Building the evidence of unknown variants

M.H.Breuning@lumc.nl

1975 - unbanded karyotype

first problem:

very short short arm of chromosome 13 in child with congenital malformations

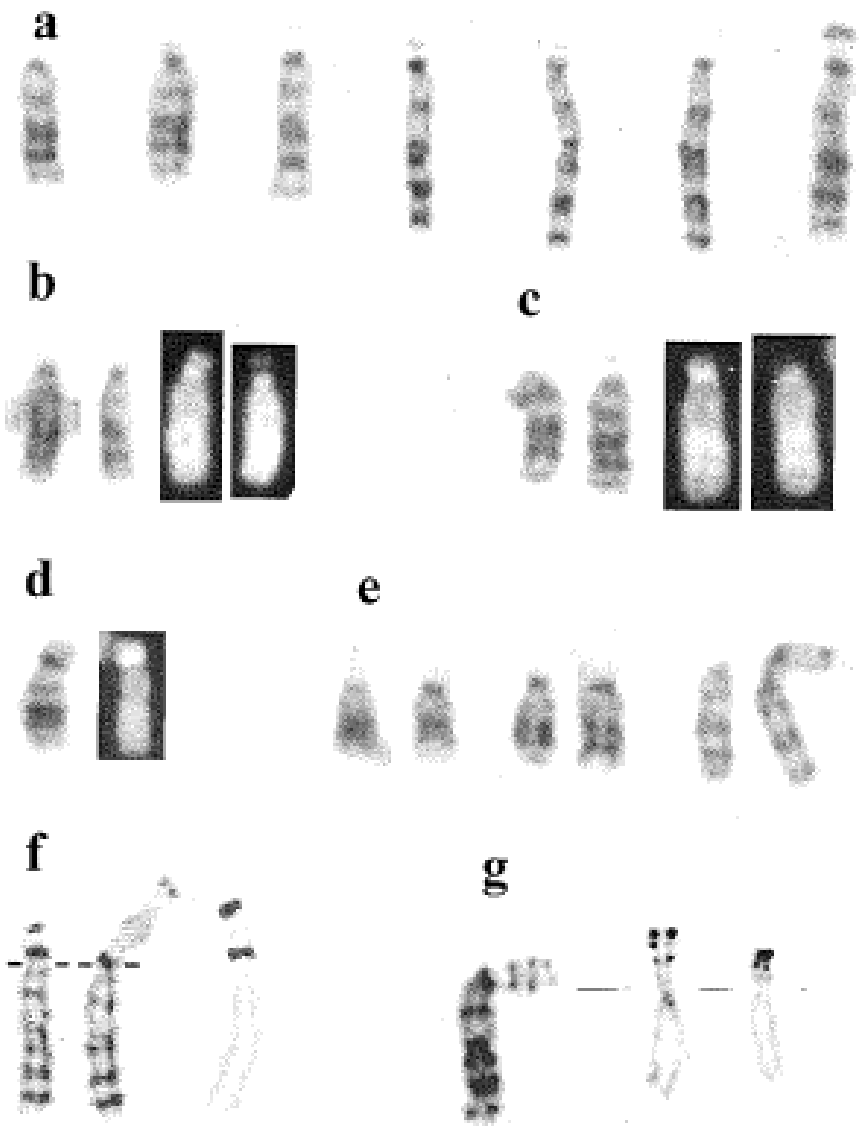
causal ?

same chromosome in normal parent

no

association ?
(partially responsable)

Chromosome 13 variants



no associations with disease known

Wyandt & Tonk, Eds: Atlas of human chromosome heteromorphisms, Dordrecht, Kluwer, 2004

HLA-B27 en de ziekte van Bechterew

THE LANCET, APRIL 28, 1973

ANKYLOSING SPONDYLITIS AND HL-A 27

D. A. BREWERTON
F. D. HART
MAEVE CAFFREY
D. C. O. JAMES

Strongest genetic disease association known, after 37 years still an enigma, not explained

Summary Using a standard microcytotoxicity technique of tissue typing, the HL-A 27 antigen was identified in 72 out of 75 patients with classical ankylosing spondylitis and in 3 out of 75 controls. The same antigen was found in 31 out of 60 first-degree relatives.

Notorious associations

- **HLA-B27 and Bechterew disease**
- **HLA-Drw3 and 4 and type 1 diabetes mellitus**
- **pericentric inversion of chromosome 9**
- **Angiotensin Converting Enzyme ins/del polymorphism**
- **etc, etc...**

Variants / Mutations in the genome....

- **can be the cause.....**

or can be

- **associated with a medical problem**

Variants, polymorphisms in the genome

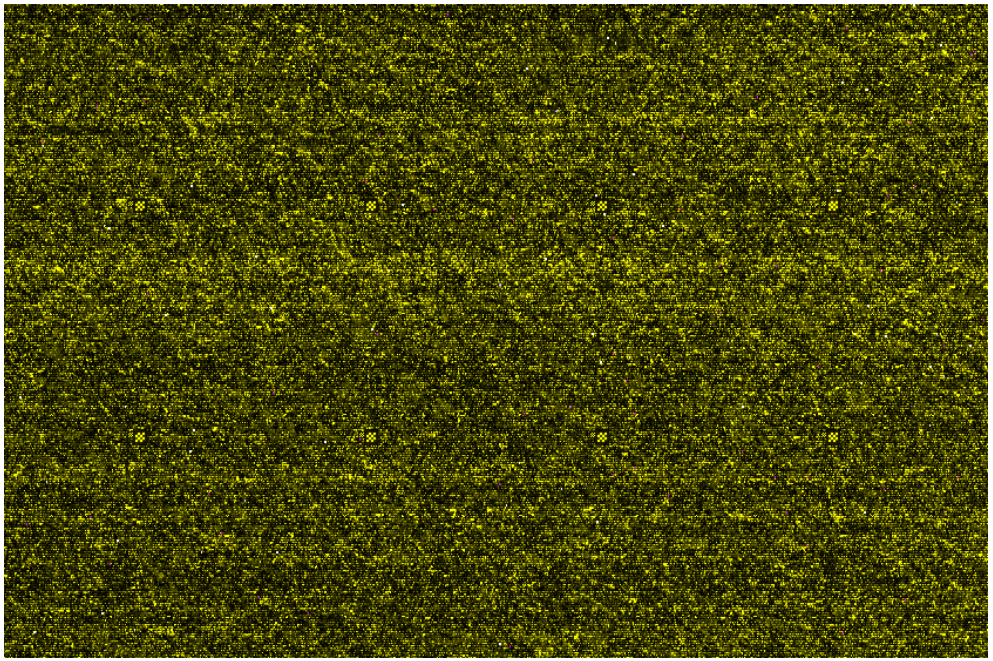
- **single nucleotide polymorphism (SNP):**

ACGCCGTAGTAGGTTAAAG.....

ACGCCATAGTAGGTTAAAG.....

1:1000 nucleotides varies - 6 million variants per genome

50 - 100 *de novo* changes in every individual !

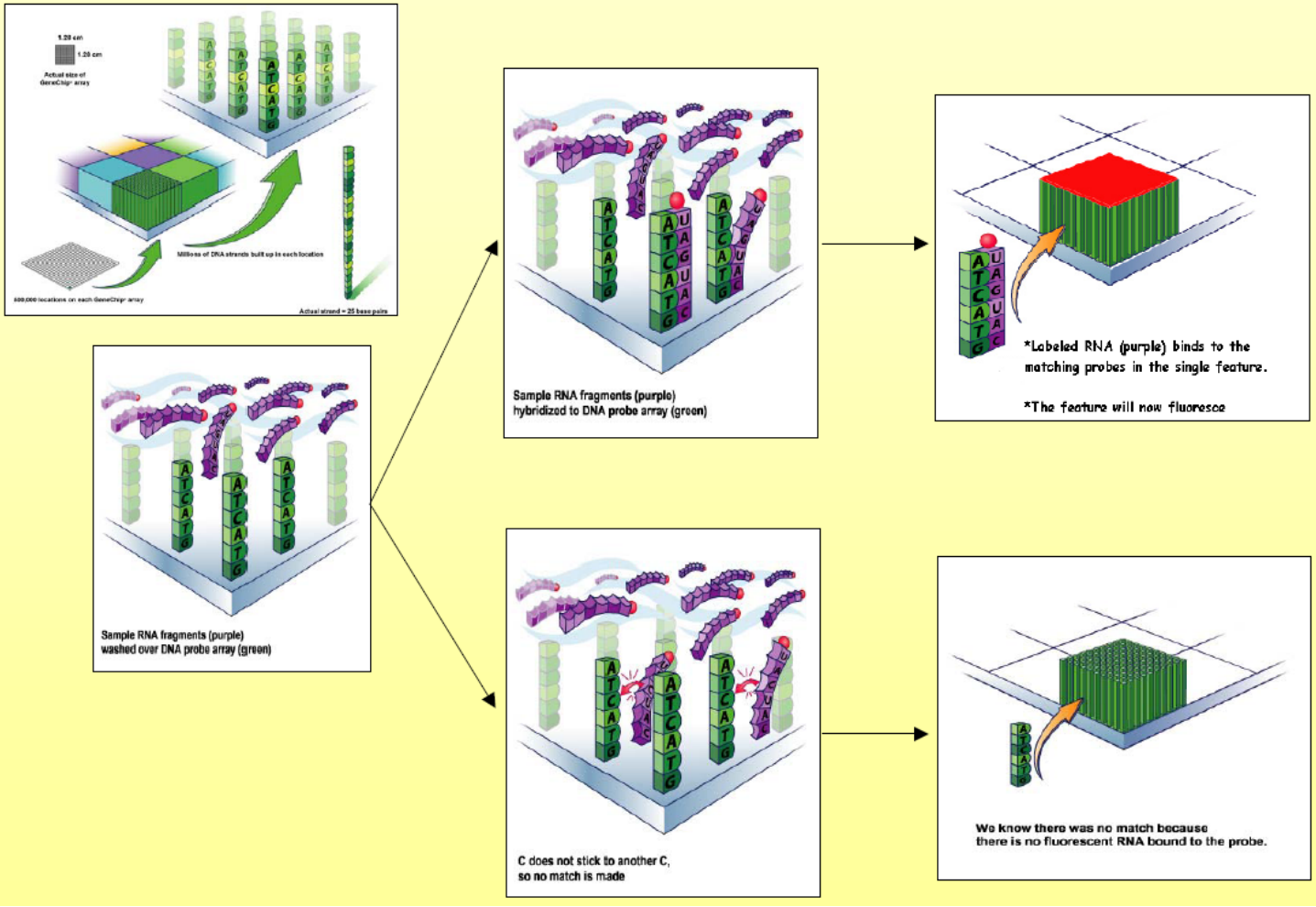


250.000 SNP's op een chip

Specific hybridization to 25-mer oligo's

SNP A/B

AA
AB
BB



Allele specific hybridization: genotype

Intensity: copy number

Monique

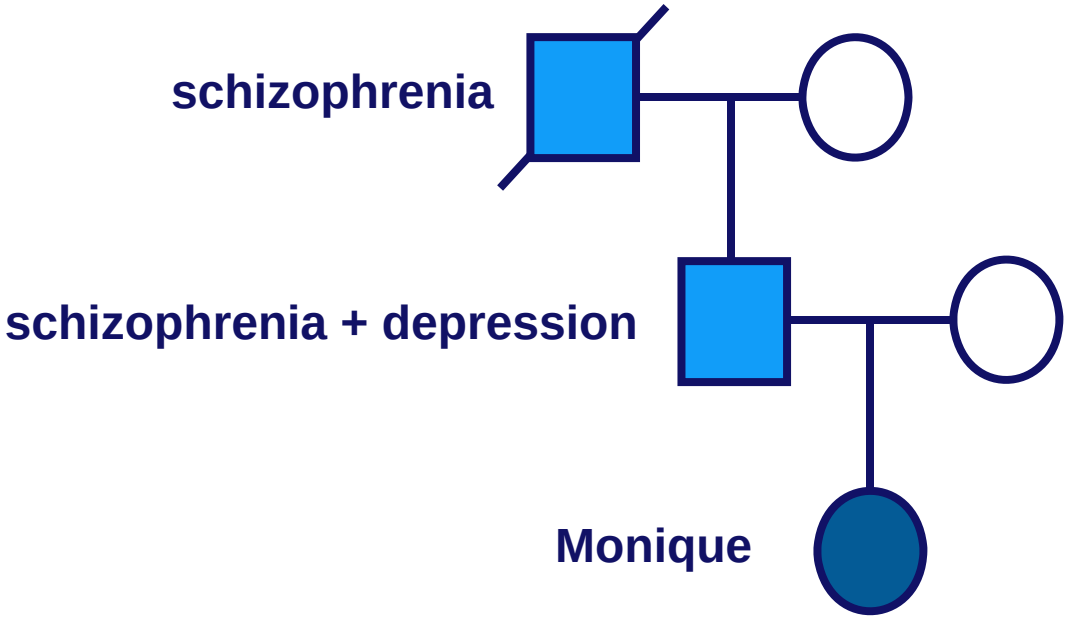
walks late, just within norm

Monique

- 3 word sentences at the age of 4
- on regular school until group 3
- pestered by other children
- vomits when she has to go to school
- tested, advice: ZMLK
- kept at home by parents
- serious behavioral problems
- 46,XX

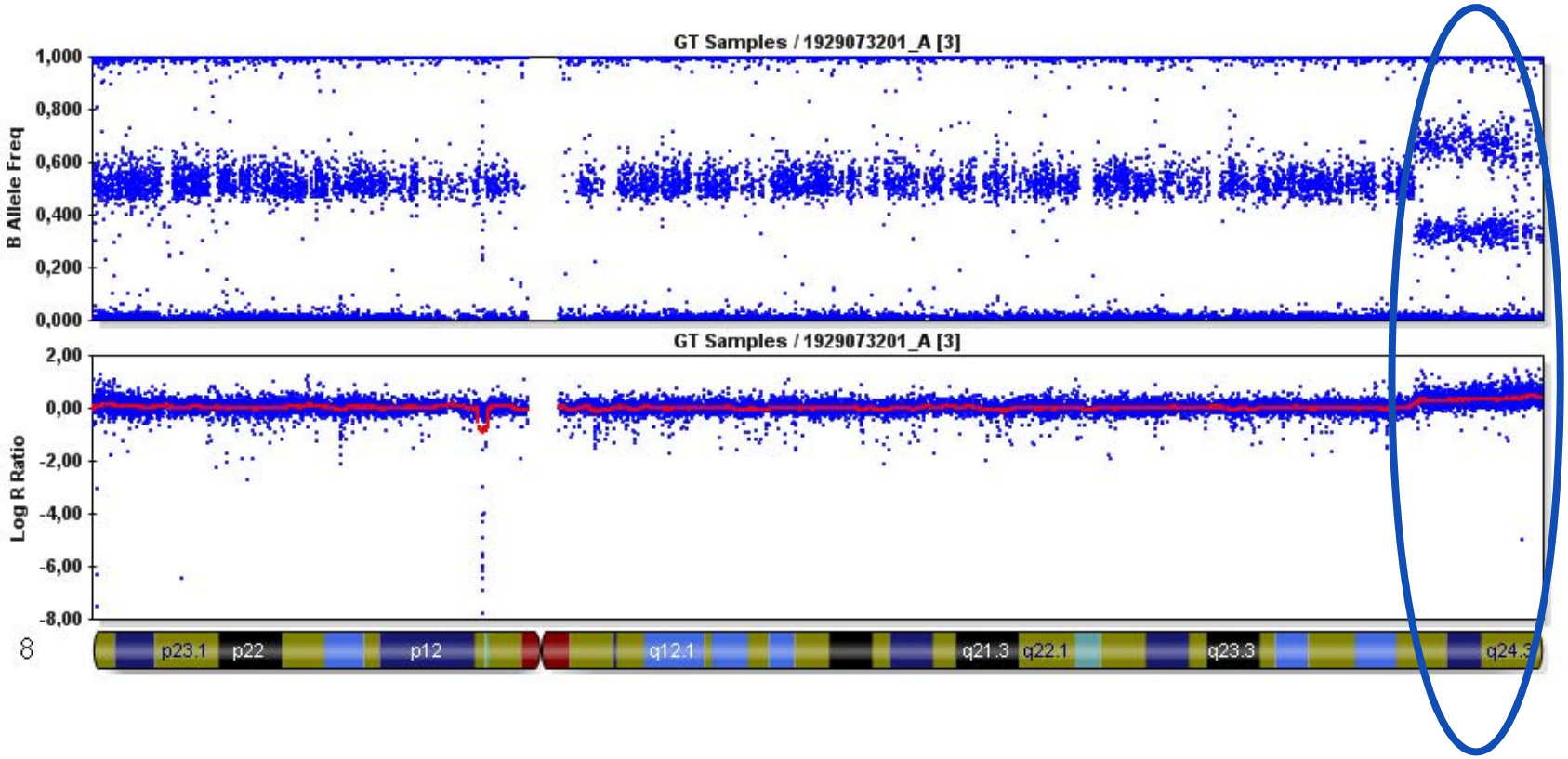
Monique with her parents

Family history



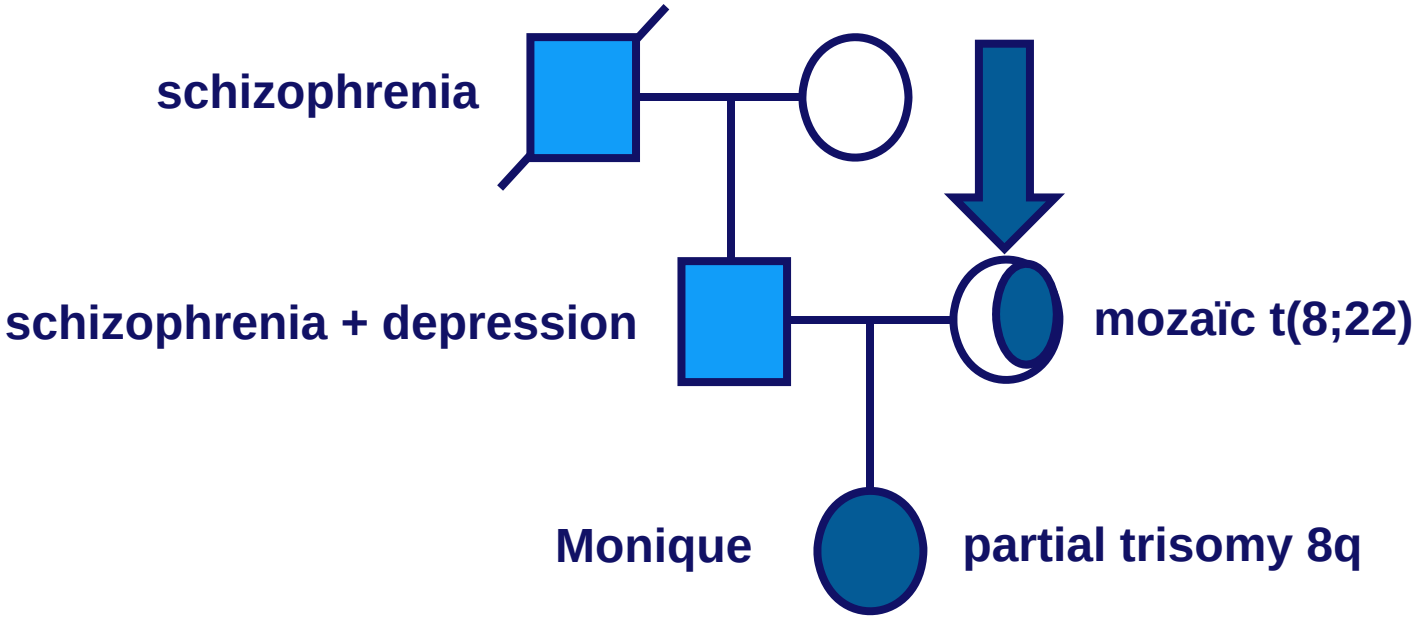
dominantly inherited psychiatric problem ?

Aberration on microarray in Monique



Duplication ~12,87 Mb in the long arm of chromosome 8

Diagnosis



~~dominantly inherited psychiatric problem ?~~

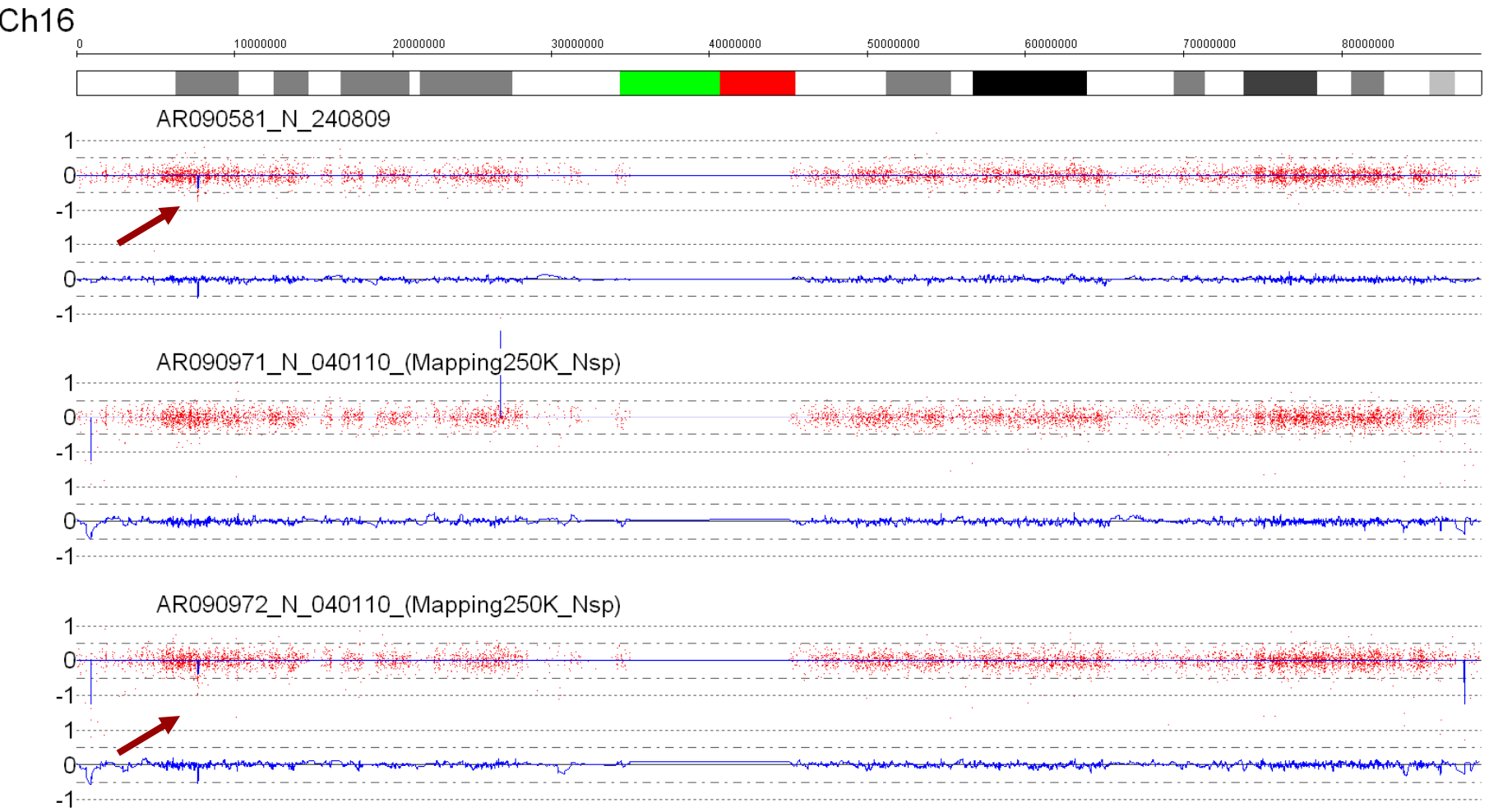
chromosome aberration coming from mom

The challenge

Our biggest problem is the interpretation of variants be it *de novo* or inherited

Some examples.....

Developmental delay, small del16p



Daughter has the same 16p13.2 del as her father

Disruption of A2BP1 – phenotype ???

J Hum Genet (2004) 49:308–311
DOI 10.1007/s10038-004-0145-4

Kavita Bhalla · Hilary A. Phillips · Joanna Crawford
Olivia L. D. McKenzie · John C. Mulley · Helen Eyre
Alison E. Gardner · Gabriel Kremmidiotis
David F. Callen

The de novo chromosome 16 translocations of two patients with abnormal phenotypes (mental retardation and epilepsy) disrupt the *A2BP1* gene

American Journal of Medical Genetics Part B (Neuropsychiatric Genetics) 144B:869–876 (2007)

Cytogenetic and Molecular Characterization of *A2BP1/FOX1* as a Candidate Gene for Autism

Christa Lese Martin,[1]* Jacqueline A. Duvall,[2] Yesim Ilkin,[3] Jason S. Simon,[4] M. Gladys Arreaza,[4] Kristin Wilkes,[2] Ana Alvarez-Retuerto,[2] Amy Whichello,[5] Cynthia M. Powell,[6] Kathleen Rao,[6] Edwin Cook,[7] and Daniel H. Geschwind[2]

We see a deletion of this gene in a normal father…..

The 16p11.2 deletion - phenotype ???

AUTISM

Kumar et al. Hum Mol Genet 17: 628-38, 2008

Weiss et al. New Engl J Med 358: 667-75, 2008

Mental Retardation

Bijlsma et al. Eur J Med Genet 52: 77-87, 2010

Obesity

Bochukova et al. Nature 463: 666-70, 2010

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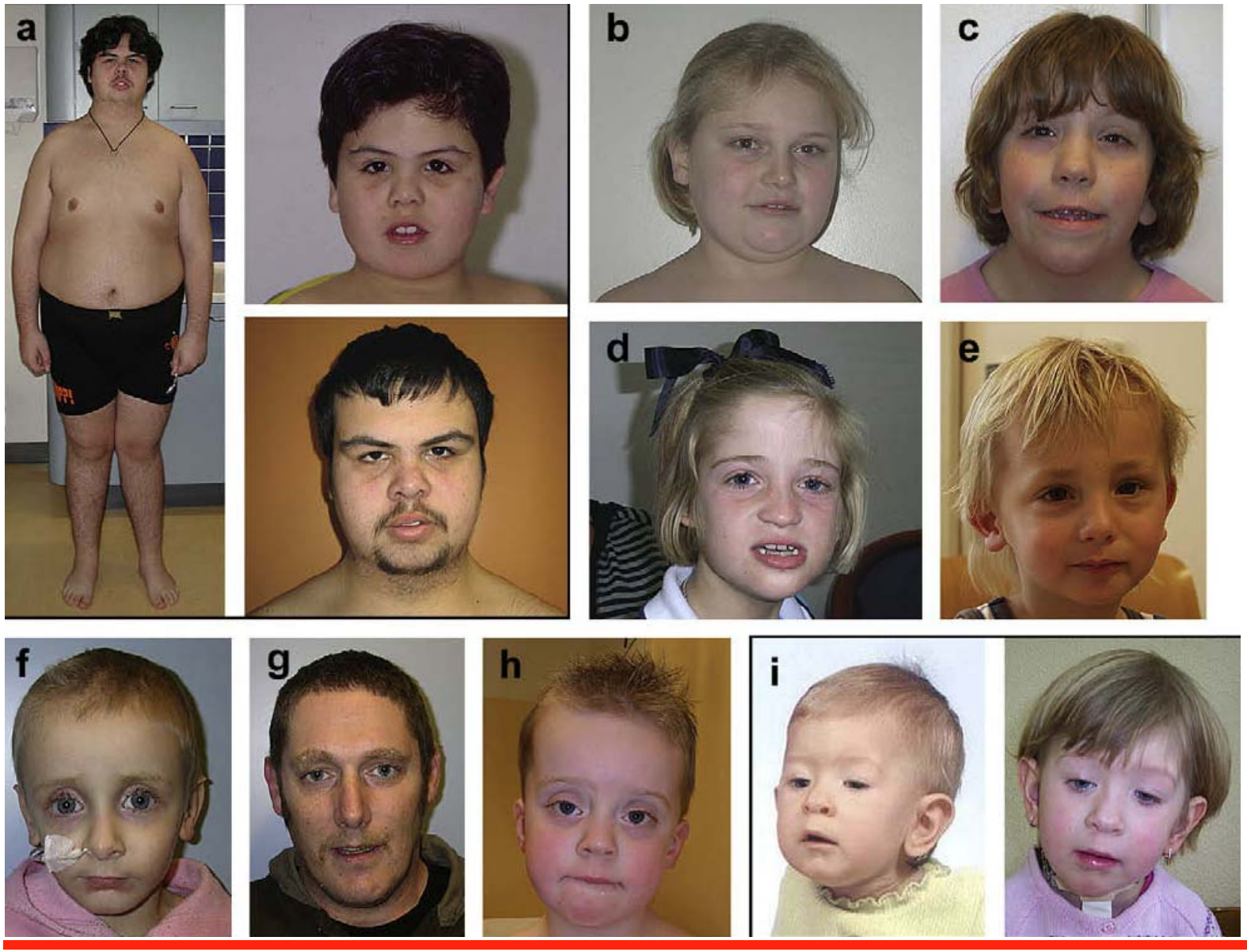

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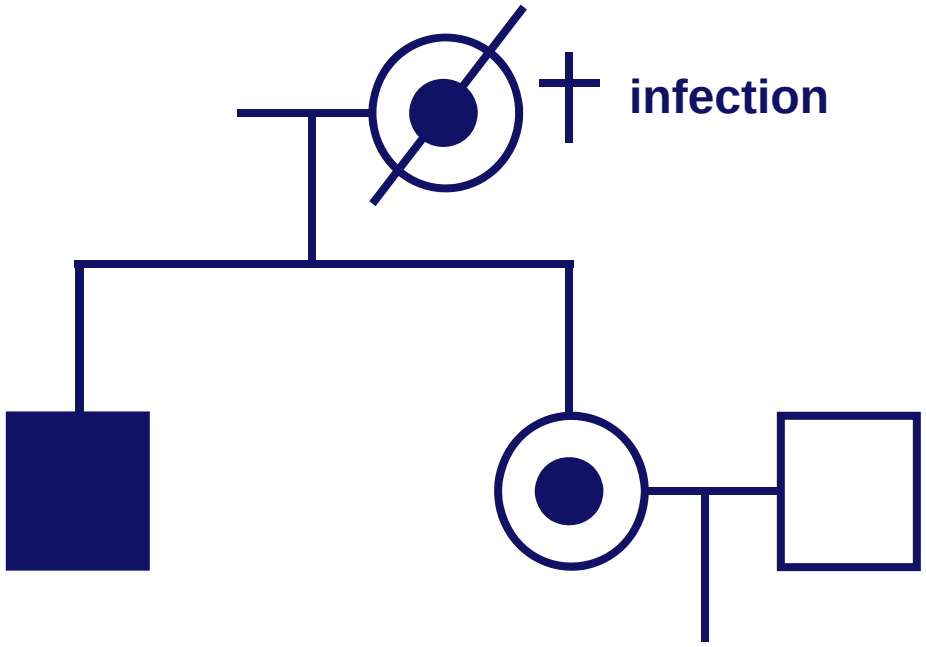
Alport Syndrome - X linked ??



DNA lab Maastricht:
(D Hellebrekers & A. van den Wijngaard)

c.4246C>T p.R1416C

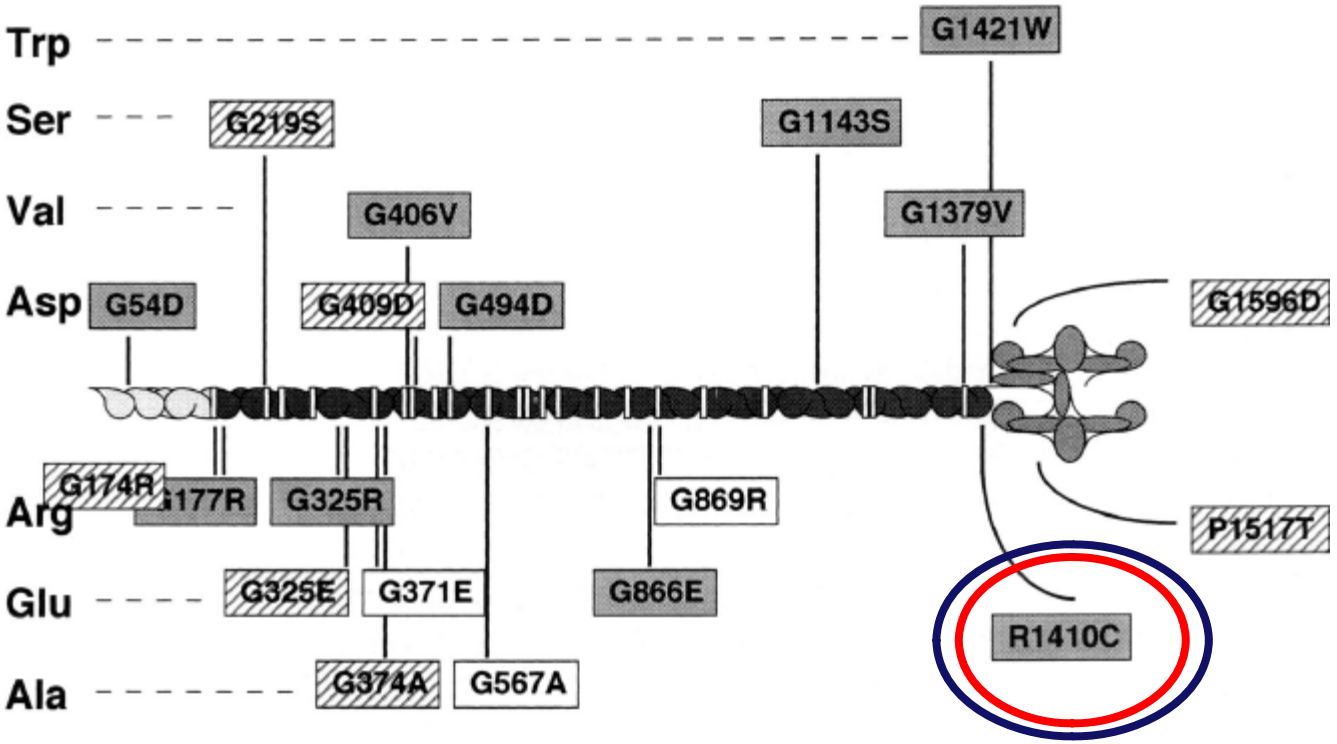
mutation pathogenic ?

refer to Renieri et al. AJHG 58:1192-1204, 1996

depicted as R1410C - same ?

'introduces Cys next to NC likely pathogenic'

Renieri et al. AJHG 58:1192-1204, 1996



Family contributed by Dr Tauro Neri from Parma, Italy

No further information on the family

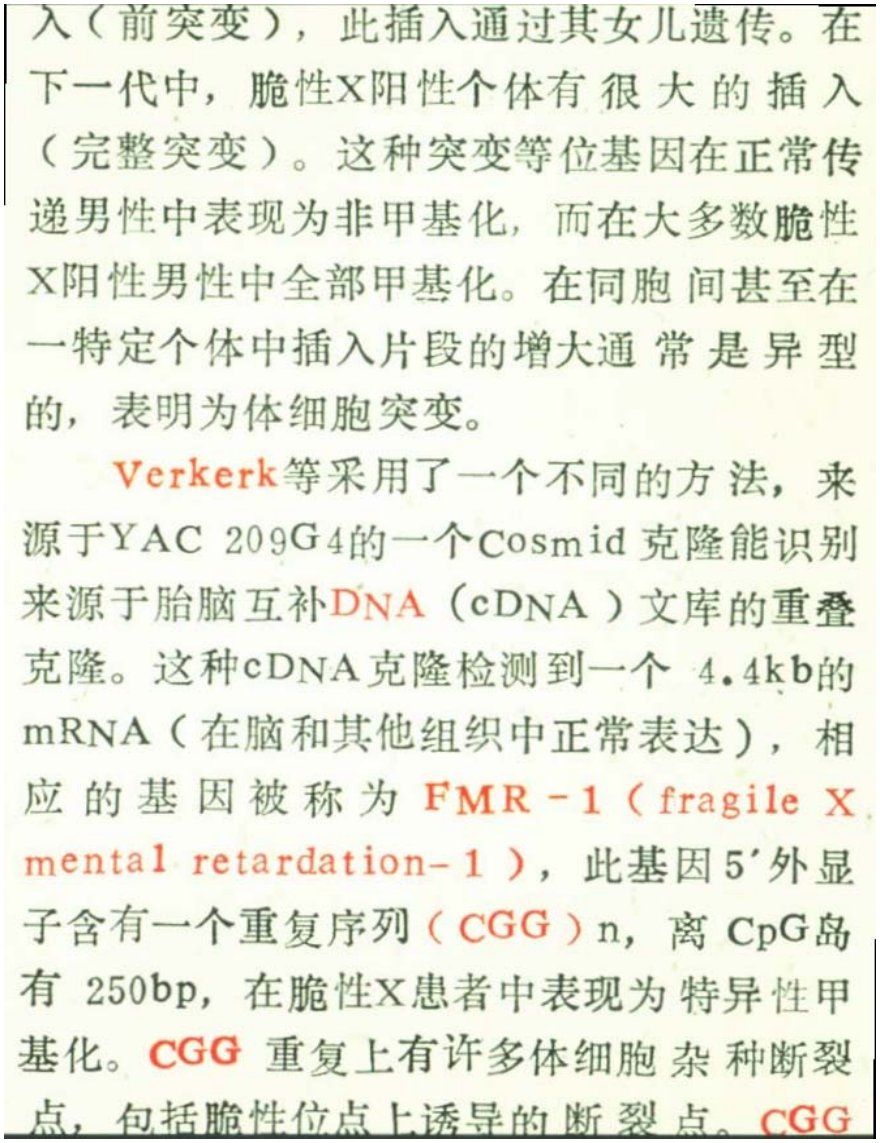
The Book of Life

- 2,91 x 10^9 basepairs
- 26.588 transcripts
- 22.000 possible genes
- 1,1 % code in exons
- 24 % in introns
- 75 % between genes

Nota bene:

each new human being carries 50 *de novo* mutations

The book of life



入（前突变），此插入通过其女儿遗传。在下一代中，脆性X阳性个体有很大的插入（完整突变）。这种突变等位基因在正常传递男性中表现为非甲基化，而在大多数脆性X阳性男性中全部甲基化。在同胞间甚至在一特定个体中插入片段的增大通常是异型的，表明为体细胞突变。

Verkerk等采用了一个不同的方法，来源于YAC 209G4的一个Cosmid克隆能识别来源于胎脑互补DNA（cDNA）文库的重叠克隆。这种cDNA克隆检测到一个 4.4kb的mRNA（在脑和其他组织中正常表达），相应的基因被称为 FMR-1（fragile X mental retardation-1），此基因5′外显子含有一个重复序列（CGG）n，离 CpG岛有 250bp，在脆性X患者中表现为特异性甲基化。CGG 重复上有许多体细胞杂种断裂点，包括脆性位点上诱导的断裂点。CGG

- **getting data is easy**
- **getting results is difficult**
- **nota bene: array and sequencing give many variants of unknown significance**
- **‘Accidental findings'**
- **Privacy**

Whole genome sequencing

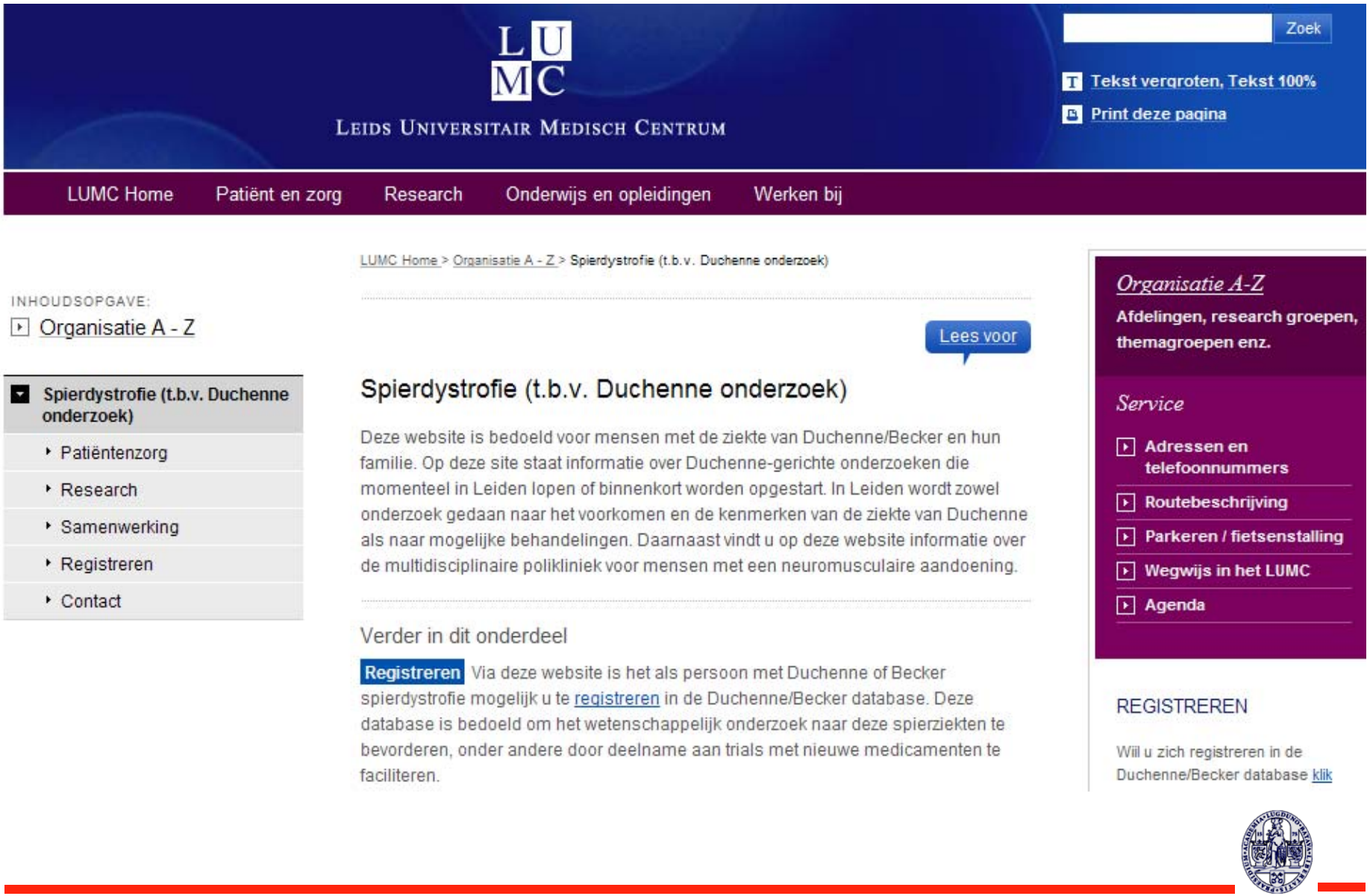
- **plus ça change, plus c' est la même chose**
- **what is new ?**
- **the enormous number of variants that we will have to characterise**

This is not a problem,

but a challenge, or opportunity

We need painstaking clinical description of case series within databases combined with molecular results, extensive DNA sampling in families

Recruiting families through the web



Public databases of mutations/variants

For all details about LOVD, see our LOVD flyer! (last updated February 24th, 2010)

List of public LOVD installations

If you are looking for a specific gene database, please also check the list of gene variant databases at the HGVS site.

Please note that the latest available build is always installed on our Leiden server. We offer free use of this LOVD installation for those interested, e.g. to maintain/start a LSDB. Contact us for more information.

Show only LOVDs with gene symbol: Show

You can also download this list to see the complete list of genes in the databases.

In total: 180,237 variants in 73,947 patients in 3214 genes in 55 LOVD installations.

http://www.dmd.nl/nmdb2/	LOVD 2.0-26	72 genes	58519 variants
Leiden Muscular Dystrophy pages	ACTA1,ACTC1,AGRN,ANO5,BAG3,BIN1,CAPN3...		7877 unique
http://chromium.liacs.nl/LOVD2/colon_cancer/	LOVD 2.0-26	9 genes	25110 variants
Colon cancer gene variant databases	APC,EPCAM,MLH1,MLH3,MSH2,MSH6,MUTYH,P...		3717 unique
http://grenada.lumc.nl/LOVD2/MR/	LOVD 2.0-26	542 genes	24712 variants
Mental Retardation database	ABCB7,ABCD1,ACE2,ACOT9,ACSL4,ACTRT1,A...		2469 unique
https://grenada.lumc.nl/LOVD2/Usher_montpellier/	LOVD 2.0-26	10 genes	8713 variants
Retinal and hearing impairment genetic mutation database	CDH23,CHM,CLRN1,GPR98,MYO7A,PCDH15,US...		1196 unique
http://grenada.lumc.nl/LOVD2/diabetes/	LOVD 2.0-26	3 genes	8082 variants
Monogenic Diabetes	GCK,HNF1A,HNF4A		308 unique
http://chromium.liacs.nl/LOVD2/	LOVD 2.0-26	33 genes	8046 variants
LOVD - Leiden Open Variation Database	ABCA13,ARG1,ASL,ASS1,ATM,ATP1A2,B3GAL...		2784 unique
http://www.genomed.org/LOVD/	LOVD 2.0-12	32 genes	5559 variants
Zhejiang University Center for Genetic and Genomic Medicine	AKAP9,ANK2,APC,BRCA1,BRCA2,CACNA1C,CA...		3168 unique
http://www.china-hvp.org/LOVD/	LOVD 2.0-12	27 genes	4898 variants
Zhejiang University Center for Genetic and Genomic Medicine	AKAP9,ANK2,APC,BRCA1,BRCA2,CACNA1C,CA...		2894 unique
https://research.cchmc.org/LOVD/	LOVD 2.0-26	47 genes	4539 variants
CCHMC - Human Genetics Mutation Database	ABCB11,ABCB4,ACADM,ACADVL,ATP8B1,CASP...		4275 unique
http://chromium.liacs.nl/LOVD2/TSC/	LOVD 2.0-26	2 genes	3881 variants
Tuberous sclerosis database	TSC1,TSC2		1605 unique
http://chromium.liacs.nl/LOVD2/cancer/	LOVD 2.0-26	2 genes	2383 variants
LOVD - Leiden Open Variation Database	BRCA1,BRCA2		990 unique

Internet

Leiden Open source Variant Databases

Eventually,
we need to have data on all varying nucleotides in the genome, whether they

cause, predispose to, protect against, or modify disease
or
are completely neutral

See poster 15.09

Discussion

Two theologists

Harry van Kruiningen