sport and Tourism, Kazan, Russian Federation.

It is assumed that genome instability can affect the lifespan. Alu-insertion is one of the causes of such instability.

The aim of the study was to search association between age and polymorphism Alu at the loci of the TEAD1 genes. Localized women intners, of PLAT, COL13A1, ACE, LAMA2 and TED1 genes. DNA of 1611 unrelated individuals from 21 to 109 years, ethnic Tatars from Russia, was genotyped. Age dynamics of the genotype and allele frequency was estimated using logistic regression analysis (SPSS18.0). Associations between age and polymorphic Alu-elements localized within introns of COL13A1 (rs9968, LAMDA2 (rs1318 and TLS19) and TED1 (rs892531) genes was found for females. Chances of achievement of longevity age above for females with COL13A1*T/*T (range 57 - 109 years, OR=1.04E, P<0.001), LAMDA2*T/* (range of 56-109 years, OR=1.016, P=0.002) and TED1*T/* (range of 78-109 years, OR=1.014, P=0.001).
years, OR=1.055, P=0.012) genotypes. Chances of achievement of longevity age below for females with COL13A1*I/D (range 57 - 109 years, OR=0.948, P<0.001) and LAMA2*D/D (range of 56-109 years, OR=0.981, P<0.001) genotypes. Association between age and a polymorphic Alu-element within an intron 16 of ACE gene (Ya5:ACE) was detected for males and females. Decrease of ACE*D/D genotype frequency was observed among long-livers males (range of 77-109 years, OR=0.953, P=0.006) and long-livers males (range of 76-109 years, OR=0.935, P=0.040). Thus, Alu-polymorphism of COL13A1, ACE, LAMA2 and TEAD1 genes, possibly, are associated with attainment of longevity age. And besides, positive association with age is traced for insertion allele of Alu-elements.

Supported by grants RFBR 13-04-01561a, 14-04-79794a, 14-04-01169a.

J17.30

Formation of the genetic structure of Uighurs in Kazakhstan

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The study of genetic structure of modern human population is one of key problems of human genetics. Genetic and demographic parameters of uighurs is presented. Marriage and migration structure of fourteen rural regions were studied. The mean value of ethnic assortative marriage was 1.68. Analysis of marriage structure suggests genetic differentiation of rural districts uighurs of region and is characterized by a high proportion of monogamous marriages (86-97%), low level and radius of migrations, the share of external migration to an average of 0.99%, high level of inbreeding from 0.0005 in Chundzha rural district to 0.00946 in Kolzhat. Positive as sortative mating on a national basis was 4.2. High index of endogamy found in Kolzhat districts (72%). The mean number of children per woman constituted 3.98. Crow index of total selection (fitot) and its components (Im, If) were 0.25, 0.04 and 0.20 respectively. The size of the portion of the population before reproduction (40.4% of the total), the prevalence of reproductive parts of the population (43.8% of total), and family size is 4.0 allow us to classify the type of growing population of uighurs.

Recent social and economic changes have led to an increase in differentiation of rural districts uighurs of Kazakhstan. Thus, detected subdivision uighur rural population on number of interacting subpopulation units, the main insulating factor that is positive assortative mating on national basis, set growing type of reproduction, described nature of migration processes, analysis of components as possible of potential population screening for uighurs in Kazakhstan.

J17.31

Random Inbreeding in Karachay

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Karachays live compactly in 4 regions of Karachay-Cherkessia (Russia). Karachays are one of the Turkic-speaking North Caucasian peoples. Values of Wright random inbreeding (a quarter of the sum of squares of frequencies of surnames) are counted on the basis of distribution of adults surnames for rank of “district” population and “Village Council” population. Both of these assessments are necessary for carrying out various types of analysis. District population rank F values are: 0.0035 (Malokarachaevsky district), 0.0014 (Prikubansky district), 0.0016 (Us-Dzhegutinsky district), 0.0015 (Karachayevsky district). The average-weight values F for “Village Council” population rank are counted separately for rural and urban populations (where urban present) and male: 0.0056 (Malokarachaevsky, rural), 0.0033 (Prikubansky, rural), 0.0042 and 0.0010 (Us-Dzhegutinsky, rural and urban), 0.0084 and 0.0016 (Karachayevsky, rural and urban), thus the general assessment of the average-weight F value for these areas without division into rural and urban people make: 0.0056, 0.0033, 0.0024, 0.0059, respectively.

J17.32

An early manifestation of LBSL syndrome, case description

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Background: Loeuencephalopathy with brain stem and spinal cord involvement and lactate elevation is an autosomal recessive disease. Mutation in gene DARS2 is associated with this syndrome. The gene is located on the long arm of chromosome 1 (1q 25.1), the disease is associated with deficiency of mitochondrial aspartyl-tRNA synthetase.

Care report: 1 year and 5 month old boy T, with static kinetic and psycho-speech development delay, excess body weight (16kg). The child is from I pregnancy against the background of threatened miscarriage. Delivery at 36-37 weeks by cesarean section. Birth weight = 3300g. At the time of examination we paid our attention at the decreased muscle tone, muscle strength tendon and peristalt reflexes are not observed, nystagmus, ataxia. Movement disorders develop further, patients become disabled by the second to fourth decade of life. In our case, the disease manifested in the heterozygous carrier by one year of life and was accompanied by obesity.

J17.33

Association analysis of haplotypes of the lepton gene promoter region with BMI in Roma population


Leptin, an adipocyte-derived protein, plays a key role in regulating energy intake and consumption. Increased circulating leptin levels, commonly found in obesity, interfere a failure to signal satiety and halt the progression of obesity. Aim of this study was to investigate the association of single nucleotide polymorphisms (SNP) and haplotypes of the lepton gene promoter region with body mass index (BMI) in Roma (Gypsy), a population known to be vulnerable to developing obesity presumably due to their life-style and genetic background.

We sequenced 1500 bp region of lepton gene promoter in the sample of 377 Roma individuals from Croatia and identified 9 polymorphic sites. Haploview software was used to assess linkage disequilibrium (LD) among polymorphic loci as well as the Unphased version 3.0.13 software for haplotype association analysis.

All nine SNPs were grouped into single LD block due to their proximity. None of the single markers (rs1349419, rs146378188, rs13245201, rs185230264, rs791614, rs12535708, rs10487506, rs17770725 and rs12535747) showed individual statistically significant association with BMI. However, two haplotypes, A-A-G-A-G-A-G-G-T-G and G-A-G-G-A-G-A-T-A, showed significant association with BMI (P=0.00561 and P=0.003214). Both p-values remained significant after 1000 permutation tests. Our data showed that haplotype association analysis provided advantage over individual SNPs analysis. Haplotypes spanning the lepton gene promoter region were found to be significant predictors of BMI in Croatian Roma.

J17.34

Germline variants of ARID5B as possible risk factors for childhood acute lymphoblastic leukemia in Latvia

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Background: Acute lymphoblastic leukemia (ALL) is the most common childhood malignancy but etiology of pediatric ALL remains poorly understood. Data from GWAS provided convincing evidence that inherited genetic variation in ARID5B contributes to childhood ALL predisposition, the strongest association was found with SNP rs10821936, some studies show strong association with whole intron. Aim. To detect possible association of SNPs in intron 3 of gene ARID5B and ALL predisposition to childhood ALL in Latvia. Material and Methods. In study were enrolled 34 ALL patients and their biological parents. In study group were determined rs10821936 and 6 other SNPs as a closest three from both sides to SNP rs10821936, where MAF > 1%. Analyses were performed using PCR, RFLP and sequencing.

Results. By TDT test strongest association were found with rs10821936 (OR=10, CI95% 1.28-7812, p=0.0066), rs10821937 (OR=0.67, CI95% 1.17-2873, p=0.0164) and almost significant with rs7908445 (OR3.33, CI95% 0.97-12.11, p=0.0525). Haplotypic analysis TDT test - with sliding window size 5: rs7923074-rs77918077-rs10821936-rs12246030-rs10821937 (AGCC) (transmitted frequency 5.959, untransmitted frequency 0.0284, p=0.0153); rs77918077-rs10821936-rs12246030-rs10821937-rs7896246 (GCCA) (transmitted frequency 0.761, untransmitted frequency 0.9877, p=0.0137). Haplotype analysis TDT test - with sliding window size 5: rs77918077-rs10821936-rs12246030-rs10821937-rs7896246 (GCCA) (transmitted frequency 0.761, untransmitted frequency 0.9877, p=0.0137).

Conclusions. 1. We have found an association with SNP rs10821936 C allele and increased predisposition
to childhood leukemia. 2. We detected risk haplotypes of childhood ALL composed of 7 SNPs located in intron 3 of ARID5B gene.

J17.35 Genetic polymorphism and mRNA levels of TNFα and TGFβ genes in patients with chronic lower limbs infections
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Introduction: The wound healing process is important medical problem which still have gain no binding results. We choose two factors, strictly involved in inflammation, and connected in proper tissue healing.

Aim of study: The purpose of the study was to investigate SNP’s in TNFα (G-308A) and TGFβ (G-747C, T-29C) genes in patients and evaluate expression of mRNA levels in comparison with controls.

Material and methods: Group of 191 patients were divided into subgroups: A) chronic leg ulcers, B) chronic non-healing wounds, C) infected ischemic necrosis of foot, D) lymphatic ulcers symptoms of dermatomyositis-admitis (DLA). A control group comprised 129 blood donors. Detection of polymorphisms was performed using the PCR-RFLP method. The level of TNFa and TGFβ gene expression was performed by Real-Time PCR.

Results: Patients in subgroups showed higher frequency of genotypes TNFα-308GG and lower of GA than in controls. TGFβ-29TT and more frequent (subgroups B and C) than in controls (p<0,001). TGFβ-74GG genotype was at highest values, in subgroup B. The GC genotype was at similar level in all subgroups being lower than in controls. TGFβ-29TT and TC genotypes were at similar level to controls. The presence of the polymorphic allele TNFα-308AA was more frequent (subgroups B and C) than in controls (p<0.001). TGFβ-74AC genotype was at highest values, in subgroup B. The GC genotype was at similar level in all subgroups being lower than in controls.

Conclusions: The presence of polymorphic alleles could predispose to increased production of mentioned proteins. Protein overexpression may impair the proper conduct of wound healing contributing to formation of ulcers.

J17.36 The study of the populations of the Volga-Ural region of Russia in the context of Eastern Eurasian mtDNA haplogroups
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Volga-Ural region is a place of encounter of different peoples who vary in both linguistic and religious grounds. An emplacement in the crossroads of two continents, Europe and Asia, had a great influence on creation of this diversity.

The aim of our study was to analyze few lines that belong to the East Eurasian component of the genetic pool in the region. We focused on the lineages of haplogroup A namely A4b and A10.

Haplogroup A4b has previously been found in the study of Derenko et al, 2008 in the Evenk and Buryat populations from Siberia. In our research, we conducted a full genome sequencing of mtDNA samples with this haplogroup in Udmurt and Beserman populations and found that mutations in the coding region do not correspond to those in the samples studied by Derenko et al, 2008. It is likely that these two lines are the two sub-branches of one subscale A4b. Although our populations showed similarity in difference with the Siberian A4b subscale on the other hand the presence of A4b in our populations itself shows some old connections with Siberian populations.

We also analyzed a full sequence of all known published examples of haplogroup A10 and compared with our results. We found that some new mutations that were not previously detected in Dolgan, Ngnanas, Nogay and Tadjik populations. It is interesting that Tajik, Tatars from Kazan and Chuvash samples are likely belong to the one subgroup of this haplogroup, indicating the recent common ancestry of these populations.

J17.37 Polymorphisms in the RIG-I-like receptors (RLRs) genes and susceptibility to multiple sclerosis in the German population
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Multiple sclerosis (MS) is an autoimmune disease of the central nervous system potentially associated with viral infection. RIG-I-like receptor genes (RIG-I, IFIH1 and LGP2) have been hypothesized as involved in the viral etiology of MS, since they are implicated in viral recognition. Previous studies have produced conflicting results regarding the association between IFIH1 gene polymorphisms and MS. Here we examined the effects of 13 single nucleotide polymorphisms (SNPs) in the RIG-I, IFIH1 and LGP2 genes on MS in the German population. The study comprised 716 patients with MS and 706 healthy control individuals. Genotyping was performed using RFLP TaqMan genotyping assays and high-resolution melting (HRM) analysis. There were no significant differences (p>0.05) between MS patients and healthy individuals for any of the investigated SNPs as well as haplotypes derived from these SNPs. Further, no differences were observed between healthy individuals and MS subgroups stratified according to disease characteristics. Our results suggest that variation in the RIG-I, IFIH1 and LGP2 does not exert a major influence on MS risk.

J17.38 Associations between MX2 polymorphism and the acquisition of HIV and co-infections in Caucasian intravenous drug users (IDUs)
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BACKGROUND: Until recently, myxovirus resistance protein 2 (MX2), a type I interferon-induced GTP-binding protein, was thought to be devoid of anti-viral activity. Latest studies indicate MX2 may be involved in blocking of HIV entry. We aimed to determine, whether C/T polymorphism of MX2 gene (rs45430) is associated with acquisition of HIV and co-infections (HCV, HBV, GBVC) in IDUs.

METHODS: Study included 345 IDUs and control group comprised of 301 blood donors (all HIV, HCV, and HBV negative). C/T polymorphism of MX2 gene was determined using TaqMan allelic discrimination assay.

RESULTS: Of IDUs, 50% were HIV+, 88% HBV+, 67% HCV+, and 33% GBVC+. In total 296 donors and 342 IDUs were successfully genotyped. C/T polymorphism was in Hardy-Weinberg equilibrium and allelic frequencies were similar in both groups (0.65 for T allele in IDUs, 0.6 for T allele in donars). No associations between C/T polymorphism and acquisition of HIV, HBV, and HCV were found. However, the prevalence of persons possessing at least one C allele was higher among GBVC compared to GBVC+ (63% vs 51%, p=0.047). Persons with at least one C allele had decreased odds of being GBVC+ (OR=0.62, 95%CI=0.39-0.98), but after adjusting for co-variables associated with GBVC status (age, HIV positivity) it did not remain significant (OR=0.66, 95%CI=0.41-1.06), which might refer to underpowered groups.

CONCLUSIONS: This is the first study to investigate associations between MX2 (rs45430) polymorphism and acquisition of HIV and co-infections in high risk population. Whether this polymorphism plays a role in GBVC acquisition needs further evaluation.

J17.39 Do polymorphisms affecting the ornithine transcarbamylase (OTC) gene affect myocardial infarction risk and blood pressure in the West Algerian population?
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Purpose: Recently, it has been shown that the rs5963409 polymorphism of the gene encoding ornithine transcarbamylase (OTC) is associated with hypertension and coronary vasomotor. On the basis of these results, we chose to study the association of two polymorphisms rs5963409, located at the 5' region of the promoter and rs1800321, located in exon 2 of the OTC gene with the risk of the occurrence of myocardial infarction (MI) and the values of systolic and diastolic blood pressures. Methods: Concerning MI, the polymorphisms were characterized in a case-control study (69 cases vs 67 age-matched controls) based on the male population originating from Oran, Algeria. The associations between the polymorphisms were assessed in an enlarged control group including 115 male subjects. Genetic characterization of two polymorphisms was performed by PCR (Polymerase Chain Reaction) followed by enzymatic digestion. Results: We neither showed associations of the rs5963409 and rs1800321 polymorphisms with variations in blood pressure values. However, we observed a significant interaction between the rs5963409 polymorphism and Body Mass Index (BMI) on the risk of the occurrence of MI. Conclusion: Our results indicate the two polymorphisms of the OTC are not associated with the MI in the West Algerian population.
J17.40
NLRP3 genotype CG increases the risk of HIV in Caucasian intravenous drug users (IDUs)
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BACKGROUND: The increased stability of NLRP3 (Nod-like receptor family, pyrin domain containing 3) by rs10754558 G could lead to earlier immune dysregulation in persons with HIV, thus associated with a higher risk of HIV infection. We examined 226 patients with MS and 200 healthy controls. Genotyping was performed by amplification and subsequent digestion with the restriction enzyme CviQII. We found significant differences of allele and genotype distributions (p=0.011 and p=0.05, respectively) between cases and controls. To our knowledge, this is the first study performed on NLRP3 in patients with MS. On the basis of the preliminary data obtained in our population, we could hypothesize an involvement of the gene in the susceptibility to the disease. In particular, the presence of the T allele, under-represented in the patients, seems to be a protective factor against MS. However, due to the small number of participants, the confirmation of the role of NLRP3 requires further studies extended to a larger sample of subjects and also to other populations.

J17.43
Variability of Genes Associated with Obesity in Populations of Russia and Neighboring Countries
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The prevalence of obesity and related diseases reached a high level throughout the world. The role of genetic factors in the disease development has been proved by numerous studies on twins and adopted children by testing candidate genes predisposing to obesity. Genome-wide association studies (GWAS) is one of the advanced methods used to detect mutations associated with overweight. We selected 20 SNPs associated with obesity with high level of significance from GWAS-catalog. Allele frequencies for these SNPs were calculated in 14 populations of Russia (Yakuts, Buryats, Tuvans, Koryaks, Mordovians, Russians, Khants, Ket, Chuvashs, Kabardinians, Karachais, Ossetians, Tatars, Udmurts and Karelains) and neighboring countries (Kazakhs, Uzbeks, Kyrgyz, Megrelians and Moldavians). Data on 7 native non-admixed populations from HapMap and “1000 Genomes” project were also included into analysis. Correlation analysis of allele frequencies and genetic diversity with geographic, ecological and climatic parameters of the study populations was performed. Allele frequency of 16 from 20 studied SNPs correlated with climate and geography. The SNP showed correlation with latitude (rs1704198, rs9299, rs10182181, rs6110577, rs9999538 and rs7603514) and with climate only (rs3101336, rs7784447, rs255906, rs2275848 and rs7447986). The revealed relationships between genetic and geo-climatic parameters may indicate the presence of positive selection on genes associated with obesity and adaptive changes in the gene structure in human populations during human dispersal out of Africa.

J17.44
The common polymorphism Val109Asp in the omentin gene is associated with daily energy intake in the Central-European population

Omentin was originally first described in 2003 and was reported to be expressed specifically in human omental adipose tissue. So far, very little is known about the relationship between the genetic variability of the omentin gene and pathophysiology of obesity. The aim of the study was to investigate two common polymorphisms in the omentin gene (rs2274908 and rs2274907) and dietary composition and anthropometric parameters of obesity in the Central European population. Material and methods: The total of 495 subjects were included into the study that were further divided into 3 groups – non-obese, obese and morbidly obese. Dietary habits were established using the 7-day food records and selected anthropometric parameters were measured. Results: There were significant differences in genotype distributions of rs2274907 between the obese and morbidly obese cohorts (P = 0.01). In the multivariate modelling, the rs2274907 polymorphism expressed independently of daily energy intake on the age and gender distribution (p = 0.03); the TT genotype being associated with the lowest average energy intake (7877 ± 2780 kcal/day) and the AA genotype with the highest daily intake of energy (8764 ± 2467 kcal/day). Significant association of the rs2274907 was also observed for the daily consumption of fat and proteins.

Conclusion: This is so far the first study to investigate the polymorphisms in the omentin gene in a large population cohort of obese and non-obese individuals. Based on our results, the rs2274907 polymorphism is associated with daily energy intake in the Central-European population.
with the daily energy intake as well as daily intake of fat and protein.

**J17.45**

**Associations between polymorphisms in PAX9 gene and congenital missing teeth**

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The congenital missing of teeth is one of the most common developmental disorders in dental pathology with values between 4.4% and 8%. Autosomal dominant hypodontia is caused by abnormalities in genes MSX1 and PAX9. PAX9 gene plays a key role in odontogenesis and gene polymorphisms were found in both biall forms but also in sporadic cases with dental agenesis. Recent studies show model PAX9 gene polymorphisms in patients with dental agenesis. Our study group consisted of 180 adolescents aged 18-22 years and one hundred controls. In this group we found a total of 10 cases (5.5%) with specific agenesis, without signs of other disorder, and no other dental anomaly, of which 7 cases were sporadic and 3 cases with familial aggregation. In families besides the index, agenesis was also found in five individuals. In two families agenesis was present in grandmother, mother and daughter. All patients were investigated clinically oral and extraoral and X-ray was performed. Selection of patients for the genetic analysis of gene PAX9 was based on the type of hypodontia clinically found, because families bearing PAX9 mutation show a pattern of clinical sign. Heterozygous mutation C503G was detected in the female proband, in her mother and grandmother. One polymorphic A240P mutation site was found, in PAX9 gene, in two sporadic cases. This variant was found in subpopulations of African American and Europe, but had no relevance to the ethnic Chinese population.

**J17.46**

**Association of the MMPs genes single nucleotide polymorphisms with gastric and duodenal ulcer in Volga-Ural region of Russia**

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Gastric and duodenal ulcer (DU and GU) has a genetic background and the aim of this study was to investigate the allele and genotype distribution of SNPs in the MMP1 (rs49437), MMP2 (rs2285053), MMP3 (rs3025058), MMP9 (rs3918242, rs17576) and MMP12 (rs2276109) genes in patients with PUD and healthy donors from Volga-Ural region of Russia. The patient group consisted of 260 individuals with PUD, the control group included 272 unrelated non-ulcer individuals with different ethnic origins (Russians, Tatars, Bashkirs). Genomic DNA was extracted from peripheral blood samples. Genotyping was performed by polymerase chain reaction - restriction fragment length polymorphism analysis. The analysis has revealed that Russians have significant higher frequency of rs49437 T/A (P=0.02; OR=1.86 and P=0.0007; OR=5.31, respectively). We have also found the association of rs49437 A/G genotype with PUD in Tatars (P=0.01; OR=1.86). Genotype rs17576 (T/C) is a marker of the increased risk of PUD development in males (P=0.005; OR=1.79), whereas rs3918242 T/T and rs17576 A/A were protective (P=0.03; OR=0.10; P=0.01; OR=0.61, respectively). The association analysis of the rs228505 (P=0.0005) and rs2276109 with PUD has not revealed significant differences between patients and healthy donors (p>0.05). Thus, we have determined statistically significant association between MMPs genes polymorphisms and peptic ulcer in Volga-Ural region of Russia.

**J17.47**

**Sequence analysis of PMS2 exon 11 and homologous region in pseudogene**

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PMS2 gene product is involved in DNA mismatch repair process. Besides the gene, there are several pseudogenes in the human genome, showing high sequence homology. To describe and distinguish sequence polymorphism in highly homologous loci which could be co-amplified in PCR assays, we have sequenced "short" 243 bp fragment containing co-amplifying regions chr7:6026682-6027488 (part PMS2 exon11 and chr7:6776854+6777096 (part of PMS2CL pseudogene) in 50 DNA samples. In 14 DNA samples, additional "long" fragment encompassing region chr7:6026682-6027488 (part of intron 10 and exon 11 PMS2) has been sequenced. The same heterozygous positions in both fragments, together with peak height differences in heterozygous positions within the short fragment allowed us to determine their location (gene/pseudogene) and to calculate MAF estimates. Altogether 6 variable positions were identified and most of them had been registered previously. The PMS2 gene and SNPs in PMS2 in public databases (UCSC). Among 1255573 (C/T) in intron 10 (MAF=0.036) and rs15024062 (A/G, Gly460Asp) in exon 11 (MAF=0.080). High frequency was determined for rs1805321 (C/T, Pro470Gly, allele T frequency 0.53) in additional 134 samples by restriction analysis. We have found that rs63750658 polymorphism (C/G, MAF=0.12) is actually located in the pseudogene (rs199351636), as well as novel SNP A/G in position chr7:6777067 (MAF=0.090). The previously known SNP rs1805320 (C/T, Gln467Glu) turned out to be different between the gene (G) and the pseudogene (T) sequences. Our results refine SNP list in the regions and emphasize careful pmc design for SNP studies in the PMS2 gene.

**J17.48**

**The association of REN gene polymorphism with athlete status and muscle mass**

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The renin-angiotensin system (RAS) is supposed to be one of the regulators of skeletal muscle growth and differentiation (Zhang et al. 2003; Johnston et al. 2011). Renin (encoded by REN gene), as a component of the RAS, activates the renin-angiotensin cascade by catalyzing the conversion of angiotensinogen to angiotensin I (Rupert, 2006). The aim of present study was to investigate the association between the intron 8 83A/G (rs2368654) polymorphism of the REN gene, athlete status and muscle mass in Russians. Two hundred and sixty-eight Russian athletes (90 females and 178 males) from different sporting disciplines were involved in the study. REN genotype and allele frequencies were compared to 151 controls (74 females and 77 males). Genotyping for the REN polymorphism was performed by RT-PCR. Muscle mass parameters were assessed by bioelectrical impedance analyzer Tanita MC 980 (Japan) in 125 athletes (44 females and 81 males). We found that the frequency of the renin allele is significantly higher in power-oriented athletes (78 vs 68%; P=0.021) compared to controls and this difference was even more pronounced in elite power-oriented athletes (89%; P=0.018). Furthermore, the REN G allele was positively correlated with fat-free mass, absolute muscle mass, muscle mass of trunk and left/right legs in elite athletes. In conclusion, we have shown that the 83A/G polymorphism of the REN gene is associated with power athlete status and skeletal muscle parameters in Russians.

**J17.49**

**The association of PPARD and PPARA gene variants with physical performance in Lithuanian elite athletes**

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Peroxisome proliferator-activated receptor (PPAR) delta and PPAR alpha (encoded by the PPARD and PPARA genes) play a role in energy homeostasis, vascular biology, mitochondrial function. Functional SNPs in the PPARA (rs4253778, intron 7 G/C) and PPARD (rs2016520, 5'-UTR region of the pseudogene) genes have been associated with mRNA and/or protein activity. The aim of this study was to determine the allele/genotype frequency distributions among 130 Lithuanian elite athletes (endurance-oriented (n=40), power-oriented (n=52), mixed endurance/power athletes (n=38)) and 175 healthy non-athletes controls. Genotyping was performed by PCR-RFLP. Results showed the genotype distribution was in Hardy-Weinberg equilibrium within all groups (P>0.05). Genotype frequency differed significantly between the male athletes and the male controls (PPARA GC/CC: 58.4/35.5/5.9% vs 76.2/22.9/0.9%; P=0.012; PPARD TT/TC/ CC: 88.1/11.9/0% vs 75.2/21.9/2.9%; P=0.031). The PPARA GC genotype was more frequent among the athletes in endurance(67.5%) and mixed sports(71.1%) than the power (50%). In power-oriented events group significantly elevated frequencies of PPARA GC and GC genotypes were determined, compared to the endurance-oriented (P=0.039) mixed athletes (P=0.005) and controls (P=0.008). These results support the positive as-
sociation of the PPARD C allele with power performance. PPARD genotype distribution in athletes with mixed endurance/power activity showed significant difference compared to controls (TT/TT/CC: 92.1±7.9% vs 53.1/46.9%/6.9%; P=0.023). In conclusion, the PPARD C allele may help athletes in power-oriented sports, and the PPARD C allele is a factor unfavourable for athletics. This finding is relevant of physical performance; it may also be informative for the targeted prevention of diseases associated with low fitness.

J17.50 A polymorphism in a possible regulatory region of PGR associated with risk of spontaneous preterm labor with intact membranes (PTB-I).

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Background. Progesterone has been used to prevent preterm deliveries by decreasing the activity of the cervix as an endogenous ligand of the progesterone receptor (PGR) and plays a critical role in the maintenance of pregnancy. The aim of this work was to investigate the association between a single nucleotide polymorphism (SNP) located in a possible regulatory region of PGR (rs1942836) and two different clinical subtypes of preterm birth: spontaneous (PTB-I) and premature rupture of membranes (PTB-PRM). This SNP has been previously studied in a heterogeneous group of PTBs. Methods. The sample included 412 triads (proband, mother, father) recruited to the Nuествa Señora de la Merced Maternity Hospital in Tucuman, Argentina. Of these triads, 200 had probands from PTB-I and 212 had probands from PTB-PRM. Genotyping was performed using Applied Biosystems Taqman probes and the Fluidigm genotyping platform. Both maternal and fetal genetic effects were analyzed using a log-linear method for analysis of case-parent-triad data (Weinberg et al. 1998). Results. We found a significant association between rs1942836 (mature effect) and PTB-I (OR: 2.01; IC 95%: 1.12-3.61; p = 0.01), but not between this SNP and PTB-PRM (OR: 1.05; IC 95%: 0.57-1.93; p = 0.85). Conclusions. These results would suggest a specific association between the PGR gene (maternal effect) and PTB-I, but not with PTB-PRM. These findings may have implications in understanding the pathophysiology of clinical subtypes of preterm birth and the potential therapeutic role of progesterone in prevention of PTB-I.

J17.51 The diversity and prevalence rate of monogenic hereditary pathology among the children of Tatarstan Republic (Russia)

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Results of prevalence rate and genetic diversity of monogenic hereditary disorders (MHDs) among the children of eight investigated Districts of Tatarstan Republic (Russia) was submitted. The total size of investigated populations was made 268894 people, including 57648 (21.4%) children. The ethnic structure of considered sample on more that 80 % is presented by Tatar population. The study was conducted on the original protocol examined by standard protocol of medical genetic research elaborated in laboratory of genetic epidemiology, Research Centre for Medical Genetics. About 350000 of 100000 MHD could be identified by this protocol. Clinical investigations were performed by pediatricians, clinical geneticists, neurologists, ophthalmologists, orthopedists, audiologist, and dermatologists, focused on diagnostic of MHD. The spectrum of MHD detected in the eight Districts comprises 158 nosologial forms - 84- autosomal dominant (AD), 54- autosomal recessive (AR) and 20- X-linked. The total prevalence rate of children's population by all types MHD - AD, AR, and X-linked, separately for urban (1:187 children) and rural (1:189 children) populations was calculated. In rural populations, the prevalence is higher 2 times higher than in urban. The total prevalence of MHD was 1:103 children. Given that this study can detect only half of hereditary diseases, the total prevalence of hereditary disorders in children more than 1,2%. In this study, we studied the prevalence of hereditary diseases among children Republic Bashkortostan, Udmurtia, Rostov Region and Chuvashia, in which the burden of hereditary diseases was 1.4%, 1.2%, 1.3% and 1.1%, respectively.

J17.52 Birth defects in Chile: implementing a National Registry and Surveillance System

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Chile is an upper middle income country, has an infant mortality rate of 7.7 per 1000 births in 2011. Birth defects (BD) are an important public health problem, account for 3.5% of the infant mortality, being the second leading cause of prematurity. Authorities of that Chilean Ministry of Health has designed a registry and surveillance system for BD (REVINACH) in stillbirths, newborns and infants less than a year in all public hospital in Chile. We report herein the characteristics of this national registry system. The Department of Statistics at Ministry of Health will be in charge of clearing, encoding, statistical analyses and dissemination of data. Data collection will be in charge of any professional that detects a BD. This registry has 3 instruments for data collection (1) Online Birth Certificate with an open field named: „Congenital Malformations Anomaly“ (2) Online database expert to collect information after birth named “REVINACH” (3) Online database to collect complex information named “Genetics Module” as a second step. All three systems are linked to a third party system involving the Civilian Registry and the National Statistic Institute. This system will be a source of information for surveillance of BD and will be essential to the planning, implementation, evaluation of public health practices and policies as well as prevention.

J17.53 Genetic predisposition to the development of restenosis in Kazakh population

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Restenosis is a narrowing of the stented vessel that occurs after stent placement. At the moment, genetic factors of restenosis were studied mostly in Caucasian population. However, ethnic variability of genetic markers is well known. The purpose was to study association of genetic variation in candidate genes of patients diagnosed with restenosis in the Kazakh population.

This case-control study included patients with a diagnosis of coronary heart disease (CHD) who developed restenosis. The control group consisted of patients with CHD who have not developed restenosis within 6 months after stenting. Genomic DNA was extracted from peripheral whole blood samples. SNP genotyping was performed on QuantStudio 12K Flex. Allele frequencies in both case and control groups were in compliance with Hardy-Weinberg equilibrium. Odds ratios (OR) and p-values have been calculated for all studied SNPs. Confidence interval (CI) was set at 95% significance level. Sampling data shows that men undergo stenting at an earlier age than women. In addition, an increased BMI was found in all patients. The results showed that known associations of polymorphisms with the risk of restenosis, shown by J.W. Verschuren in the European population, were not observed in Kazakh population. However, a statistically significant association with the risk of restenosis was found with fibrinogen beta-chain gene polymorphism rs1800790 (OR - 2.1, P-value - 0.019) and thrombomodulin (THBD) gene polymorphism rs1042579 (OR - 1.7, P-value - 0.011).

It can be inferred that both polymorphisms are likely to be predictors of restenosis, while THBD polymorphism is specific for Asian population.

J17.54 Dimorphism -23HphI of the INS gene (rs689): association with type 1 diabetes in several populations of the Russian Federation

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After HLA, the next strongest genetic association with Type 1 diabetes (T1D) is seen for the INS gene. The aim of this study is cross-ethnic group comparisons of frequencies and analysis of associations with T1D -23HphI INS dimorphism (in promoter region of the gene, the rs689) in Bashki, Byurt, Udmurt, Yakut and Russian ethnic group of the Russian Federation. Case-control design was applied for assessment of 528 patients with T1D and 374 healthy sex and ethnic matched individuals. Allele identification was performed with RFLP or Real-Time PCR technique. Association of genetic markers with pathology was evaluated according to odds ratio index (OR), association was considered statistically significant when p-value<0.05. Allele A and genotype AA of rs689 are associated with T1D in Russian (Odds=2.5 and 2.7 respectively), Bashki (Odds=5 and 6 respectively), Udmurt (Odds=2.4 and 2.7 respectively).
and 2.6 respectively) and Yakut (0.63 and 4 respectively) populations. Allele T and genotype T of rs689 are protective markers in these populations (0.16>OR<0.4). Association of rs689 with TID has not been identified in Buryat population (with low incidence of TID). Cross-ethnic comparison of frequencies of alleles and genotypes showed statistically significant differences. Buryat population differs from all other examined populations by much higher frequency of allele A (87% vs. 69-75%; p<0.006) and of genotype AA (77% vs. 45-60%; p<0.01). It is concluded that in the Buryat ethnic group rs689 is not diabetogenic marker unlike all other examined groups.

J17.55
Spinocerebellar ataxias (SCAs) in Europe: updating current situation
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Spinocerebellar ataxias (SCAs) represent a clinically, genetically and pathologically heterogeneous group of rare hereditary untreatable neurodegenerative disorders, which affect the cerebellum and its connections. As far as we known, there are more than 30 subtypes reported in literature. In Europe, it’s estimated that one to three per 100 000 individuals suffer from a SCA subtype, but this number could vary among different ethnic and geographic groups. Epidemiological data on SCAs is considered scarce and fragmentary with relatively low frequency within a population usually being better known. The goal of this study was to systematically compile and update the available information regarding prevalence of SCAs, as well as several indicators, namely number of published reports, expert centres, diagnostic tests, patient’s organizations, available biobanks, as well as performed and ongoing clinical trials. Data were collected considering literature reports from 2000 until 2013, and using several databases, such as Orphanet and EuroGeneTest. Updating SCAs current data could be an important tool for needs assessment in specialized molecular diagnosis resources, planning future health and community services and better resources allocation, being helpful to evaluate if the available resources are in accordance with the real requirements in different European countries.

J17.56
The SHBG gene polymorphism (rs12150660) is associated with elite power athlete status and muscle mass
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Testosterone regulates muscle mass and strength, bone mass, fat distribution and the production of red blood cells. Sex hormone-binding globulin (SHBG) is the key protein responsible for binding and transporting of testosterone. SHBG regulates its bioavailability and therefore its effects in the body. Polymorphism at the SHBG gene locus (rs12150660 G/T) has been associated with testosterone concentrations. Since individuals with the TT genotype have higher serum testosterone concentrations in comparison with carriers of the G allele (data from GWAS), we hypothesized that the polymorphism could affect the production of red blood cells. Sex hormone-binding globulin (SHBG) regulates its bioavailability and therefore its effects in the body. Polymorphism at the SHBG gene locus (rs12150660 G/T) has been associated with testosterone concentrations. Since individuals with the TT genotype have higher serum testosterone concentrations in comparison with carriers of the G allele (data from GWAS), we hypothesized that the polymorphism could affect the production of red blood cells. Since testosterone has been shown to be a significant predictor of muscle mass, the effect of the SHBG genotype on muscle mass and strength was investigated in a group of power athletes and control group. The study group consisted of 284 participants of Olympic and International Games and the remaining 129 athletes were national–level athletes. The DNA was isolated from peripheral blood lymphocytes and genotyped using TaqMan method. All statistical analyses were performed using Statistica ver. 10. The frequencies of the T allele in power-oriented athletes (n=143, 20.3%; P=0.7462), endurance-oriented athletes (n=220, 15.0%; P=0.2054) and a whole cohort of athletes (17.1%; P=0.5078) were not significantly different from controls (18.8%). However, the frequency of the T allele in elite power-oriented athletes (n=65, 26.2 vs. 12%, P=0.0061) was significantly higher as compared with elite endurance-oriented athletes (n=58). Furthermore, correlation analysis showed positive association between the T allele and muscle mass in male elite athletes. It is hypothesized that the carriage of the T allele may give some advantage for strength and power performance. The aim of the study was to investigate the association between the SHBG G/T polymorphism, athlete status and muscle mass. A total of 363 Russian athletes and 130 controls were genotyped using RT-PCR. Muscle mass was measured by body composition analyzer Tanita MC-980.

J17.57
Extreme Carrier Frequency Of The Splice Site IVS1+1G>A Mutation In GJB2 Gene In The Eastern Siberia Is Comparable To Carrier Frequency Of The Sickle Cell Anemia In Africa
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This study presents data on the carrier frequencies of the splice site mutation IVS1+1G>A in GJB2 gene, causing an autosomal recessive form of deafness among various ethnogeographical groups of Yakut population and in a random sample of Yakut individuals. 350 DNA samples of hearing individuals from various ethnogeographical groups of Yakut population: Central group (n=60), Buryat group (n=60), Northern group (n=60), and random samples of Yakut individuals (n=170) were obtained from the DNA Bank of the Department of Molecular Genetics of Yakut Research Center of Complex Medical Problems of RAMS (Yakutsk, Russian Federation). The detected average carrier frequency of IVS1+1G>A mutation in Yakut population (n=350) was - 10.3%. Extremely high carrier frequency of the splice site mutation IVS1+1G>A in GJB2 gene in Yakut population is comparable to carrier frequency of the sickle-cell anemia in Africa, that may indicate a possible selective advantage of carriers of this IVS1+1G>A mutation in a subarctic climate. This study was supported by RBRF (#12-04-00342-a, #12-04-098520, #14-04-00174-a), SB RAS Integration Project #92 «Ethnogenesis of indigenous peoples in Siberia and North Asia: comparative, historical, ethnico-social and genomic analysis», the Sakha Republic President grant for Young Researchers for 2014 (RP#80), RAS Program «Fundamental Sciences for Medicine» (#30 for 2013-2015), and «Scientific and Educational Foundation for Young Scientists of Republic of Sakha».

J17.58
Variations in nuclear genes are associated with elite sport performance in the Polish population
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Objectives: Single nucleotide polymorphisms are the most common type of human genetic variation. It is widely recognized that genetic factors located in mitochondrial and nuclear genomes influence sport performance. The aim of our study was to assess whether selected nuclear DNA variants are associated with athlete performance in the Polish population.

Methods: The study group comprised 413 unrelated elite athletes and the control group consisted of 451 unrelated sedentary individuals. The athlety was stratified into three subgroups: the power athletes (n=188) and the endurance ones (n=225). The study group included 284 participants of Olympic and International Games and the remaining 129 athletes were national–level athletes. The DNA was isolated from peripheral blood lymphocytes using standard procedures. Genotyping of 10 nuclear DNA variants (ACE, rs4341; ACTN3, rs1801579; GABBR1, rs12599456; CHRNA3, rs4950; AGT, rs6699; FAAH, rs524420; PARC, rs10081282; TFAM, rs1937; TFAM, rs1606604; FCNLCA1, rs8195797) was conducted using TaqMan method. All statistical analyses were performed using Statistica ver. 10.

Results: We showed that six polymorphisms were associated with outstanding results in power (TFAM,rs 2306604, FAAH, ACE, ACTN3) or endurance sports (CHRNA3, GABBR1). Gender and sport level of athletes were also significant.

Conclusion: Our study indicates that in the Polish population genetic background could influence sport performance.

J17.59
Gene pool of the Kazakhs according MTDNA and of ten X-chromosomal STR markers
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The aim of the present research was: to study the polymorphism of mitochondrial DNA and 10 X-linked microsatellites (DYS387, GTA1720D5, DYS7132, DXS9898, DYS7423, DYS837, DYS101, DXS6809, DYS6799, HPRTB) in population of Kazakh.

Genetic and geographic analysis of the genetic structure of Kazakh populat
null
Amitogenomic phylogeny of haplogroups U2e and U3: revealing the
associated with the geographic distances between studied populations. The
the tribe of Tore and the Great Zhuz (0.021). These genetic distances are
the Small Zhuz (0.393), whereas the shortest distance was found between
groups of Kazakh tribes. The most distant ones were the tribe of Sunak and
haplogroup frequencies to assess the genetic similarity among studied
Population pairwise FST values were calculated from the Y-chromosomal
Characteristics of Y-chromosome Polymorphisms in Kazakh
J17.65

Population pairwise FST values were calculated from the Y-chromosomal haplogroup frequencies to assess the genetic similarity among studied groups of Kazakh tribes. The most distant ones were the tribe of Sunak and the Small Zhuz (0.393), whereas the shortest distance was found between the tribe of Tore and the Great Zhuz (0.021). These genetic distances are associated with the geographic distances between studied populations. The distribution of Y-chromosomal haplogroups is strongly correlated with the tribal-clan structure of Kazakhs. Presence of certain haplogroups at high frequency at particular tribes is in favor of the hypothesis that many tribes go back to one biological founder, confirming the link between Kazakh family tree Shuiezhyn with the genetic composition.

J17.66

A mitogenomic phylogeny of haplogroups U2e and U3: revealing the phylogenetic signals for population expansions in the Slavs prehistory

To resolve the phylogeny of some uncommon and poorly studied West Eurasian mitochondrial DNA (mtDNA) haplogroups, we sequenced 32 U2e and 19 U3 complete mitochondrion of Central and Eastern Europeans (Greeks, Slovaks, Poles, Russians, Ukrainians and Belarusians) and re-analysed the available at the present time data on 74 U2e and 80 U3 complete mtDNAs. Molecular dating suggests that the coalescence time estimates are ~21 and ~35 thousand years (ky) for haplogroups U2e and U3, respectively. Detailed analysis of about 500 Slavic complete mitogenomes belonging to different haplogroups allowed us to identify a number of lineages that seem specific for Europe. A European (U2e1b1a1b1, U2e1b1b, U3a1a, H5a1f, U5a1a1c1, U5a1e1, U2e2a1a, U4a2d, H5a2, U2e2a1d and U5a1b1b). These subhpalpogroups consist of similar haplotypes revealed in different ethnic groups of modern Slavs, thereby proving the existence of ethnolinguistic community of Slavs through DNA testing. Evolutionary age of Slavic-specific subhaplogroups is calculated to approximately 3.9 ky (from 2.3 to 5.9 ky, according to the mutation rate proposed by Soares et al. (2009) for the entire mtDNA molecule). This indicates that the ancestors of modern Slavs inhabited areas of Central and Eastern Europe from the times of Bronze and Iron Ages, i.e. earlier than it was estimated on the basis of archaeological, historical and linguistic data. This study was supported by Russian Foundation for Basic Research (grant 14-04-00131) and the Program of Presidium of Russian Academy of Sciences (grant 12-1-2013).

J17.67

Incidence of alpha globin gene defect in the Lebanese population: a pilot study

Inherited hemoglobin disorders are the most common monogenic defects described worldwide. It is well established that Mediterranean and Near Eastern populations are at high risk for thalassemias in general, and for α-thalassemia in particular. Prenatal as well as premartial screening programs in countries with high prevalence have already been founded. In this study, we aim at assessing the incidence of alpha thalassemia deleterious alleles in the Lebanese population. Methods: DNA was extracted from 200 newborns dried blood cards remaining from routine neonatal screening at the American University of Beirut Medical Center. DNA samples were screened for the 21 most common α-globin deletions and point mutations reported worldwide, through multiplex Polymerase Chain Reaction (PCR) and Reverse-Hybridization technique. Results: The carrier rate of α-thalassemia in our sample population was 8% which is higher than that reported from Jordan (2-4%). This finding is comparable to Mediterranean countries (Israel: 5-9%, Greece: 7%, Adana-Turkey: 7.5%) but lower than that reported in other Arab countries (UAE: 16.5%; Oman: 38.6%; Saudi Arabia: 50%). Two mutations were detected: the 3.7del single gene deletion (75%) and the non-gene deletion 2 VS1 [1nt] (25%). These mutations are common worldwide. Interestingly, the -α 4.2 and MED mutations, particularly common in Arab and Middle Eastern populations, were absent in our survey. Conclusion: This study is the first dedicated to investigate α-thalassemia incidence in Lebanon. Data obtained demonstrates a high carrier rate in a relatively, highly consanguineous population. These results may impact premartial and newborn screening policies in our country.

J17.68

5-HTT genotype and sexual behavior traits in healthy female subjects

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Serotonergic neurotransmission affects a large range of behaviors, from food intake to sensory processing and motor activity; cognition, emotion regulation, social behavior and most important to our proposal, reproductive activity. A key regulator for serotonergic neurotransmission is the serotonin transporter (5-HTT), which removes serotonin released into the synaptic cleft. The 5-HTT protein is encoded by a single gene, SLCTA4. Transcriptional activity of this gene is modulated by several variations, including a repetitive sequence, the SLCTA4 linked polymorphic region (5-PLR). Specifically, the 5-HTTLPR short allele (s) has reduced transcriptional efficiency compared with the long allele (l), and individuals carrying the s allele tend to have, among other, an exaggerated amygdala response to threatening visual stimuli, biased processing of emotional information, likely resulting from altered functional connectivity within a corticolimbic circuit, impulsivity and increased anxiety related temperamental traits.

We used a new instrument for the measurement of sexual behavior: “Sex-Promiscuity-Infidelity-Questionnaire” on a cohort of 154 female students recruited at the University of Chieti, in order to test the possible interactions between carriers individuals and sexual behavior. Through an Exploratory Factor Analysis (EFA) we explored the factor structure of the questionnaire, composed by three factors: “Demographic Inquiries”, “Sexual Promiscuity” and “Sexual Instability”. Furthermore the EFA showed significant carri- ers’ factor loadings on sexually instability behavior factor (~0.35).

Our findings support the possible association between serotoninergic neurotransmission, mediated by 5-HTTLPR affect, and the sexual behavior in female healthy subjects.
Several mutations in the IL12B, IL12RB1, IFNGR1, IFNGR2, STAT1, and IKBKG genes lead to the development of rare syndrome of atypical familial mycobacteriosis (OMIM # 209955). We set out to test whether rare variants and polymorphisms in these genes can predispose to tuberculosis. These genes are frequently mutated in consanguineous families from Russian populations (304 patients, 265 controls) and Tuvinians (238 patients, 263 controls). First, we carried out the screening for most common mutation variants of the genes (IL12RB1 Gln32Ter, Gln376Ter, Arg123Trp; IFNGR1 Ile877Thr; 4-bp Del NTB818, 1-bp Del NTB818; IFNGR2 2-bp Del 278AG, Thr168Asn, 663Del27; STAT1 Leu706Ser, Gln463His, Gln520Gln). All these variants appeared as "wild-type". Then we performed the search for rare variants of the studied genes in 173 individuals suffering from aggressive forms of TB. Direct sequencing did not reveal any mutations causing atypical familial mycobacteriosis; however, 15 previously established single nucleotide polymorphisms were identified (IL12RB1 rs11086087, rs1157934, rs17852635, rs940152, rs24461312, rs177882555, and rs3746190; IL12B rs1979766; IFNGR1 rs2234711, rs17181457, rs7749390, rs11754268, and rs11914; IFNGR2 rs17983129; and STAT1 gene rs2066797). Using "case-control" design, we identified an association between the rs2066797 variant of the STAT1 gene and TB in Russians (p = 0.02). Association of this polymorphism with TB was detected for the first time. Our results suggest that rare mutations responsible for atypical familial mycobacteriosis are unlikely associated with TB.

**J17.70 Pathway-VEGAS: A post-GWAS method in genetic epidemiology**

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Genome-wide association studies (GWAS) have revolutionised the field of gene mapping. As the GWAS field matures it is becoming clear that for many complex traits, a proportion of the missing heritability is attributable to common variants of individually small effect. Detecting these small effects individually can be difficult and statistical power would be increased if relevant variants could be grouped together for testing. We propose grouping markers together based on pre-specified biological pathways. We have implemented this pathway-based test in the program Pathway-VEGAS. The method is based on prior calculation of gene-based p-values using the existing Versatile Gene-based Association Study (VEGAS) software. Pathway-VEGAS uses the gene-based p-values to construct a pathway test based on a set of pre-specified pathways. The method appropriately takes into account situations where neighbouring genes are present in the same pathway - results for relevant regions are calculated by accounting for linkage disequilibrium between markers using simulations from the multivariate normal distribution. Pathway size is taken into account via a re-sampling approach. Importantly, since the approach only requires summary data, the method can be easily applied in all GWASs, including meta-analysis, singleton based, family based and DNA-pooling based designs. We found statistically significant findings using pathway-VEGAS on a number of traits including endometriosis, height and educational attainment. The approach identifies biologically relevant pathways, offering insights not possible with single marker approaches.

**J17.71 Epidemiology of consanguineous families in Autism**

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Analyses of large autism datasets have provided statistical and functional evidence for the role of rare point mutations (O’Roak, 2012, Sanders, 2012; Yu, 2013) and transmitted de novo copy number variants (CNVs) (Morow, 2008; Pinto, 2010; Levy, 2011; Sanders, 2011), and offer crucial insights into the diverse genetic mechanisms that can lead to Autism Spectrum Disorders (ASDs). Here we present CNV analysis for a cohort of 183 consanguineous families with one or more children affected with ASD. We provide new insights into the genetic architecture of ASD as our cohort is uniquely enriched for recessive loss of function variants. We follow up findings and draw comparisons with additional large ASD and control datasets; the Simons Simplex and the Autism Genetic Resource Exchange (AGRE) cohorts. Consanguineous individuals suffer from highly consanguineous populations across these cohorts, we demonstrate that ascertainment can lead to selection of different underlying genetic mechanisms causing ASDs. These differences are reflected in metrics such as the affected male:female ratio and the relative contribution of de novo CNVs versus inherited homozgyous deletions. Specifically, we find that de novo CNVs play a significant role in non-consanguineous families with a single affected child (p < 0.04), but a lesser role in multiplex families, and they are no more common in ASD cases than controls in multiplex consanguineous families. In contrast, we present the strongest statistical evidence (p = 0.013) to date that homozygous deletions, are a major contributor to ASD disease burden in consanguineous families, contributing to as much as 5-10% of cases.

**J17.72 Cost-effective designs for genotype imputation in sequencing based genome-wide association studies**

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Genotype imputation is now an essential tool in genetic studies to systematically infer missing and untyped genotypes. Accordingly, progressively larger reference panels are being chosen based on whole-genome sequencing in various populations. Developing general guidelines for optimally cost-effective imputation designs, however, requires evaluation of performance issues that include the relative utility of population-specific compared to general multi-population reference panels; genotyping with various array scaffolds; and population differences in haplotype structure. Here we compared the effectiveness of a Sardinian specific (SardSeq) panel, derived from whole-genome sequencing of 2,120 Sardinians, to the 1000 Genomes Project (1000G) reference panels, using combinations of two genome-wide and three custom arrays as baseline genotype, in both Sardinians and other Europeans. In Sardinians, the SardSeq panel provided better coverage and genotypic impute 1000G accuracy for different ethnic backgrounds. Here we showed the cost-effectiveness of this panel could be advantageous in other Europeans. We expect our results to be useful in planning future studies and in current sequencing efforts that are not part of the 1000 Genomes Project.

**J17.73 Association of single nucleotide polymorphism in SLC30A8 gene with aging in Tehran lipid and glucose study**

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Background: Genome-wide association studies have identified thousands of variants that are associated with numerous phenotypes. One such variant, rs13266634, in Solute Carrier family 30 (zinc transporter), member 8, has been consistently associated with numerous phenotypes. One such variant, rs13266634, in Solute Carrier family 30 (zinc transporter), member 8, has been consistently associated with numerous phenotypes. One such variant, rs13266634, in Solute Carrier family 30 (zinc transporter), member 8, has been consistently associated with numerous phenotypes.

Conclusion: Our findings showed the association between the presence of G-allele with aging was tested using plink software. As the results, we showed that rs13266634 in SLC30A8 gene has association with aging in Tehran lipid and glucose study (p = 0.0025).

**J17.74 Lack of association between the rs2304391 GABRR2 polymorphism and Temporal Lobe Epilepsy**

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ABSTRACTS PUBLISHED ABSTRACTS
Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the brain, acting primarily via the GABA receptors. Multiple lines of evidence indicate that dysfunction of GABA B receptors may be involved in the etiology of temporal lobe epilepsy (TLE). The aim of this study was to determine whether a single nucleotide polymorphisms (SNPs), +8A>G (rs2304391) at 3’UTR of subunit 2 of the GABA B receptor gene (GABBR2), contributes to TLE. We used a case-control approach comparing the frequency of the above-mentioned GABBR2 polymorphism between patients with TLE and controls. We enrolled 303 consecutive patients (167 men; median age: 27.4 ± 18.10) and 271 healthy controls (137 women; mean SD age: 64.20 ± 17.06), matched for age, sex and ethnicity. All patients had a diagnosis of non-lesional TLE, based on comprehensive clinical, neuroradiological, electroencephalographic, and brain MR evaluations. All patients and controls were Caucasian and were born in Italy. Patients and controls gave written informed consent prior to participation in the genetic studies. Patients and controls were genotyped for detection of the rs2304391 polymorphism using TaqMan Allelic Discrimination assays, on an Applied Biosystems PCR platform. The genotype distributions for both healthy subjects and epileptic patients were consistent with the Hardy-Weinberg equilibrium. Analysis of genotype or allelic frequencies between patients and controls showed no statistically significant difference, moreover the polymorphism did not influence severity and age at onset of epilepsy. Our data suggest that GABBR2 gene polymorphism does not act as a susceptibility factor for sporadic non-lesional TLE.

J17.75 Gene-gene interaction analyses of variants in the GABA system and alcohol use A. Ulgen1, M. Le2
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Epistatic or gene-gene interaction refers to the phenomenon that the joint effect of two genes is different from the sum of the individual effects of each gene. Recently, gene-gene interactions have been implied as a potential solution to the problem of missing heritability in the detection of genes for complex disorders. Furthermore, combinatorial approaches based on principal components analysis with the generalized multifactor dimensionality reduction method to study G × G and G × E interactions uniting nuclear families and unrelated individuals have been proposed. We apply methods of G × G interaction to a sample of 7224 individuals who have been phenotyped for their drinking and alcohol abuse and genotyped on 970 SNPs in 34 GABA system genes including 20 GABA receptor subunit genes. Results from applying both combinatorial approaches, PGMdR and Random Forest approaches based on classification trees, to the alcohol use data will be presented. We further discuss and compare the power to detect these interactions using these two methods and the resulting outcome. This should shed light into the functionality of these variants implied in the GABA receptor signaling pathway.

J17.76 Unsupervised Clustering Analysis of Gene Expression in an extended pedigree from Norfolk Island Population A. J. Rodriguez Acevedo1, M. Benton1, R. Lea1, A. Goldiner1, L. Griffiths1
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Norfolk Island belongs to the Commonwealth of Australia and it comes from a settlement of 194 inhabitants resettled from the Pitcairn Islands in 1856. All of them were descendants of nine “Bounty” mutineers’ men and twelve Tahitian women. The Island has been isolated and strict immigration and quarantine legislation restrict new burden from migration. In an attempt to understand and identify gene interactions in this unique population we performed a microarray analysis using an Illumina HumanHT-12 v4 array in venous blood samples from 335 related individuals. In order to compare the transcriptome structure from Norfolk Island population, we have taken previously published microarray data from the Atlanta CHDWB study. An unsupervised clustering analysis using k-means and principal component analysis (PCA) were performed using R. PCA showed that, in the Norfolk Island population, approximately 22% of the total variation is explained by its first principal component against 14% of variation in the CHDWB population. On the other hand, the k-means analysis identified 24 clusters in the Norfolk Island population and 18 in the CHDWB population where approximately 31% of the transcripts could not be assigned to any of the clusters due to the lack of correlation. Enrichment analysis and Network visualization was performed. Our study supports a homogeneous structure of the Norfolk Island population and suggests a specific set of genes to explain different phenotypic traits. It also contributes to the identification of possible biomarkers in common diseases such as migraine and cardiovascular disease, common pathologies in the Norfolk Island population.

J18.01 Chromosomal microarray analysis in paediatric patients with cognitive impairment/behavioural abnormalities/epilepsy and congenital anomalies: well-known syndromes, novel syndromes, parental contribution. A clinical perspective R. Fischer1, F. Papadou2, F. Nisarc2, R. Ortolani1, M. Masciopinto1, E. Piccinno1, M. Venedem1, D. Palumbo1, P. Palumbo1, L. Zelante1, M. Carella1
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Chromosomal microarray analysis (CMA) has emerged as a powerful new tool to identify genomic abnormalities associated with a wide range of developmental disabilities and congenital anomalies. The detection of submicroscopic copy number variant (CNVs), not visible by conventional karyotyping, helped the genetic diagnosis in our paediatric patients who had already received, with normal results, standard karyotype or FISH. Furthermore, since 2008, 212 patients aged 2 months-16 years, were analyzed using SNP-array technology and clinically significant submicroscopic imbalances were detected in 70 cases (33%). De novo imbalances were identified in 26 children (12%), while inherited imbalances were recognized in 34 children (16%). In 10 patients it was not possible to study both parents. In 59 patients (27.8%) a CNV was carried on the phenotype. For these cases, investigations of potential microduplication syndromes and chromosomal region with a likely pathogenic correlation were identified. In 11 patients (5%) two or more CNVs were present (2 CNV = 7 patients; 3 CNVs = 4 patients): parents’ analyses revealed that the rearrangements were inherited and contributed to child’s clinical phenotype. Clinicians who evaluate children with developmental disabilities and congenital anomalies have the role to attempt to establish a genetic diagnosis of these cases. Furthermore, and particularly when parents or family members becomes a routine recommendation, given that incidental CNVs findings have a significant impact on risk counseling for future pregnancies and other family members at risk.

J18.02 Bioethical, Humanistic And Technocratic Resources Of Enhancing Of Civilian Control Of Human Genetics S. Sprincean1, M. Sprincean1,2
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Background: Civilian control on human genetics represents one of main practical leverages to increase benefits and reduce risks for society as well as for healthcare system at global, local and community levels, in context of scientific and technological progress in genetic research, for molleration of general-social acceptance rate of the most ambiguous biotechnological innovations. The approach of civilian control in decision-making for better exploitation of human genetics potential for sustainable and long-term improving of healthcare, individual and global security and innovation climate in society, represents a new mainstream in development of medicine, public healthcare, genetic research and human neuro- and bio- enchantment. Materials and methods: The study used statistical data analysis and synthesis, observation, social survey, individual questioning. Results: There are several categories of resources to increase importance for a global welfare.

J18.03 Clinical outcome and genetic counselling in a rare complex small supernumerary marker chromosome (dup(9p21.2)-pter) and
Constructing a family tree. Frequent mistakes and new needs

M. O. Mkheidze

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Abnormal unique complex small supernumerary marker chromosomes (sSMC) have been found in 0.9% of patients, making the correlation between the patient’s phenotype and genotype particularly difficult. Some characteristic syndromes have been described; i.e., Pallister Killian ([i(12p)], Cat-eye ([i22p–q]) and Emanuel [der(22)t(11;22)]. Genetic counselling for these new marker chromosomes may be challenging, both for the patients and the parents, particularly when the SMC is a derivative of a maternally inherited translocation, resulting in partial trisomy of two chromosomes. The authors present the case of a 9 month female, referred for karyotyping with high resolution banding presenting with microcephaly, slow physical development and facial dysmorphism. Classical cytogenetics revealed an abnormal sSMC. The father’s karyotype was normal but the mother had a t(9;21) balanced translocation. Analysis of Fluorescence in situ hybridization (FISH -whole chromosome painting and centromeric probes, Cytog3) and Multiplex Ligation-dependent Probe Amplification (MLPA, MRC Holland) results in the proband allowed us to conclude that she has partial trisomy for chromosomes 9 (9p21–pter) and 21 (pter–21q11). Final karyotype: 47,XX,+mar(der(21)t(9;21)(p21;q21);D13Z1/D21Z1+,wcp21)+1mat. mlpa 9p21psubtel(P036E1, P070E2)x3

As in the present case some publications have noted that sSMC resulting from apparently balanced translocations present in one of the progenitors will influence the phenotype by the presence of specific partial trisomies. As far as we know this is the first case found with an sSMC that originates in chromosomes 9 and 21. The authors emphasise the need for good characterisation of these markers in order to define a reasonable/probable clinical outcome and allow appropriate counselling for the family.

J18.04 Inconsistency between molecular and clinical data in subjects with a D4Z4 reduced allele should prompt to further investigations


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Out of 171 consecutive myopathic index cases recruited from the Italian National Registry for FSHD and carrying a D4Z4 reduced allele (DRA) with 4-8 repeats, we identified 148 subjects completely fulfilled the clinical diagnostic criteria for FSHD and 23 subjects that presented myopathic features suggestive of an alternative diagnosis. In this group we observed a wide clinical heterogeneity, including 1) involvement of muscles that are not usually affected 2) sparing of muscles that are typically affected in FSHD, 3) non autosomal dominant inheritance, 4) additional clinical features. Molecular analysis of the 4q35 subtelomeric haplotype did not reveal any molecular element enabling us to differentiate these patients from classical FSHD cases. Further investigations in two patients with overlapping syndromes allowed us to identify additional mutations, respectively a heterozygous CAV-3 gene mutation and a haplotypic transition of mitochondrial tRNA-Leu.

In an elderly woman the muscle biopsy resulted suggestive for the diagnosis of inflammatory myopathy with inclusion bodies. Three cases presented a bent spine syndrome: histological and immunohistochemical analysis of muscle biopsies failed to detect other pathological conditions and additional genetic investigations were negative. Co-matching QTLs highlighted our difficulty to re-evaluate the significance and the predictive value of DRA, not only for research but also in clinical practice. Further clinical and genetic analysis of FSHD families will be extremely important for studies aiming at dissecting the complexity of FSHD. This approach will favour correct diagnosis and genetic counselling.

J18.05 Constructing a family tree. Frequent mistakes and new needs

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Analyzing more than 1500 pedigrees some frequent mistakes were shown: a) the absence of a horizontal line for an offspring b) symbol location between generation lines, c) displacement of children birth order, d) wrong numbering of generations, e) incorrect numbering of symbols in each generation. Human infertility is an important problem. 10 to 15 percent of couples all over the world are infertile. Assisted reproductive technology (ART) is modern effective therapy for overcoming infertility. To draw a pedigree with ART it is necessary to use adequate standard genetic symbols that are present in the family trees that are drawn with different symbols are compared. Symbols suggested are founded by the author.

J18.06 Legislative and ethical peculiarities of human genetic data protection

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Genetic science related to the individual as the main subject of the research is exposed to a wide range of ethical and legal issues. From the developments in genetic science other sciences have evolved, thanks to which the modern world is able to protect the genetic information of data, and to receive the sanction while ignoring the laws of such data. However, there are still many problems related to the protection of personal genetic information, data protection standards, and the inviolability of an individual, the assurance of freedom and privacy of information.

Genetic discrimination is strictly forbidden by international conventions and declarations (Convention on Human Rights and Biomedicine, Additional Protocol to the Convention on Human Rights and Biomedicine, concerning Biomedical Research, Directive 95/46/EC of the European Parliament), which means that discrimination on grounds of personal genetic information (diseases, abnormalities) is not available in all areas, including employment and insurance. However, individuals face some problems unable to get a job due to the publicity and information disclosure to their employers, or increasing the amount of insurance premiums. It is essential to protect genetic rights, because of that any form of discrimination against a person on grounds of his genetic heritage is prohibited and intervention in the health field or disclosure of such information may only be carried out after the person concerned has given free and informed consent to it.

J18.07 The impact of genetic counselling on prevention of mental retardation in south of Iran

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Introduction: Mental retardation has high prevalence in south of Iran because of consanguineous marriages. As uncurable nature, prevention is the best way. This study was designed to evaluate the impact of genetic counselling on prevention of mental retardation. Methods: This case control study was done between 2010-2013 and 120 women with mental retarded children were participated. All of them had another pregnancy after their mental retarded child. 60 of them had pregnancy after genetic counselling (case group) and 60 women had pregnancy without doing genetic counselling (control group). The study data was analyzed using SPSS19. RESULTS: In case group mean maternal age was 33±4.9 and mean age of mental retarded children was 7.8±4.5 years.In control group it was 34±5.2 for mothers and 10±2.9 for mental retarded children. Genetic counselling before pregnancy was protective factor for having mental retarded child (Odds Ratio 4.261, 95% confidence interval 1.312–13.834). 71.7% of parents in case group and 55% in control group had consanguineous marriages. Screening tests in pregnancy were done in 78.3% of mothers in case group and 21.7% in control group. Down syndrome was the most common cause of mental retardation in both groups. 3.3% of mothers in case group were referred for abortion (diseases, abnormalities) is not available in all areas, including employ- ment and insurance. However, individuals face some problems unable to get a job due to the publicity and information disclosure to their employers, or increasing the amount of insurance premiums. It is essential to protect genetic rights, because of that any form of discrimination against a person on grounds of his genetic heritage is prohibited and intervention in the health field or disclosure of such information may only be carried out after the person concerned has given free and informed consent to it.

J18.08 Hereditary Hyperterrinemia Cataract Syndrome in two Italian patients

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We describe two unrelated Italian patients with a combination of congeni-
tal nuclear cataract and hyperferritinaemia without iron overload. Affected patients had normal serum iron, transferrin saturation, and transaminases, but high serum ferritin levels, 1819 and 823 ng/ml, respectively. The first patient was referred to us with the suspicion of thalassemia trait. Only asking a detailed family medical history by telephone (at the age of 15) for cataract was referred. Pedigree analysis showed a brother with cataract. After this, in the brother hyperferritinaemia levels were discovered too. The second one was referred, during pregnancy, because of the presence of cataract and hyperferritinaemia. Pedigree analysis showed bilateral cataract and hyperferritinaemia in several relatives. The association of hyperferritinaemia with congenital cataract and positive family history led us to consider the diagnosis of Hyperferritinaemia-Cataract Syndrome (HCFS). HHCS is an autosomal dominant syndrome characterized by cataract and high ferritin serum levels, not related to iron overload. The syndrome is caused by heterozygous mutations in the iron-responsive element (IRE) of the ferritin light chain gene (FTL). Direct sequencing of SUTR of FTL showed, in the first patient, an heterozygous deletion of 16 nucleotides (-176, -162 and 16 nt, NM_000146.3) and, in the second one, an heterozygous missense mutation (-163C-G), both causative of HHCS (Lusci et al, 2013). We conclude that in patients with recurrent congenital cataract and hyperferritinaemia without iron overload, HHCS should be explored in order to provide genetic counseling and targeted follow up.

J18.09
Assessment of diagnostic criteria for HNPCC by comparative study of overall survival of CRC patients

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HNPCC is a clinical diagnosis, based on the Amsterdam criteria I (ACI) or revised Amsterdam criteria II (ACII). Due to cultural, socio-economical reasons and small size of families, in clinical practice often is hard to fulfill ACII and obtain an appropriate diagnosis for patients diagnosed with CRC. Therefore, based on different publications several modified criteria are created in use. Studies also showed overall survival of CRC patients with HNPCC is better than sporadic CRC patients. The aim of this study is to evaluate diagnostic criteria for HNPCC used in clinical practice by comparative study of overall survival of CRC patients. The retrospective case-record data of 1423 patients were analysed. Patients were classified in eight groups by clinical diagnosis, family anamnesis, age of diagnosis, stages by TNM, criteria matching. HNPCC group was selected based on ACII. The mortality was analyzed using the Cox proportional hazards models. The diagnosed group, age, and stratified tumour stages were tested with multivariable linear regression models adjusted for covariates. Patients group matching to ACII criteria (HNPCC) did show significantly better survival prognosis vs. any other patient group with cancer anamnesis in family and CRC patients without reported cancer anamnesis in family (sporadic CRC). All groups, except HNPCC, did not reach statistical significance for better overall survival compared to sporadic CRC group. Our data suggest that despite small families and lack of appropriate family anamnesis, ACII criteria is still the most sensitive clinical diagnostic guideline for HNPCC if microsatellite instability testing is not available.

J18.10
Patient’s use of the Internet for Medical Genetics Informations

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Clinical experience suggests that the Internet is increasingly becoming an important resource for patients seen in medical genetics. Patient-provider relationships will probably change and medical providers will face new challenges as patients obtain health informations from the Internet. Objectives To determine the percentage of patients enrolled in a medical genetics practice who use the Internet to obtain health information, to describe the type of information and to evaluate patient’s perception of the quality of this information. Methods: A prospective study was performed about patient use of the Internet before attending a medical genetics appointment. We analysed 61 questionnaires assessing: frequency of patient use of the Internet for medical genetics informations, perceptions of the quality of this information, if patients discuss this with their doctors. Results: Results show that 72.13 % (44/61) of patients have access to the Internet, of which 63.6 % (28/44) reported searching for genetic informations. 85,71% (24/28) of the patients found the information useful. Of those using the Internet for medical genetics informations 46,4% (13/28) did not discuss this informations with their doctors: Conclusions: Medical genetics care providers should recognize that patients are using the Internet as a source of medical and health information and should be prepared to offer suggestions for Internet resources and to assist patients in evaluating the quality of medical information available on the web sites. The role of medical genetics professionals is changing as a result of the development of the Internet.

J18.11
Genetic counseling for a pregnant woman with Marfan syndrome

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Marfan syndrome is well known autosomal dominant disorder of connective tissues and is caused by mutation of FBN1. Most cases are inherited from parents but 25% cases are caused by de novo mutation. A pregnant lady aged 27 with Marfan syndrome was referred to genetic outpatient at 10 gestational weeks. She visited to us with her younger brother. Her main concern was the risk of Marfan syndrome for her baby, however she did not realized that neonatal presentation of severe and rapidly progressive disease in multiple organ systems. She knew she could take genetic test, but she was not into it and prenatal test, either. She admitted maternal ward from 25 weeks because of her heart condition. She needed keeping rest and monitoring with cardiovascular team. At 27 weeks, Valsalva sinus expanded to 43mm and complained slight dyspnea gradually. At 31 weeks, we decided to perform Caesarean section because the circulating plasma volume usually increases at 30-32 weeks in pregnancy. She delivered a girl weighed 1532g with Apagar score 5/5. The girl is now 2 years old and does not show any symptoms. Generally, pregnancy should only be considered after appropriate counseling from a geneticist or cardiologist familiar with this condition. However this lady did not have any counseling before pregnancy because the cardiologist asked her not to be pregnant. It is important to consider how we could share the genetic information with female patients who is not allowed to conceive, but might happen to be pregnant.

J18.12
The application of the Illumina NextSeq500 sequencing platform to whole human, exome and RNA sequencing

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Here we will present data and analyses obtained on the Illumina NextSeq500 sequencing platform with whole human genome resequencing of clinically relevant samples, together with data from indexed exome and RNA samples. Further developments by Illumina of their sequencing by synthesis chemistry together with advances in instrumentation design have enabled the production of the first desktop high throughput sequencing system. The NextSeq500 is capable of producing up to 120 Gb of high quality data with 400 million tags in <29 hours from paired 150 cycle runs. Shorter paired and single 75 cycle runs are also possible together with the availability of mid throughput configuration delivering 40G from 130 million tags with paired 150 cycle reads. The system has a number of innovative features including the use of: Miniaturized optics Novel 2 channel chemistry Dry flowcell Simplified workflow with reagent cartridges Connectivity to BaseSpace for cloud analysis or an onsite version enables simple secure storage and manipulation of data. Data can be analyzed using a variety of tools relevant to the sample such as RNA/GE or the Illumina ISAAc pipeline for DNA samples. RNA data can be analyzed by the Tophat and Cufflinks applications.

J18.13
Silver-Russell syndrome - novel (epi)genotype-phenotype correlations

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Silver-Russell syndrome (SRS) is a congenital imprinting disorder with hypomethylation of ICR1 on chromosome 11p15.5 or maternal uniparental disomy of chromosome 7 (mUPD7), characterized mainly by severe pre- and postnatal growth retardation, relative macrocephaly and a small triangular face. Retrospective and prospective assessment included 77 SRS patients (36 fema-
Two supernumerary marker-chromosomes in a healthy woman: a J18.15 type correlation is further discussed. Our knowledge this mutation has not been described yet. Phenotype / genotype correlation characterized by short stature, delayed bone age and speech development, and typical facial dysmorphism. The face of FHS is the most distinctive feature, and all are tightly clustered within the final exon, with the exception of a small SMC. Here we present two patients with typical FHS facial features, although they slightly differ one from the other. In the first patient a nonsense mutation (c.1166delC) was detected. This is a recurrent mutation described in several other patients. The parents of the patient did not harbour this mutation, thus supporting its de novo origin. A frame shift mutation (c.7257_7258delAA (p.Gln2419fs*23)) was detected in the second case. To our knowledge this mutation has not been described yet. Phenotype / genotype correlation is further discussed.

Supported by 00064203 and C2.16/1.36002/24022.

J18.16

The meaning of informed consent in paternity testing

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The following objectives have been set: to frame the concept and the requirements for informed consent, to analyse the legal acts of the Republic of Lithuania covering the issues regarding informed consent, to analyse types of and indications for paternity testing and to name legal and ethical problems arising from application of the informed consent. The concept of informed consent is based on the principle of autonomy. The obligation of doctors to inform patients is inseparable from the requirement to receive informed consent. The two parts are mandatory for any medical procedures and interventions. The main requirements for the informed consent include rationality, sufficient and clear information, free will and the form of consent conforming to the legal acts. However, the informed consent is not an absolute requirement as the patient has a right to remain uninformed. Additionally, under certain circumstances it might be impossible to

to its low frequency and size it was impossible to determine its origin (likely from constitutive heterochromatin). Our observations are compared with similar case reports. Supported by the GAUK-264811 and TACR-TA01010931.
Teaching workshops of Medical Genetics to students and patient, correct presentation and interpretation of genetic information communicate, establishment of confidence and partnership between doctor and patient. The correct interpretation of the results of the conducted genetic tests is crucial for the beneficial effect from the counseling. Characteristics of the patient’s presentation and interpretation of the results of the conducted genetic tests is crucial for the beneficial effect from the counseling. Conclusion: Genetic counseling requires abilities and approach to communicate, establishment of confidence and partnership between doctor and patient, correct presentation and interpretation of genetic information with respect for autonomy and the right of personal choice of the patient.

Teaching workshops of Medical Genetics to students

Today the major guide for educators in establishing their teaching goals and objectives is Bloom’s Taxonomy of the Cognitive Domain revised by Krathwohl. From only knowing the specific concepts, students should then develop more and new skills, so that they could analyze, synthesize and even critically evaluate their own work or their colleagues. During each workshop that I taught from October 2013 to January 2014 students had to apply their knowledge to either create an original presentation (short story) or solve different problems. 100 first year students from the Medical and Pharmacy University ”Carol Davila”, Bucharest, Romania.

The study demonstrates the achieved ability in understanding and interpreting genetic information of the medical students.

Predictive genetic testing in families with growth hormone deficiency: An Indian experience

Growth is a multifactorial and complex process, yet its pattern is predictable in children. Deviations from normal pattern results in short stature which has serious psychological and social repercussions. Growth hormone (GH)/insulin like growth factor-I (IGF-1) axis has an established role in somatic growth regulation. Alterations impairing synthesis, secretion or biological action of GH results in a pathological phenotype called Growth Hormone Deficiency (GHD). GHD is eminently treatable, if supported by an early diagnosis and timely commencement of recombinant GH therapy which is highly expensive and prescribed over several years. In addition to the stigma of short stature, this exorbitant remedy is considered a burden by the patients in developing countries. High risk of inheritance in the next generation is also a matter of great concern. The present report on 100 families describes the impact of the disorder and socio-economic concerns which are a decisive factor in opting for treatment. Families with one child affected with GHD were ardent to discern the recurrence in subsequent pregnancies based on which were willing for termination. The solution to this problem in developing countries is predictive genetic testing enabling early detection for treatment and prenatal diagnosis for prevention. Timely genotyping can be provided if the genetic spectrum is known in the population as variations in mutation type and prevalence are documented worldwide. Equipped with this knowledge identification of common mutations will be easier and faster reducing anguish, improving risk perception, providing quality life and thereby eliminating the burden of morbidity.

Population genetic carrier screening for cystic fibrosis, fragile X syndrome and spinal muscular atrophy: Exploring experiences of carriers identified through the VGGS Reproductive Genetic Carrier Screening program

With advancing genetic technologies, carrier screening for multiple inherited conditions can now be offered within the population. This research aimed to explore how women experience undergoing carrier screening for three common inherited conditions: cystic fibrosis (CF), spinal muscular atrophy (SMA) and the X-linked disorder fragile X syndrome (FXS). Through the Victorian Clinical Genetics Services Reproductive Genetic Carrier Screening program. Adopting a qualitative approach using phenomenology as the theoretical framework, the study utilised in-depth semi-structured interviews, which were transcribed verbatim. The transcripts were coded using thematic analysis to identify emerging themes. Eight female participants took part: five received a carrier result for SMA and three for CF. The majority of participants were pregnant during screening and described the decision to have the test as straightforward. Participants experienced emotional responses such as anxiety and stress whilst waiting for their partner’s test result and also completed online research to find out more about the relevant condition during this time. Participants were in favour of population carrier screening, preferably offered prior to conception. The findings of this study elucidated that genetic counsellors (GCs) play an essential role within this program to adequately support couples after they receive a carrier result given the varying consent processes undertaken prior to screening. The implementation of Internet resources and GC facilitated guidance to access reliable online information is crucial to help empower couples and assist the coping process. improving awareness of the availability of population carrier screening within the community will also help improve knowledge levels and facilitate preconception screening.

The utility of incidental findings seen through the eyes of parents with children in genetic research

The use of new generation sequencing technologies has added complexity to the issues surrounding the return of research results to participants. Guidelines are in flux in both the research and clinical settings. In ongoing scientific and bioethical discussions about a possible “duty” to return incidental findings (or not), the criteria of clinical utility plays a central role. Some authors have been arguing for a multi-dimensional concept of utility, including utility as perceived by parents as well (Foster et al., 2009, Gosses et al., 2010, Bollinger et al., 2012). But what does the concept of “utility of an incidental finding” actually entail from a research participant’s standpoint? Our presentation aims to shed light on this issue, drawing on semi-structured in-depth interviews conducted in Quebec (Canada) with parents of children undergoing oncology treatment and participating in research. Following an overview of the concept of clinical utility under professional guidelines, we will analyse the multifaceted reasons as revealed behind the willingness of parents to know their child’s incidental findings in four case-scenarios. Parents expressed very practical reasons, even though they do not necessarily rely on risk factors or on the “clinical” actionability of results. Finally, we propose some limits to the adoption of this broader concept of utility by parents in hopes of informing bioethics guidelines on return of research incidental findings.

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Zbtb16 gene: P06.29-S
ZBTB18: P08.80-M
ZDHHC15: P11.136-M
Zearalenone: P15.18-M
ZEB2: J11.32
zebrafish: C05.5, C15.5, P04.21-S, P04.66-M
Zellweger spectrum disorder:
P06.48-M
ZIC1: C10.6
ZIC3: P05.38-M
zinc metabolism: J12.097
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