KATHOLIEKE UNIVERSITEIT LEUVEN

Practical challenges that copy number variation and whole genome sequencing create for genetic diagnostic labs

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ESHG
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When and how to introduce arrays and whole genome sequencing

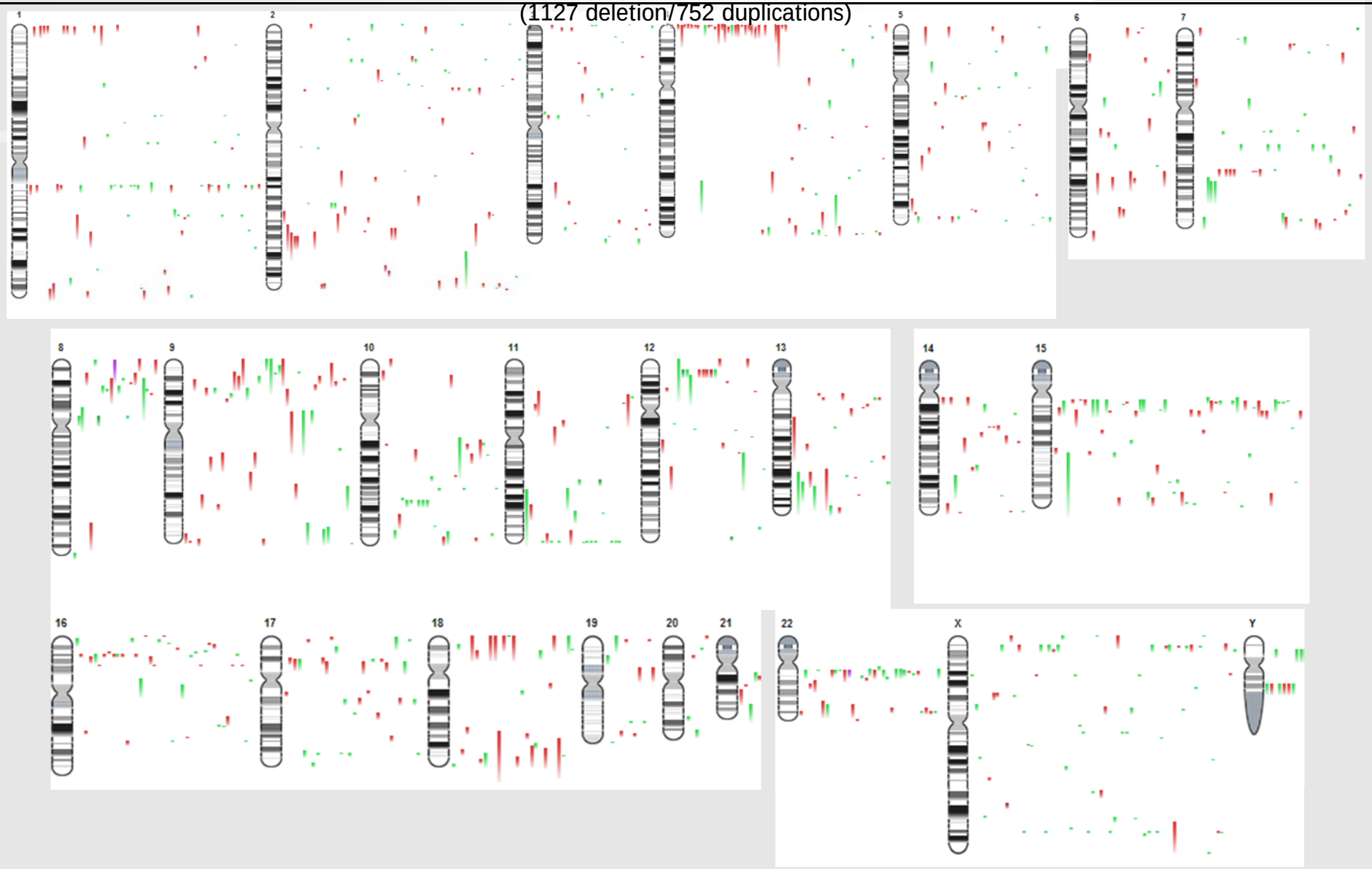
1. Analytical validity?
2. Clinical utility?
3. Clinical validity?

Analytical validity

- Arrays:
  - Proof-of-principle: 1997
  - Reliable detection of single copy variations: 2003-2004
- Full genome sequence
  - Proof-of-principle: 2009
    - Estimated 30.000-300.0000 SNP artefacts
  - Reliable calling of all nucleotides/CNVs ….

Clinical utility for MCA/MR
CNVs as cause of developmental disorders:
> 100 new syndromes in 5 years

(1127 deletion/752 duplications)



Clinical utility array CGH

Emerging patterns of cryptic chromosomal imbalance in patients with idiopathic mental retardation and multiple congenital anomalies: a new series of 140 patients and review of published reports

B Menten*, N Maas*, B Thienpont, K Buysse, J Vandesompele, C Melotte, T de Ravel, S Van Vooren, I Balikova, L Backx, S Janssens, A De Paepe, B De Moor, Y Moreau, P Marynen, J-P Fryns, G Mortier, K Devriendt, F Speleman, J R Vermeesch

J Med Genet 2006;43:625–633. doi: 10.1136/jmg.2005.039453

Array CGH in patients with learning disability (mental retardation) and congenital anomalies: updated systematic review and meta-analysis of 19 studies and 13,926 subjects

Gurdeep S. Sagoo, MSc, PhD[1], Adam S. Butterworth, BA, MSc[2], Simon Sanderson, MRCP, FFPH[3], Charles Shaw-Smith, MA, PhD[4], Julian P. T. Higgins, BA, PhD[2], and Hilary Burton, MA, FFPH[1]

The performance of CGH array for the detection of cryptic constitutional chromosome imbalances

J Schoumans, B-M Anderlid, E Blennow, B T Teh, M Nordenskjöld

J Med Genet 2004;41:199–202. doi: 10.1136/jmg.2003.013920

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www.nature.com/ejhg

POLICY

Guidelines for molecular karyotyping in constitutional genetic diagnosis

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Jacqueline Schoumans[5], Roberto Ciccone[6], Fra
Jill Clayton-Smith[9], Conny Van Ravenswaaij[10]
Philippos C Patsalis[12], Helen Firth[13], Koen Dev

on molecular karyotyping
ith developmental delay
le congenital anomalies
setting

Consensus Statement: Chromosomal Microarray Is a First-Tier Clinical Diagnostic Test for Individuals with Developmental Disabilities or Congenital Anomalies

David T. Miller,[1,*] Margaret P. Adam,[2,3] Swaroop Aradhya,[4] Leslie G. Biesecker,[5] Arthur R. Brothman,[6] Nigel P. Carter,[7] Deanna M. Church,[8] John A. Crolla,[9] Evan E. Eichler,[10] Charles J. Epstein,[11] W. Andrew Faucett,[2] Lars Feuk,[12] Jan M. Friedman,[13] Ada Hamosh,[14] Laird Jackson,[15] Erin B. Kaminsky,[2] Klaas Kok,[16] Ian D. Krantz,[17] Robert M. Kuhn,[18] Charles Lee,[19] James M. Ostell,[8] Carla Rosenberg,[20] Stephen W. Scherer,[21] Nancy B. Spinner,[17] Dimitri J. Stavropoulos,[22] James H. Tepperberg,[23] Erik C. Thorland,[24] Joris R. Vermeesch,[25] Darrel J. Waggoner,[26] Michael S. Watson,[27] Christa Lese Martin,[2] and David H. Ledbetter[2,*]

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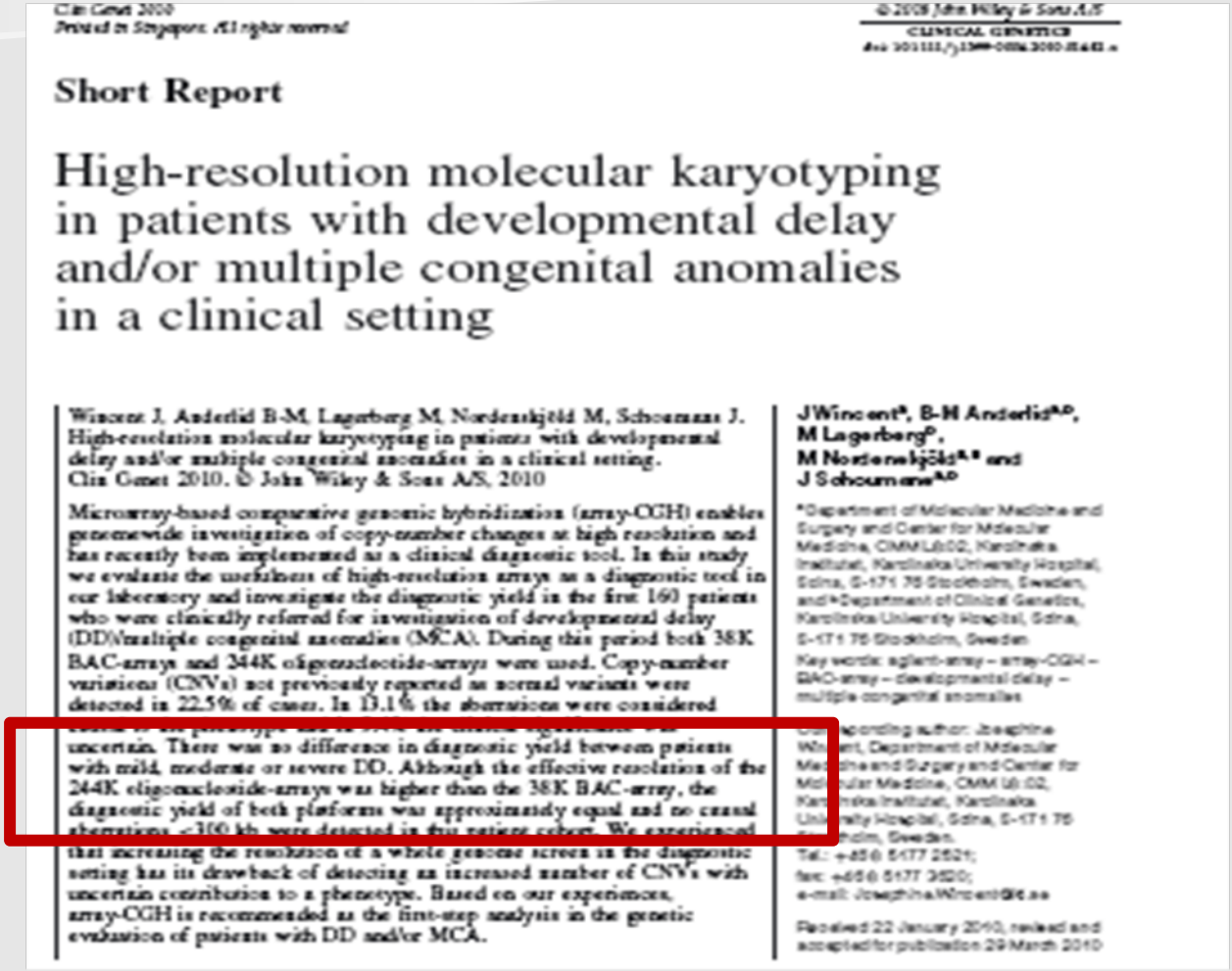
Clinical utility (2008-...)

- Traditional constitutional cytogenetic applications:
  - Mental retardation/ multiple congenital anomalies: yes!
  - Prenatal? (clinical issues?) yes!
  - Miscarriages? (mosaicisms?) yes!
- Other medical disciplines?
  - Neurology/ Psychiatry? yes!
    - Autism
    - Schizophrenia
  - Isolated heart defects? ?
  - Multifactorial diseases? ?
    - Infectious diseases
    - Gastrointestinal diseases
  - Monogenic diseases? ?
  - .... All medical disciplines?

Clinical validity?



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CLINICAL GENETICS

Short Report

High-resolution molecular karyotyping in patients with developmental delay and/or multiple congenital anomalies in a clinical setting

Wincent J, Anderlid B-M, Lagerberg M, Nordenskjöld M, Schoumans J. High-resolution molecular karyotyping in patients with developmental delay and/or multiple congenital anomalies in a clinical setting. Clin Genet 2010. © John Wiley & Sons A/S, 2010

Microarray-based comparative genomic hybridization (array-CGH) enables genomewide investigation of copy-number changes at high resolution and has recently been implemented as a clinical diagnostic tool. In this study we evaluate the usefulness of high-resolution arrays as a diagnostic tool in our laboratory and investigate the diagnostic yield in the first 160 patients who were clinically referred for investigation of developmental delay (DD)/multiple congenital anomalies (MCA). During this period both 38K BAC-arrays and 244K oligonucleotide-arrays were used. Copy-number variations (CNVs) not previously reported as normal variants were detected in 22.5% of cases. In 13.1% the aberrations were considered [illegible] uncertain. There was no difference in diagnostic yield between patients with mild, moderate or severe DD. Although the effective resolution of the 244K oligonucleotide-arrays was higher than the 38K BAC-array, the diagnostic yield of both platforms was approximately equal and no causal aberrations <100 kb were detected in this patient cohort. We experienced that increasing the resolution of a whole genome screen in the diagnostic setting has its drawback of detecting an increased number of CNVs with uncertain contribution to a phenotype. Based on our experiences, array-CGH is recommended as the first-step analysis in the genetic evaluation of patients with DD and/or MCA.

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Key words: agilent-array – array-CGH – BAC-array – developmental delay – multiple congenital anomalies

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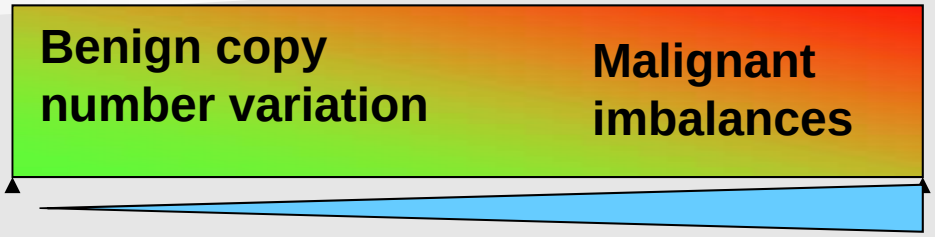
Clinical VALIDITY?

Clinical significance of anomaly?

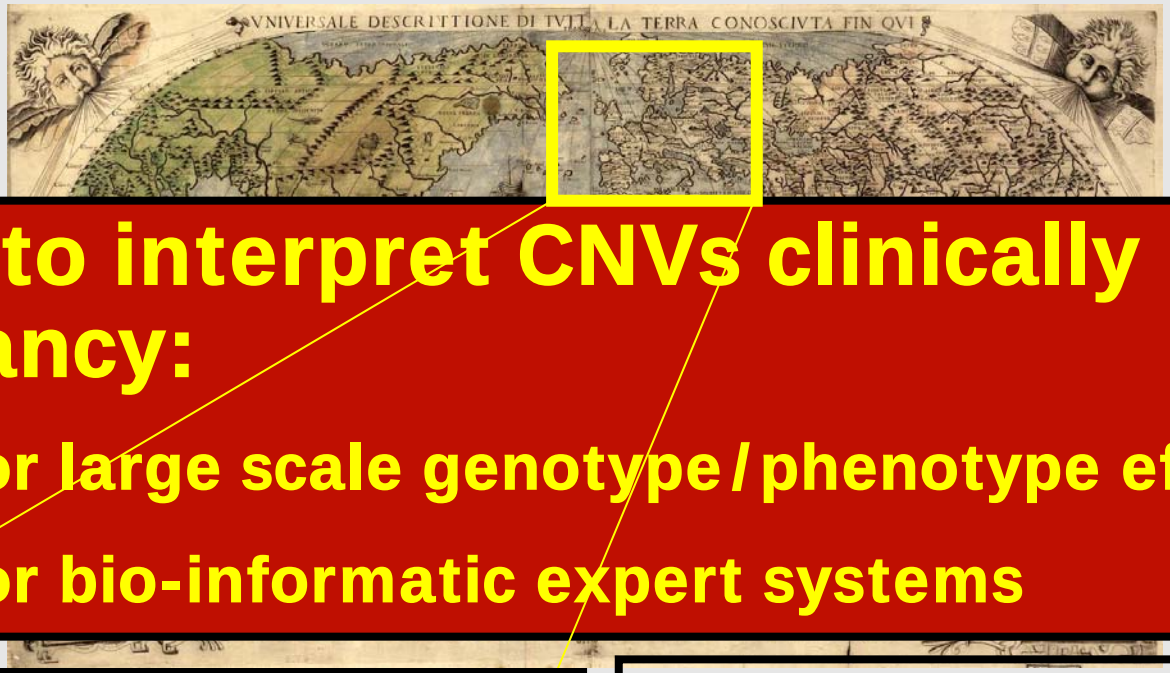


1 bp Deletion or duplication size **10 Mb**

We are all copy variable!!

With ever increasing resolution, the boundary between bening and pathogenic CNVs becomes blurred!

Current status of CNV validity



Ability to interpret CNVs clinically is in it's infancy:

- **Need for large scale genotype/phenotype efforts**
- **Need for bio-informatic expert systems**

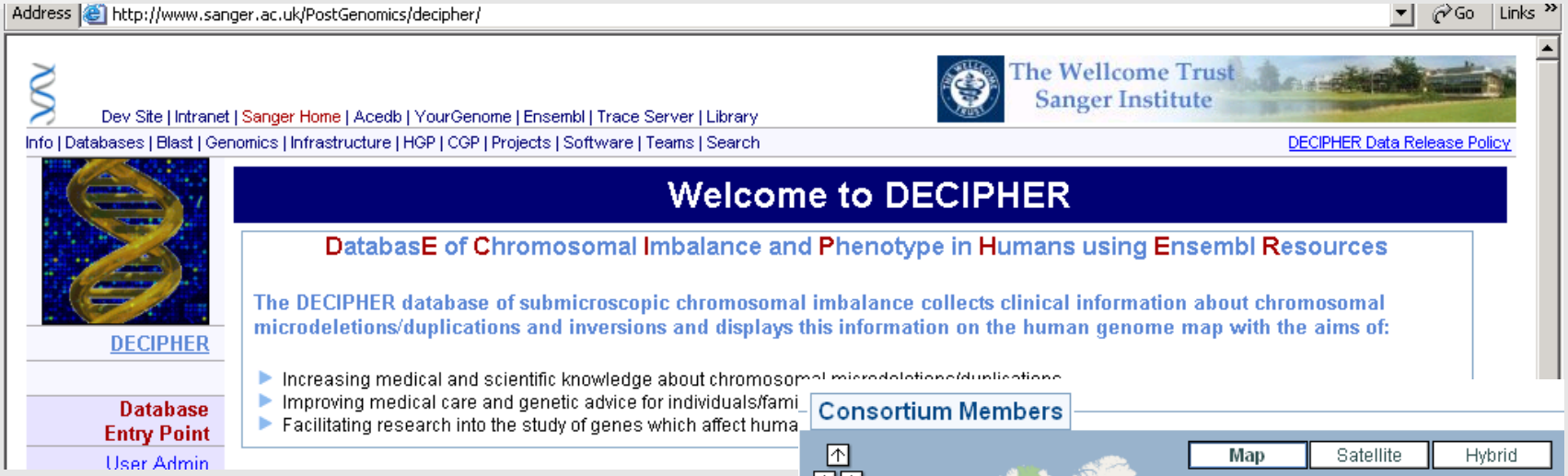
Europe:

Higly penetrant recurrent CNVs

Rest of the world:

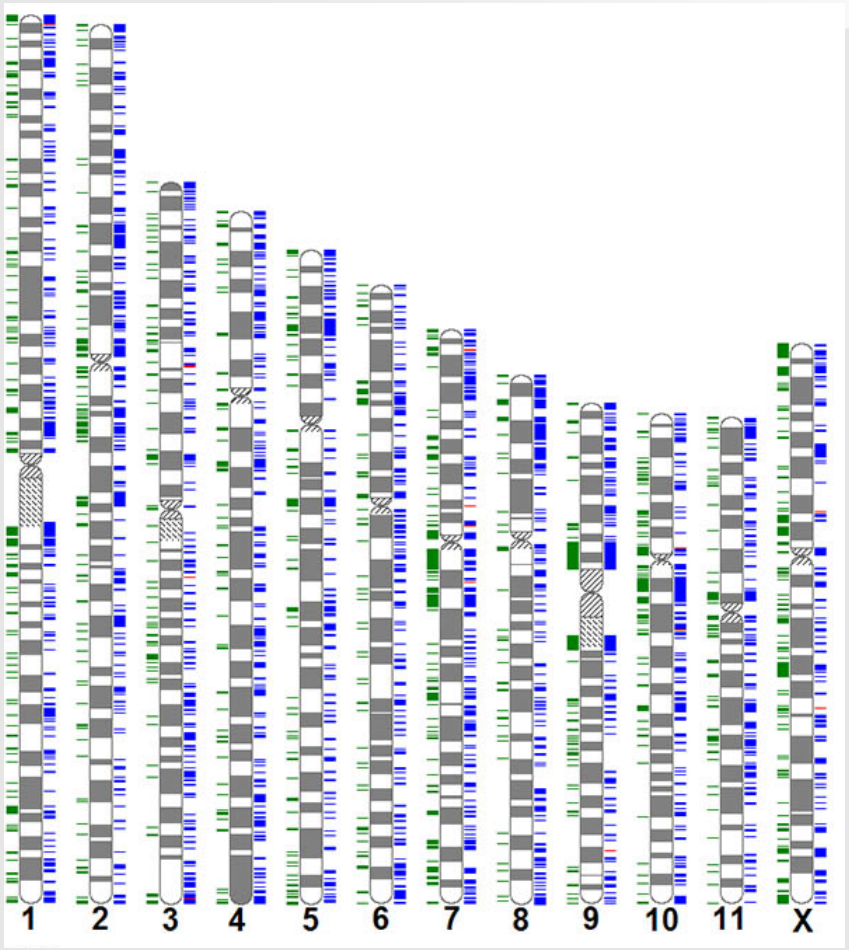
Rare CNVs with variable penetrance & expressivity

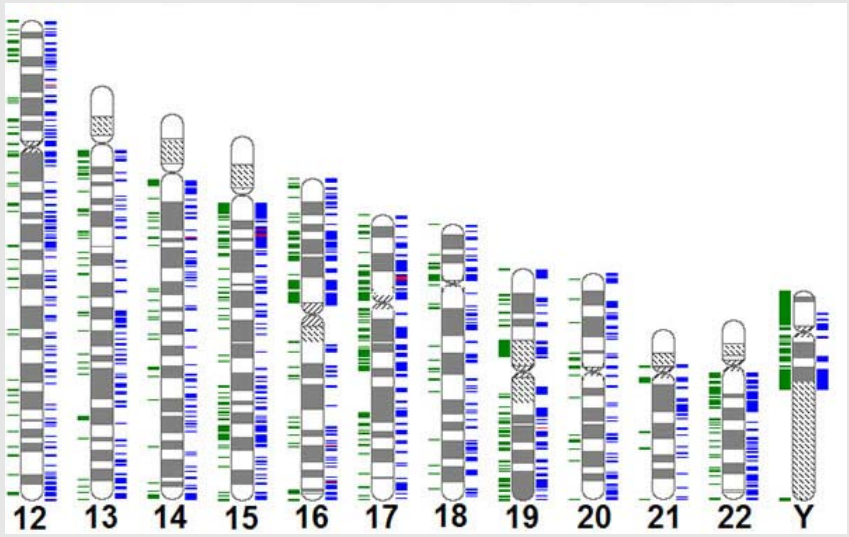
Identifying recurrent imbalances and phenotypes



ECARUCA

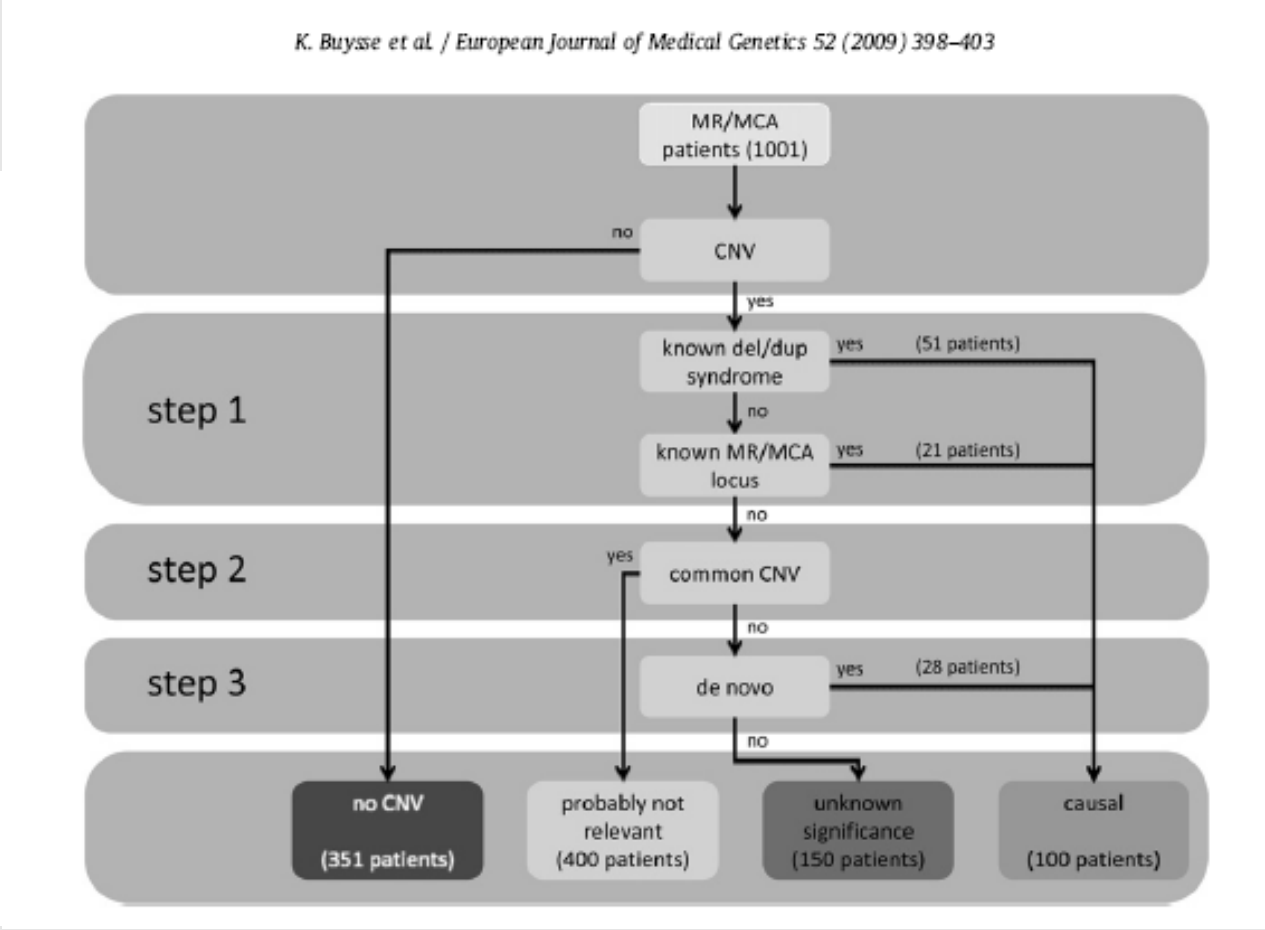
Genome variation Database: Map all "benign" variation





Database of genomic variants

Interpretation scheme for CNV screen in patients



Lessons (2)

De novo does not mean causal

Since multiple de novo events occur each generation, we will always identify novel events in full genome sequencing

Lessons

Proper clinical evaluation is and will be essential for proper data interpretation!

Use common knowledge for interpretation first

Clinical VALIDITY?

Clinical significance of anomaly?

- Traditional constitutional cytogenetic applications:
  - Mental retardation/ multiple congenital anomalies:
    - For larger (>1 Mb) CNVs — High (~75%)
    - For smaller CNVs — Low

Because the huge genetic heterogeneity of MCA/MR the clinical validity of full genome sequencing will remain low untill large phenotype-genotype databases establish associations between genes and phenotypes!

Lessons (3)

Molecular cytogeneticists are doing research and a little diagnosis

MCA/MR screening in a diagnostic setting should only be performed with known loci associated with disease

= complex molecular test

Challenges/solutions for clinical implementation of genome sequencing

- Analytical validity
  - Establish IQA and EQA (CEQAS/Eurogentest)
- Clinical utility:
  - De novo imbalances are not necessarily causal
  - Inherited imbalances can be causal
  - Phenotypic variability
    - Establish genotype/phenotype databases (f.e. ECARUCA/DECIPHER/Literature)
    - Mine the genome only for the genes with known disease association.
- Clinical validity
  - Logaritmic increase in number of syndromes
  - CNVs/ SNps become risk factors
    - Bioinformatic support in data interpretation
    - Large scale association studies needed
    - Counseling by experts!

But....

For DISCUSSION

Clinical validity becomes irrelevant!

Genome sequencing will be performed without a clinical question. We will mine the genome, dependent on the clinical question.

We should determine, when in a lifetime the genome should be sequenced, where to store the information and who has when access to which information.