Array in daily practice promises and pitfalls
Technical state of the art

Joris Vermeesch

K.U.Leuven

May 2011, ESHG, Amsterdam, The Netherlands

Postnatal diagnosis of patients with MCA/ID

European Journal of Human Genetics (2007), 1–10
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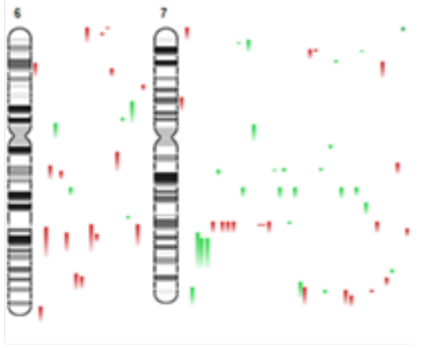
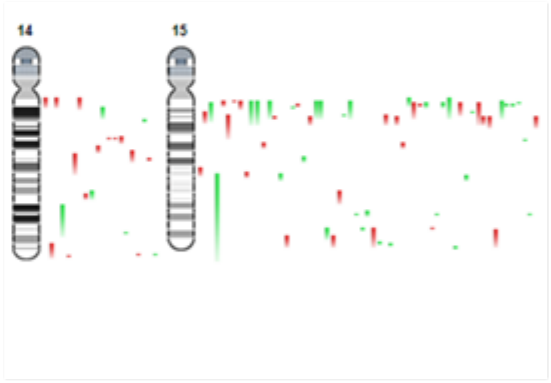
www.nature.com/ejhg

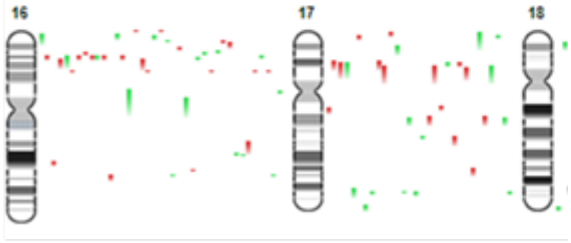


POLICY

Guidelines for molecular karyotyping in constitutional genetic diagnosis

Joris Robert Vermeesch*,[1], Heike Fiegler[2], Nicole de Leeuw[3], Karoly Szuhai[4], Jacqueline Schoumans[5], Roberto Ciccone[6], Frank Speleman[7], Anita Rauch[8], Jill Clayton-Smith[9], Conny Van Ravenswaaij[10], Damien Sanlaville[11], Philippos C Patsalis[12], Helen Firth[13], Koen Devriendt[1] and Orsetta Zuffardi[6]





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

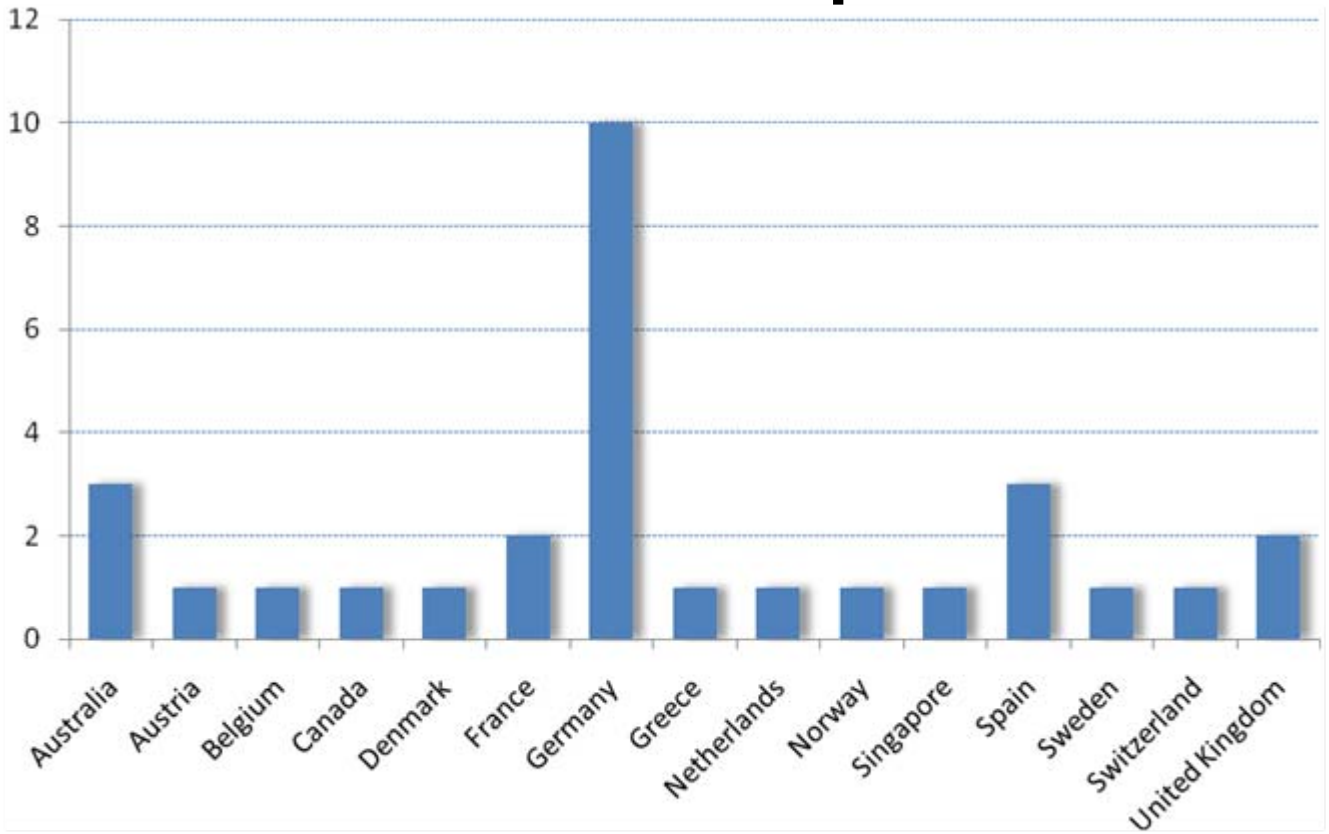
David T. Miller,[1,*] Margaret P. Adam,[2,3] Swaroop Aradhya,[4] Leslie G. Biesecker,[5] Arthur R. Brothman,[6] Nigel P. Carter,[7] Deanna M. Church,[8] John A. Crolla,[9] Evan E. Eichler,[10] Charles J. Epstein,[11] W. Andrew Faucett,[2] Lars Feuk,[12] Jan M. Friedman,[13] Ada Hamosh,[14] Laird Jackson,[15] 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,*]

The American Journal of Human Genetics *86*, 749–764, May 14, 2010

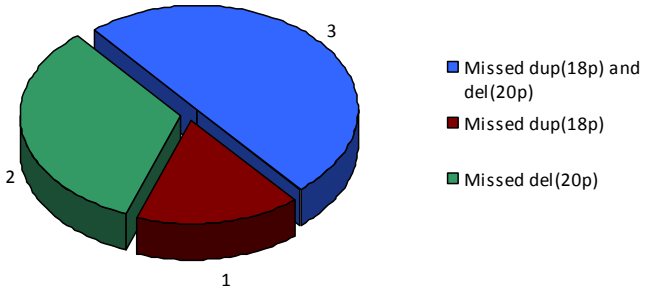
First external quality control scheme

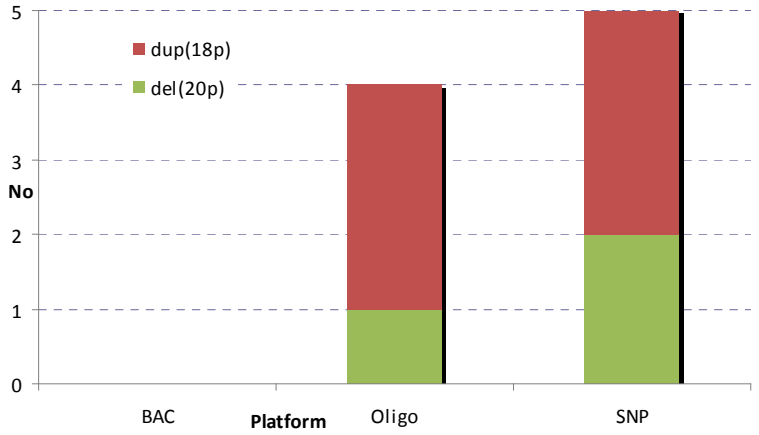
First external quality control scheme in 2010

30 Participants



Genotyping errors in 6 labs!



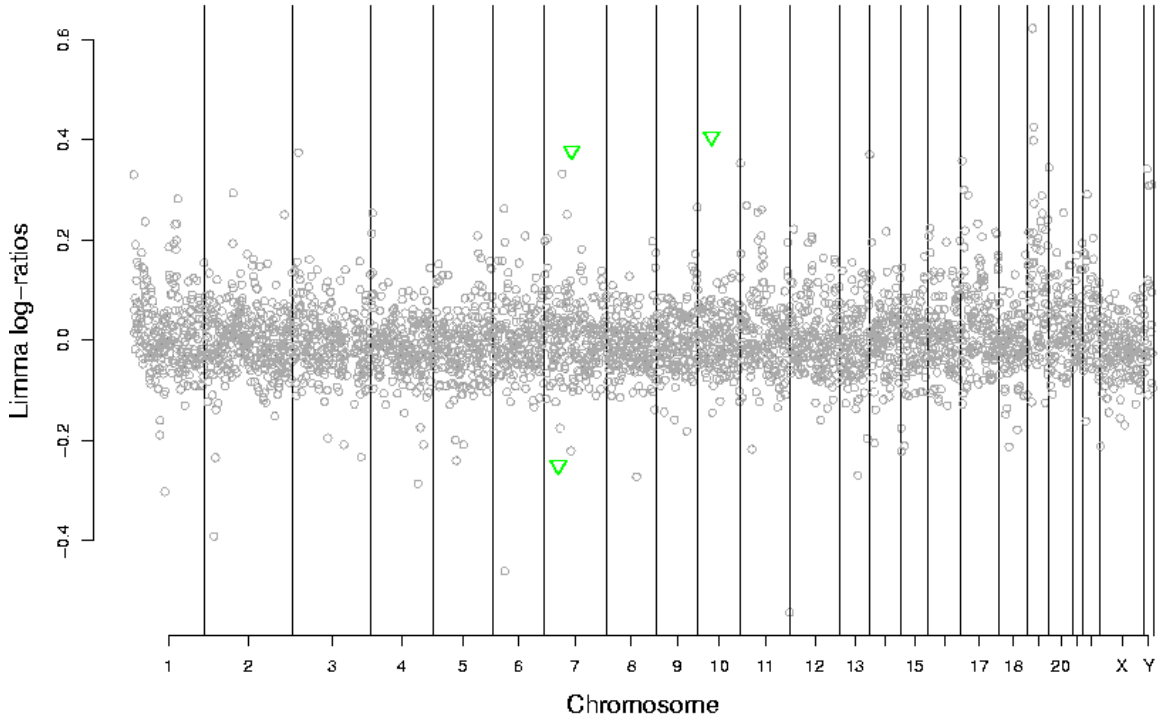


By platform

Sizes:

- 9.3 Mb duplication
- 1.7 Mb deletion

Technical aspects

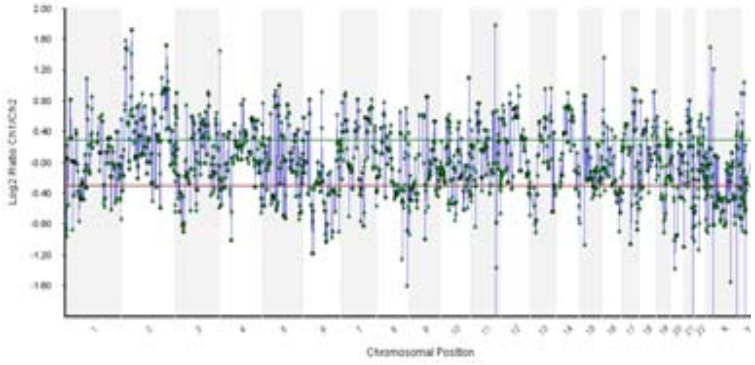


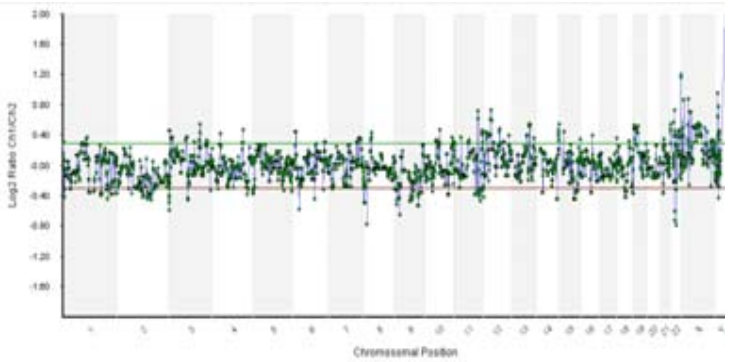
Is this a normal or an abnormal molecular karyotype?

Answer depends on premises:

- Technical premises
  - Array quality
  - Thresholding/statistics ?
  - Reference sample
- Biological premises
  - Polymorphisms?
  - ...

Standard deviation





Problem: number of false positives dependens on variation of intensity ratios

Different treshholding methods

- Floating Segmentation algoritms
- Hidden Markov
- CNAT,CNAG

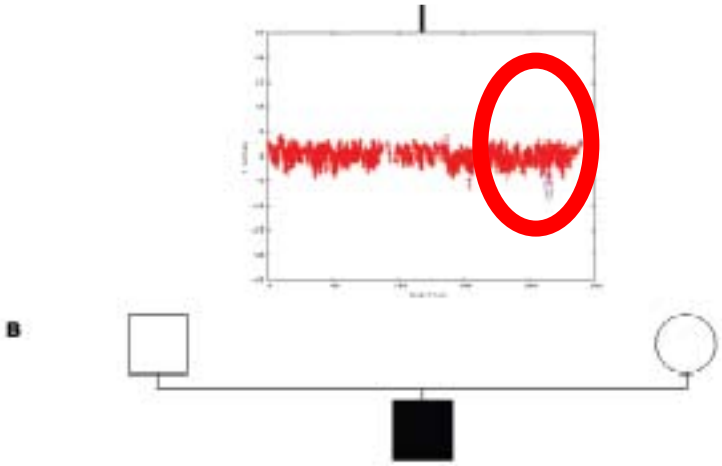
- But every method has its limits..

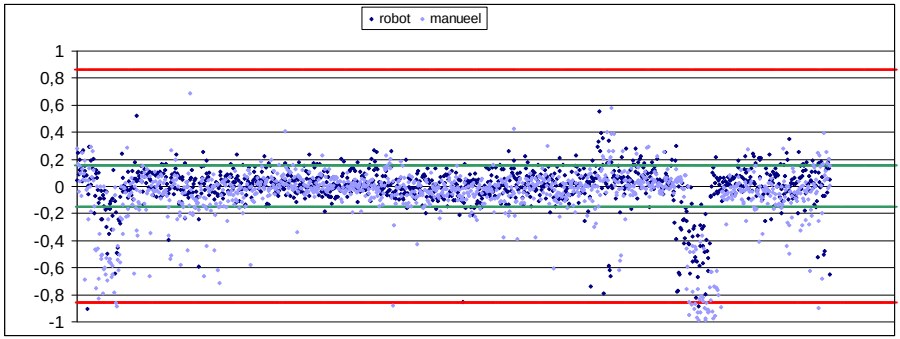
Due to statistics

Due to biology

- Paralogous sequences
- Sequence variation
- Underlying rearrangements
- ....

Dynamic range





Factors influencing dynamic range:

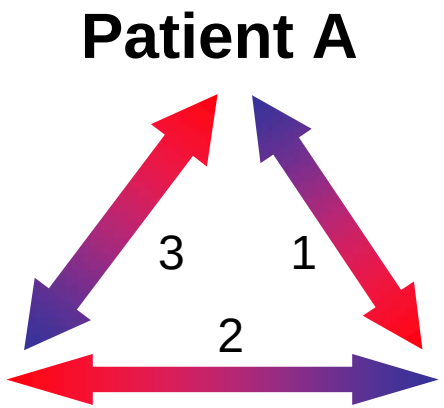
- BAC amplification quality
- Hybridisation conditions
- CotI quality
- ……

Friedman et al., AJHG, 2007

Reference material

- DNA from normal individual
  - Who's normal?
- DNA from a mixture of individuals
  - How many?
  - Which?
  - Value?
- DNA from other patients
  - When?
  - Three way hybridisations
- DNA from same individual (for acquired disorders only)

Loopdesign

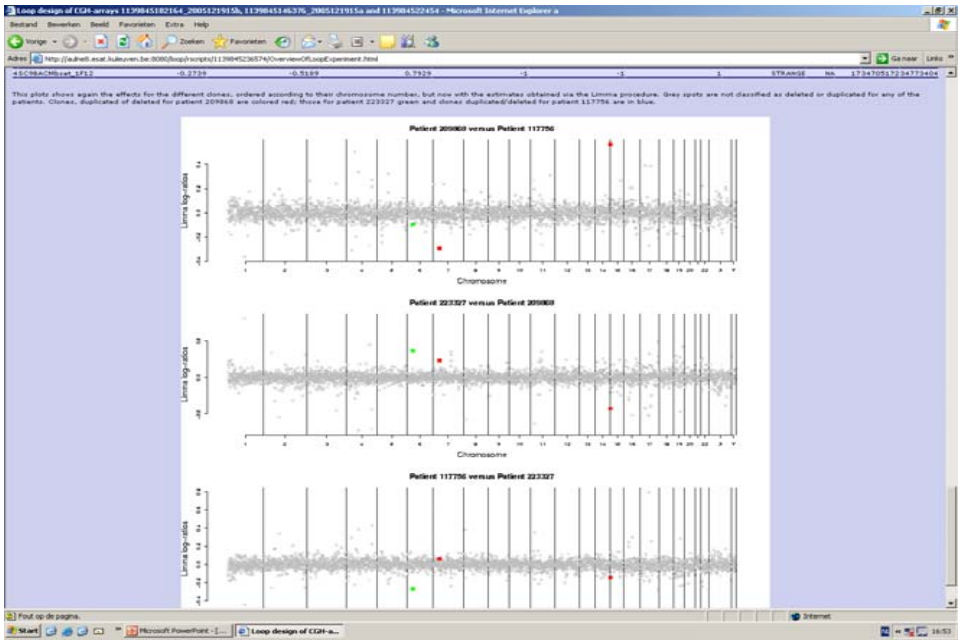


1
2
3

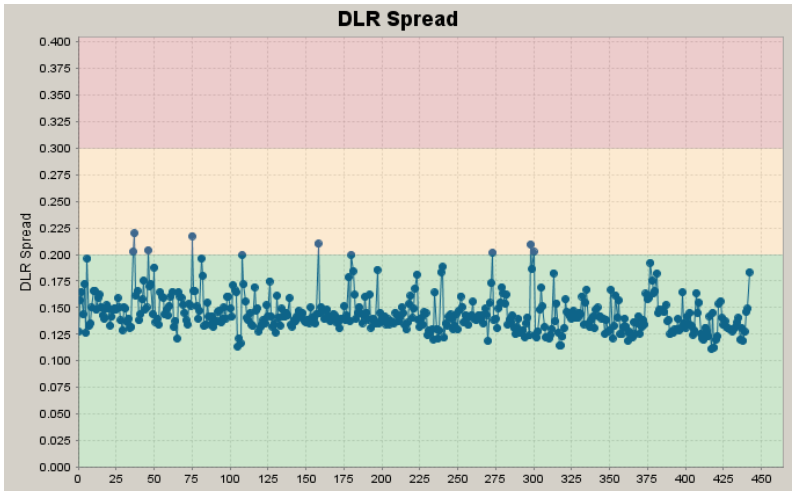
Cy3 ➔ Cy5

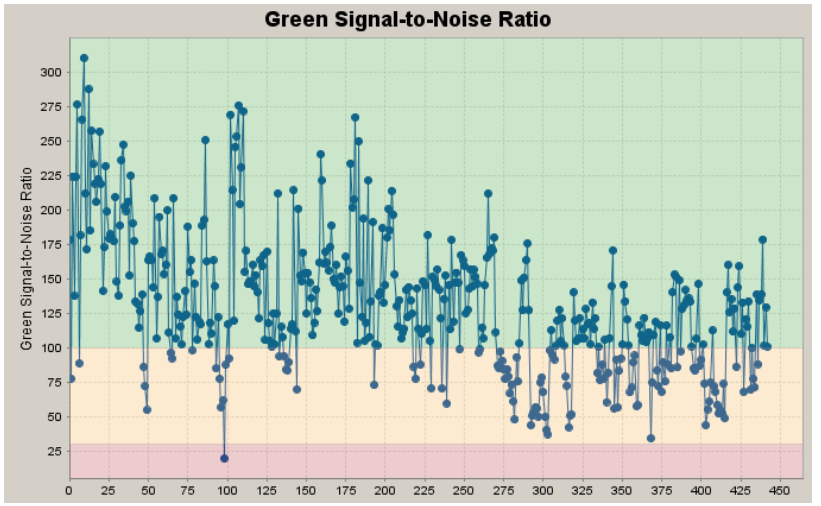
Patient A ➔ Patient C
Patient B ➔ Patient A
Patient C ➔ Patient B

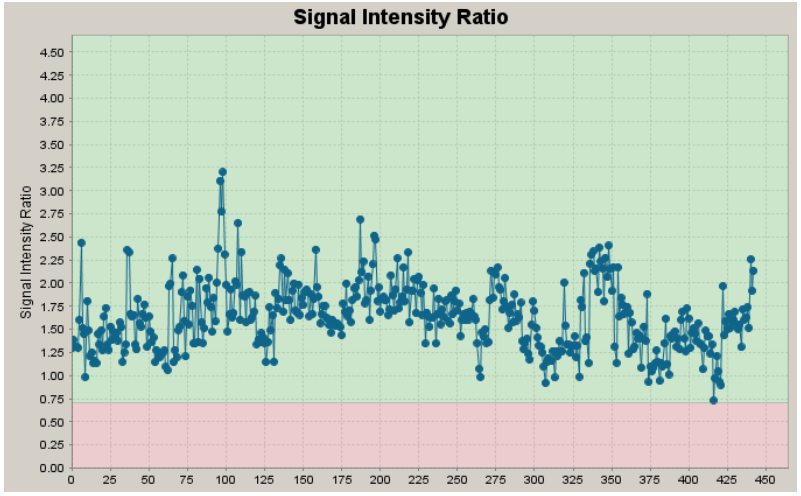
! Only patients with different phenotypes

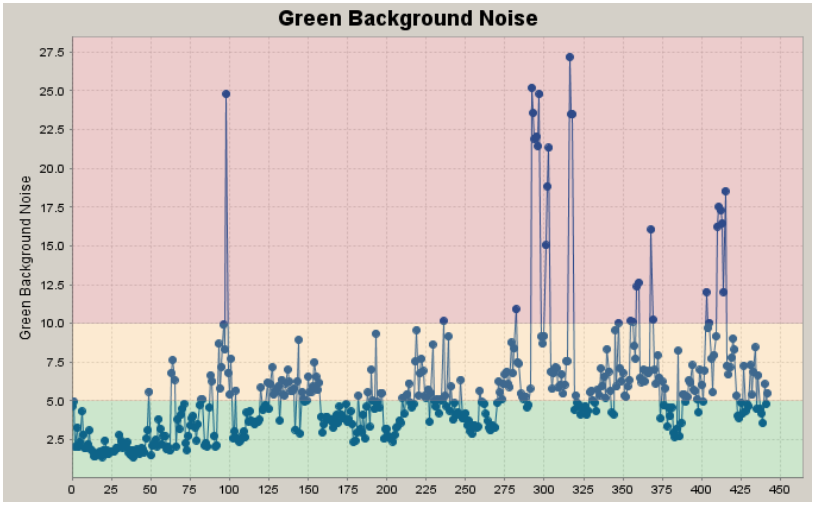


Longitudinal QA is important





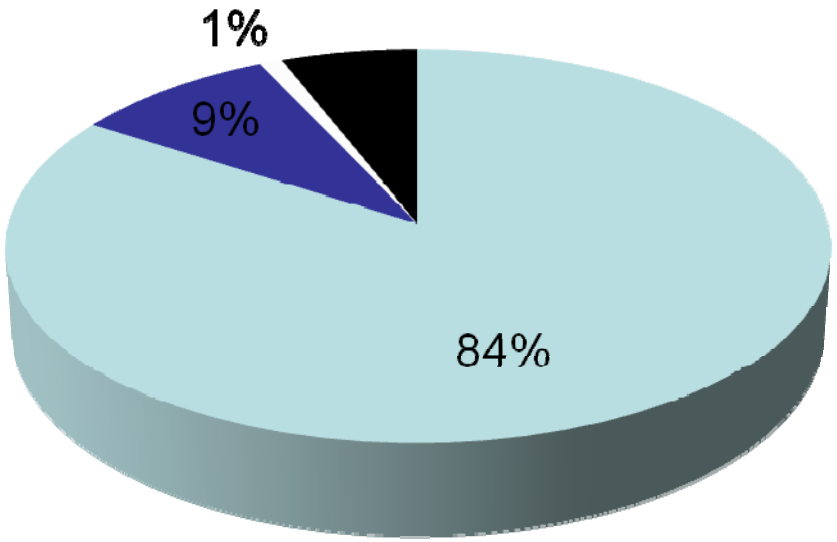




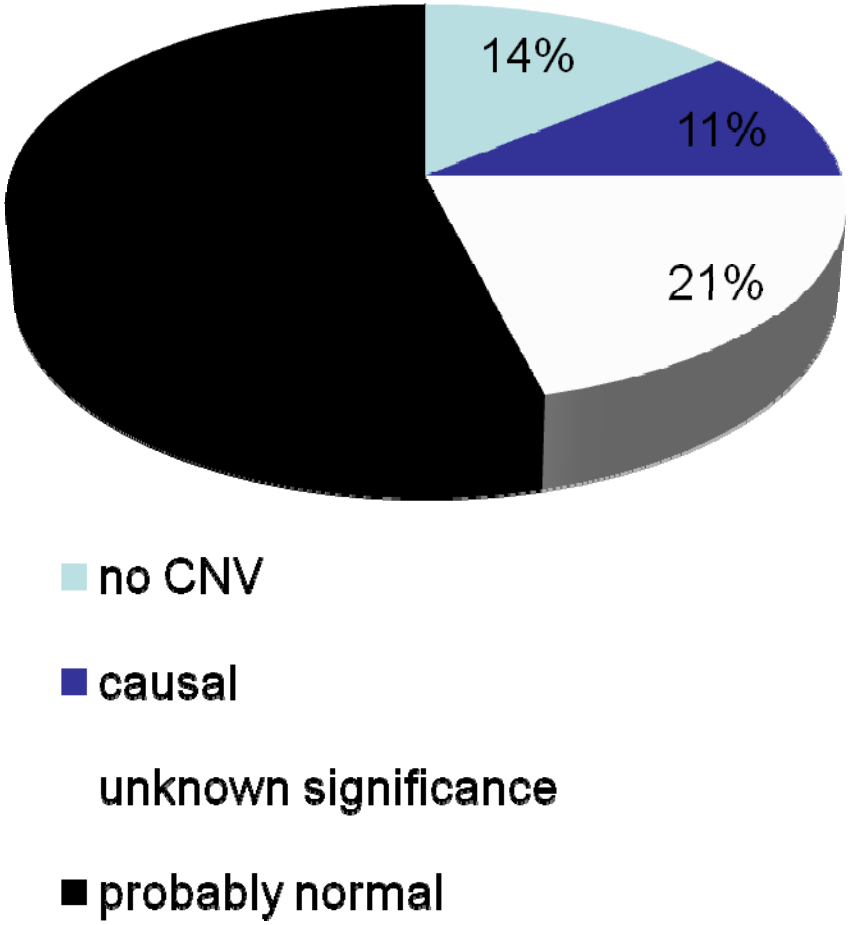
Practical technical issues

- Ideal resolution?
- Degree of mosaicism one can/needs to be able to detect?
- To SNP or not to SNP?
- Is conventional cytogenetics still necessary?

1 Mb BAC array

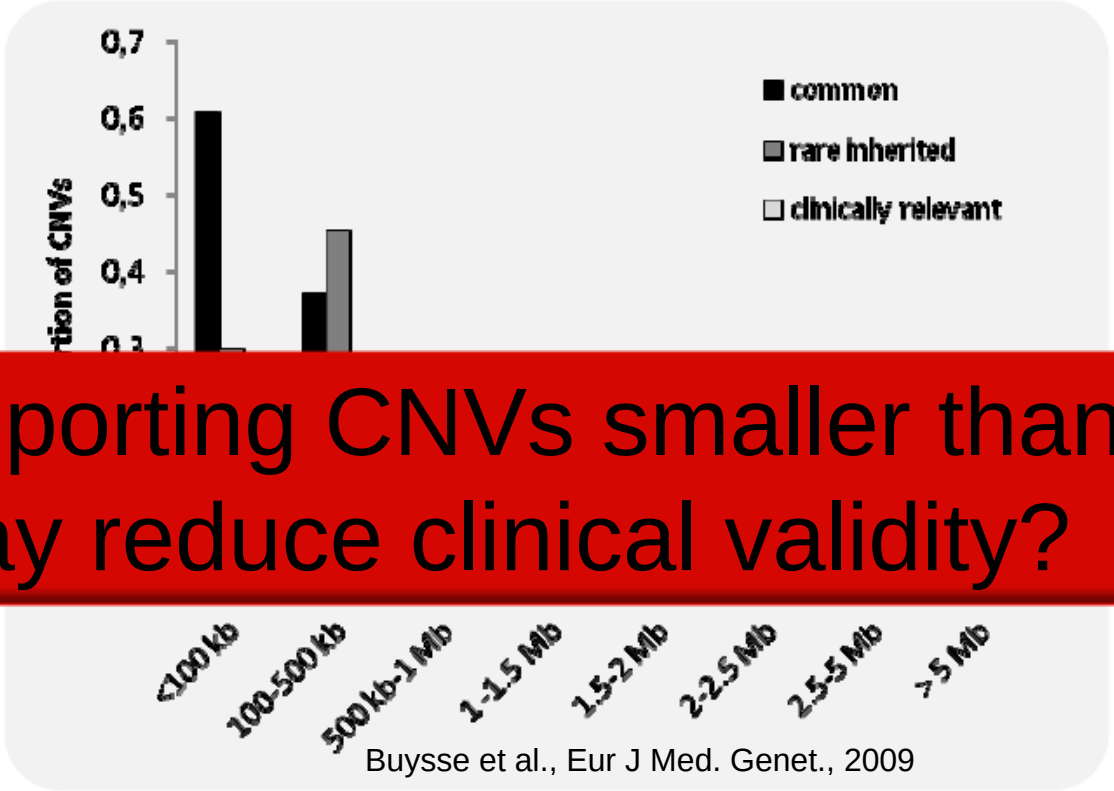


44 K oligonucleotide array



Buysse et al. Eur J Med. Genet., 2009

Is there a clinical valid minimum resolution?

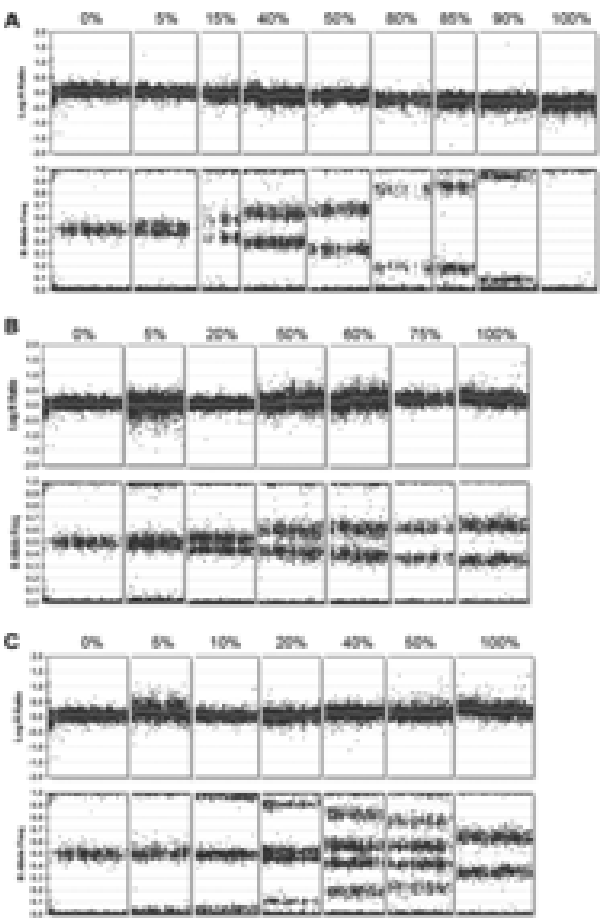


Buysse et al., Eur J Med. Genet., 2009

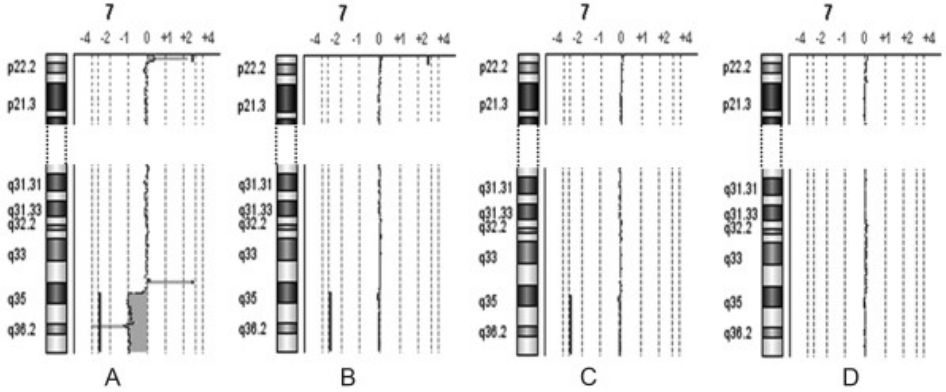
Reporting CNVs smaller than 200 kb may reduce clinical validity?

Itsara et al., 2010: Rare CNVs smaller than 200 kb are equally frequent in control and patient population

mosaicism



Conlin L K et al. Hum. Mol. Genet. 2010;19:1263-1275



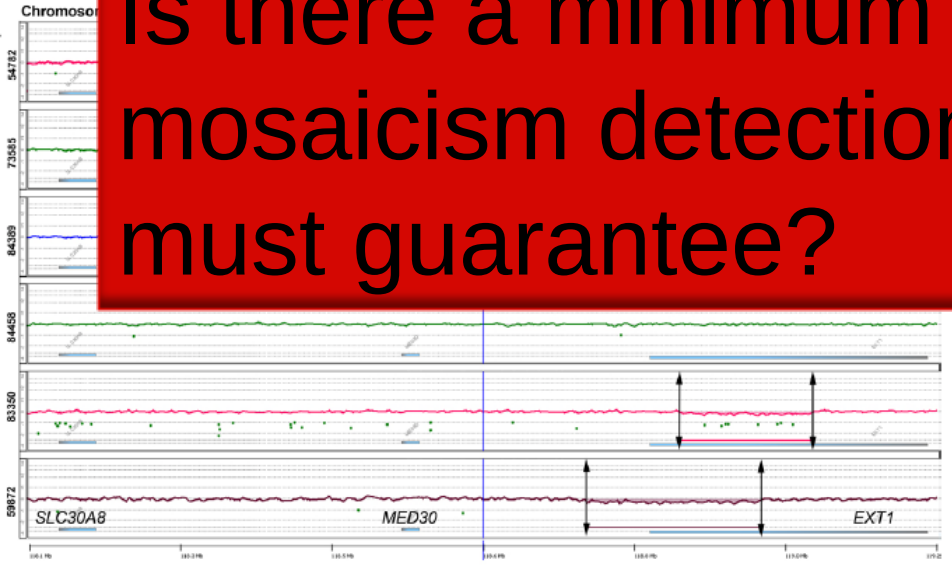
CGH partial profiles of chromosome 7 in patient 1. A) Patient's 100% DNA. B) Synthetic mosaicism at 10% level, C) 8%, D) 7%. Valli *et al. Molecular Cytogenetics* 2011 **4**:13

Clinical utility/validity

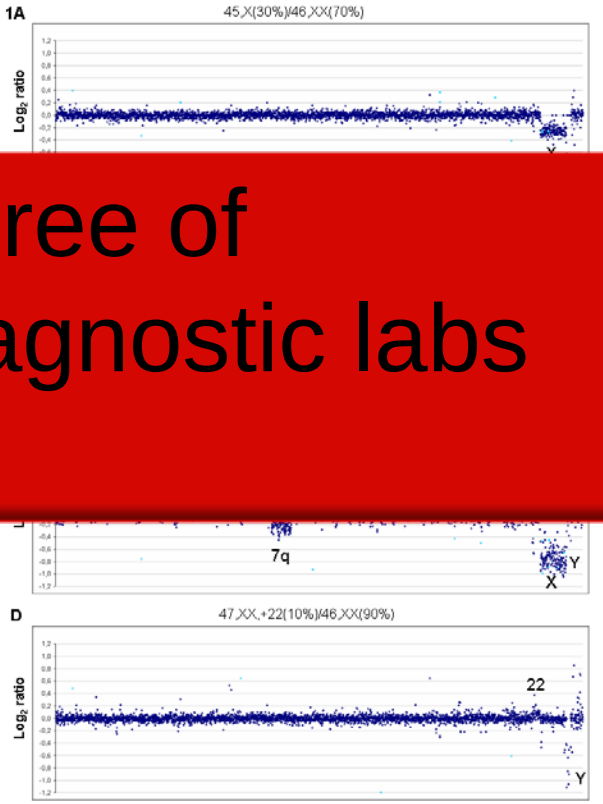
Tiling Resolution Array-CGH Shows That Somatic Mosaic Deletion of the *EXT* Gene is Causative in *EXT* Gene Mutation Negative Multiple Osteochondromas Patients

OFFICIAL JOURNAL HGV HUMAN GENOME VARIATION SOCIETY www.hgvs.org

Károly Szuhai[1#*], Ivy Jennes[3#], Danielle de Jong[1], Judith V.M.G. Bovée[2], Malgorzata Wiweger[2], Wim Wuyts[3#], and Panc



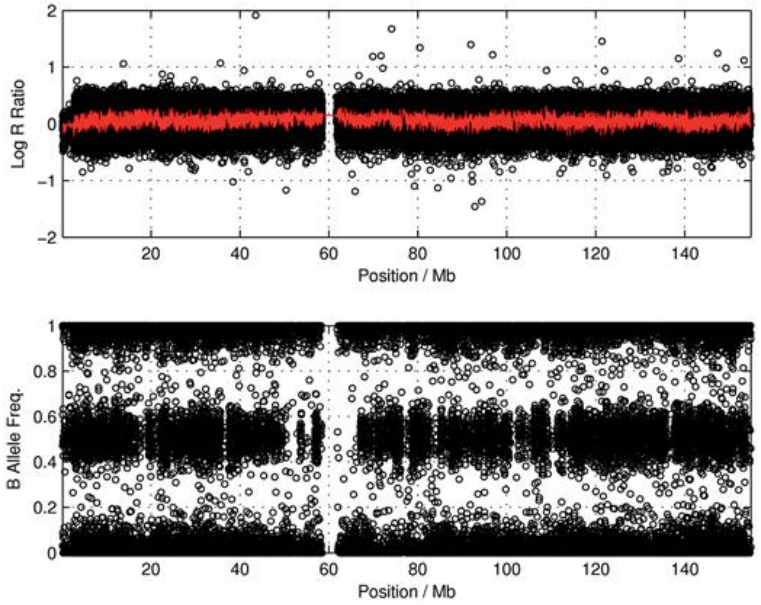
Miscarriages:
Array CGH is first tier test

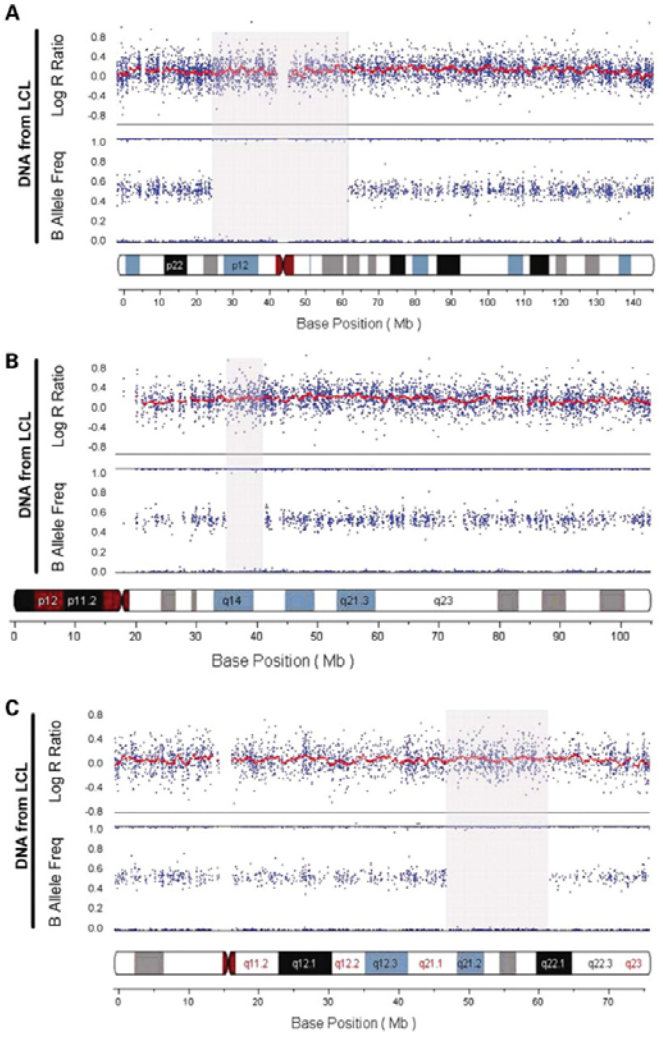


Robberechts et al., Gen. Med.. 2009

Is there a minimum degree of mosaicism detection diagnostic labs must guarantee?

To SNP or not to SNP





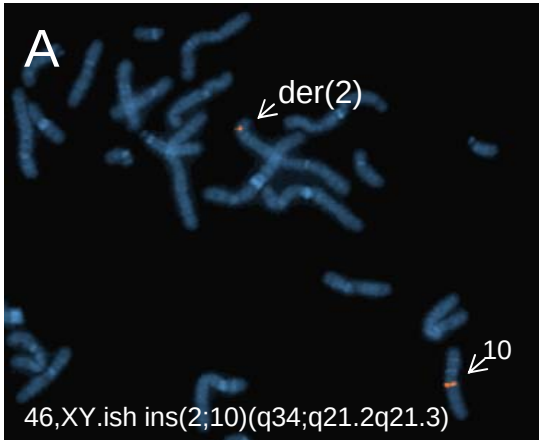
Simon-Sanchez J et al. Hum. Mol. Genet. 2007;16:1-14

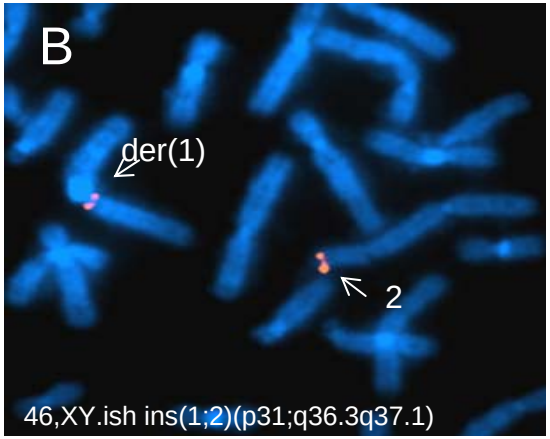
Clinical Utility/validity?

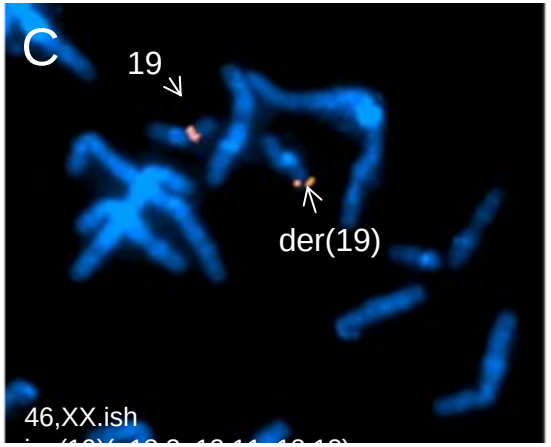
- Increased power to detect deletions/duplications
- Extra power to detect (low grade) mosaicism
- Ability to detect regions of homozygosity
  - (but is this clinicaly relevant/usefull?)
- Information on UPDs
  - (but what is the frequency? Can UPD be deduced from the phenotype?)
- Information on origin of CNV
  - (clinically not relevant...?)

Need for cytogenetic follow-up?

•insertional translocations underlie approximately 2.1% of the apparently *de novo,* interstitial CNVs!
•Parental testing is warranted! Can only be detected by FISH!







Nowakowska et al., submitted

Need for cytogenetic follow-up?

- Pericentromeric imbalance
  - Could be due to presence of marker
- Mosaicism
  - Determine degree of mosaicism/confirmation
- Parental follow-up for terminal deletions and duplications
  - could be due to balanced translocation in parents.
- Parental follow-up in miscarriages/prenatal/postnatal trisomies of acrocentric chromosomes
  - could be due to Robertsonian translocations in parents.
- Parental follow-up for de novo non-recurrent translocations
  - could be due to insertional translocation

Clinical utility (2008-…)

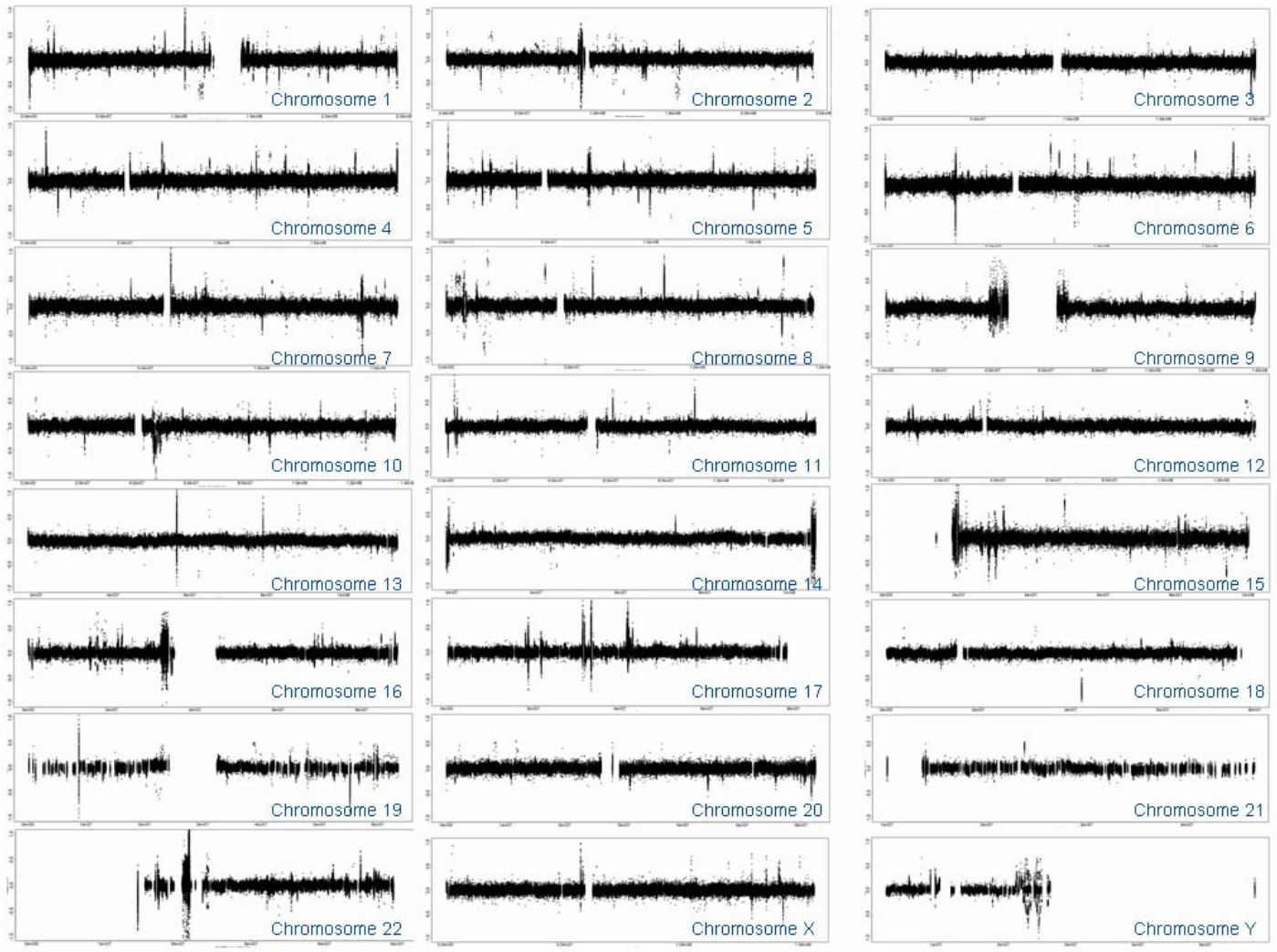
- Traditional constitutional cytogenetic applications:
  - Mental retardation/ multiple congenital anomalies: yes!
  - Prenatal? (clinical issues?) yes!
  - Miscarriages? (mosaicisms?) yes!
- Other medical disciplines?
  - Neurology/ Psychiatry? yes!
    - Autism
    - Schizophrenia
  - Isolated heart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

Clinical VALIDITY?

Clinical significance of anomaly?

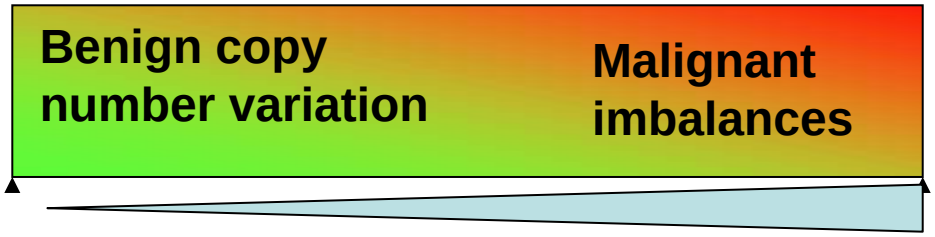
- Traditional constitutional cytogenetic applications:
  - Mental retardation/ multiple congenital anomalies:
    - For larger (>1 Mb) CNVs — High (~75%)
    - For smaller CNVs (<200 kb) — Low
  - Prenatal? (clinical issues?) (>1 Mb)
    - Abnormal ultrasound — High
    - Normal ultrasound — Low
  - Miscarriages? (mosaicisms?)
    - chromosomal aneuploidies — High
    - Small imbalances — Low
- Other medical disciplines?
  - Autism? — Low
  - Neurology/ Psychiatry? — Low
  - Isolated heart defects?
  - Multifactorial diseases?
  - …. All medical disciplines?

We are all copy variable



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!

The challenge: Which imbalances are causal for the phenotype?

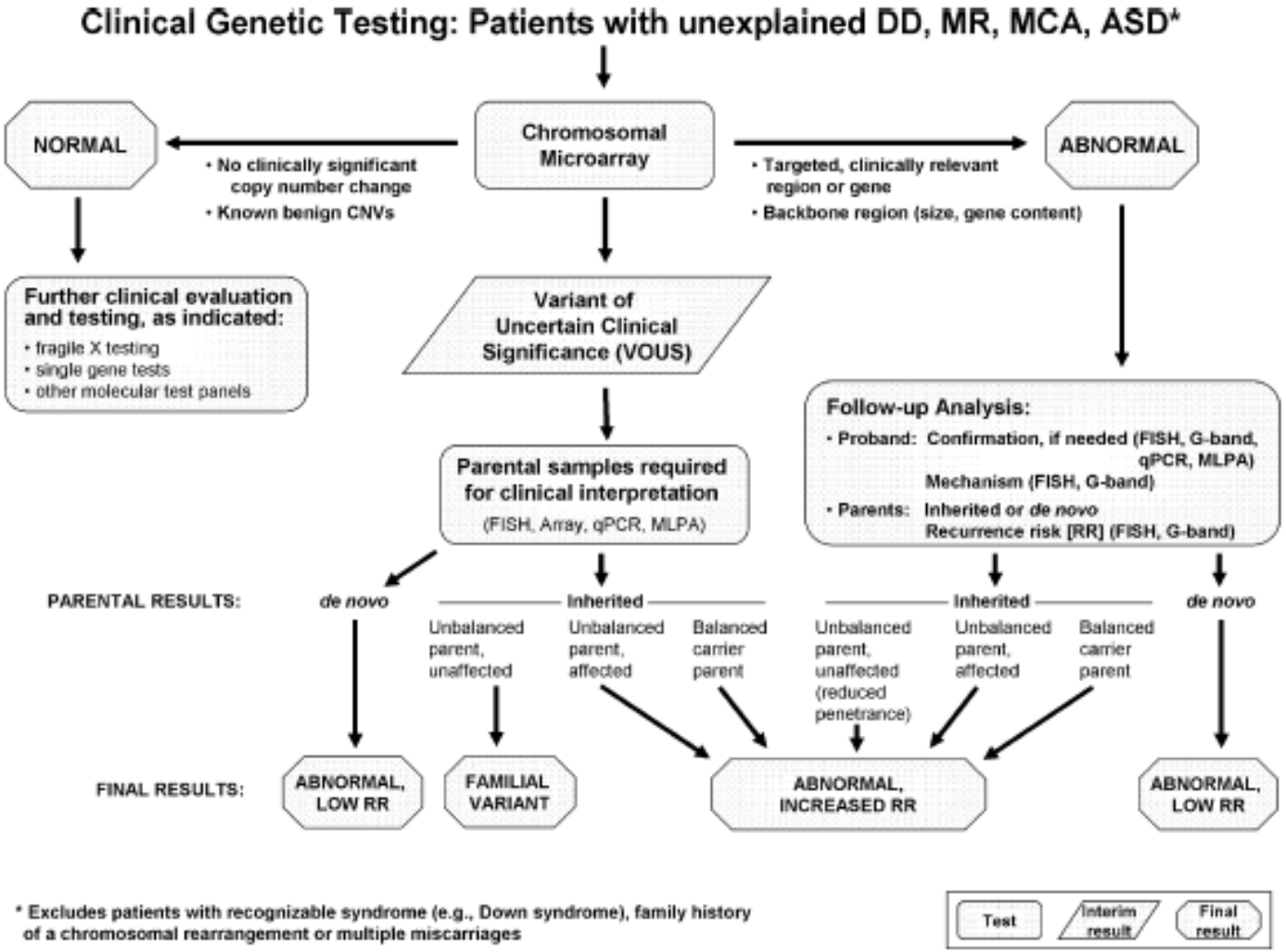


Figure 3. Algorithm for CMA Testing in Patients with Unexplained DD, MR, MCA, and ASD

Miller et al., Am.J.Hum.Gen. 2010

The challenge: Which imbalances are causal for the phenotype?

Conventional wisdom:

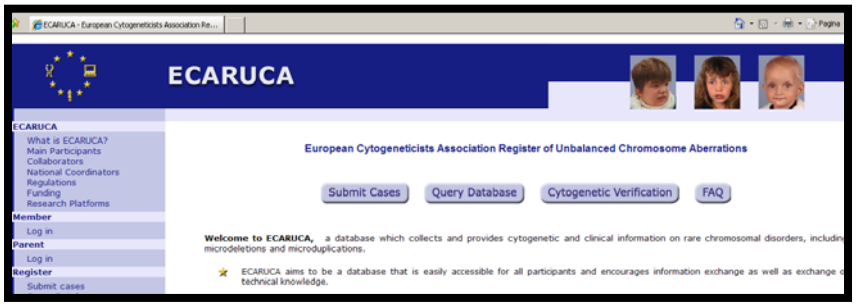
Recurrent imbalances with same phenotype are causal

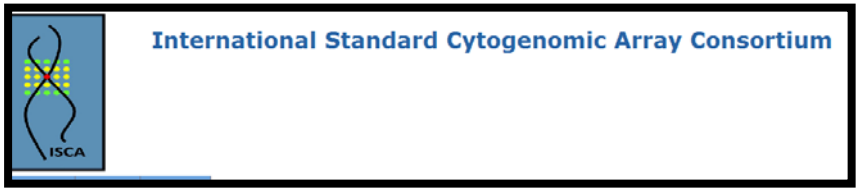
The larger the size, the more likely causal

Population embedded CNVs are benign

Inherited imbalances are benign while *de novo* imbalances are causal

Identifying recurrent imbalances and phenotypes





Limitations

- Little information on CNVs associated with prenatal phenotypes
- As a consequence, for many CNVs the outcome is unclear

Solutions

- Large scale collection of all genotypes & phenotypes!
- Require submission of phenotype and genotype to public repository upon publishing.

The challenge: Which imbalances are causal for the phenotype?

Conventional wisdom:

Recurrent imbalances with same phenotype are causal

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Inherited imbalances are benign while *de novo* imbalances are causal

The challenge: Which imbalances are causal for the phenotype?

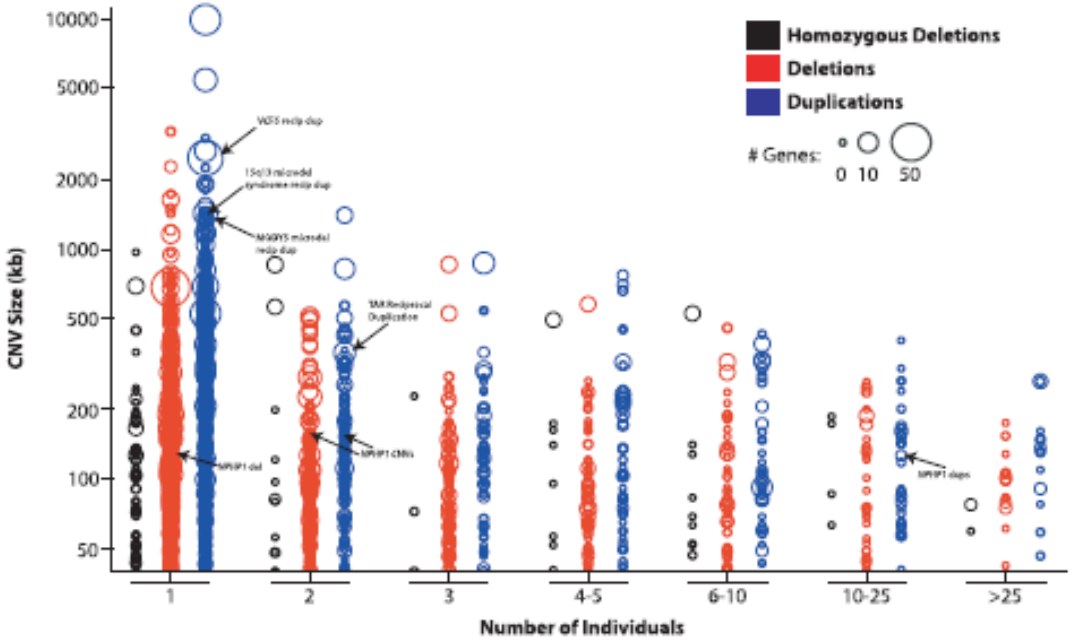
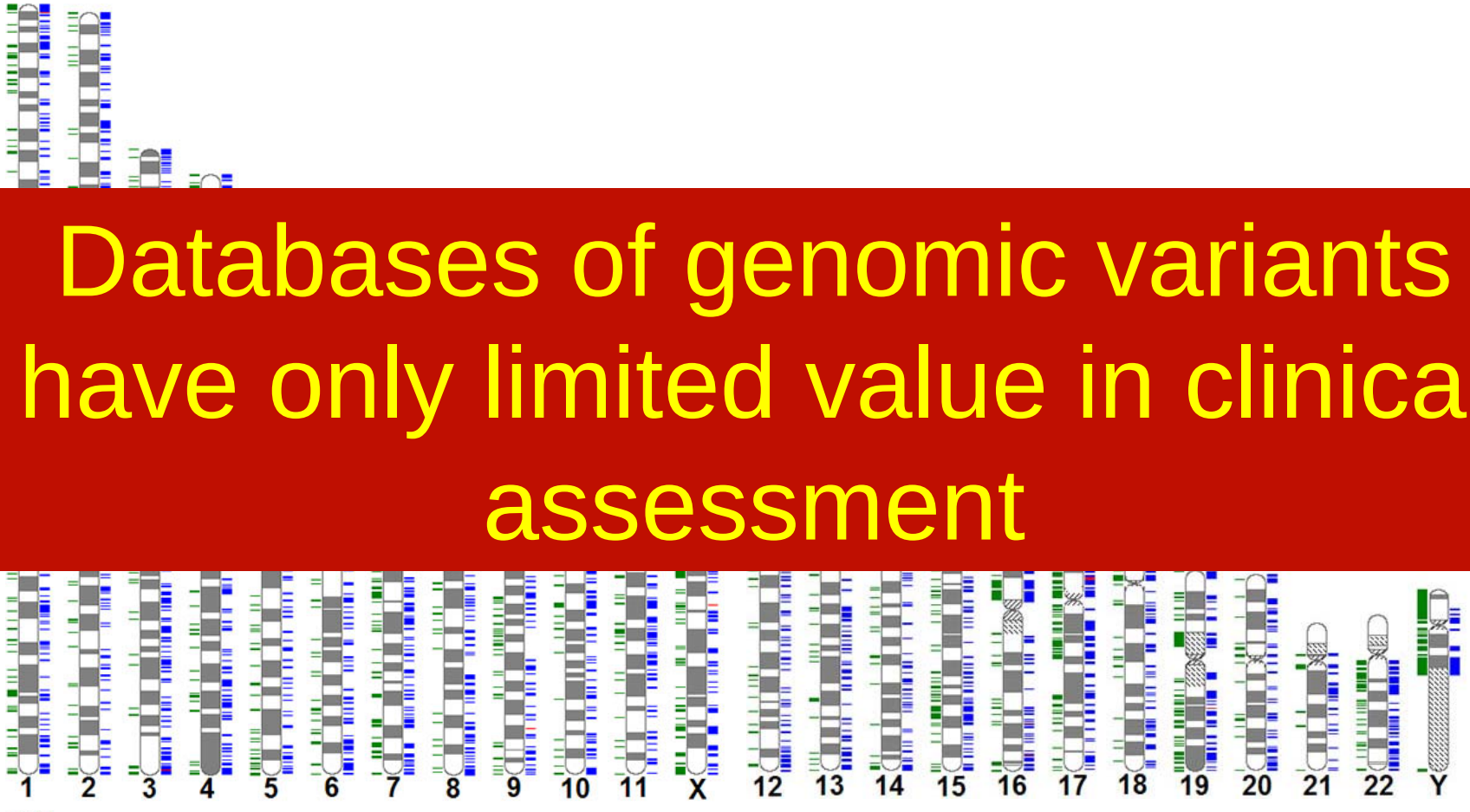


Figure 4. CNV Length, Gene Content, and Frequency Distributions
CNVs were plotted according to event type (color), length (*y* axis), frequency in the population (*x* axis, number of individuals from n = 2493), and number of RefSeq genes affected (circle size). To facilitate comparison across different platforms, events from different individuals were considered the same if their putative breakpoints were within 50 kb of one another. CNVs related to previously reported disease-causing variants are highlighted.

Size alone is not a good determinant!

Genome variation Database: Map all “benign” variation



Databases of genomic variants have only limited value in clinical assessment

- *Database of genomic variants May 2008*
- *Redon et al. Nature, 2008*

Mendelian CNVs:
a paradigm shift in (cyto)genetics

Inherited apparently benign CNVs *CAN* cause disease

"Mendelian CNVs" is the term coined here to indicate benign CNVs which can cause disease dependent on either copy number state, inheritance pattern or genetic and environmental background.

Mendelian CNVs: New wine in old bottles

- Autosomal recessive
- Autosomal dominant
- X-linked
- Imprinted CNVs
- Variable expressivity and incomplete penetrance

The challenge:
Which imbalances are causal for the phenotype?

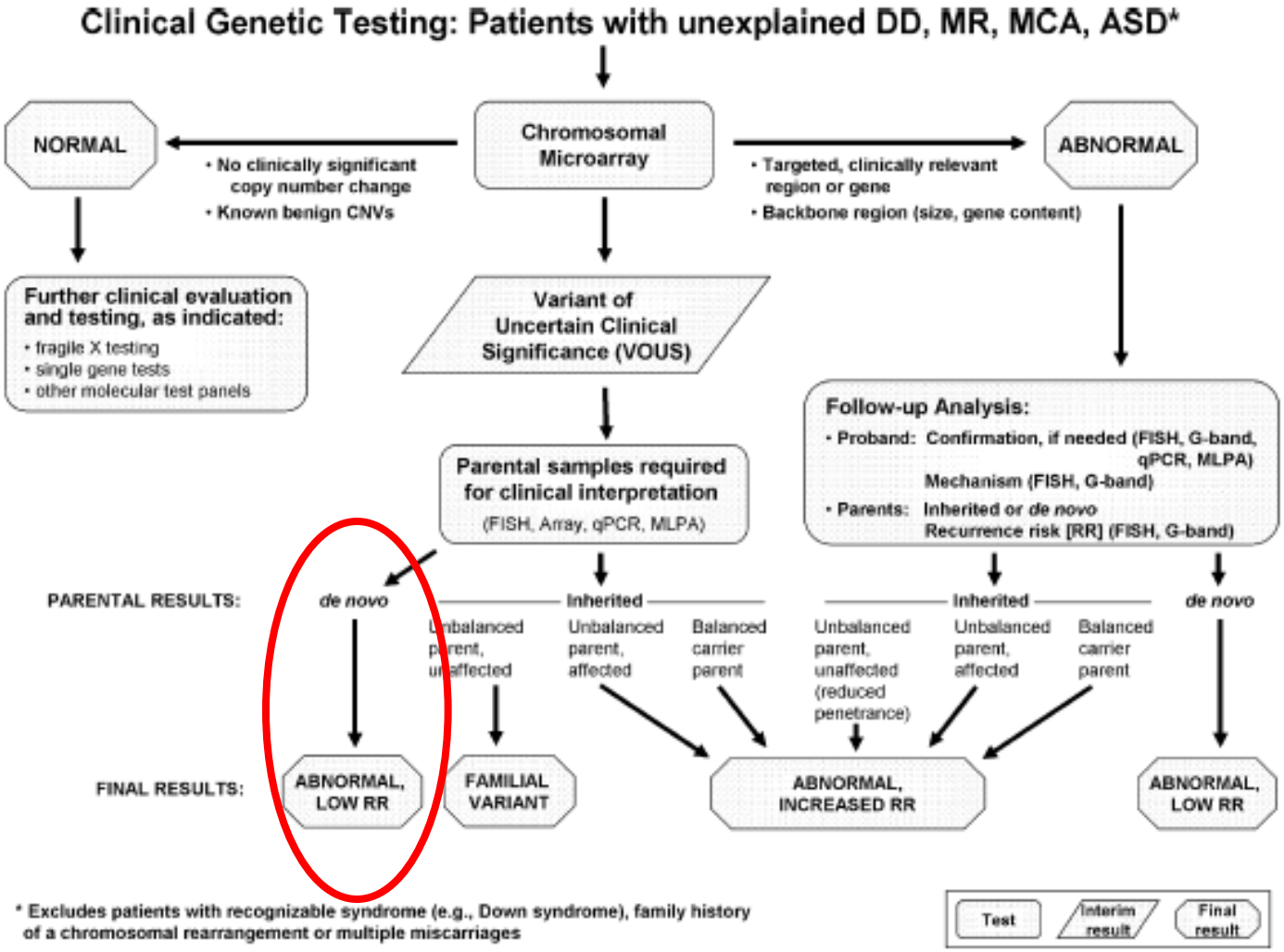
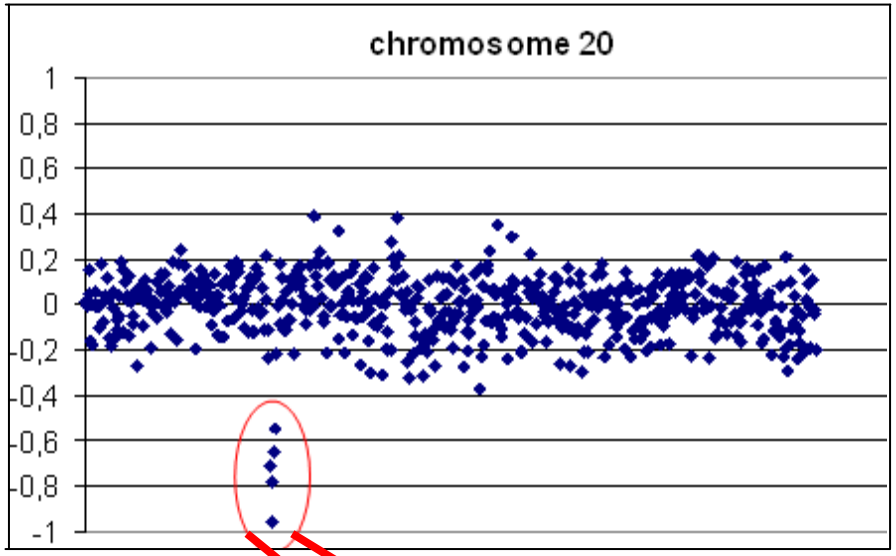
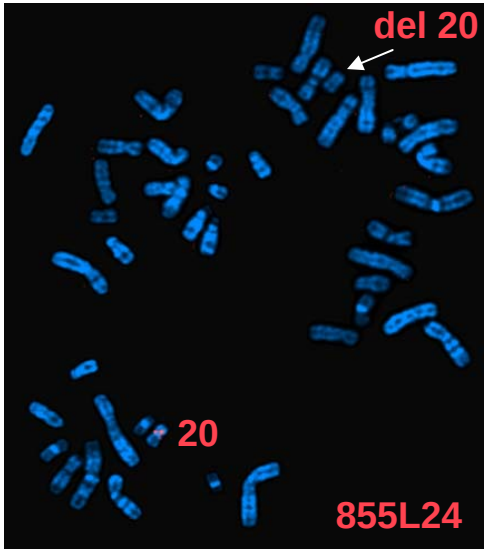


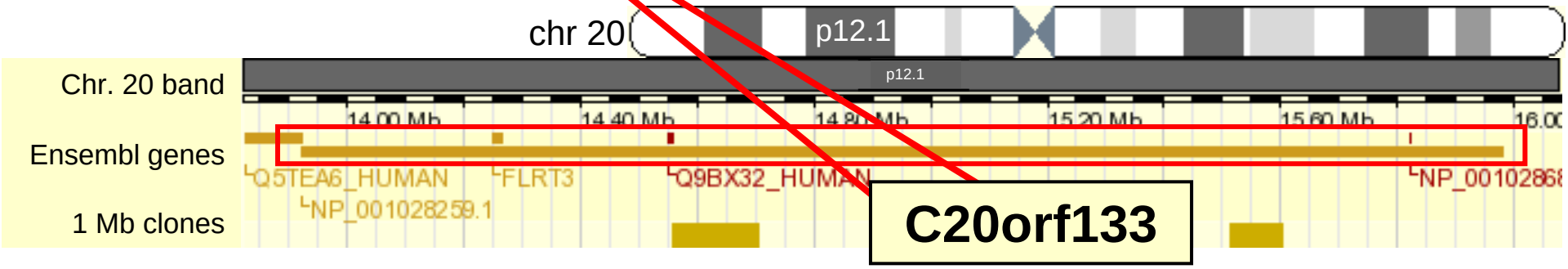
Figure 3. Algorithm for CMA Testing in Patients with Unexplained DD, MR, MCA, and ASD

Miller et al., Am.J.Hum.Gen. 2010

De novo deletion in C20orf133 cause for Kabuki syndrome?







deletion ~250 kb

Appr-1-p processing enzyme family:
- DNA repair
- Chromatin biology

Maas et al., J. Med.Gen., 2007

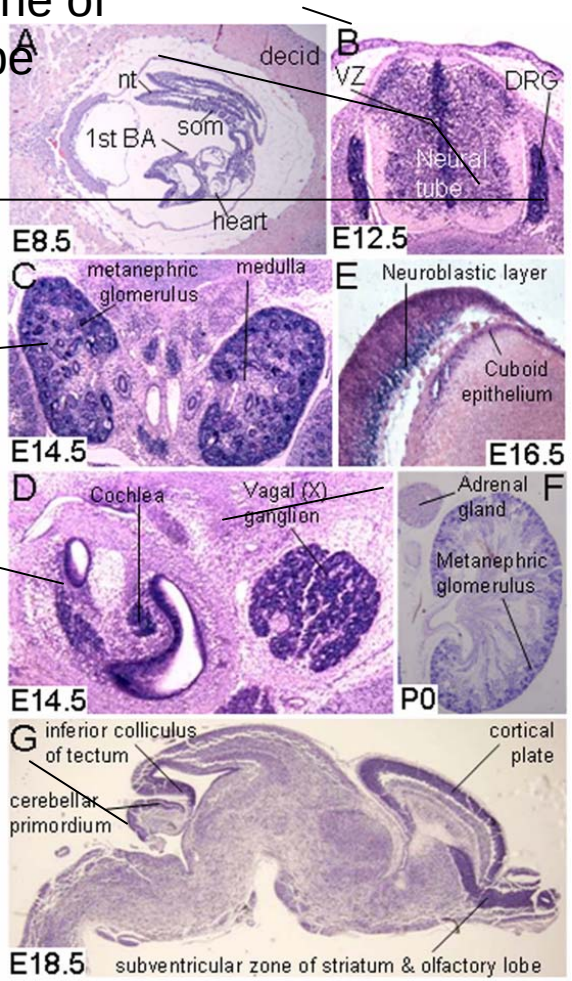
C20orf133

ventricular zone of the neural tube

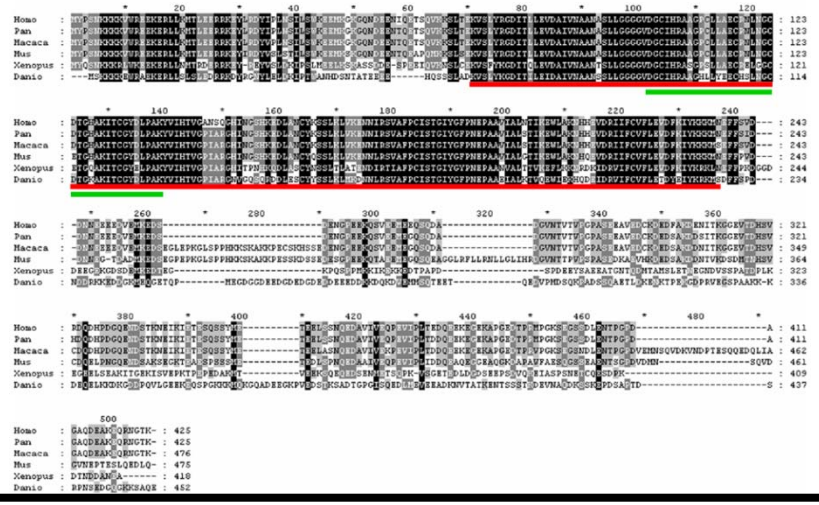
dorsal root ganglion

kidney

inner ear



conservation among different species



nature genetics

Exome sequencing identifies *MLL2* mutations as a cause of Kabuki syndrome

Sarah B Ng[1,7], Abigail W Bigham[2,7], Kati J Buckingham[2], Mark C Hannibal[2,3], Margaret J McMillin[2], Heidi I Gildersleeve[2], Anita E Beck[2,3], Holly K Tabor[2,3], Gregory M Cooper[1], Heather C Mefford[2], Choli Lee[1], Emily H Turner[1], Joshua D Smith[1], Mark J Rieder[1], Koh-ichiro Yoshiura[4], Naomichi Matsumoto[5], Tohru Ohta[6], Norio Niikawa[6], Deborah A Nickerson[1], Michael J Bamshad[1–3] & Jay Shendure[1]

Sequencing of MLL2 shows de novo mutation in this patient!!

An estimated 1 out of 5 CNVs between 60 & 500 kb are benign!

Itsara et al., Genome Research, 2010

- De novo CNV mutation rate: 2.5/100 live births
- An fourfold increase of de novo CNVs in autism spectrum patients

•=> 1/5 de novo CNVs is benign

For smaller CNVs this frequency is likely higher!

Van Ommen al. Nature Gen. 2005:

- Extrapolation of the frequency of CNVs in the Duchenne Muscular Dystrophy

•1 deletion every 8 generations and a duplication of 1/50 generations

Vermeesch et al., EJHG, in press

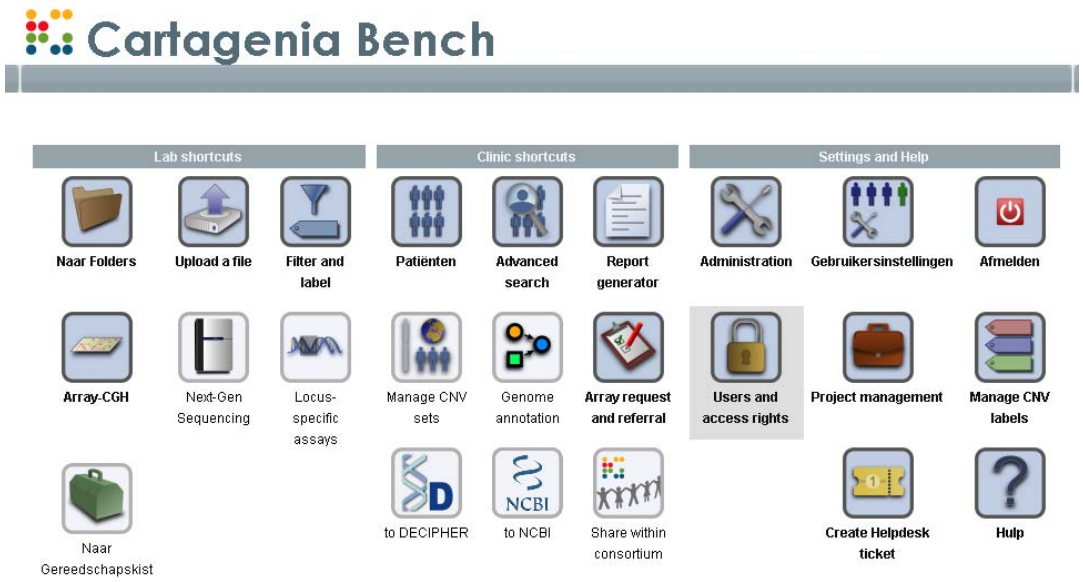
Needs for the community

Evidence based CNV data

Curated database for pathogenic and benign CNVs?

Needs for bioinformatic support

The quantity of information cannot be reproducibly interpreted and requires bioinformatic support



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- **Thomy de Ravel**
- **Hilde Peeters**
- **Eric Legius**
- **Gert Matthijs**