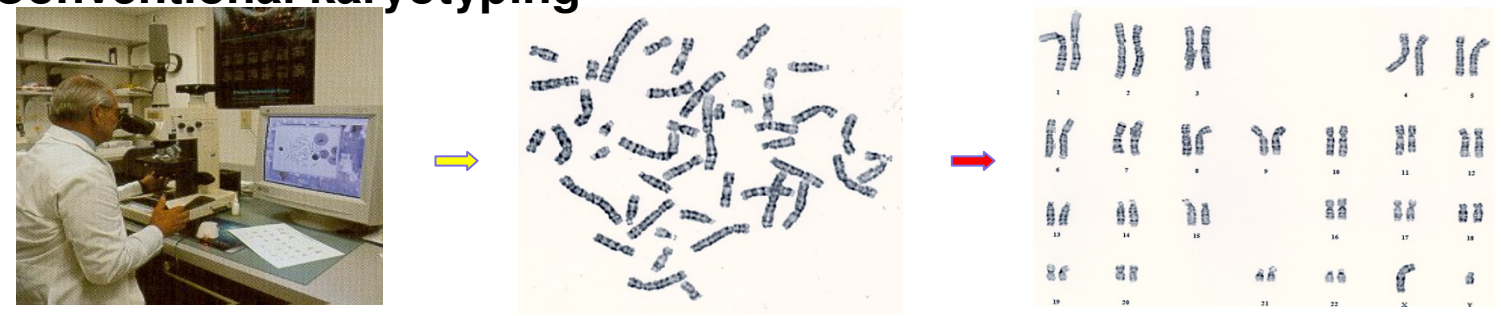

# Molecular karyotyping: From postnatal to preimplantation genetic diagnosis

Joris Vermeesch,
Center for Human Genetics
K.U.Leuven, Belgium

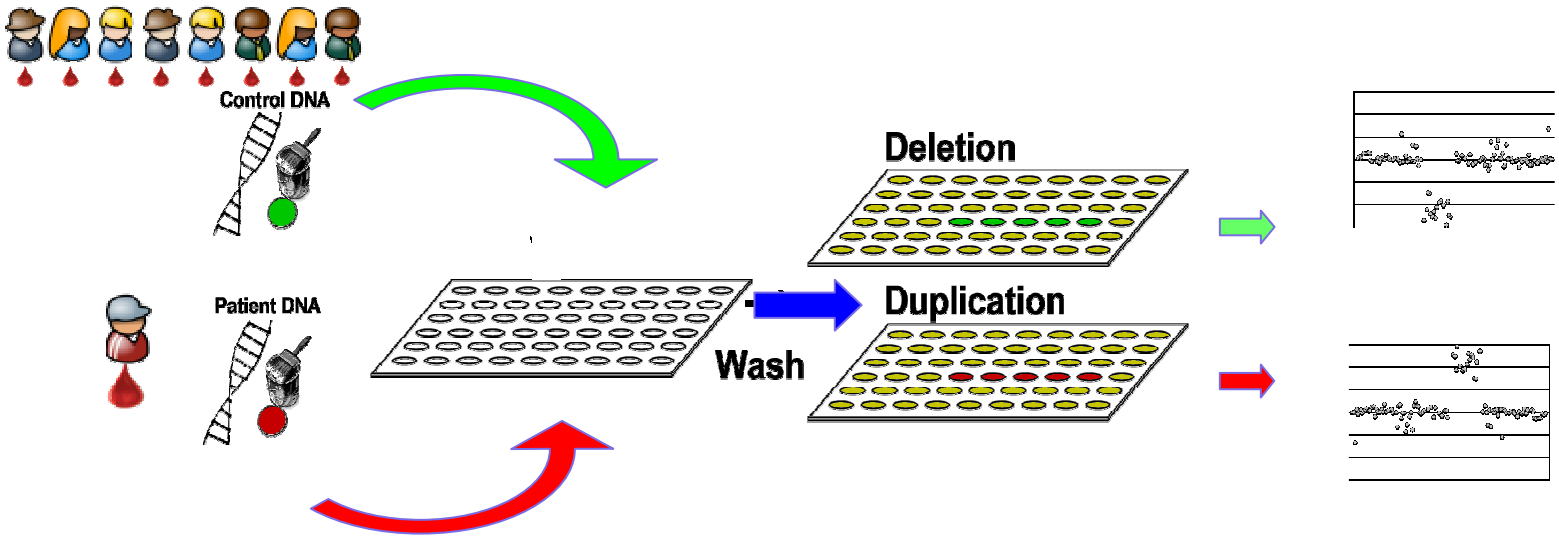
Salisbury
July, 5-6, 2010

# The array revolution

## Conventional karyotyping

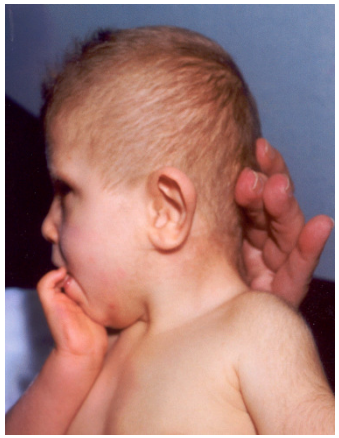


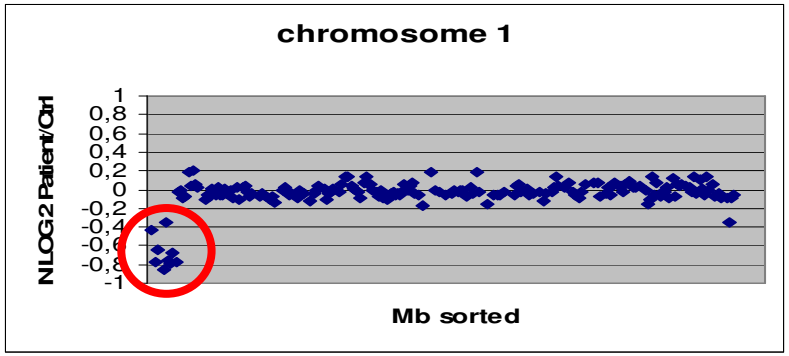
## Molecular karyotyping: DNA microarrays



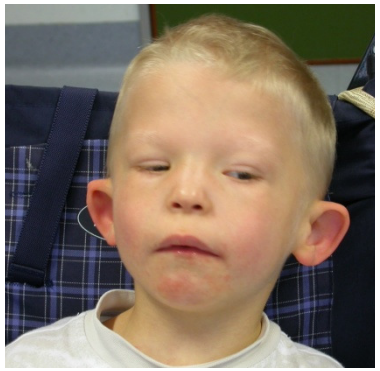
# Very high incidence of submicroscopic imbalances

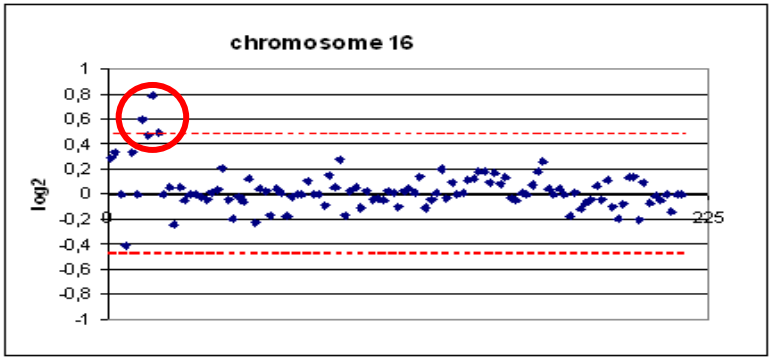
## Deletions

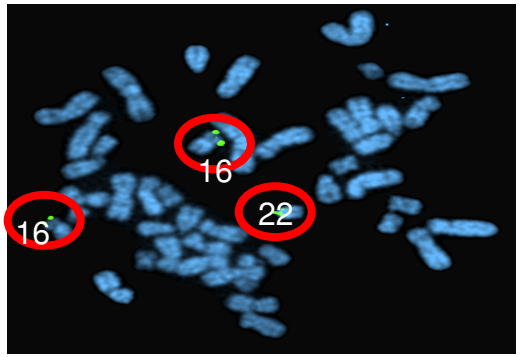




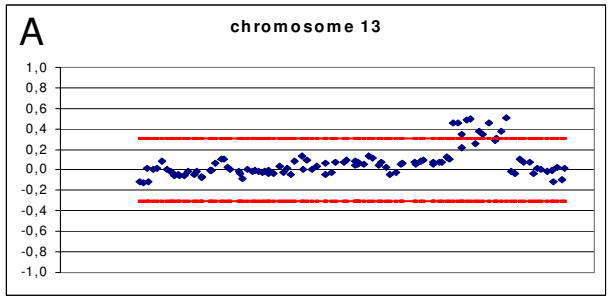

## Duplications

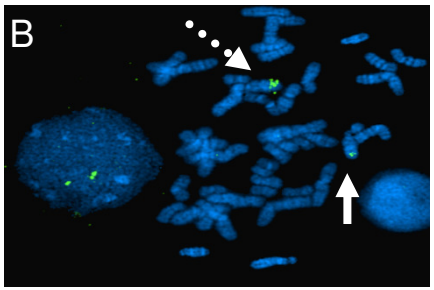


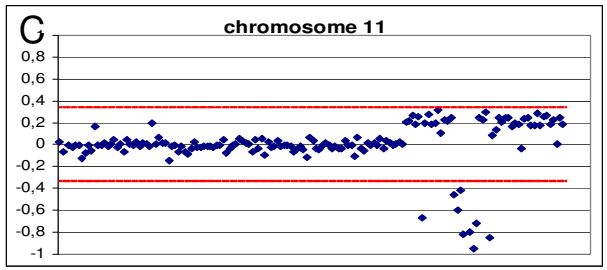


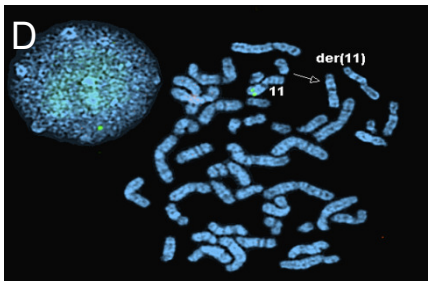



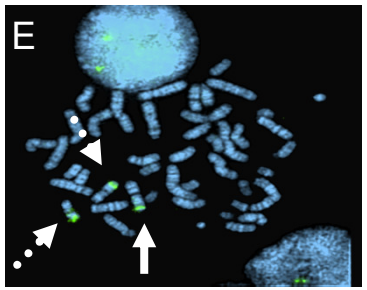
# Beware of mosaics

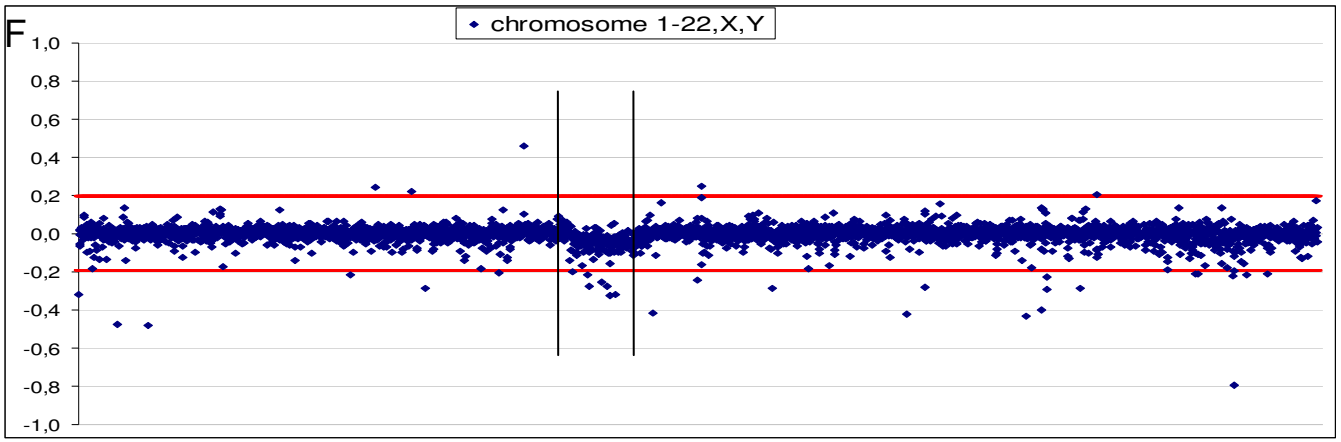


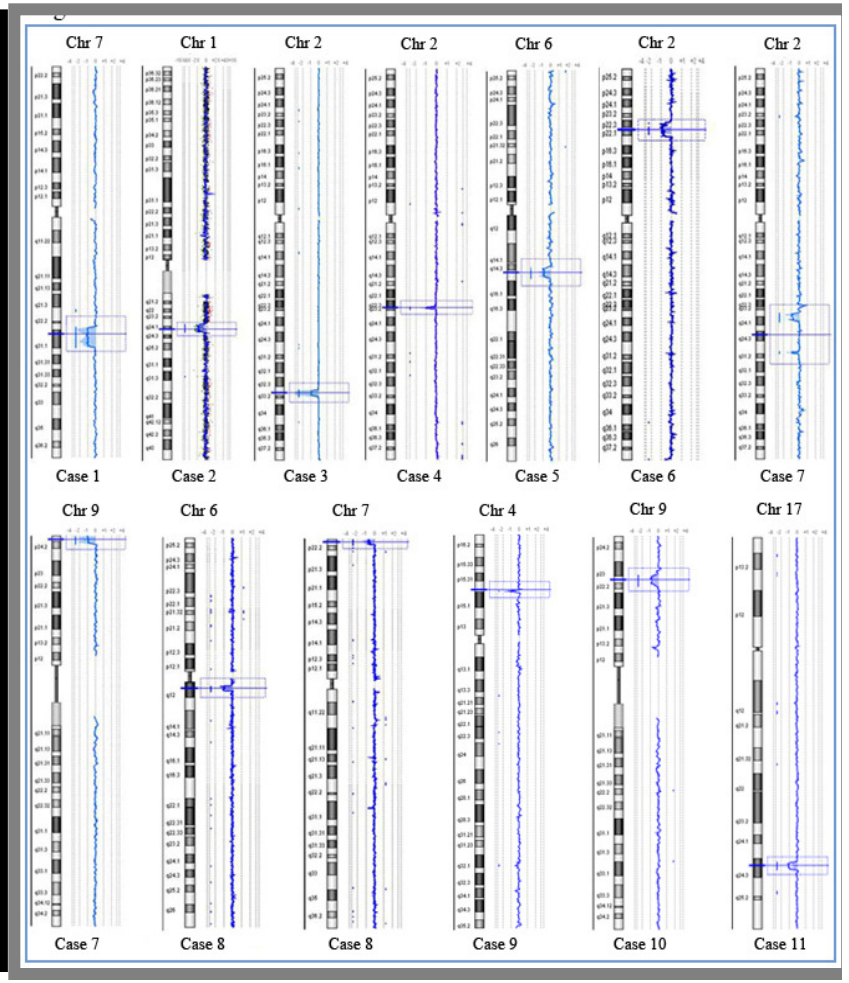

**As low as 5% mosaicism can be detected!!**

**Sensitivity > karyotyping**

Menten et al., J.Med.Gen., 2005

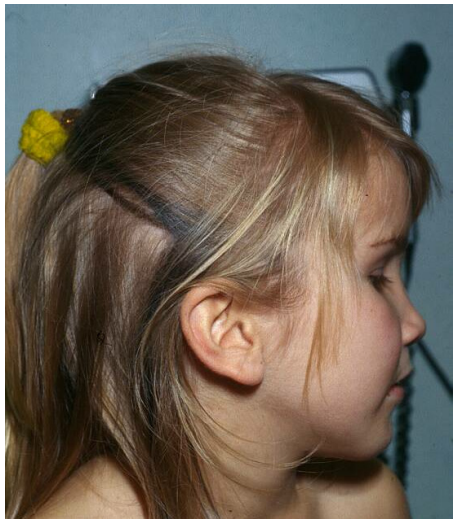
# Apparently balanced translocations: the majority is unbalanced!!!

- 59 cases
  - 41 apparently balanced translocations:
    - 27 patients : 40% (11/27) unbalanced
      - 22% (6/27) with deletions at the translocation breakpoints
      - 18% (5/27) with complex rearrangements
    - 14 fetuses: all normal
  - 18 complex rearrangements: 16/18 (89%) unbalanced
    - 13 patients
    - 3 fetuses
    - 2 females with repeated abbortions

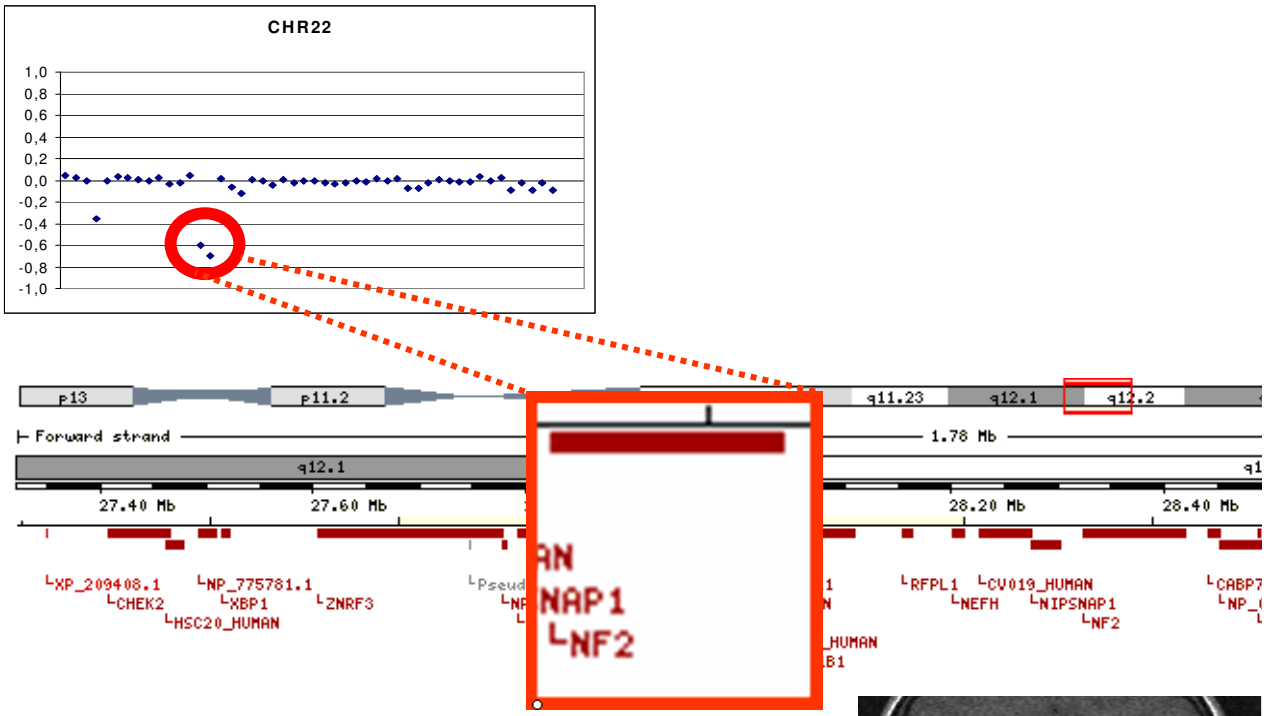


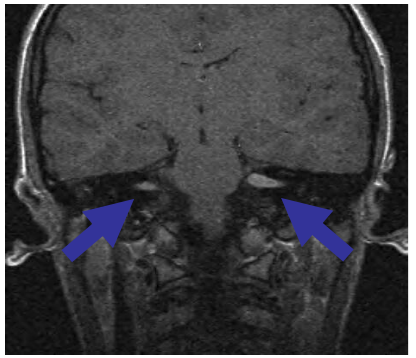

De Gregori et al., J. Med. Gen. 2007

# From diagnosis to prognosis



- Patient
  - Pulmonary valve stenosis
  - Cleft uvula
  - Mild dysmorphism
  - Mild learning difficulties
  - High myopia



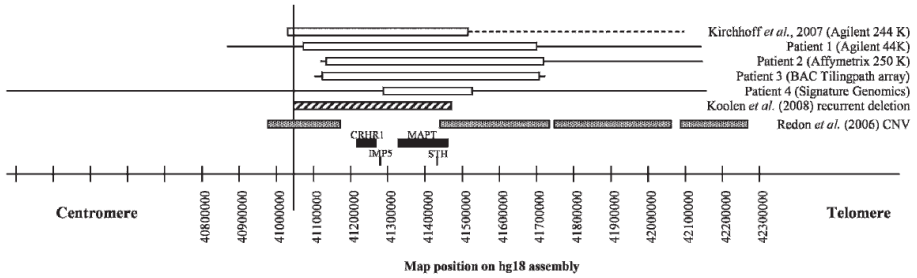
# For all recurrent deletion syndromes the reciproce duplication is now identified

JMG

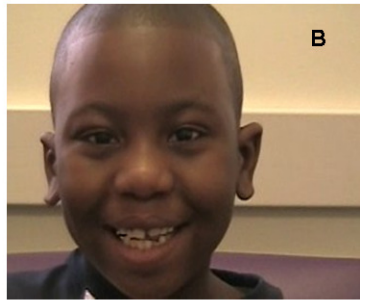
## 17q21.31 microduplication patients are characterised by behavioural problems and poor social interaction

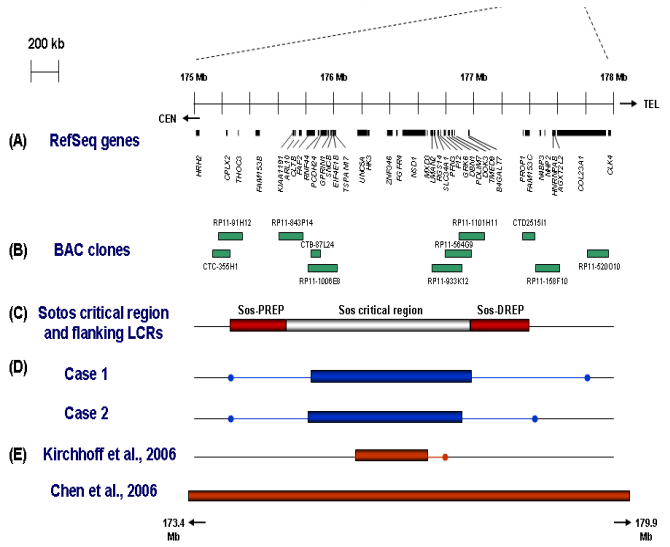
B Grisart, L Willatt, A Destrée, J-P Fryns, K Rack, T de Ravel, J Rosenfeld, J R Vermeesch, C Verellen-Dumoulin and R Sandford






## A syndrome of short stature, microcephaly and speech delay is associated with duplications reciprocal to the common Sotos syndrome deletion

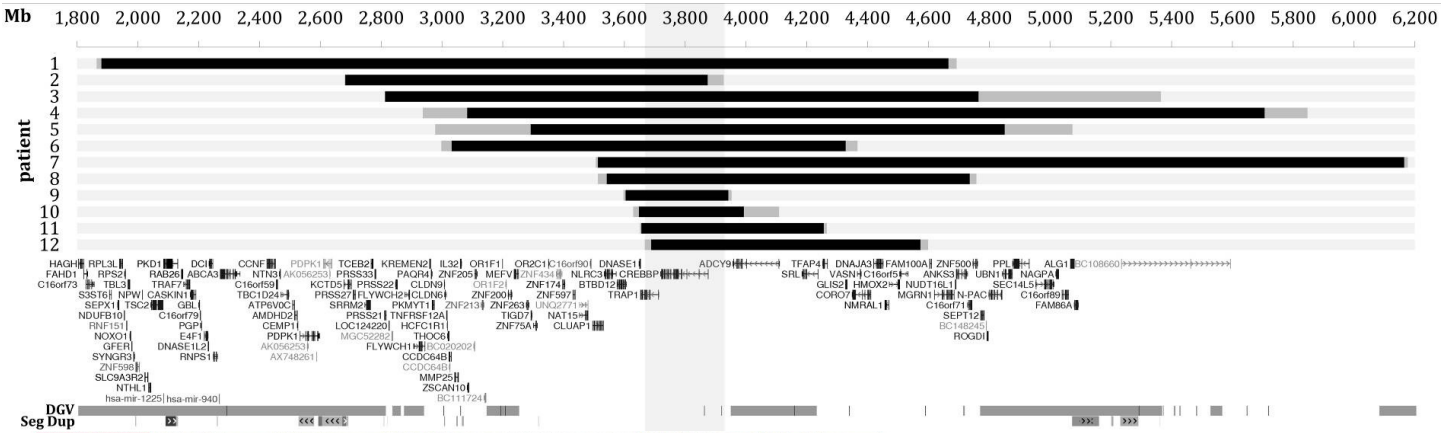



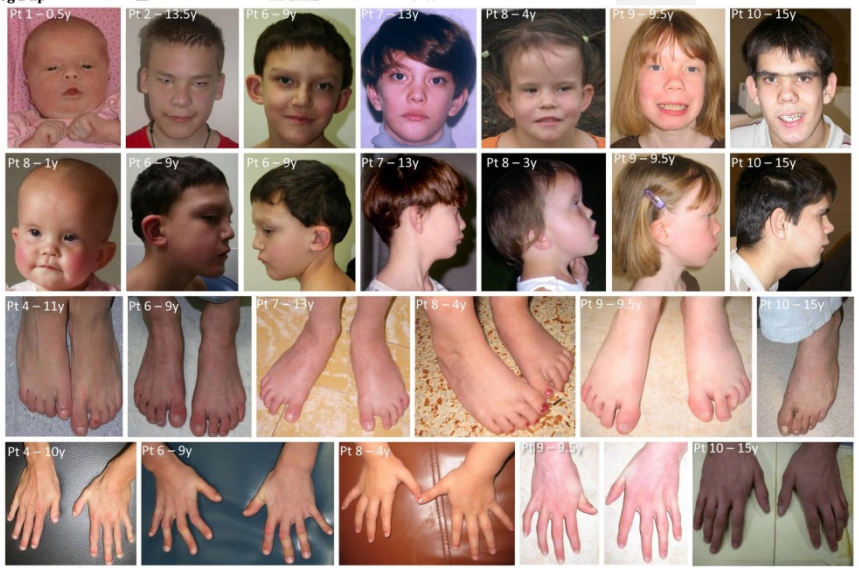




Franco et al., Eur.J. Hum. Gen., in press

# Accumulation of non-recurrent imbalances leads to the functional identification of genes

## Duplications of the critical Rubinstein Taybi deletion region on chromosome 16p13.3 cause a novel recognizable syndrome





***Authors:***

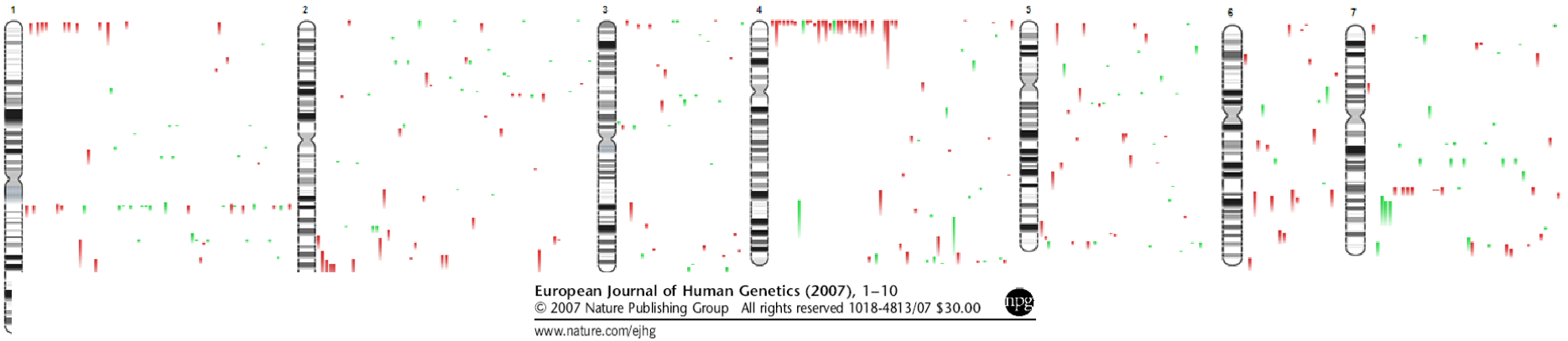
Bernard Thienpont[1], Frédérique Béna[2], Jeroen Breckpot[1], Nicole Philip[3], Björn Menten[4], Hilde Van Esch[1], Emmanuel Scalais[5], Jessica M. Salamone[6], Chin-To Fong[7], Jennifer L. Kussmann[8], Dorothy K. Grange[9], Jerome L. Gorski[8], Farah Zahir[10], Siu Li Yong[11], Michael M. Morris[2], Stefania Gimelli[2], Jean-Pierre Fryns[1], Geert Mortier[4], Jan M. Friedman[10], Laurent Villard[12], Armand Bottani[2], , Joris R. Vermeesch[1], Sau Wai Cheung[13] & Koen Devriendt[1]



*J.Med.Gen., in press*

# CNVs as cause of developmental disorders:
# > 500 new syndromes in 5 years

(1127 deletion/752 duplications)



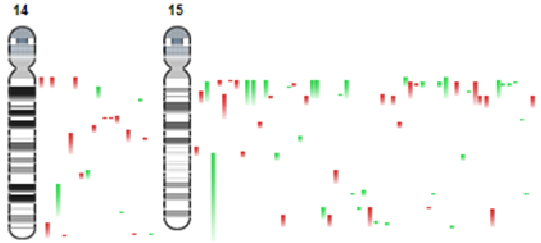

European Journal of Human Genetics (2007), 1–10

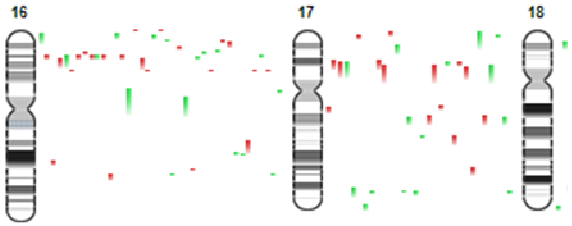
www.nature.com/ejhg

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]



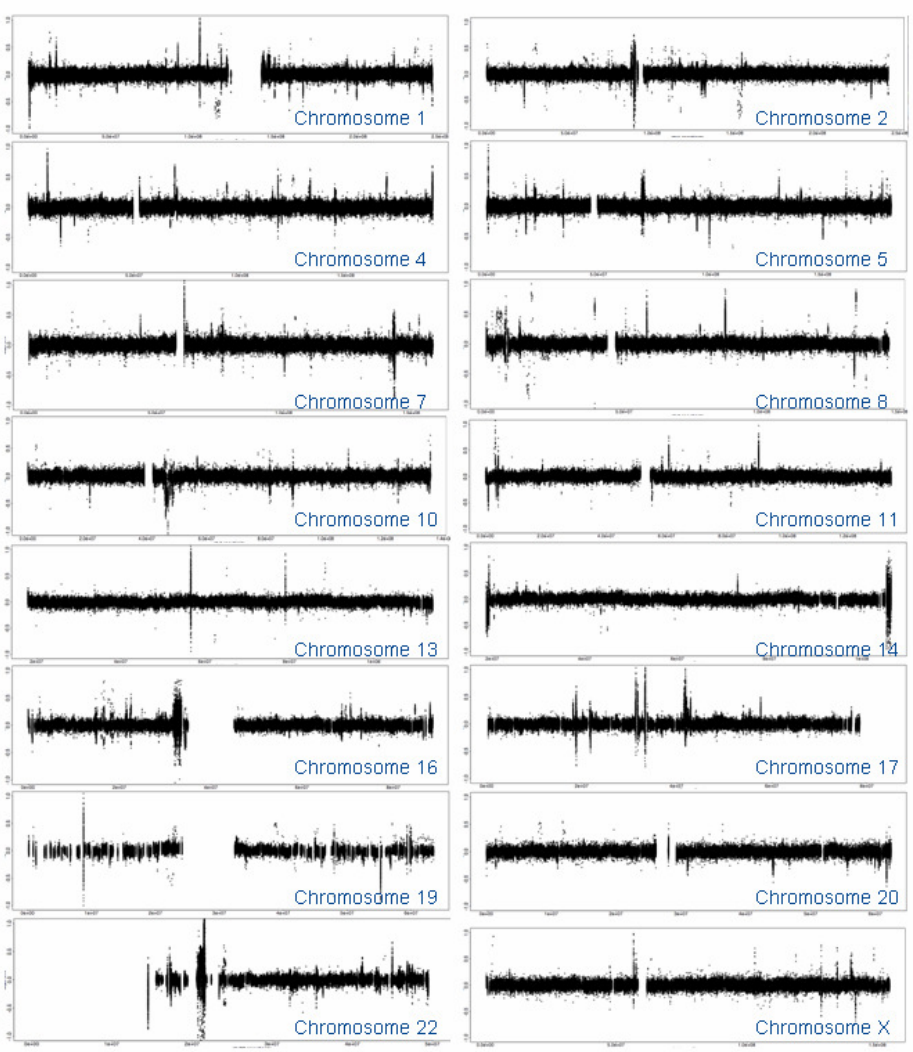



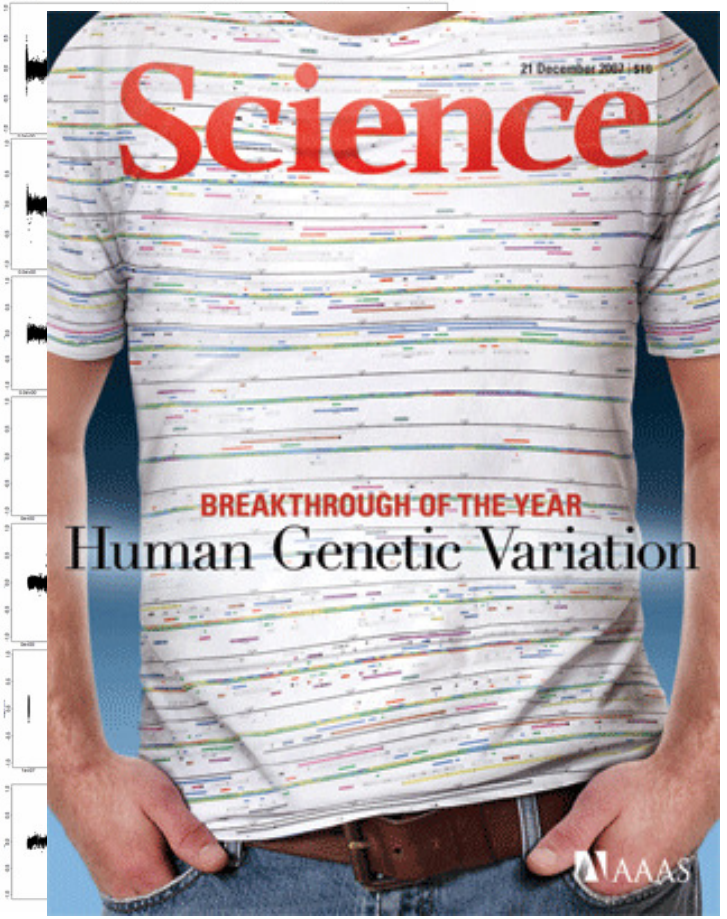

## Consensus Statement: Chromosomal Microarray Is a First-Tier Clinical Diagnostic Test for Individuals with Developmental Disabilities or Congenital Anomalies

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

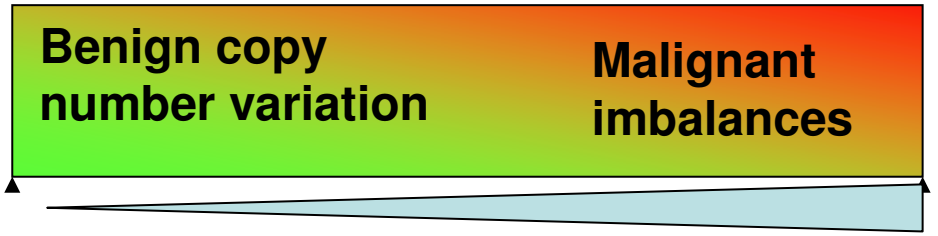
# The bad news:
# we are all copy variable



# 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?

***Conventional wisdom:***

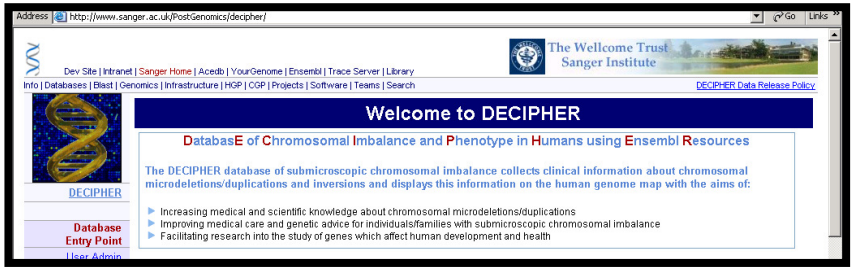
Recurrent imbalances with same phenotype are causal

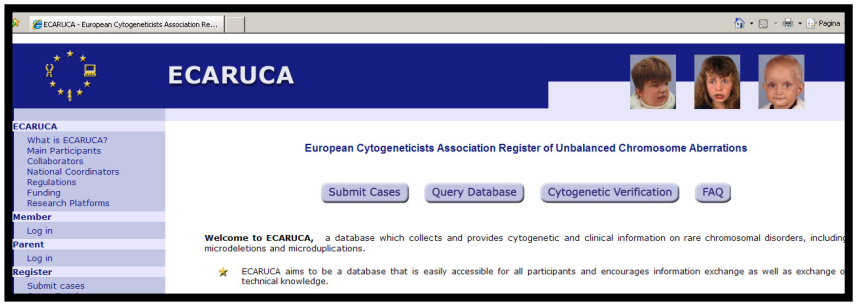
The larger the size,  the more likely causal

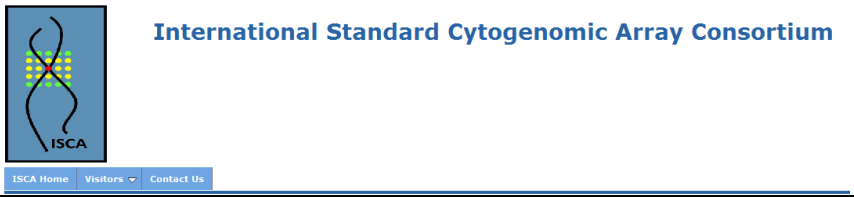
Population embedded CNVs are benign

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

# Identifying recurrent imbalances and phenotypes




**Limitations**

- Only imbalances believed to be causal are collected
- Depend on goodwill of laboratories (lot of information lost)
- Phenotyping is labour intensive

**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

The larger the size, the more likely causal

Population embedded CNVs are benign

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

# The challenge: Which imbalances are causal for the phenotype?

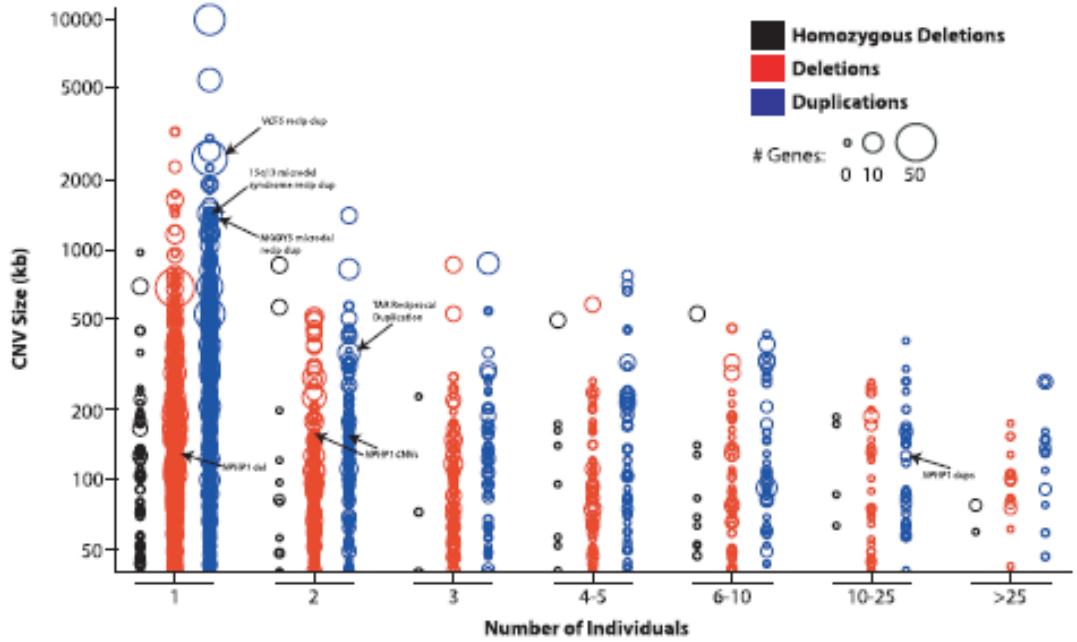



**Figure 4. CNV Length, Gene Content, and Frequency Distributions**
CNVs were plotted according to event type (color), length (*y* axis), frequency in the population (*x* axis, number of individuals from n = 2493), and number of RefSeq genes affected (circle size). To facilitate comparison across different 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!

# The challenge: Which imbalances are causal for the phenotype?

***Conventional wisdom:***

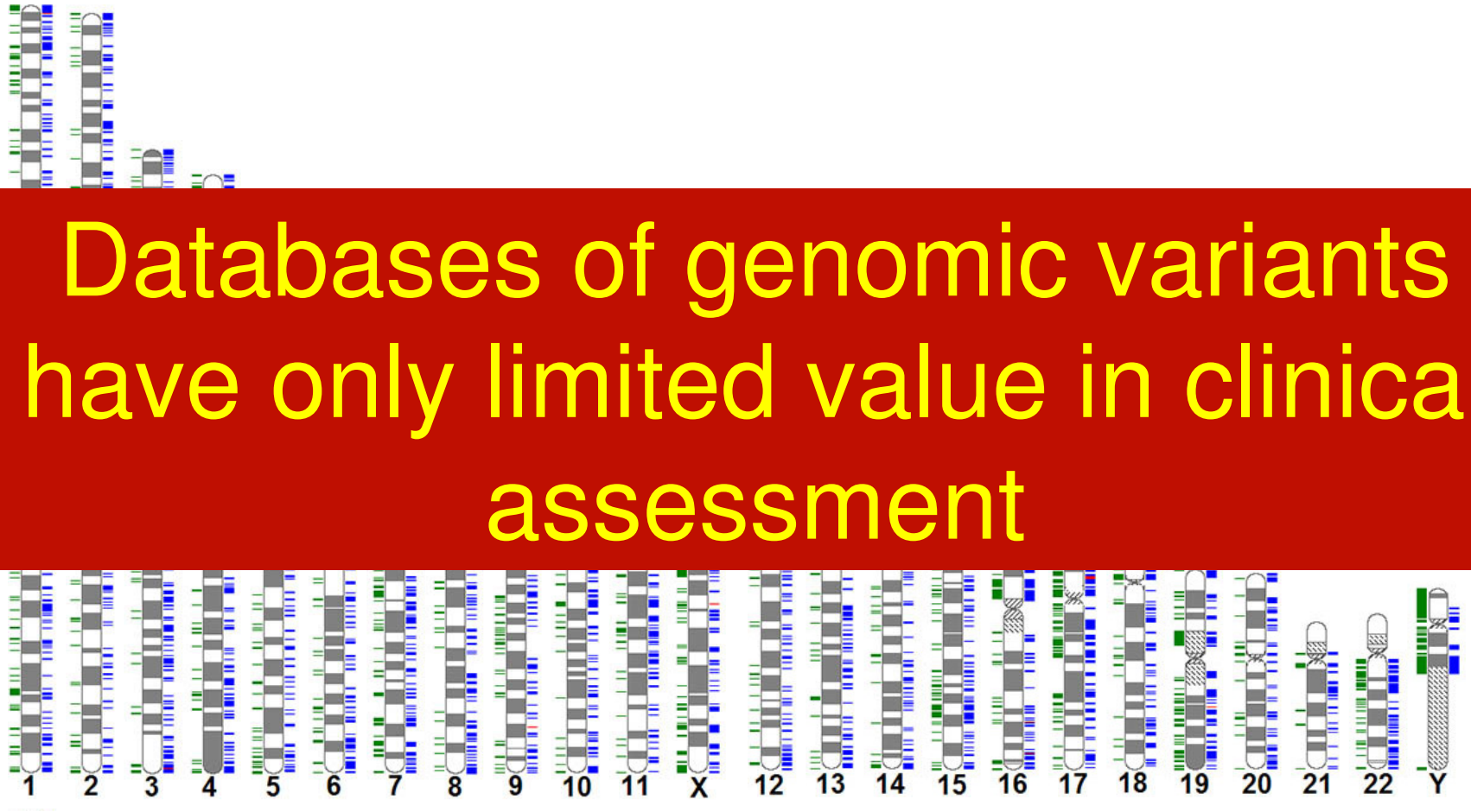
Recurrent imbalances with same phenotype are causal

The larger the size,  the more likely causal

Population embedded CNVs are benign

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

# 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*

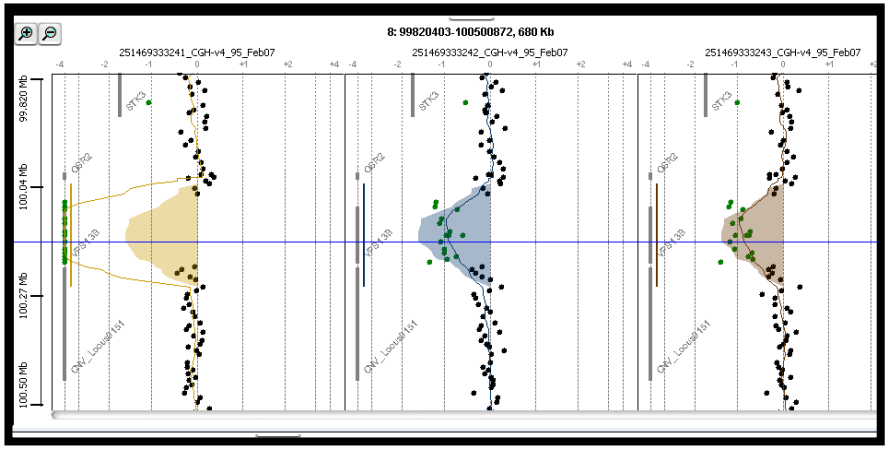
# Mendelian CNVs:
# a paradigm shift in (cyto)genetics

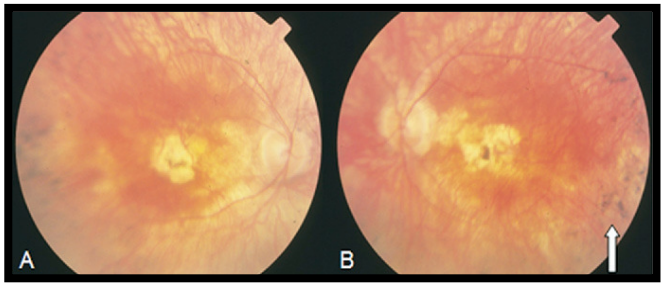
**Inherited apparently benign CNVs *CAN* cause disease**

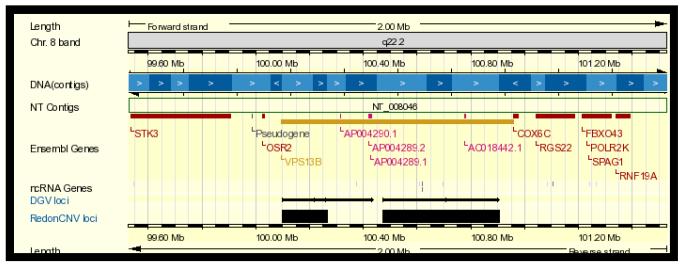
***"Mendelian CNVs"*** is the term coined here to indicate benign CNVs which can cause disease dependent on either copy number state, inheritance pattern or genetic and environmental background.

# Autosomal recessive CNVs: e.g.Cohen syndrome

- Autosomal recessive inheritance: mutations in VPS13B (*COH1*)
- Phenotype
- mild to severe MR
- microcephaly
- Truncal obesity
- Characteristic face
- Specific behavior
- Retinal dystrophy , high myopia (retinal detachment, cataract)

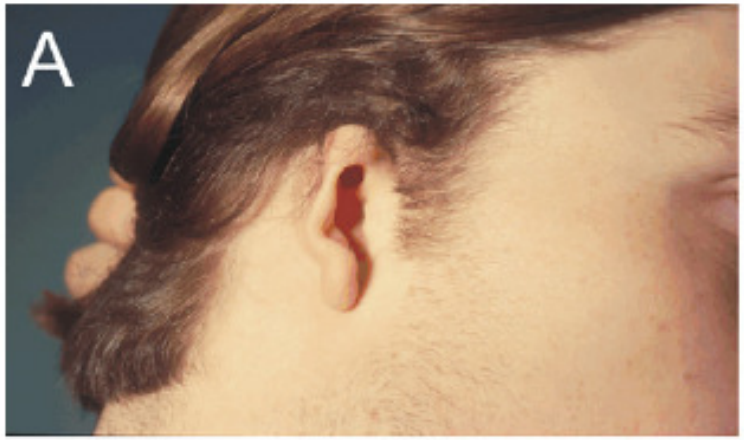


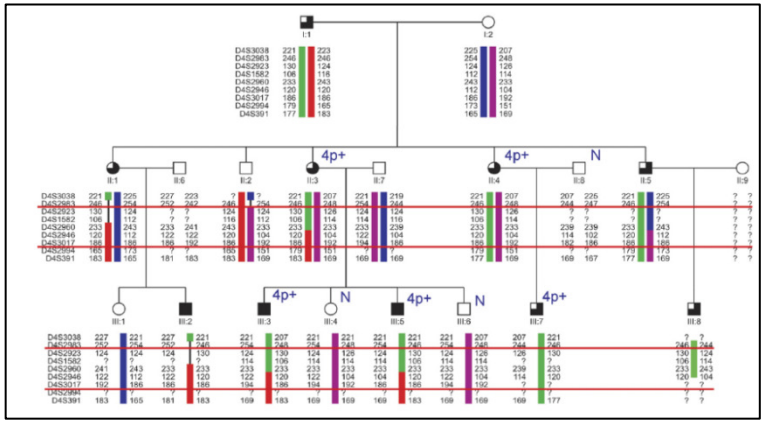



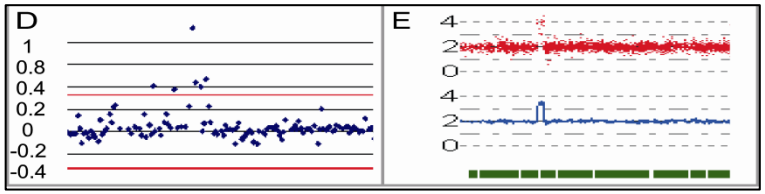



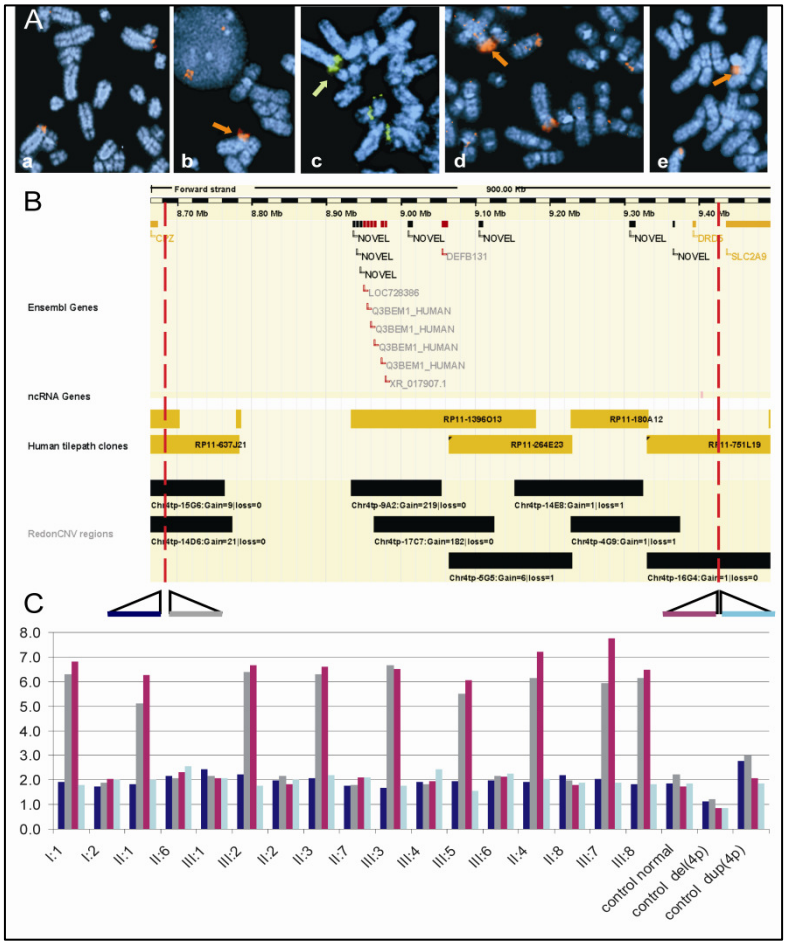

*Balikova et al., Hum. Mutation, in press*

# Autosomal dominant CNVs:
# Five tandem copies of ORGC cause microtia




Autosomal-Dominant Microtia Linked to Five Tandem Copies of a Copy-Number-Variable Region at Chromosome 4p16

Irina Balikova,[1] Kevin Martens,[1] Cindy Melotte,[1] Mustapha Amyere,[2] Steven Van Vooren,[3] Yves Moreau,[3] David Vetrie,[4] Heike Fiegler,[4] Nigel P. Carter,[4] Thomas Liehr,[5] Miikka Vikkula,[2] Gert Matthijs,[1] Jean-Pierre Fryns,[1] Ingele Casteels,[6] Koen Devriendt,[1] and Joris Robert Vermeesch[1,*]

The American Journal of Human Genetics *82*, 181–187, January 2008

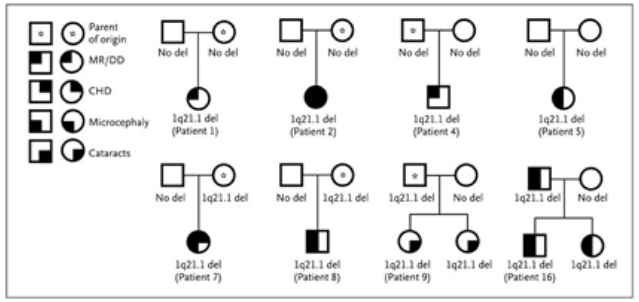
# CNVs as risk factor for MR/CA (variable penetrance and expressivity)

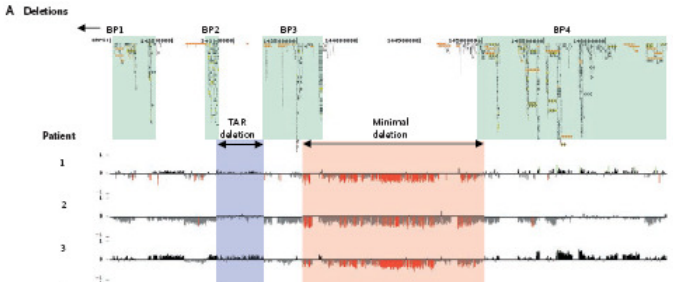
The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

## Recurrent Rearrangements of Chromosome 1q21.1 and Variable Pediatric Phenotypes

H. Mefford, A. Sharp, C. Baker, A. Itsara, Z. Jiang, K. Buysse, S. Huang,



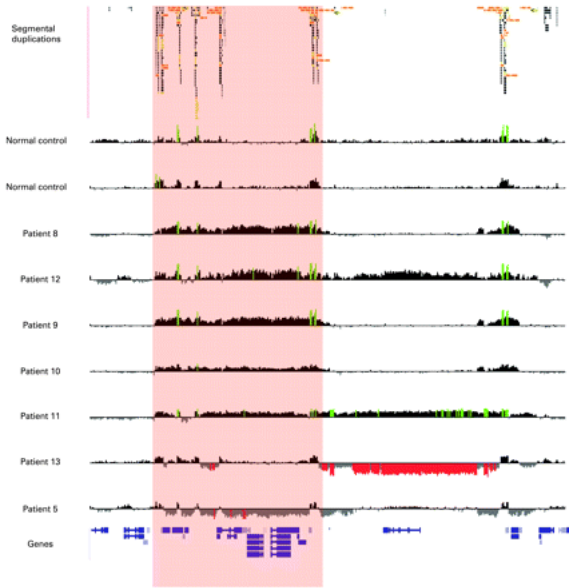




| Deletion | Duplication |
|---|---|
| 25/5218 patients | 9/5218 patients |
| 0/4737 controls | 1/4737 controls |
| P = $1.1 \times 10^{-7}$ | P = 0.02 |

JMG ONLINE

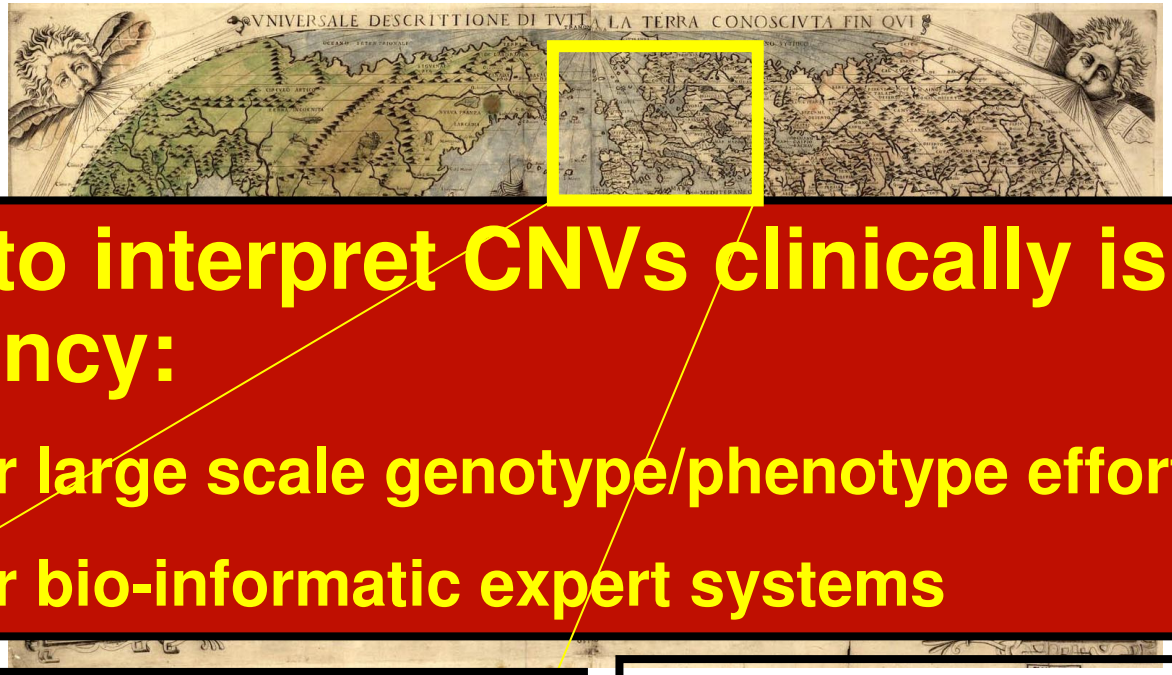
## Recurrent reciprocal deletions and duplications of 16p13.11: The deletion is a risk factor for MR/MCA while the duplication may be a rare benign variant

Femke D Hannes, Andrew J Sharp, Heather C Mefford, Thomy de Ravel, Claudia A Ruivenkamp, Martijn H Breuning, Jean-Pierre Fryns, Koen Devriendt, Griet Van Buggenhout, Annick Vogels, Helen H Stewart, Raoul C Hennekam, Gregory M Cooper, Regina Regan, Samantha JL Knight, Evan E Eichler and Joris R Vermeesch




| Deletion | Duplication |
|---|---|
| 5/1026 patients | 5/1026 patients |
| 0/2014 controls | 5/1682 controls |
| P =0.0048 | No Difference |

# Messages from postnatal diagnosis




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

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

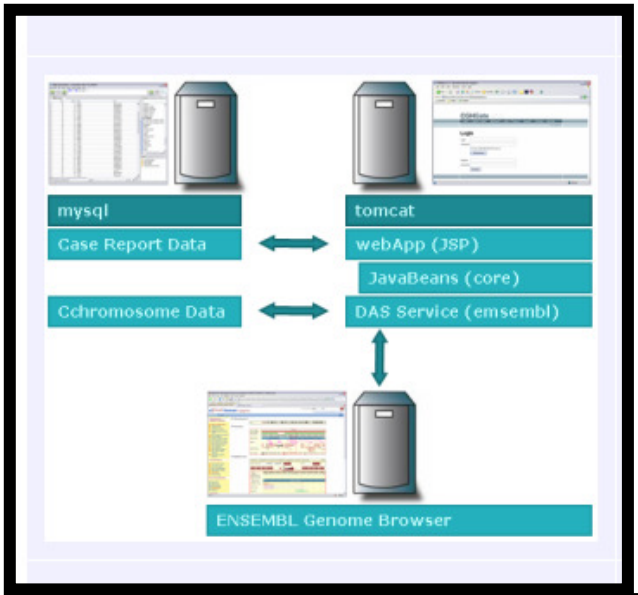
Higly penetrant recurrent CNVs

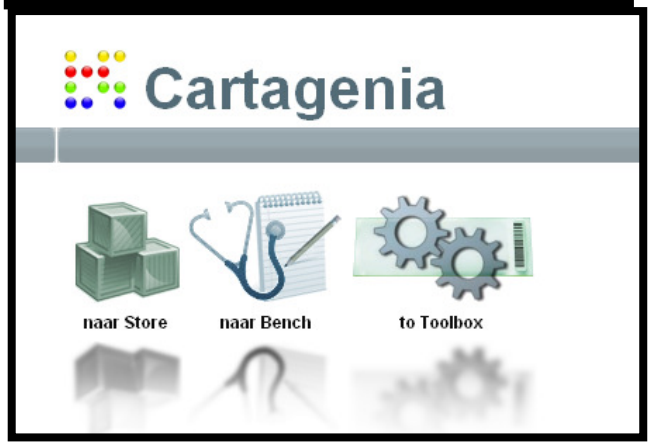
Rest of the world:

Rare CNVs with variable penetrance & expressivity

# Need for bioinformatic tools for interpretation






*www.cartagenia.com*

# Towards prenatal diagnosis

## Microarray Analysis of Cell-Free Fetal DNA in Amniotic Fluid: a Prenatal Molecular Karyotype

Paige B. Larrabee,[1] Kirby L. Johnson,[2] Ekaterina Pestova,[3] Madhuri Lucas,[3] Kim Wilber,[3] Erik S. LeShane,[2] Umadevi Tantravahi,[4] Janet M. Cowan,[2] and Diana W. Bianchi[2]

Divisions of [1]Newborn Medicine and [2]Genetics, Department of Pediatrics, Tufts–New England Medical Center, Tufts University School of Medicine, Boston; [3]Vysis, Inc., Downers Grove, IL; and [4]Department of Pathology, Women and Infants' Hospital, Providence, RI


## Prenatal detection of unbalanced chromosomal rearrangements by array CGH

L Rickman, H Fiegler, C Shaw-Smith, R Nash, V Cirigliano, G Voglino, B L Ng, C Scott, J Whittaker, M Adinolfi, N P Carter and M Bobrow

*J. Med. Genet.* 2006;43;353-361; originally published online 30 Sep 2005; doi:10.1136/jmg.2005.037648


## High resolution array analysis: diagnosing pregnancies with abnormal ultrasound findings

Matthew Tyreman, Kristin M Abbott, Lionel R Willatt, Richard Nash, Christoph Lees, Joanne Whittaker and Ingrid Simonic

**No technical problems!**

# Towards prenatal diagnosis?

Right to have “normal” baby

TERRA INCOGNITA

How to deal with

- Variable expressivity and penetrance?
- Unclassified variants?
- Late onset disorders?
- Unexpected finding in foetus?
- Unexpected finding in parents?

What is “normal”

# Prenatal diagnosis for abnormal ultrasound?


**High resolution array analysis: diagnosing pregnancies with abnormal ultrasound findings**

Matthew Tyreman, Kristin M Abbott, Lionel R Willatt, Richard Nash, Christoph Lees, Joanne Whittaker and Ingrid Simonic

*J. Med. Genet.* published online 17 May 2009; doi:10.1136/jmg.2008.065482

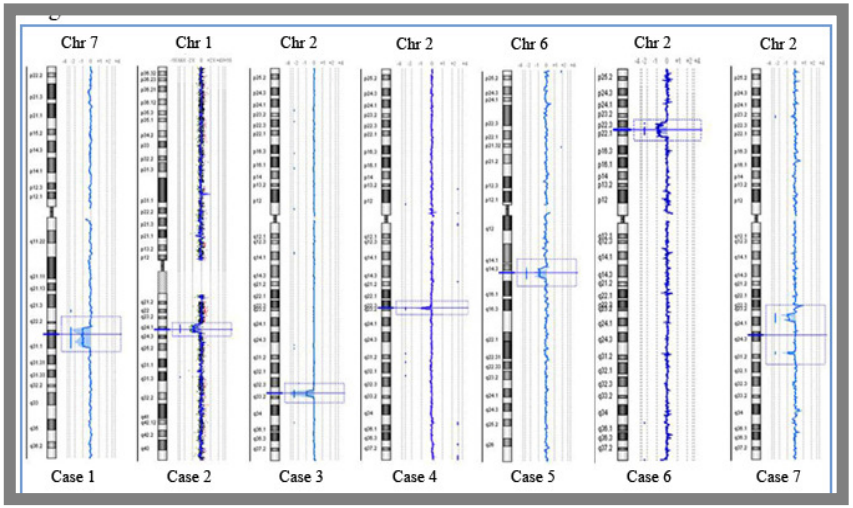
Strategy in Leuven (approved by ethical committee)

- Only foetuses with abnormal ultrasound and at least two signs
- Interpretation by both a cytogeneticist & clinical geneticist
- Report only relevant findings
- No connection between the original data and patients!

# Prenatal diagnosis

## When conventional karyotyping shows chromosomal anomaly

(apparently balanced translocation, marker chromosomes,..)

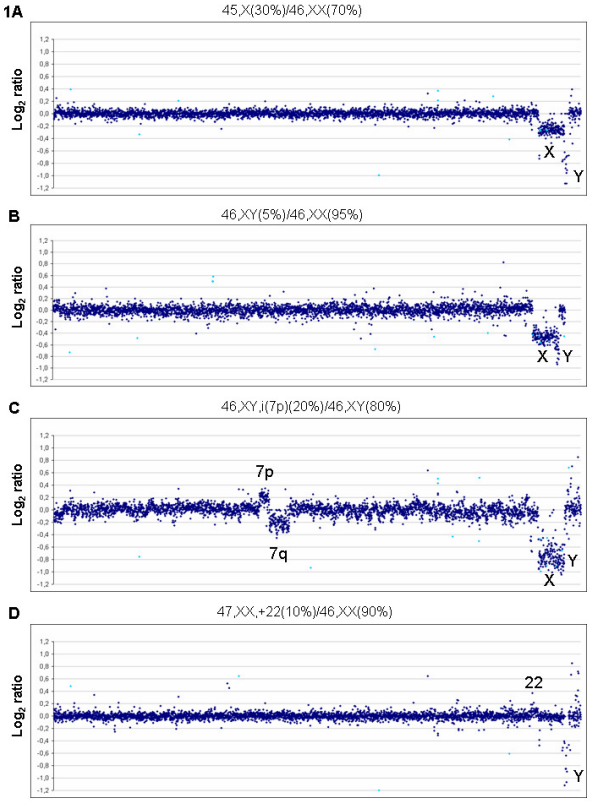



**Cryptic deletions are a common finding in "balanced" reciprocal and complex chromosome rearrangements: a study of 59 patients**

M De Gregori, R Ciccone, P Magini, T Pramparo, S Gimelli, J Messa, F Novara, A Vetro, E Rossi, P Maraschio, M C Bonaglia, C Anichini, G B Ferrero, M Silengo, E Fazzi, A Zatterale, R Fischetto, C Previderé, S Belli, A Turci, G Calabrese, F Bernardi, E Meneghelli, M Riegel, M Rocchi, S Guerneri, F Lalatta, L Zelante, C Romano, M Fichera, T Mattina, G Arrigo, M Zollino, S Giglio, F Lonardo, A Bonfante, A Ferlini, F Cifuentes, H Van Esch, L Backx, A Schinzel, J R Vermeesch and O Zuffardi

*J. Med. Genet.* 2007;44;750-762; originally published online 31 Aug 2007;

## Miscarriages

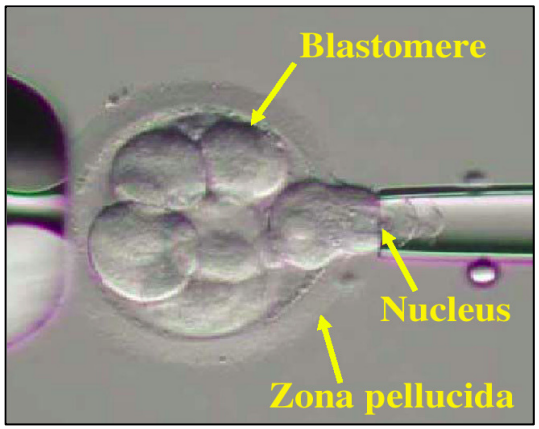


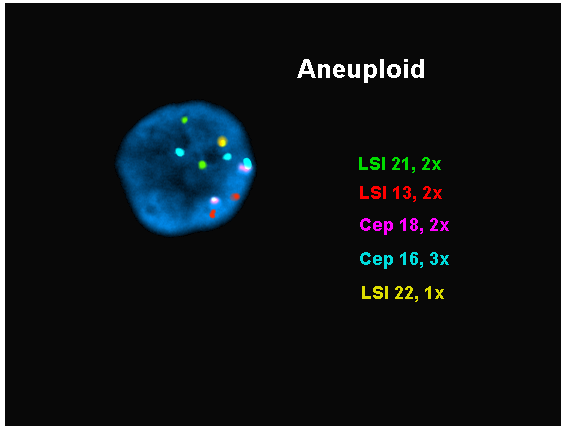

*Robberechts et al., Gen. Med.. 2009*

# Towards single cell array CGH?

Why?

# Chromosomal anomalies are a major cause of reproductive failure



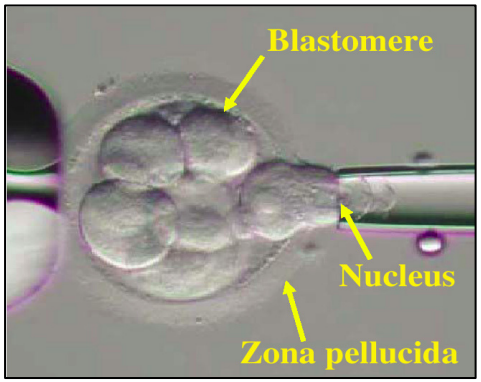

FISH shows 45-70% embryos with aneuploidy =>screen against abnormal embryos

**Main disadvantage: Only some loci !**

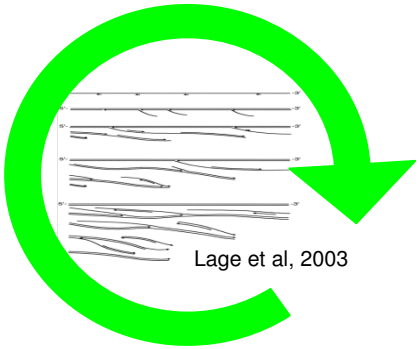
# Towards pre-implantation genetic diagnosis?

Is it technically possible?

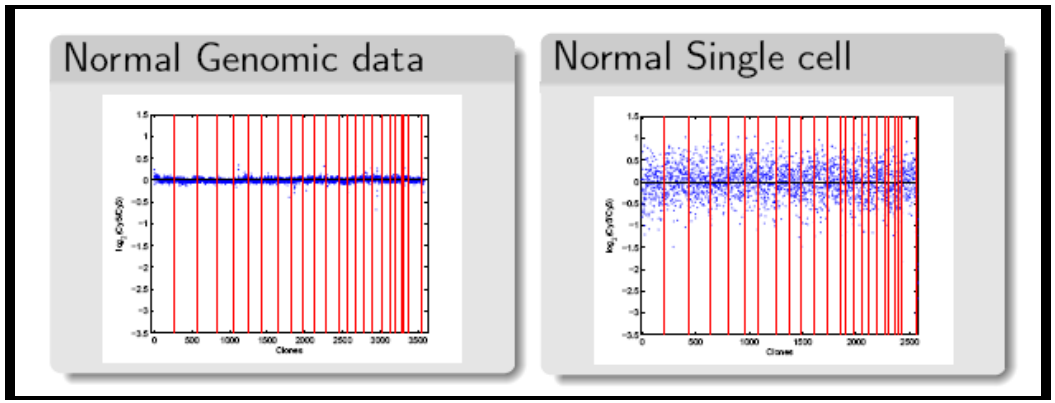
# Single cell array comparative genomic hybrization using arrays



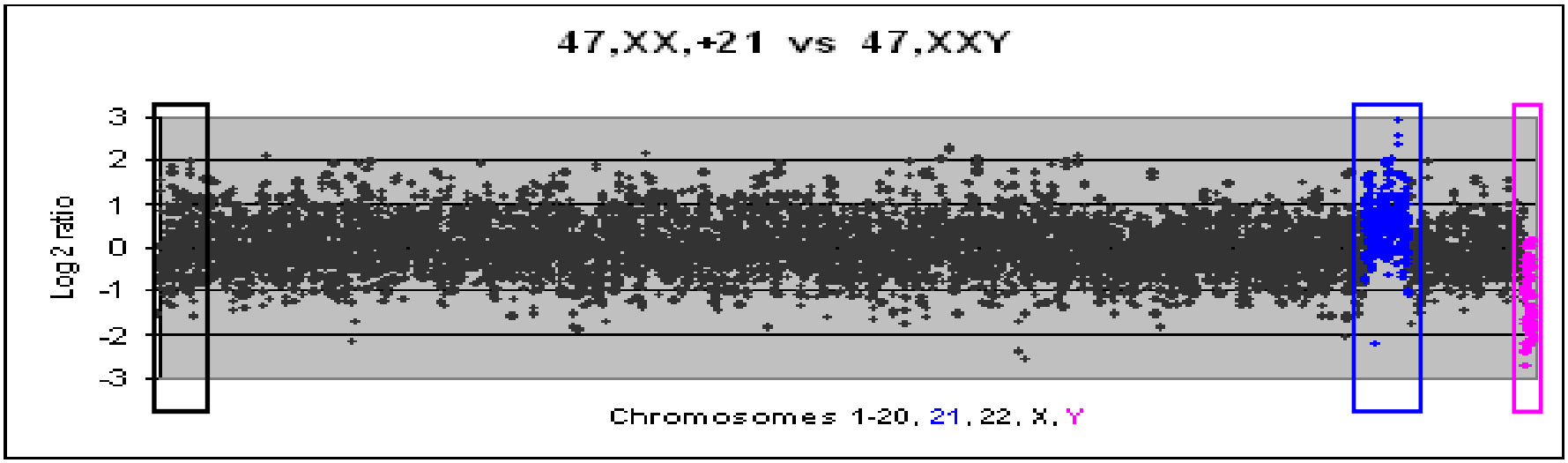

Single blastomere
7 pg DNA

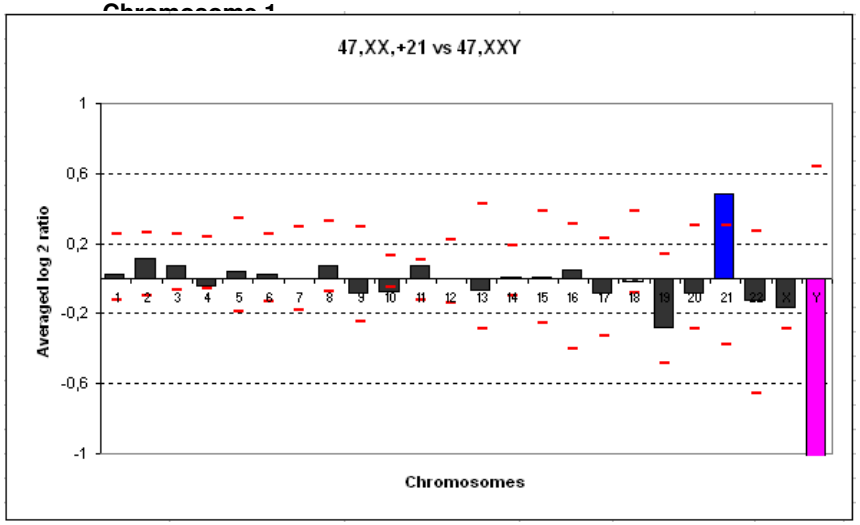


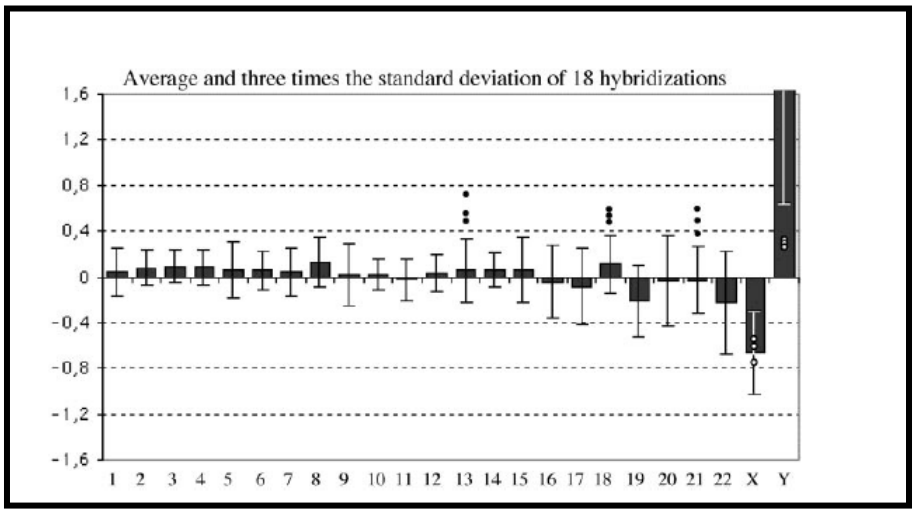

Genomi Phi
2.5 µg DNA



# Single cell array CGH

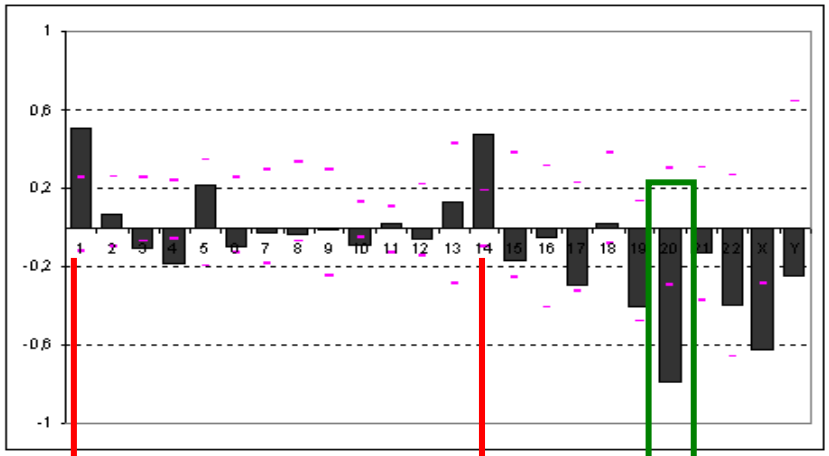


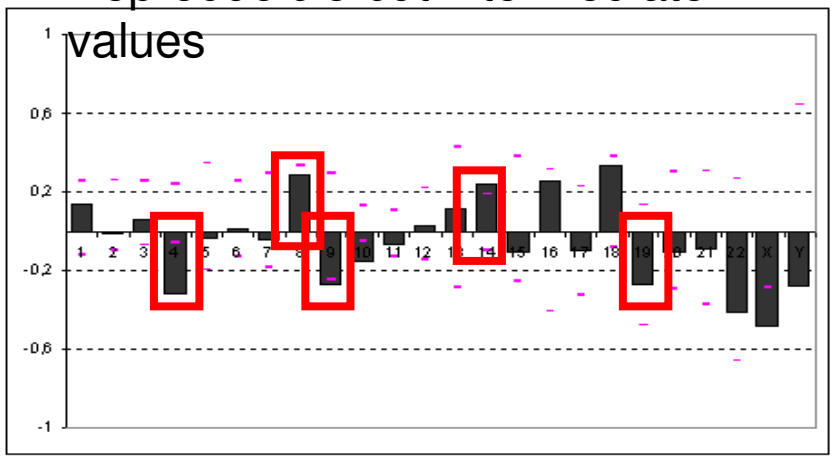



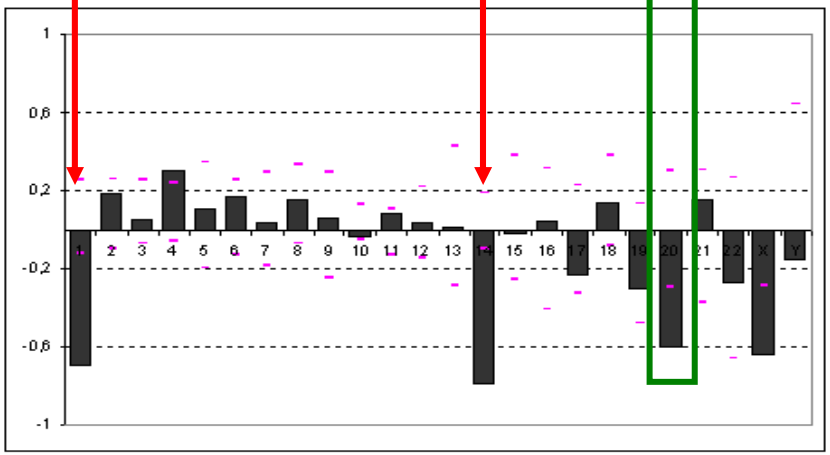

*Le Caignec et al, Nucleic Acids Res 2006*

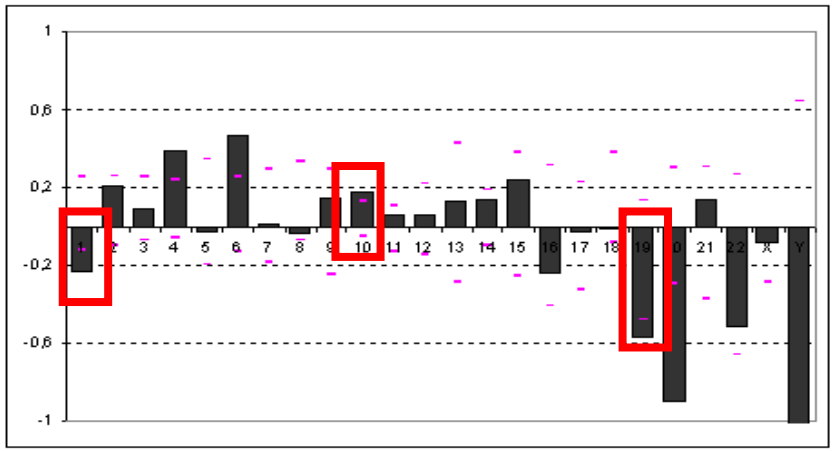
# What is the accuracy?





Reproducible but intermediate values

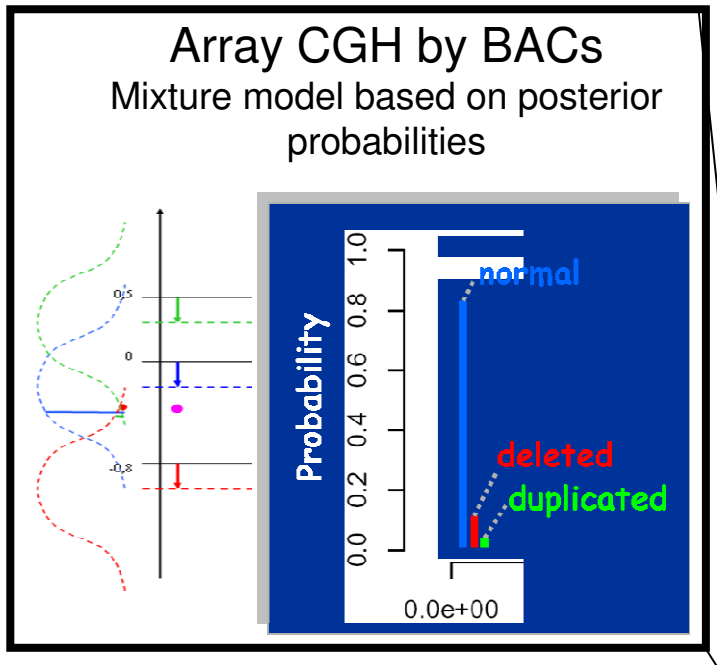


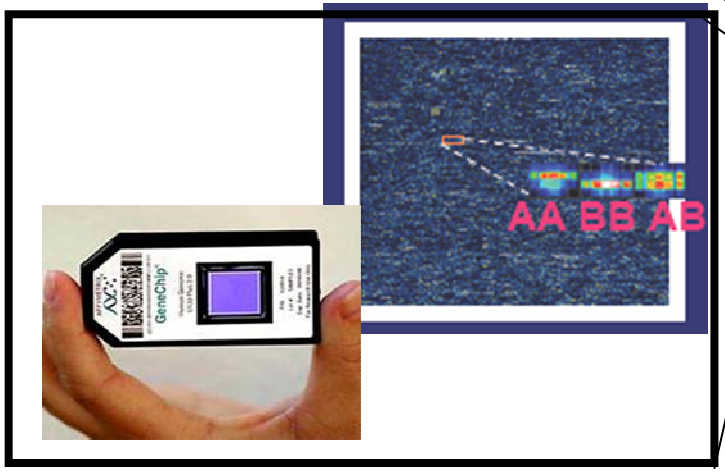


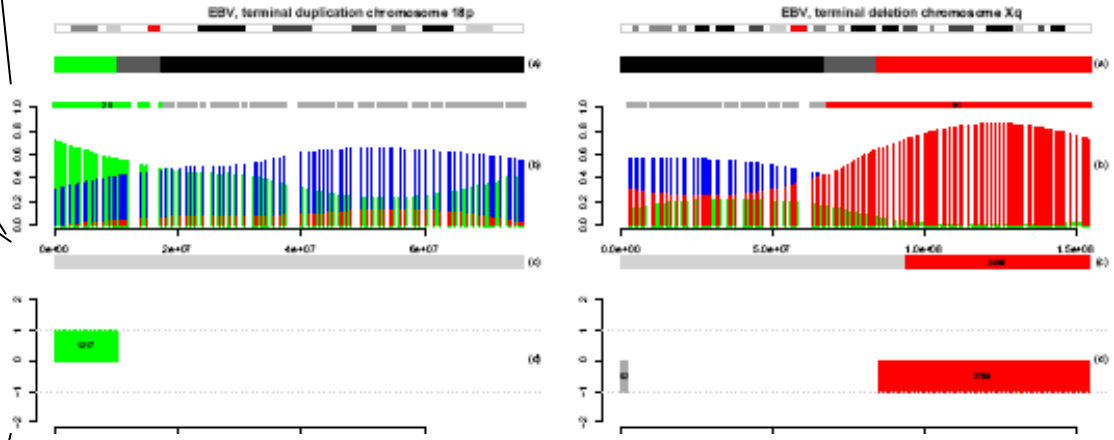
Non disjunction ?

Anaphase lag ?

# New methodology:
# Combine array CGH and SNP array data



# Analysis of human embryos : study design

PGD patients

Inclusion criteria :

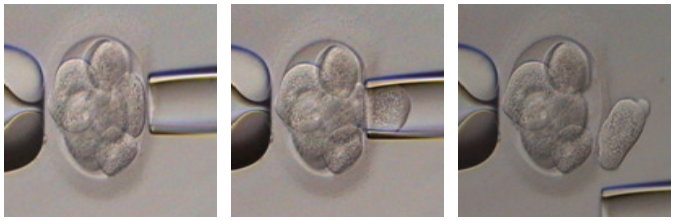
PGD for sex-selection or microdeletion

Age ≤ 35 years

Normal karyotype

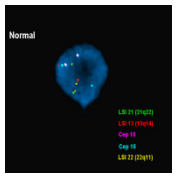
Good quality embryos

Biopsy of 1 or 2 blastomeres

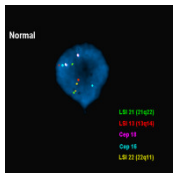


FISH

Correct allele

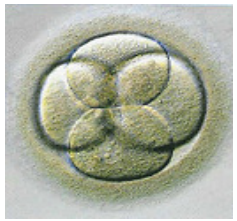



Wrong allele



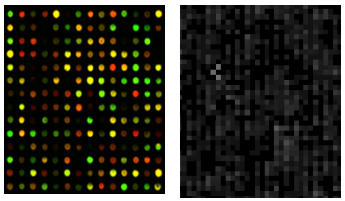

ET

Good morphology



Bad morphology

aCGH and SNP on remaining blastomeres



Data analysis

Validation

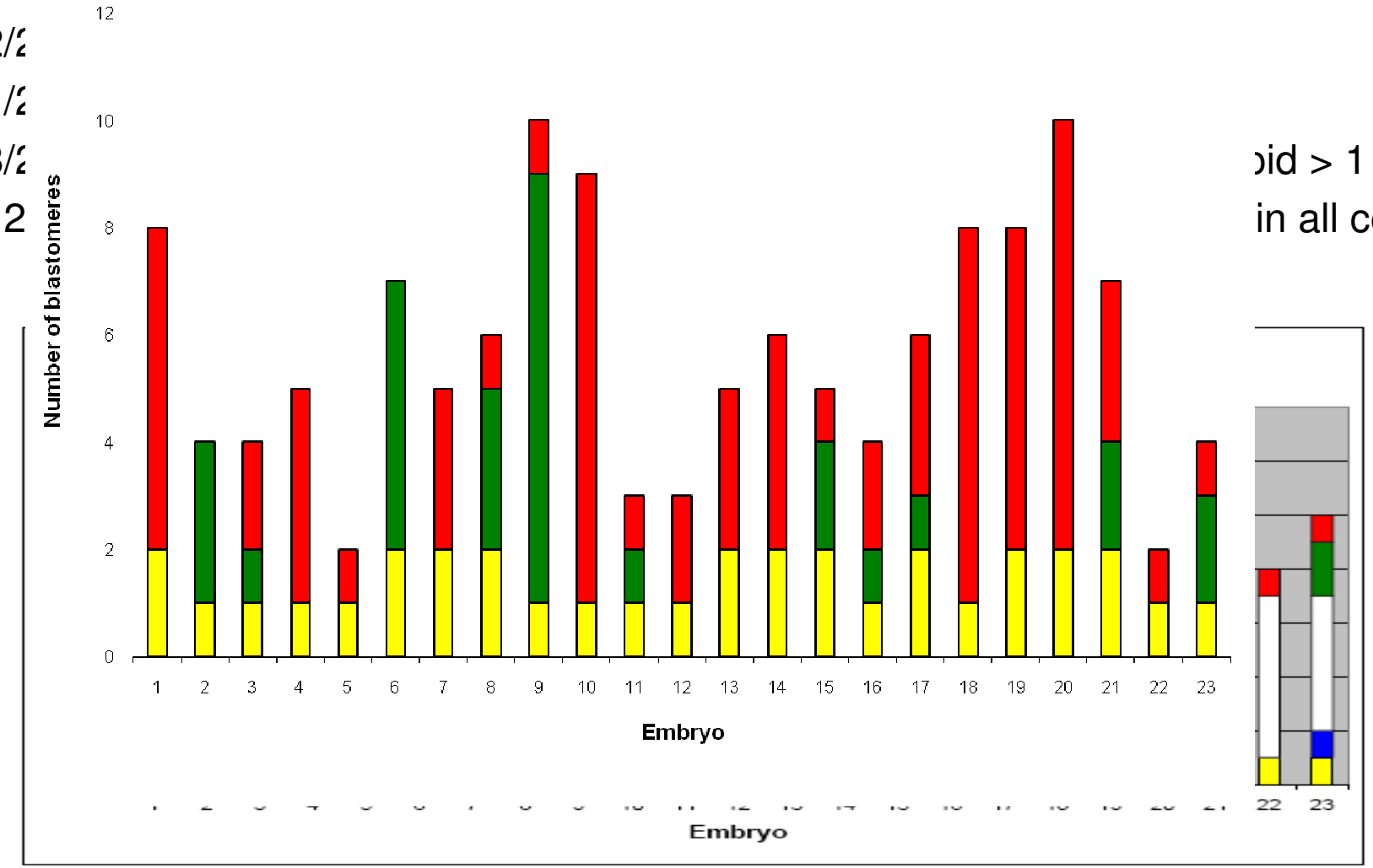
Biological meaning

# The majority of human cleavage stage embryos contain chromosomally imbalanced blastomeres

- 2/2
- 1/2
- 8/2
- 12

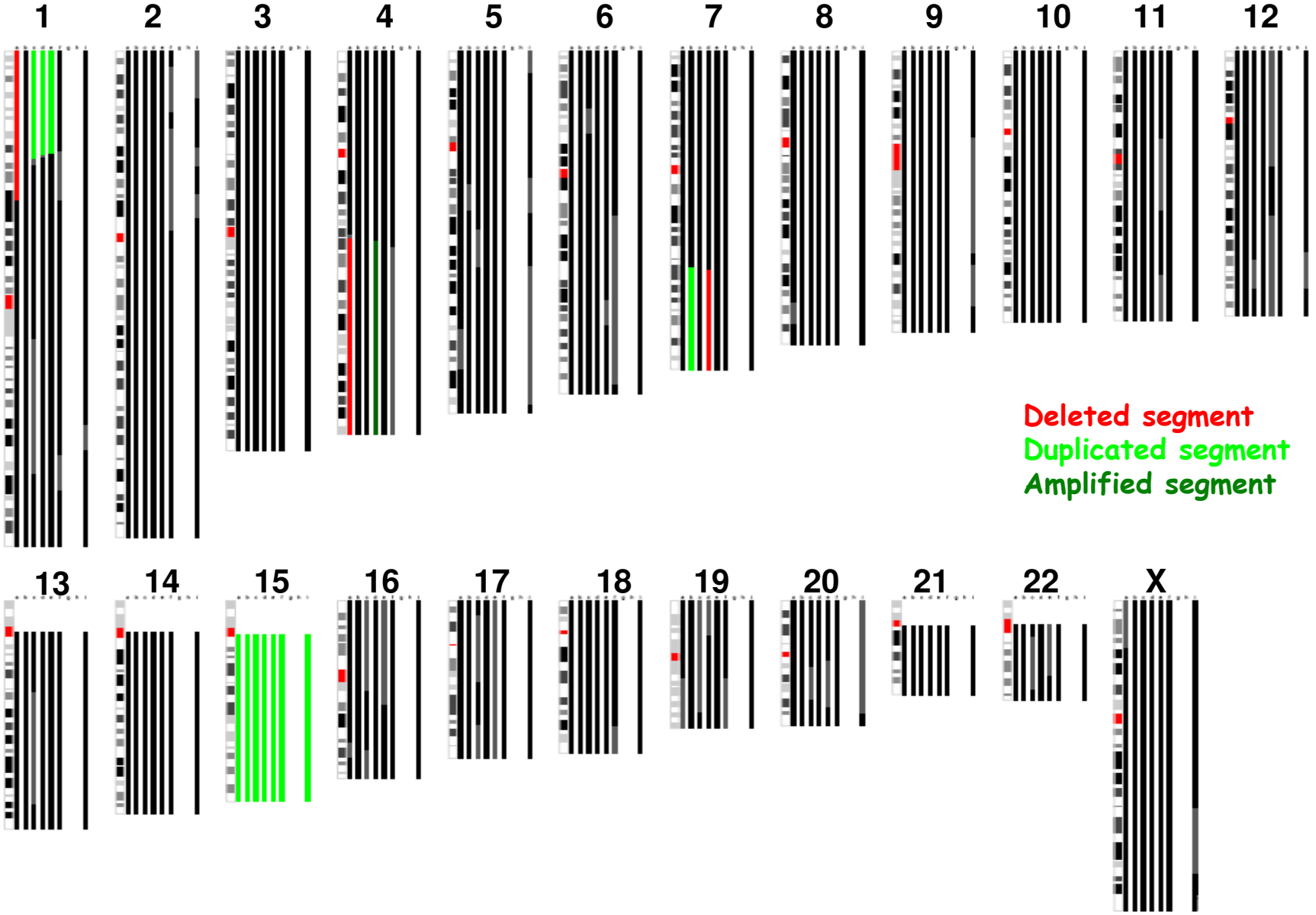
oid > 1

in all cells)

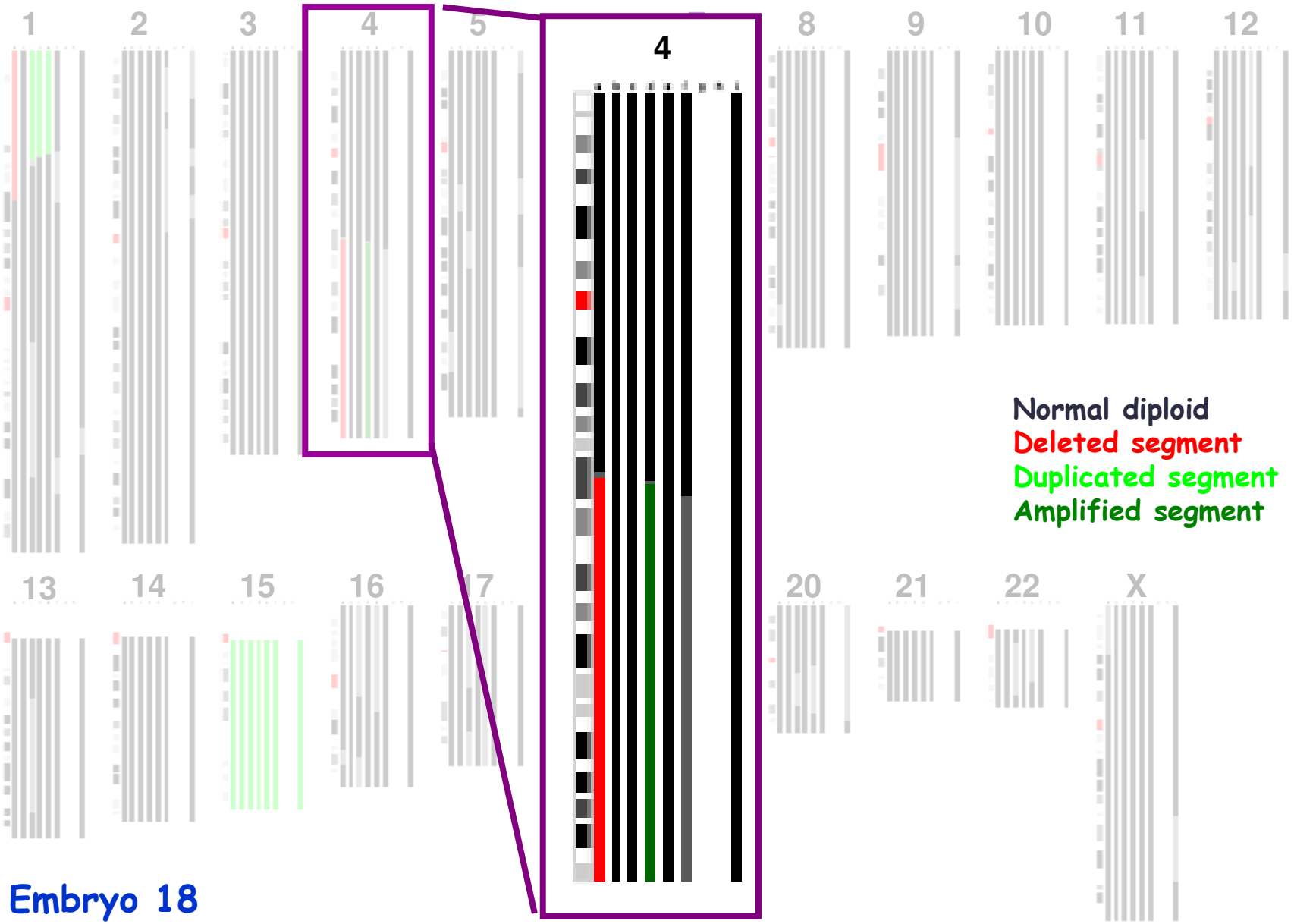



FISH / array : normal diploid / array : abnormal

# Overview of the chromosomal status of all blastomeres of embryo 18



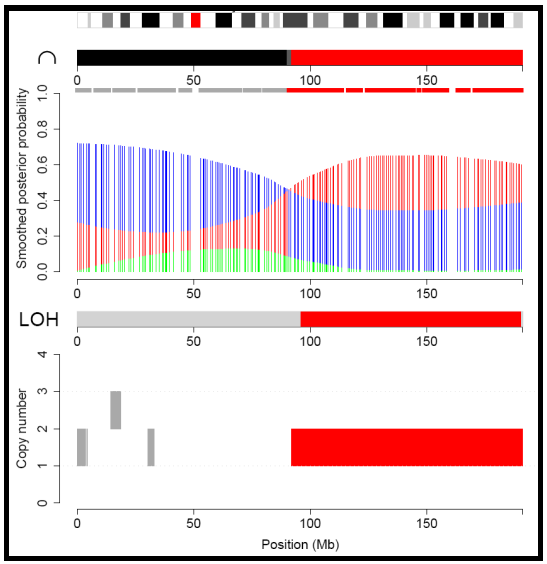
# Simple terminal imbalances are terminal deletions, duplications or amplifications



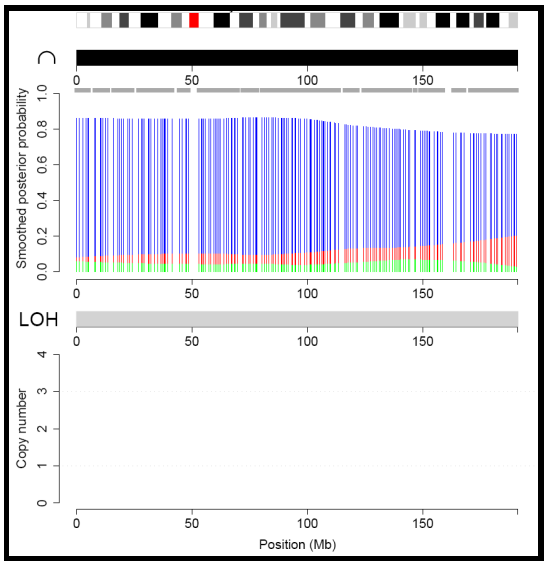
# Simple terminal imbalances detected in 39% embryo's

Chromosome 4

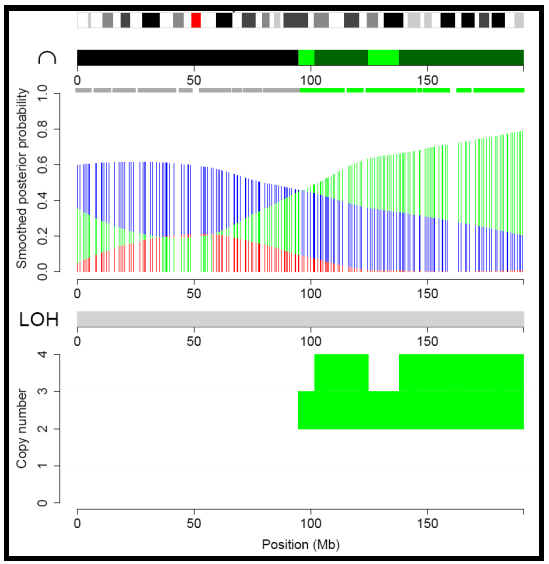
Blastomere "a"

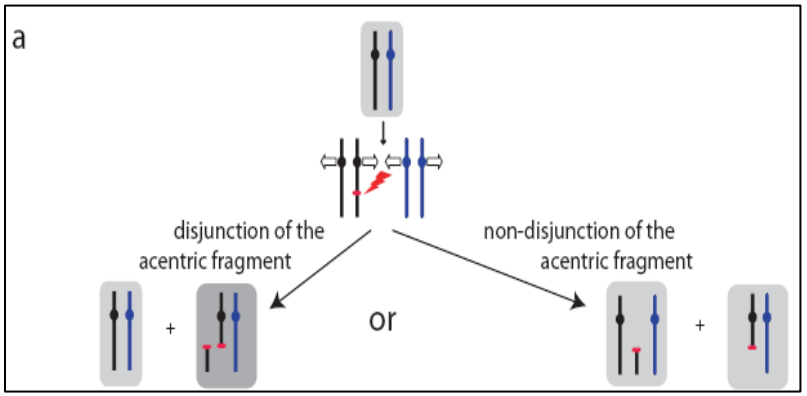



5 blastomeres

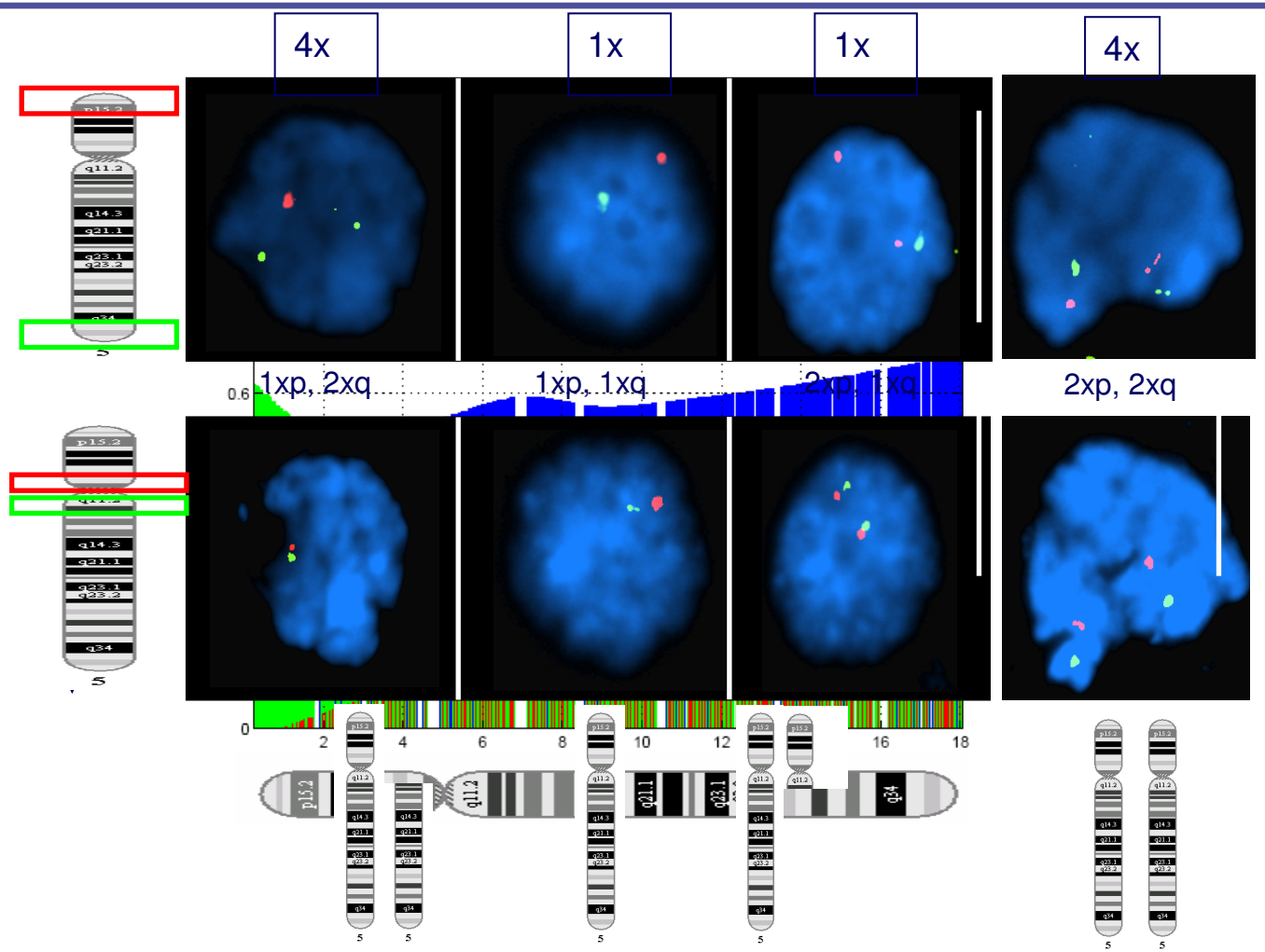



Blastomere "d"

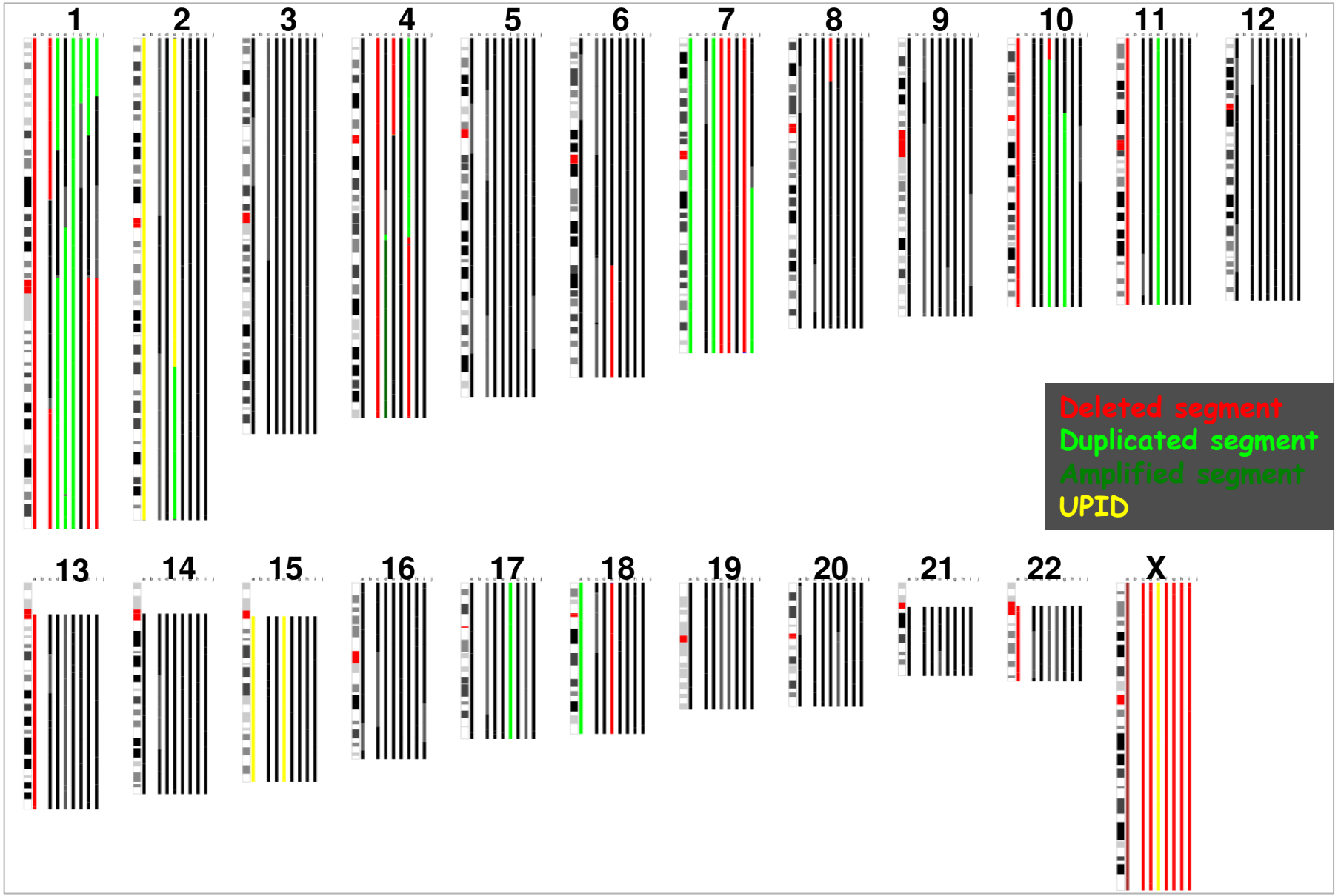


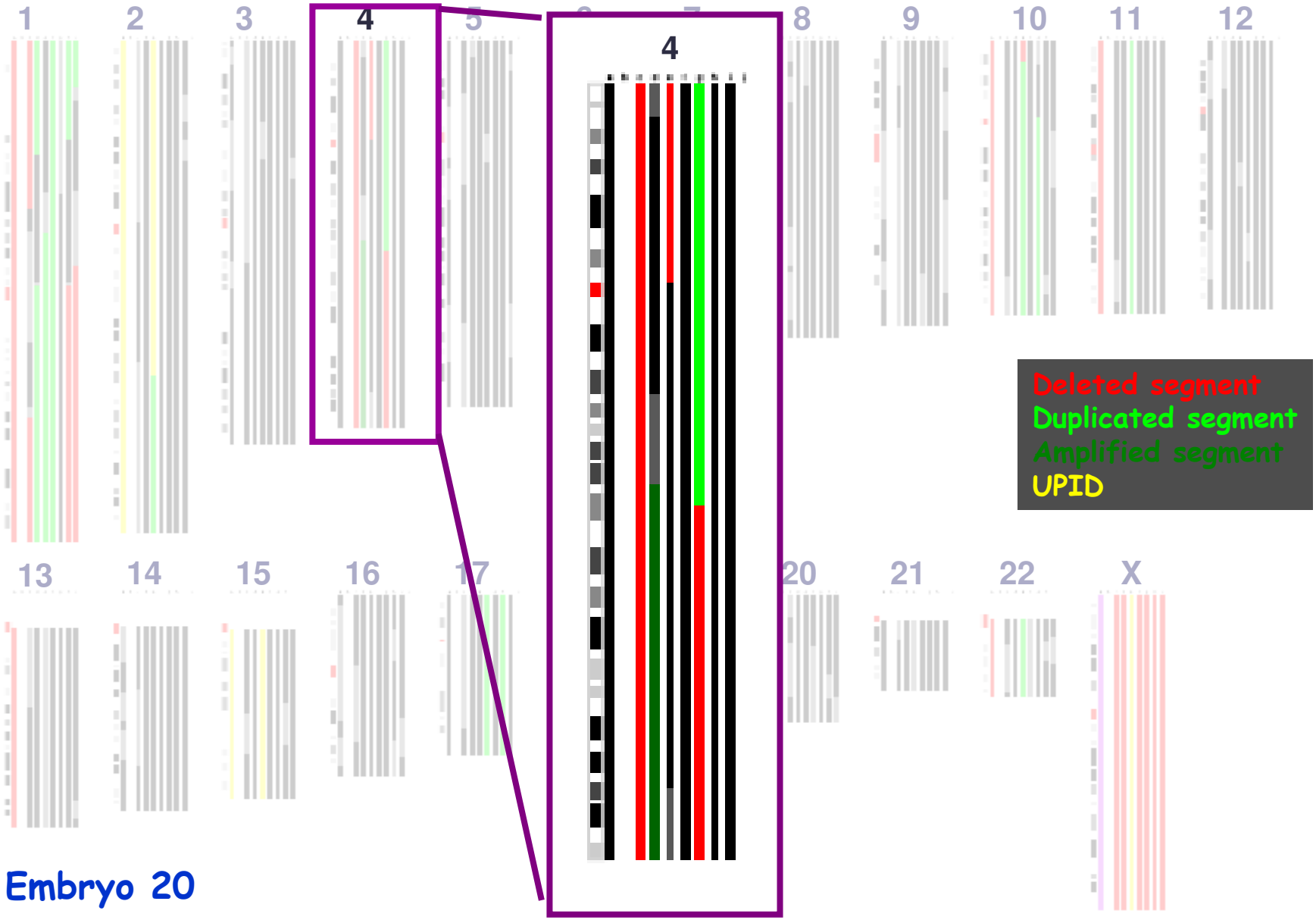
# Embryo 39: chromosoom 5



# 17% of IVF embryos contain complex rearrangements

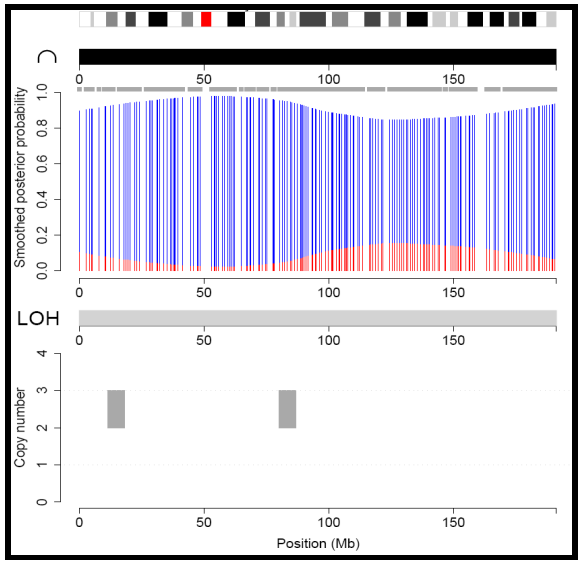


# 17% contain complex rearrangments

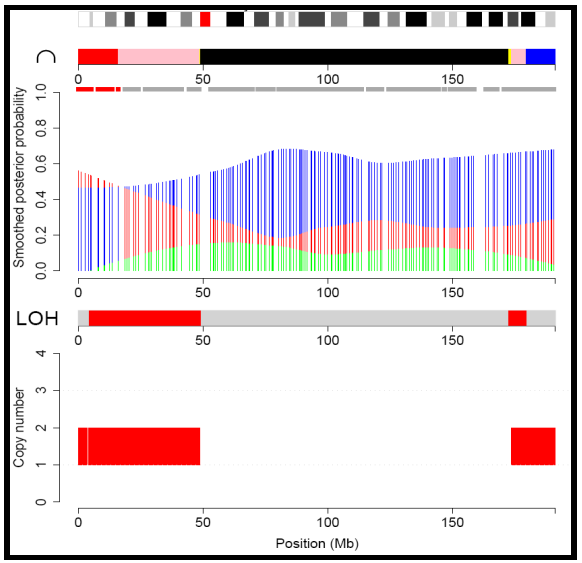


# Complex terminal imbalances are terminal imbalances accompanied by aneuploidies for the same chromosome

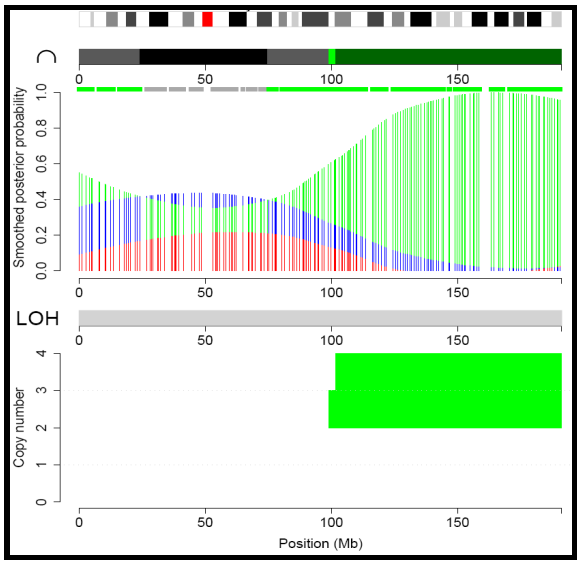
Blastomere "a and f"



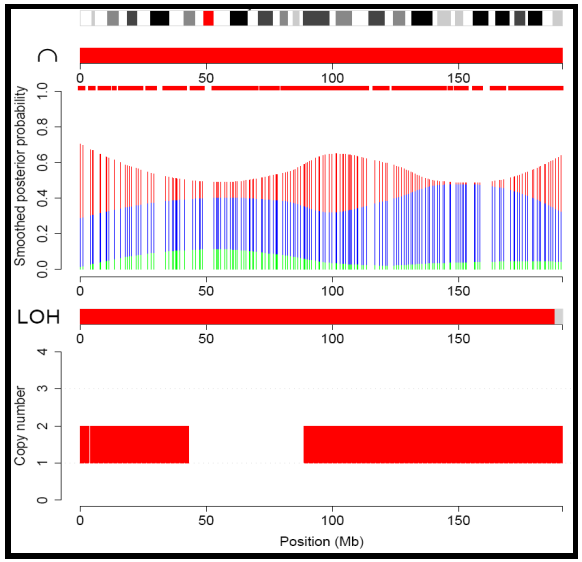

Blastomere "e"



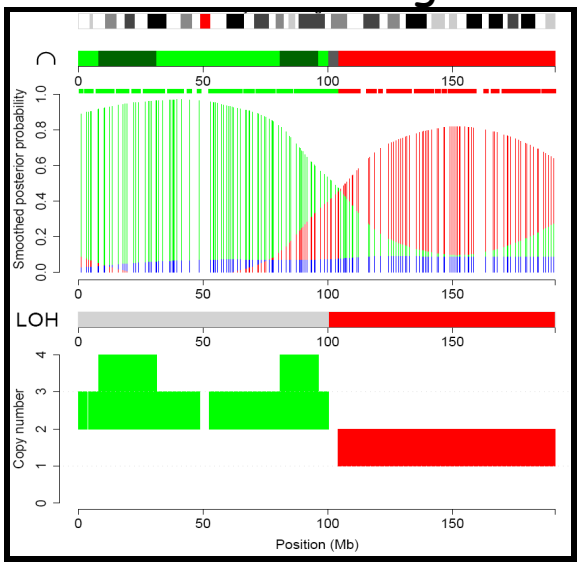

Blastomere "d"

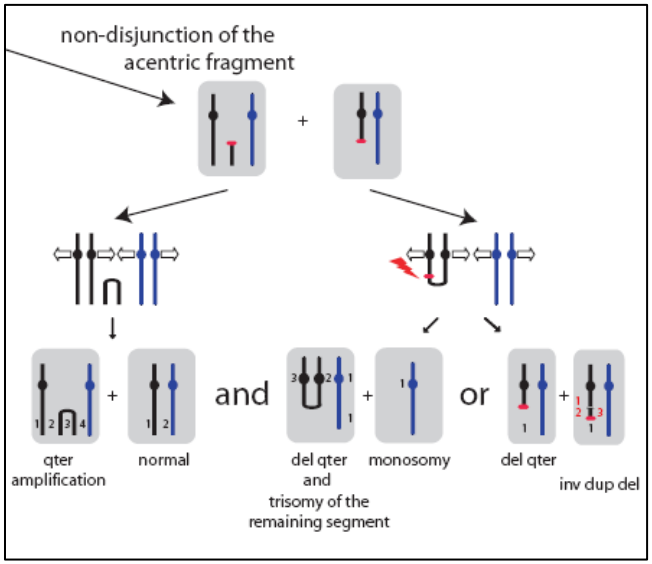



Blastomere "c"

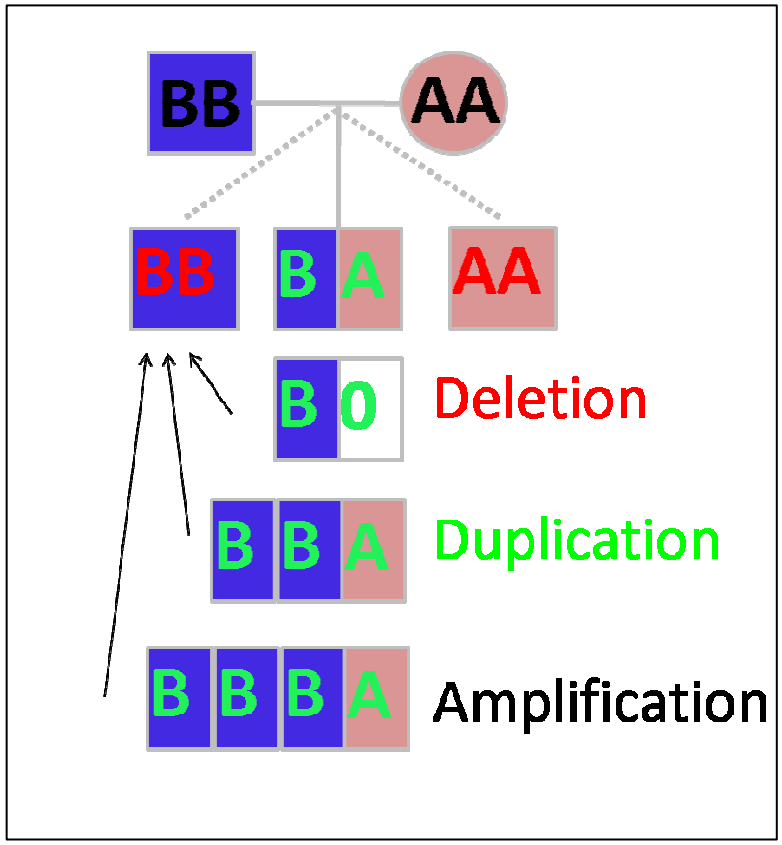


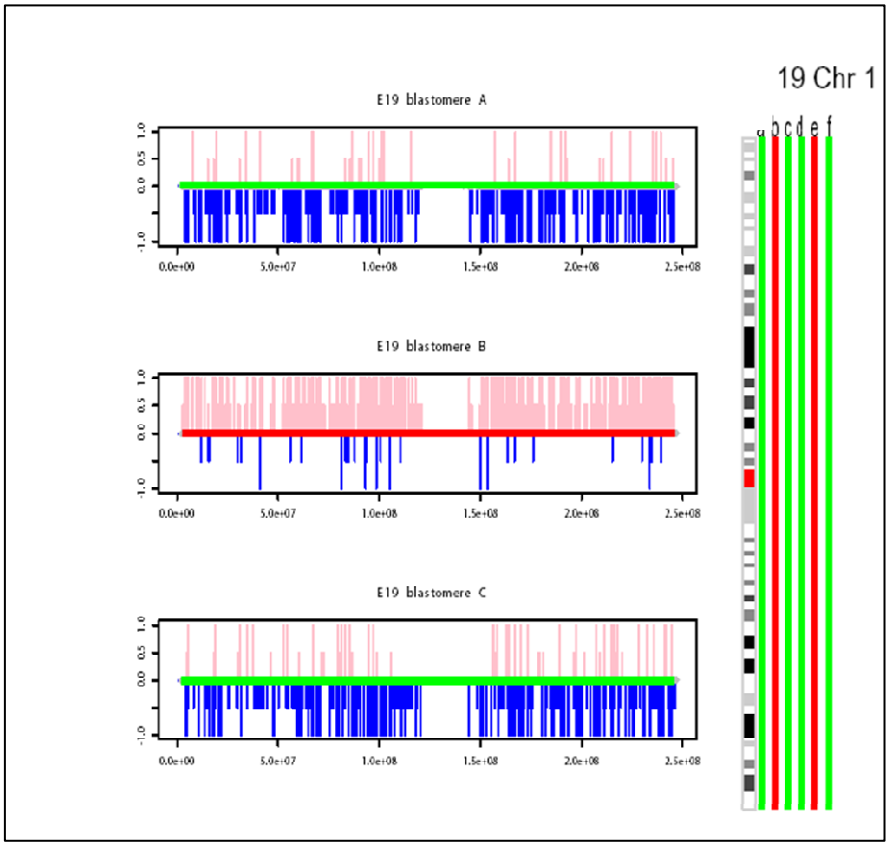

Blastomere "g"



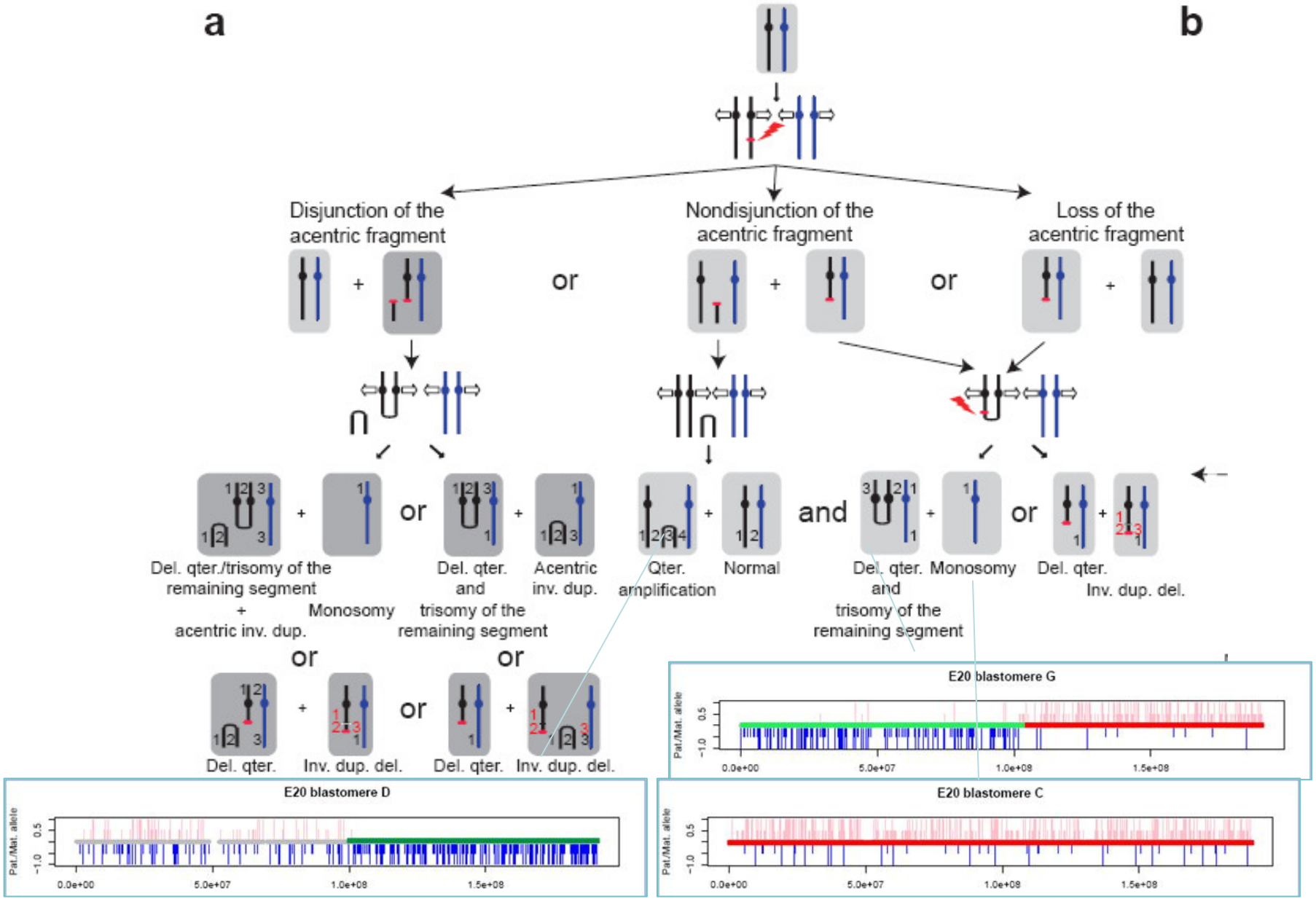



# Parent of origin algorithm

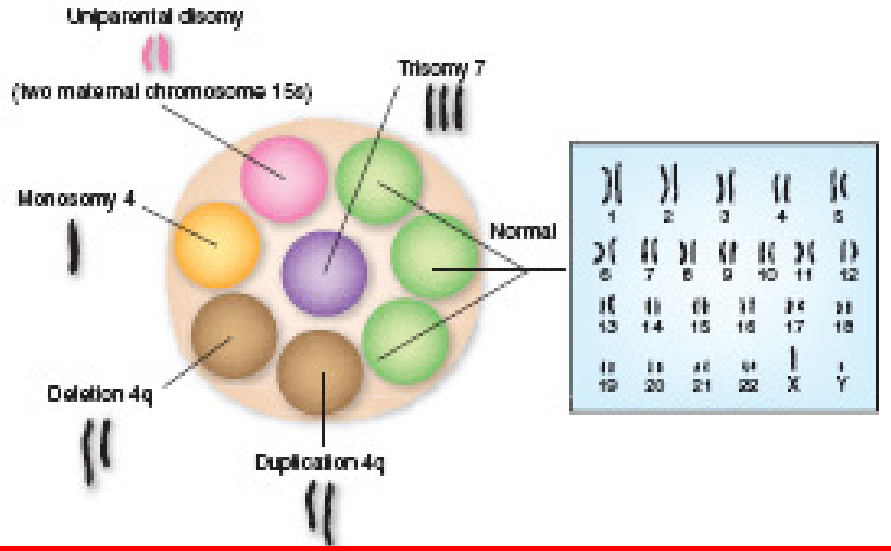


# Mechanics behind terminal imbalances in human embryogenesis



# Embryos are chromosomally unstable




**PGD for aneuploidy screening is useless because**

- **mitotic error rate is higher than meiotic error rate**
- **One cell is not representative of whole embryo**

nature medicine

Chromosome instability is common in human cleavage-stage embryos

Evelyne Vanneste[1,2,9], Thierry Voet[1,9], Cédric Le Caignec[1,3,4], Michèle Ampe[5], Peter Konings[6], Cindy Melotte[1], Sophie Debrock[2], Mustapha Amyere[7], Miikka Vikkula[7], Frans Schuit[8], Jean-Pierre Fryns[1], Geert Verbeke[5], Thomas D'Hooghe[2], Yves Moreau[6] & Joris R Vermeesch[1]

# Acknowledgments


**K.U.Leuven**

- ***Clinical geneticists K.U.Leuven***
  - Jean-Pierre Fryns
  - Koen Devriendt
  - Hilde Van Esch
  - Thomy de Ravel
  - Gert Matthijs
- ***Bioinformatics ESAT K.U.Leuven***
  - Yves Moreau
  - Steven Van Vooren
  - Peter Koninckx
- *Center for biostatistics*
  - Geert Verbeke
  - Michelle Ampe
- *Leuven University fertility Center*
  - Thomas Dhooghe
  - Sofie Debrock

**UCL**

- Miikka Vikkula

**VUB Center for medical genetics**

- Inge Liebaers
- Karen Sermon



**K.U.Leuven *Molecular cytogenetics***

- **Thierry Voet**
- **Evelyne Vanneste**
- Irina Balikova
- Niels Van der Aa
- Bernard Thienpont
- Caroline Robberecht
- Ilse Vanhevel
- Paul Brady
- Femke Hannes


Position for postdoc!