UMC St Radboud

Copy number variation detection on Affymetrix microarrays

implementation in the BeNeLux countries

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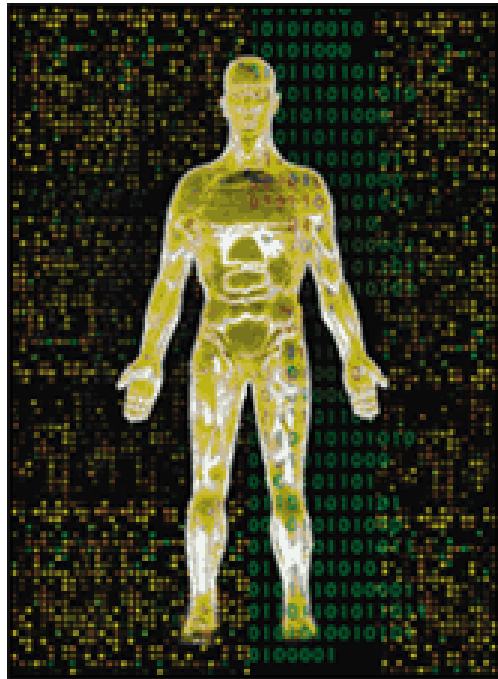


Layout presentation

- General introduction
- Mental retardation and arrays
- Using the 250K Affymetrix SNP array for diagnostics
- Developments

HISTORY OF NIJMEGEN

OLDEST CITY OF THE NETHERLANDS

STICHTING NOVIOMAGUS.NL

UMC St Radboud

Department of Human Genetics part of the RUNMC

- Largest department of Human Genetics in Netherlands
- From bed to bench side in one building: Clinical genetics, genome diagnostics, genome facilities and genome research
- Offer genome diagnostics for all possible diseases to local population of 3 million people as well as external diagnostics requests (16-17,000/year)
- Offer genome facilities (sequencing/microarrays) to all scientists in University as well as external collaborators
- Wide variety of research topics: Focus on genetics underlying brain disorders. Excellent clinical cohorts!

www.humangenetics.nl

Organization in the Netherlands

- License system for genetic testing

-8 clinical genetic centers are allowed to offer post- and pre-natal genetic testing, no commercial labs.

University Medical Center Groningen (Agilent *(Illumina)*)
University Medical Center Amsterdam (Agilent)
Free University Medical Center Amsterdam (Agilent/*Affymetrix*)
University Medical Center Leiden (Affymetrix)
Erasmus Medical Center Rotterdam (Agilent/Affymetrix *(Illumina)*)
University Medical Center Utrecht (Agilent *(Bluegnome)*)
University Medical Center Maastricht (Affymetrix)
University Medical Center Nijmegen (Affymetrix)

-Uniform way of re-imbursement from the insurance companies (postnatal/prenatal) based on agreed numbers of tests per year

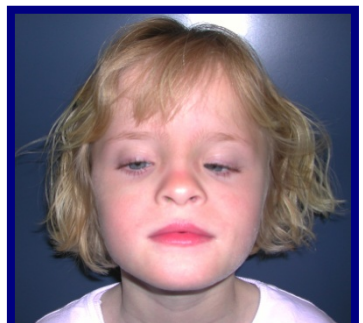
Cytogenetic diagnostics for patients with MR/MCA

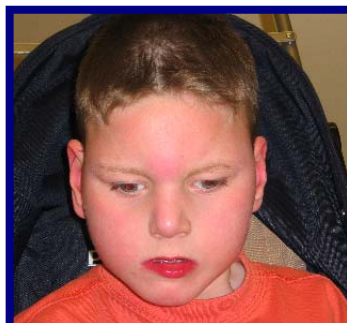
Mental retardation (IQ<70) in 2 % of the population

Dept Human Genetics Nijmegen annually 1200 MR +/- MCA patients for (cyto)genetic diagnostics

- Mild—IQ 50-75
- Moderate—IQ 35-55
- Severe—IQ 20-40
- Profound—IQ <20-25

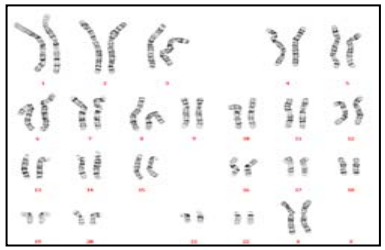
Majority of patients not isolated MR but part of more Complex phenotype MR+/MR+MCA

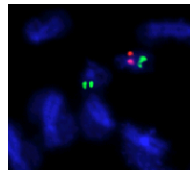




Diagnostic workflow for patients with mental retardation (MR) and / or Multiple Congenital Anomalies (MCA)

Karyotyping and /or FISH

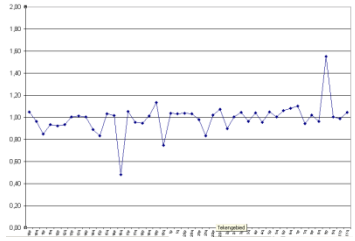




Aberrant (~7%)

Normal

Subtelomere MLPA analysis [1]



Aberrant (~5%)

Normal (~90%)

In parallel:

→ DNA diagnostics

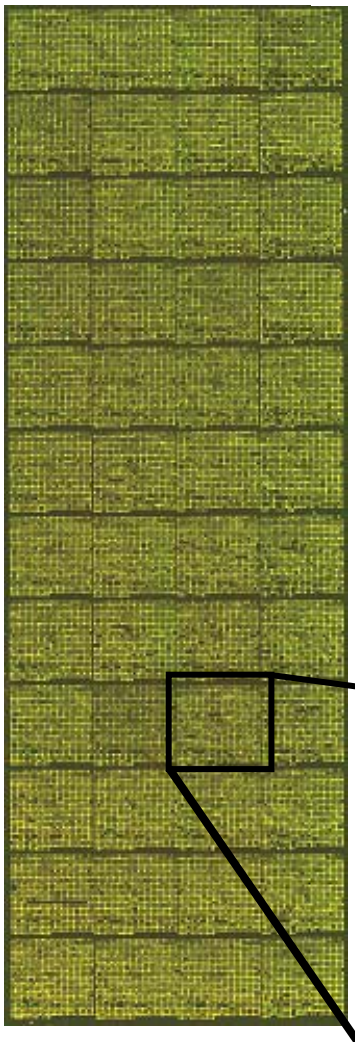
FraX PWS-AS

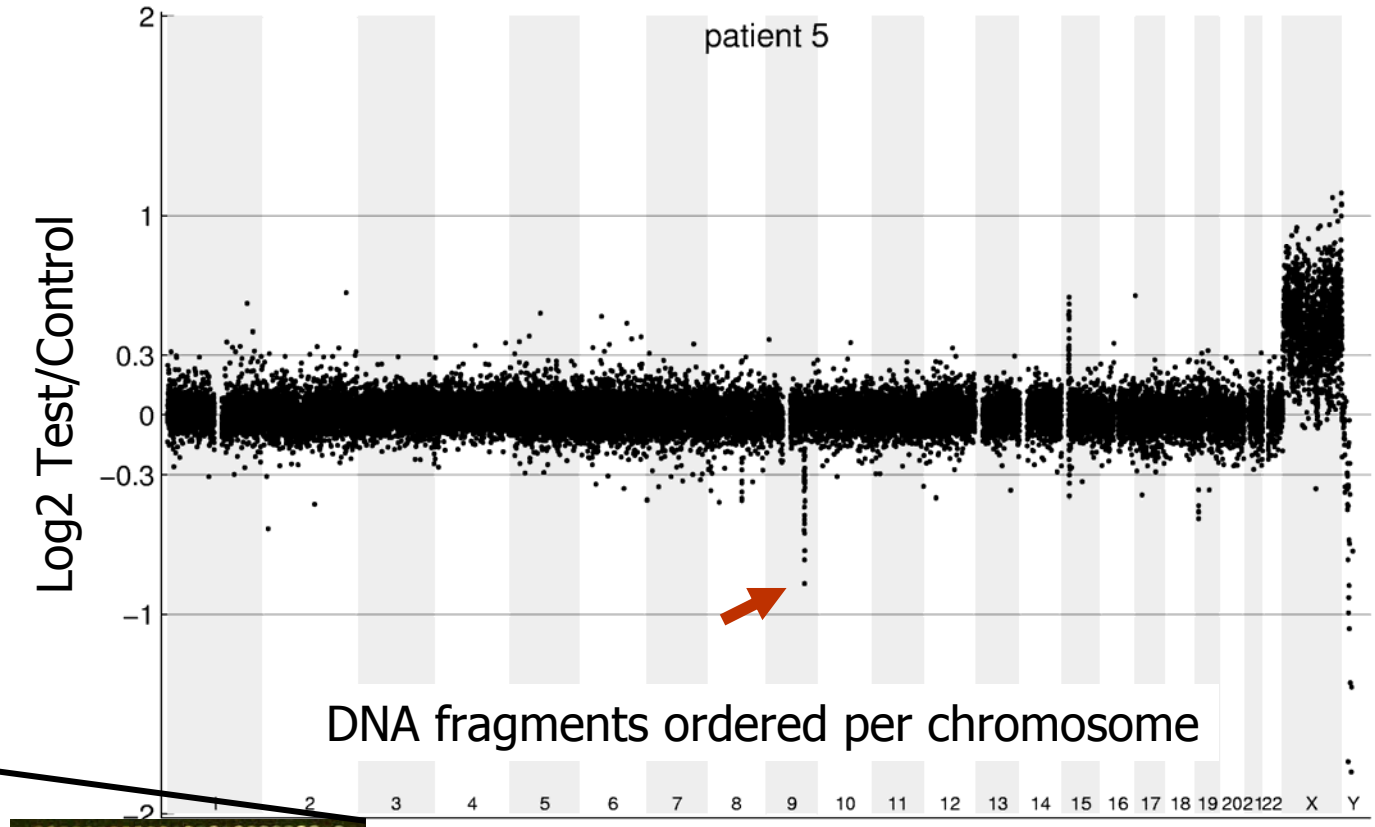
RETT Mitochondrial

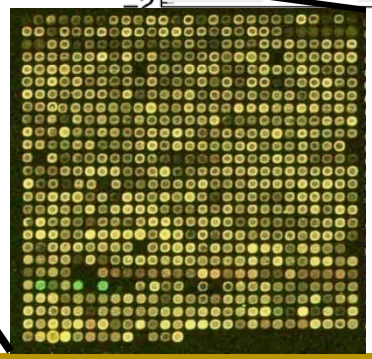
[1] DA Koolen et al J Med Gen 2004

CNV detection in 2005; Home-made 32k BAC arrays

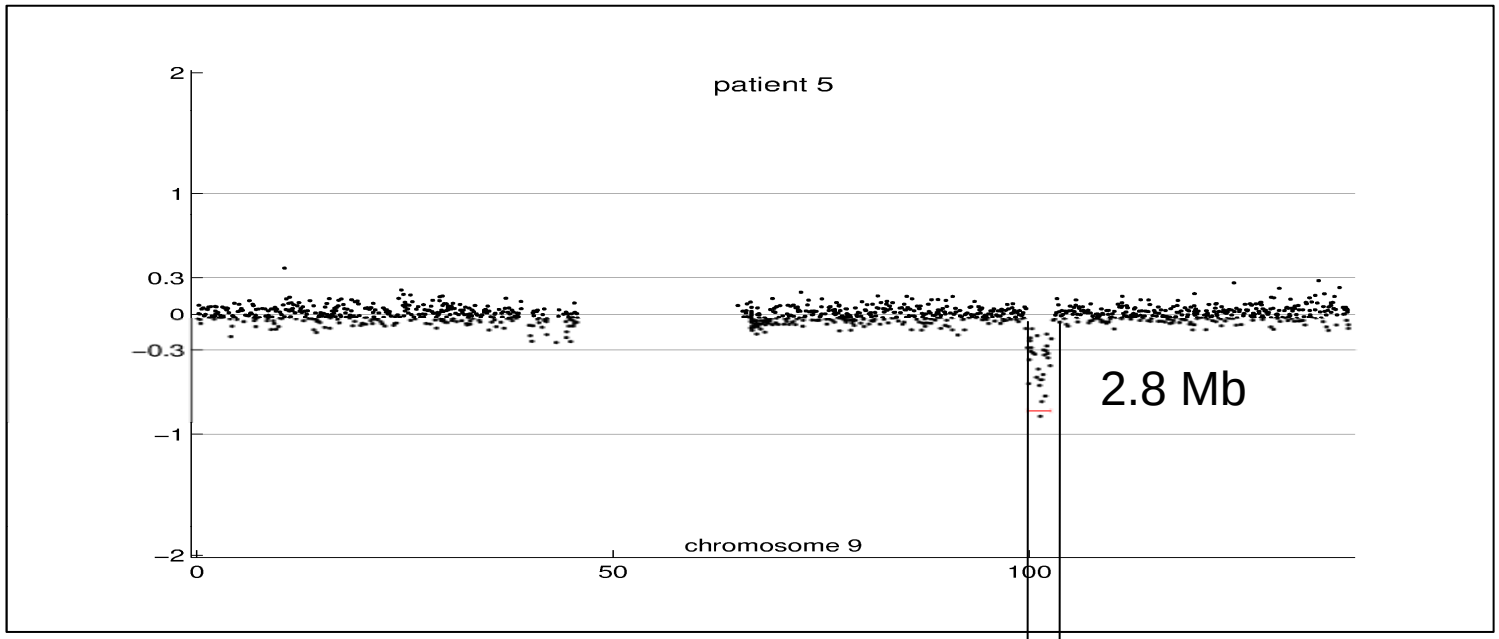
after regular karyotyping and STD-MLPA



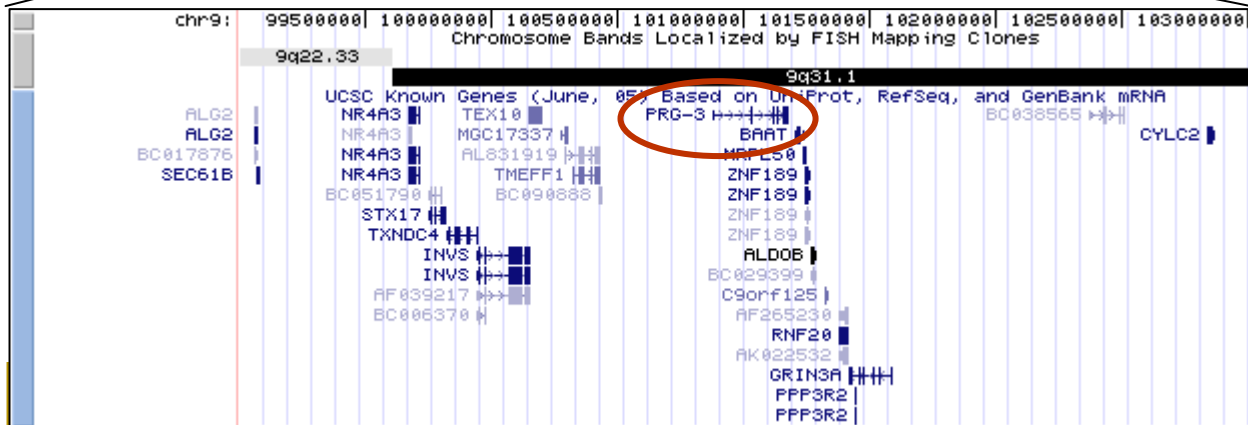




Detail of Copy Number Variation on chromosome 9

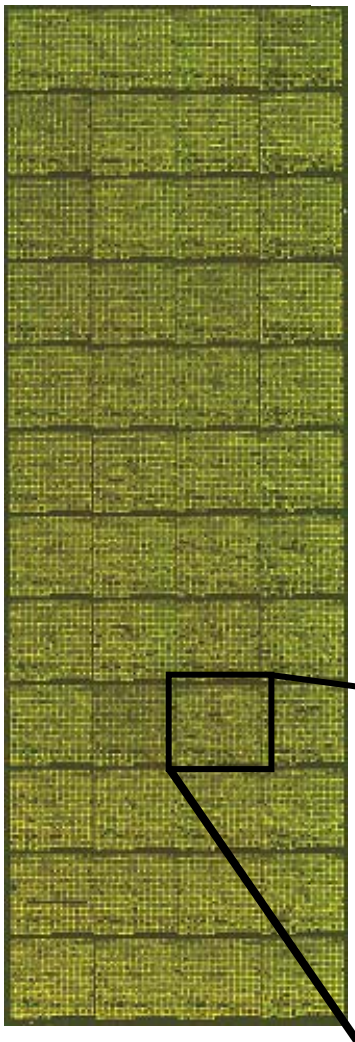


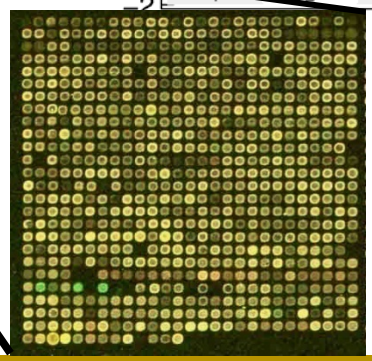
Plasticity-related gene 3 is involved in neuronal plasticity.
This gene is strongly expressed in brain. It shows dynamic expression regulation during brain development and neuronal excitation.

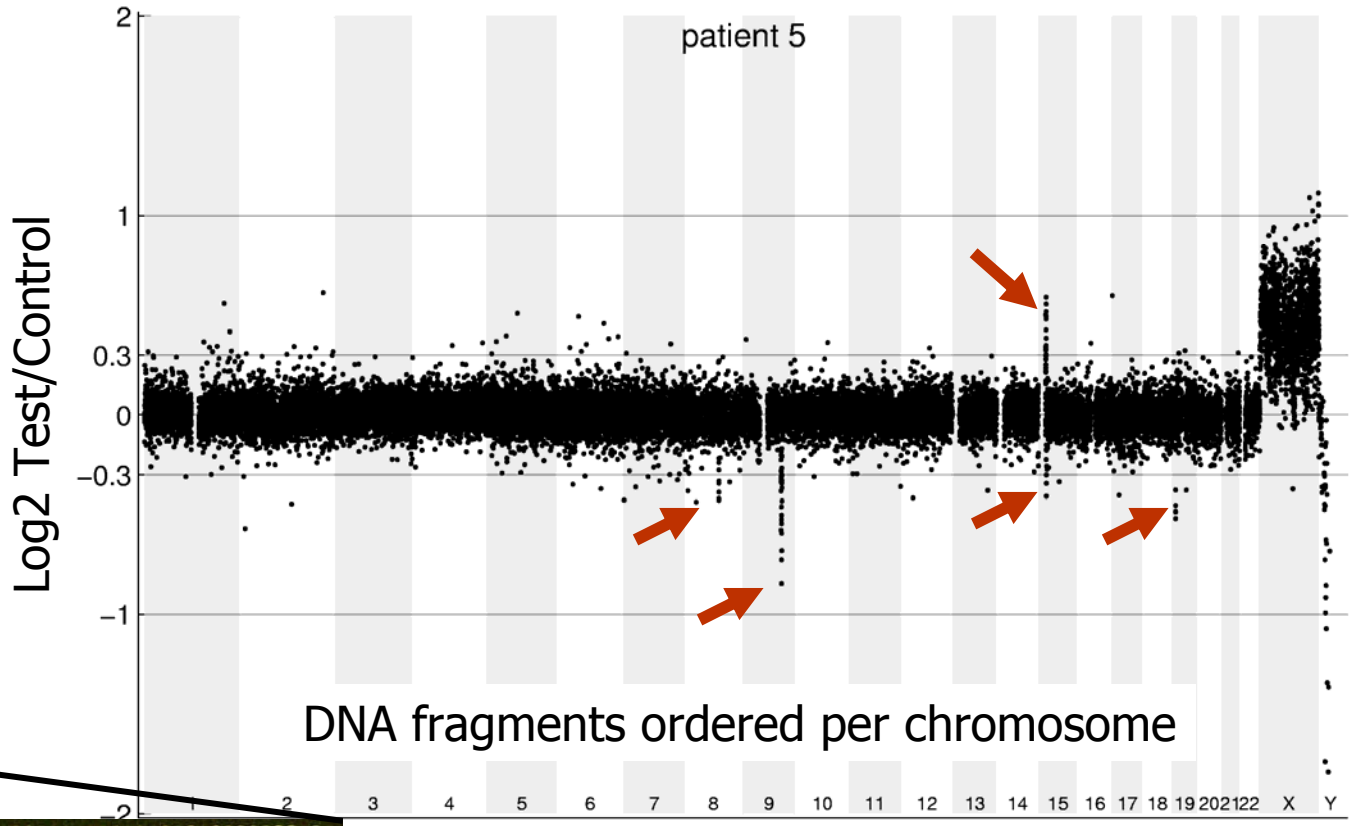


CNV detection in 2005; Home-made 32k BAC arrays

after regular karyotyping and STD-MLPA

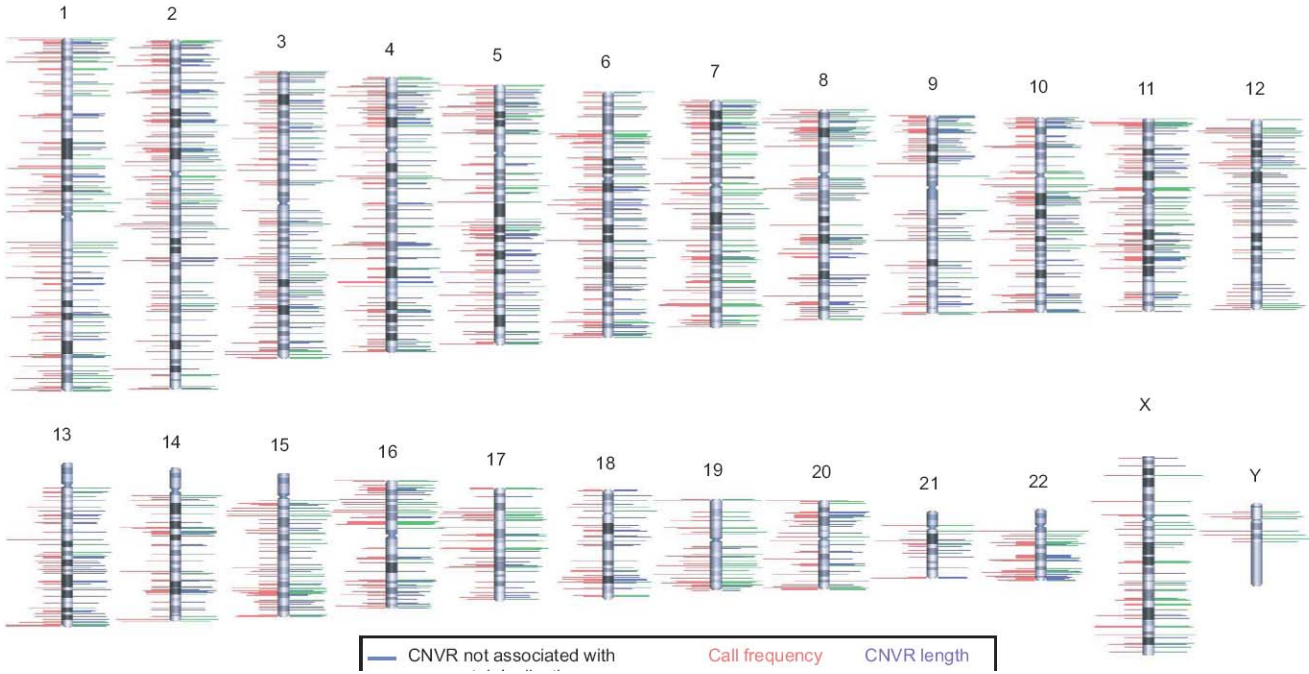






Apparently benign CNVs are common

- Benign, mostly inherited CNVs > 1 kb occur all over genome



Redon *et al.* Nature 2006

Availability of good control data and parental samples is crucial for data interpretation!

Genomic microarrays in mental retardation

Application and validation of clinical use

Am. J. Hum. Genet. 77:606–616, 2005

Diagnostic Genome Profiling in Mental Retardation

Bert B. A. de Vries,[1,*] Rolph Pfundt,[1,*] Martijn Leisink,[2] David A. Koolen,[1] Lisenka E. L. M. Vissers,[1] Irene M. Janssen,[1] Simon van Reijmersdal,[1] Willy M. Nillesen,[1] Erik H. L. P. G. Huys,[1] Nicole de Leeuw,[1] Dominique Smeets,[1] Erik A. Sistermans,[1] Ton Feuth,[3] Conny M. A. van Ravenswaaij-Arts,[1] Ad Geurts van Kessel,[1] Eric F. P. M. Schoenmakers,[1] Han G. Brunner,[1] and Joris A. Veltman[1]

Departments of [1]Human Genetics, [2]Biophysics, and [3]Epidemiology and Biostatistics, Nijmegen Centre for Molecular Life Sciences, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands

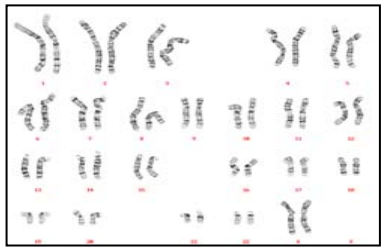
- 100 patients with unexplained MR, normal karyotype
- Hybridize DNA to 32k BAC array
- Test parental DNAs to determine inheritance
- Validate findings by FISH/MLPA/QPCR

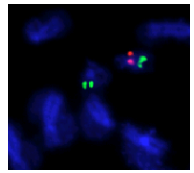
→ Rare *de novo* CNVs in 10% of cases!!!!!!!

Diagnostic workflow for patients with mental retardation (MR) and / or Multiple Congenital Anomalies (MCA)

2005-2008

Karyotyping and /or FISH

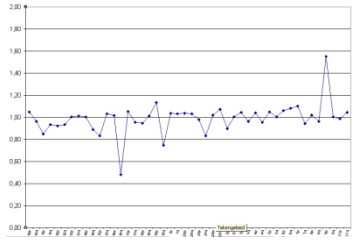




Aberrant (~7%)

Normal

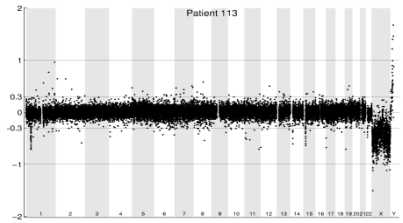
Subtelomere MLPA analysis [1]



Aberrant (~5%)

Normal

Genome wide array (CGH) analysis [2]



Aberrant (~10-13%)

Normal (~ 75%)

In parallel:

→ DNA diagnostics

FraX PWS-AS

RETT Mitochondrial

[1] DA Koolen et al J Med Gen 2004

[2] BBA de Vries et al Am J Hum Gen 2005

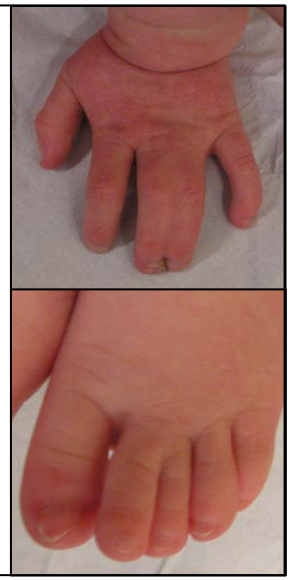
Started with diagnostic application of 500K SNP array in 2007

CNV analysis with SNP arrays has a lot of diagnostic advantages

- Sample mix-up can be detected in trio data
- Non-paternity can be detected in trio data
- Parent-of-origin of aberrations can be determined in trio data
- Uniparental disomies can be detected in single and/or trio data
- Incomplete trio's can still be conclusive (de novo?)
- Consanguinity can be detected
- Homozygosity mapping for recessive loci is possible
  (Walker Warburg, Leigh, hereditary retinal dystrophies)
- Can be combined with SNP-based association study

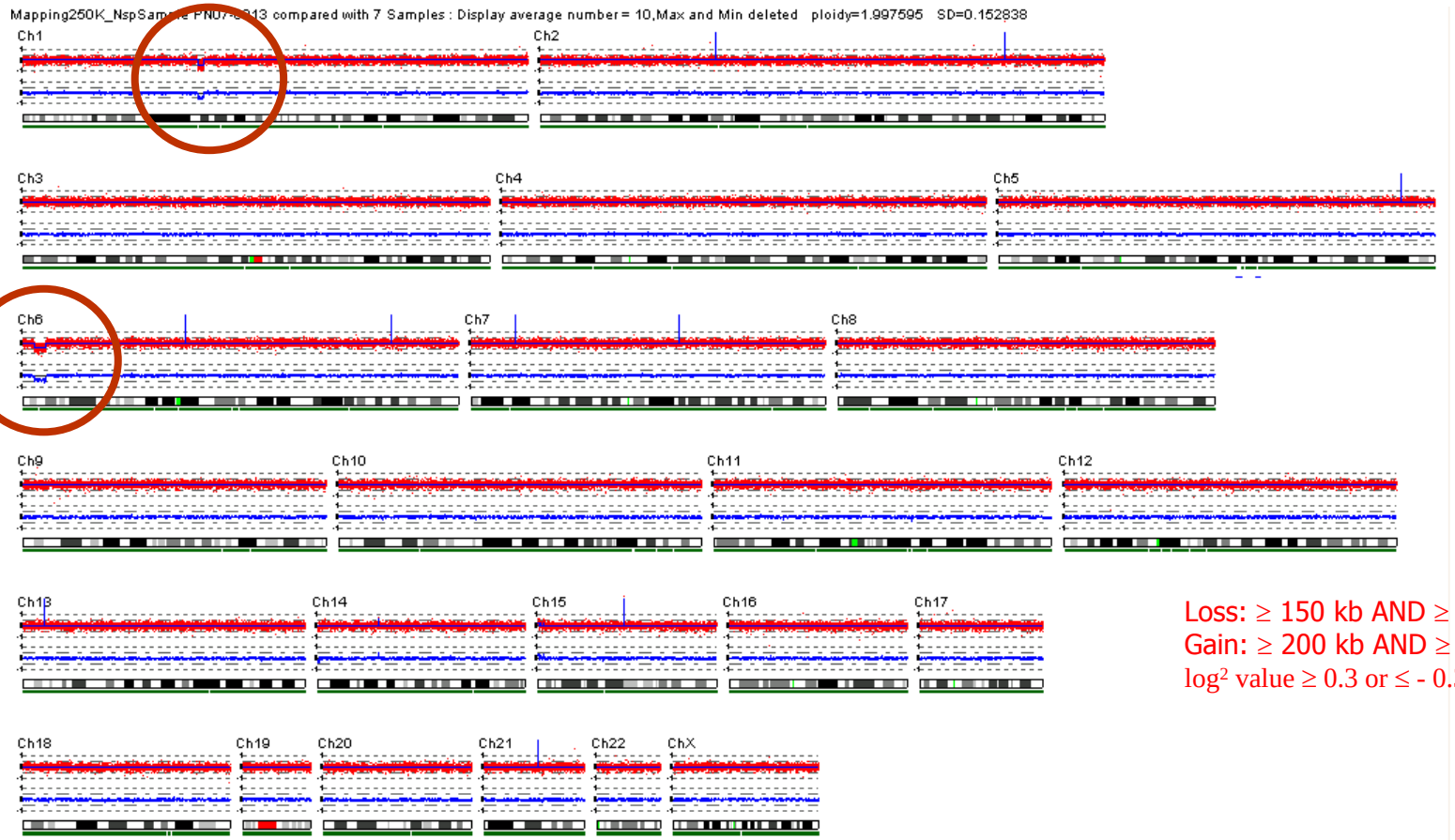
Example SNP array procedure:

Patiënt PN07-0013



Girl dob. 08/2004
Moderate mental retardation
Hypertelorism
ASD (hart defect)
Syndactyly
Cutis marmorata

Whole genome view:



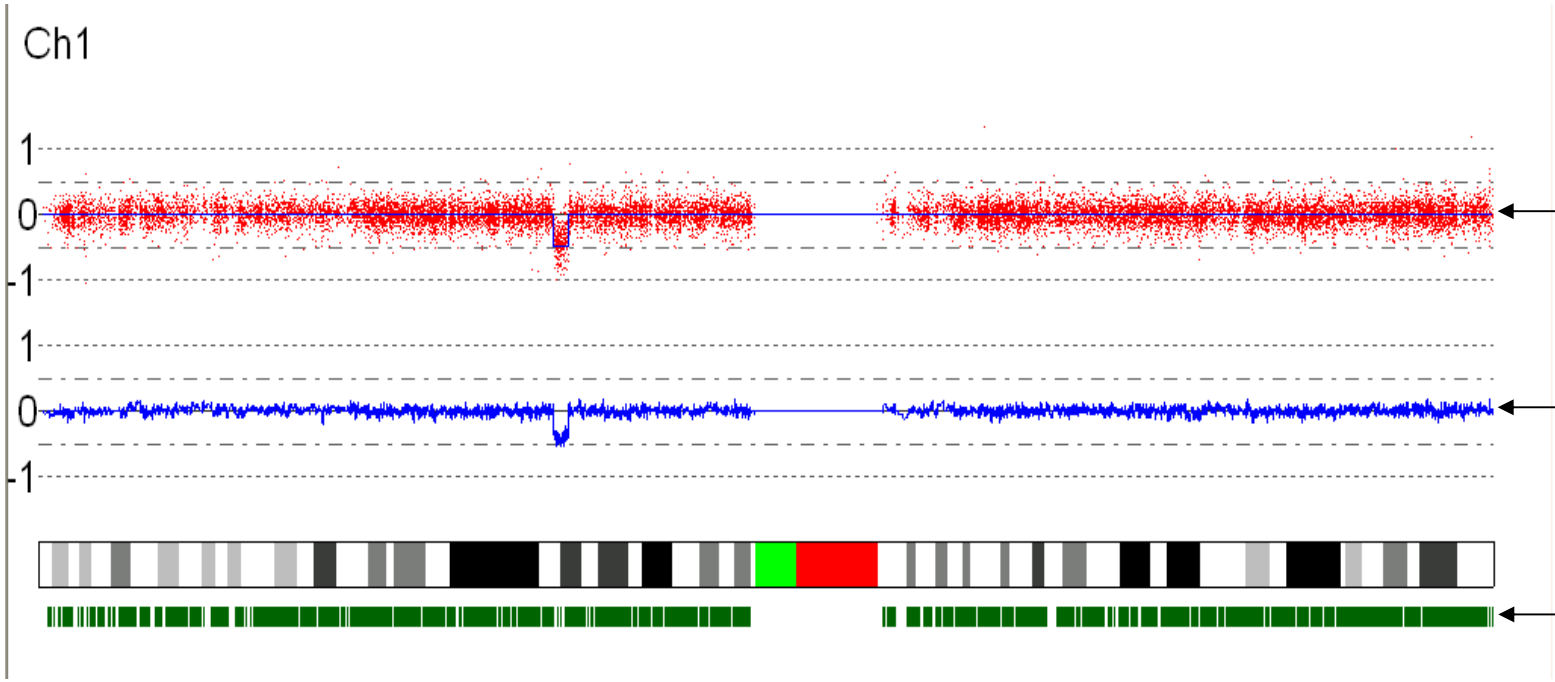
Loss: ≥ 150 kb AND ≥ 5 SNPs
Gain: ≥ 200 kb AND ≥ 7 SNPs
$\log^2$ value ≥ 0.3 or ≤ - 0.38

A copy number variant (CNV) is considered a normal genome variant IF the same or similar CNV has been encountered several times in unaffected individuals:

i) In ≥ 2 in-house analysed controls (unaffected parents)

ii) In ≥ 3 controls reported elsewhere (i.e. in http://projects.tcag.ca/variation/)

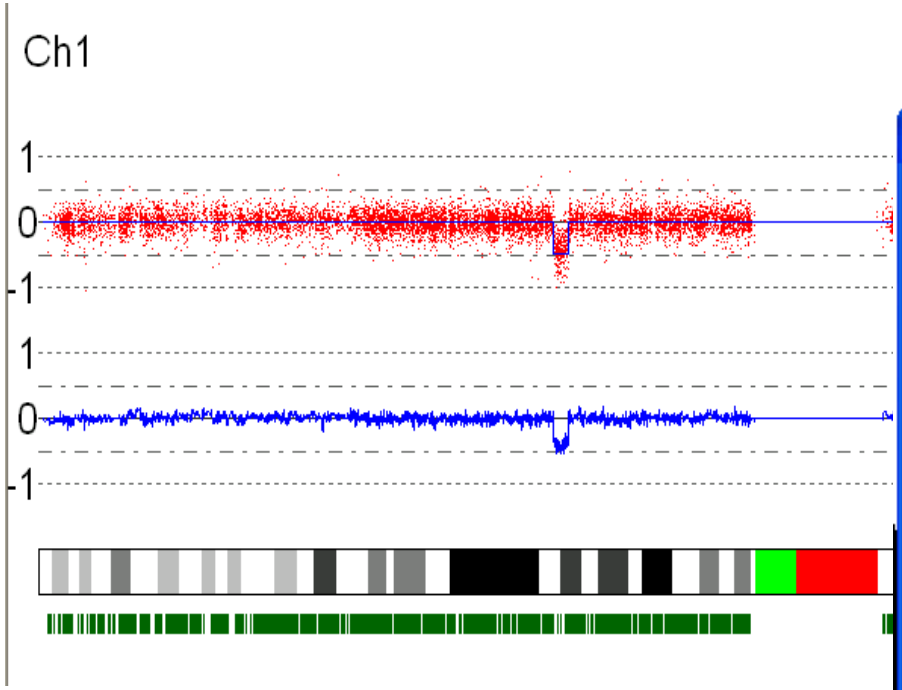
Zoom of chromosome 1

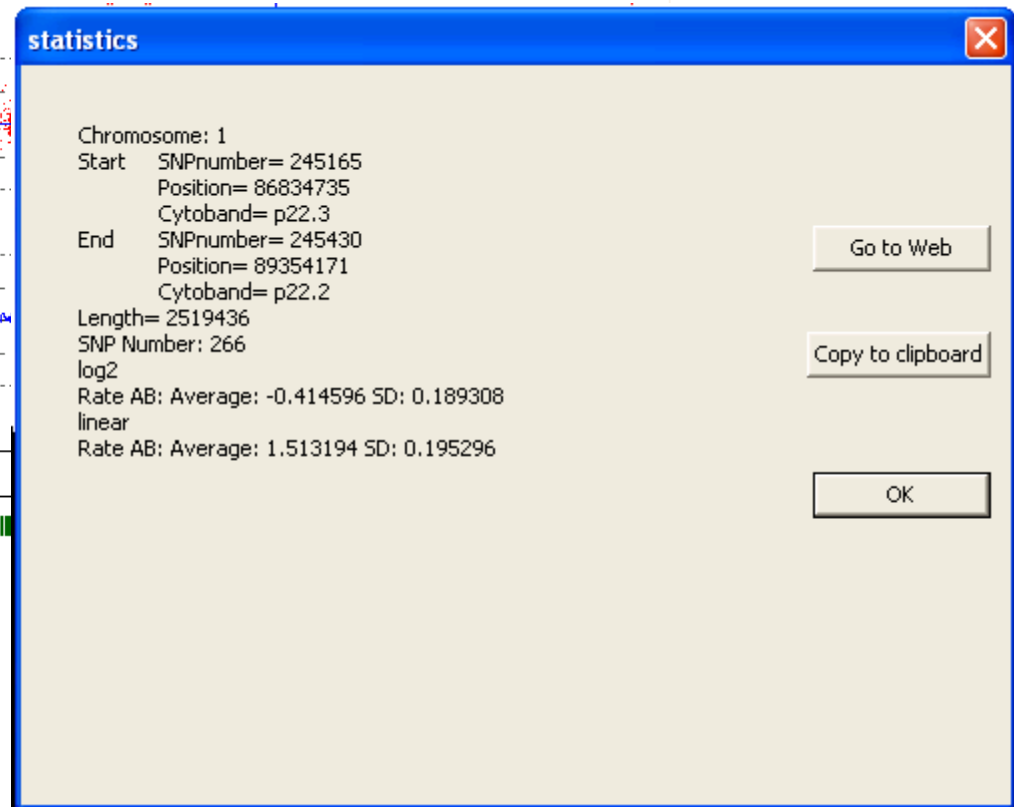


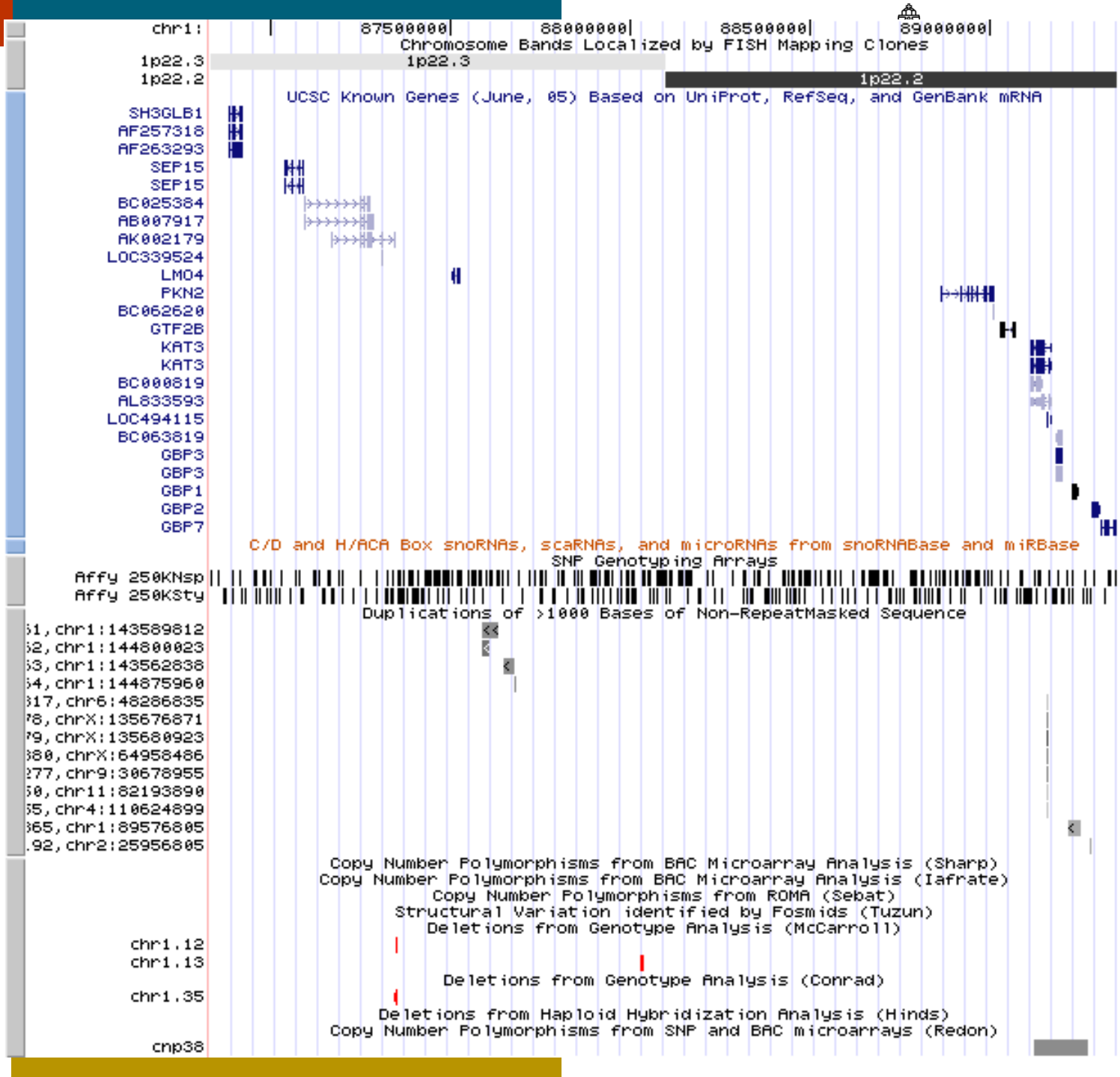
log(T/R) per SNP

10 SNP average

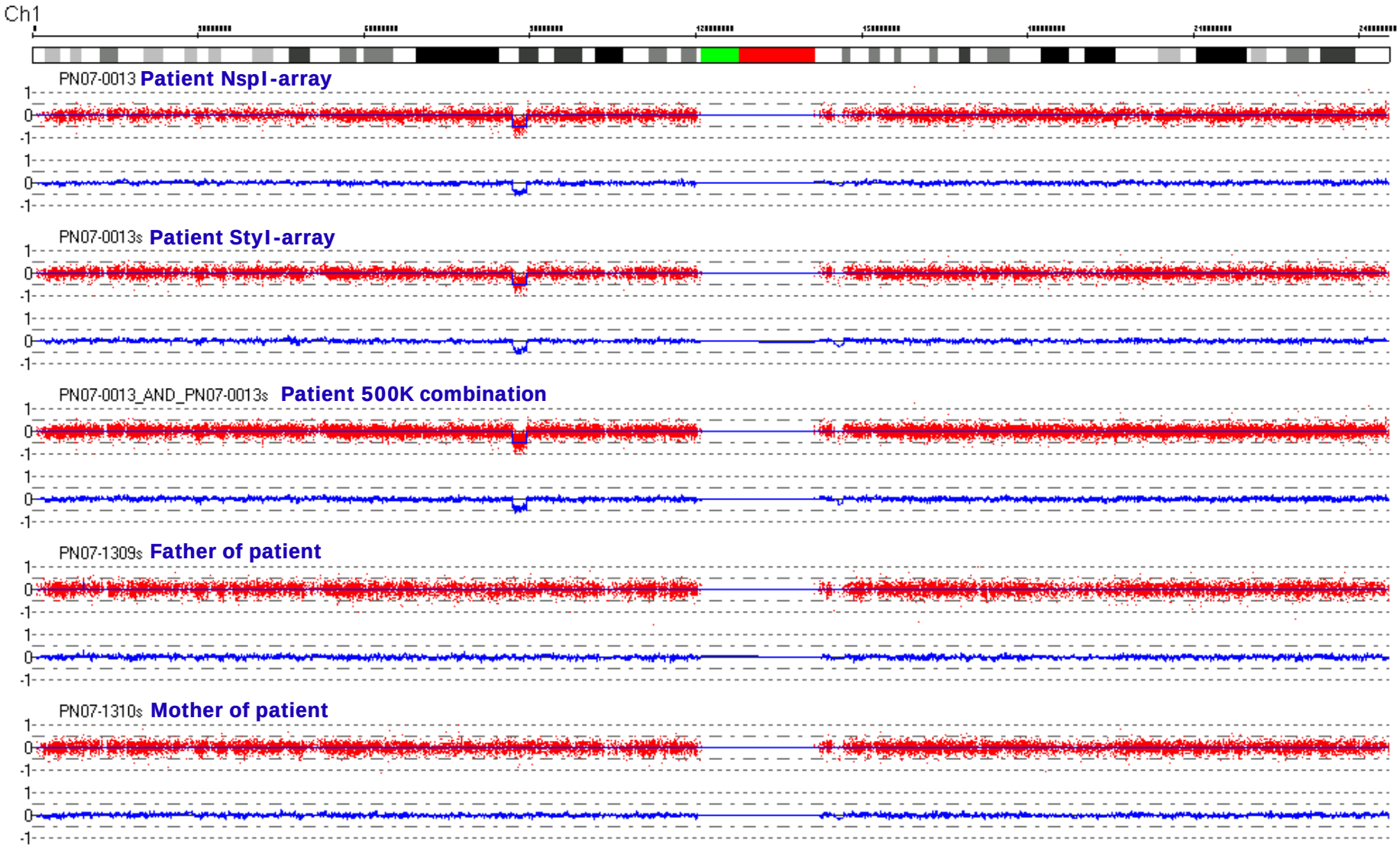
Hetero-Of Homozygous


Ch1
1
0
-1
1
0
-1


statistics
Chromosome: 1
Start SNPnumber= 245165
Position= 86834735
Cytoband= p22.3
End SNPnumber= 245430
Position= 89354171
Cytoband= p22.2
Length= 2519436
SNP Number: 266
log2
Rate AB: Average: -0.414596 SD: 0.189308
linear
Rate AB: Average: 1.513194 SD: 0.195296
Go to Web
Copy to clipboard
OK


chr1:
87500000
88000000
88500000
89000000
Chromosome Bands Localized by FISH Mapping Clones
1p22.3
1p22.3
1p22.2
1p22.2
UCSC Known Genes (June, 05) Based on UniProt, RefSeq, and GenBank mRNA
SH3GLB1
AF257318
AF263293
SEP15
SEP15
BC025384
AB007917
AK002179
LOC339524
LMO4
PKN2
BC062620
GTF2B
KAT3
KAT3
BC000819
AL833593
LOC494115
BC063819
GBP3
GBP3
GBP1
GBP2
GBP7
C/D and H/ACA Box snoRNAs, scaRNAs, and microRNAs from snoRNABase and miRBase
SNP Genotyping Arrays
Affy 250KNsp
Affy 250KSty
Duplications of >1000 Bases of Non-RepeatMasked Sequence
51,chr1:143589812
52,chr1:144800023
53,chr1:143562838
54,chr1:144875960
317,chr6:48286835
78,chrX:135676871
79,chrX:135680923
380,chrX:64958486
277,chr9:30678955
50,chr11:82193890
55,chr4:110624899
365,chr1:89576805
.92,chr2:25956805
Copy Number Polymorphisms from BAC Microarray Analysis (Sharp)
Copy Number Polymorphisms from BAC Microarray Analysis (Iafrate)
Copy Number Polymorphisms from ROMA (Sebat)
Structural Variation identified by Fosmids (Tuzun)
Deletions from Genotype Analysis (McCarroll)
chr1.12
chr1.13
Deletions from Genotype Analysis (Conrad)
chr1.35
Deletions from Haploid Hybridization Analysis (Hinds)
Copy Number Polymorphisms from SNP and BAC microarrays (Redon)
cnp38

Profile of the patient is compared to the profile of the parents for the region of interest:



46,XX.arr snp 1p22.2p22.3(SNP_A-2080422-SNP_A-1918382)x1 dn.

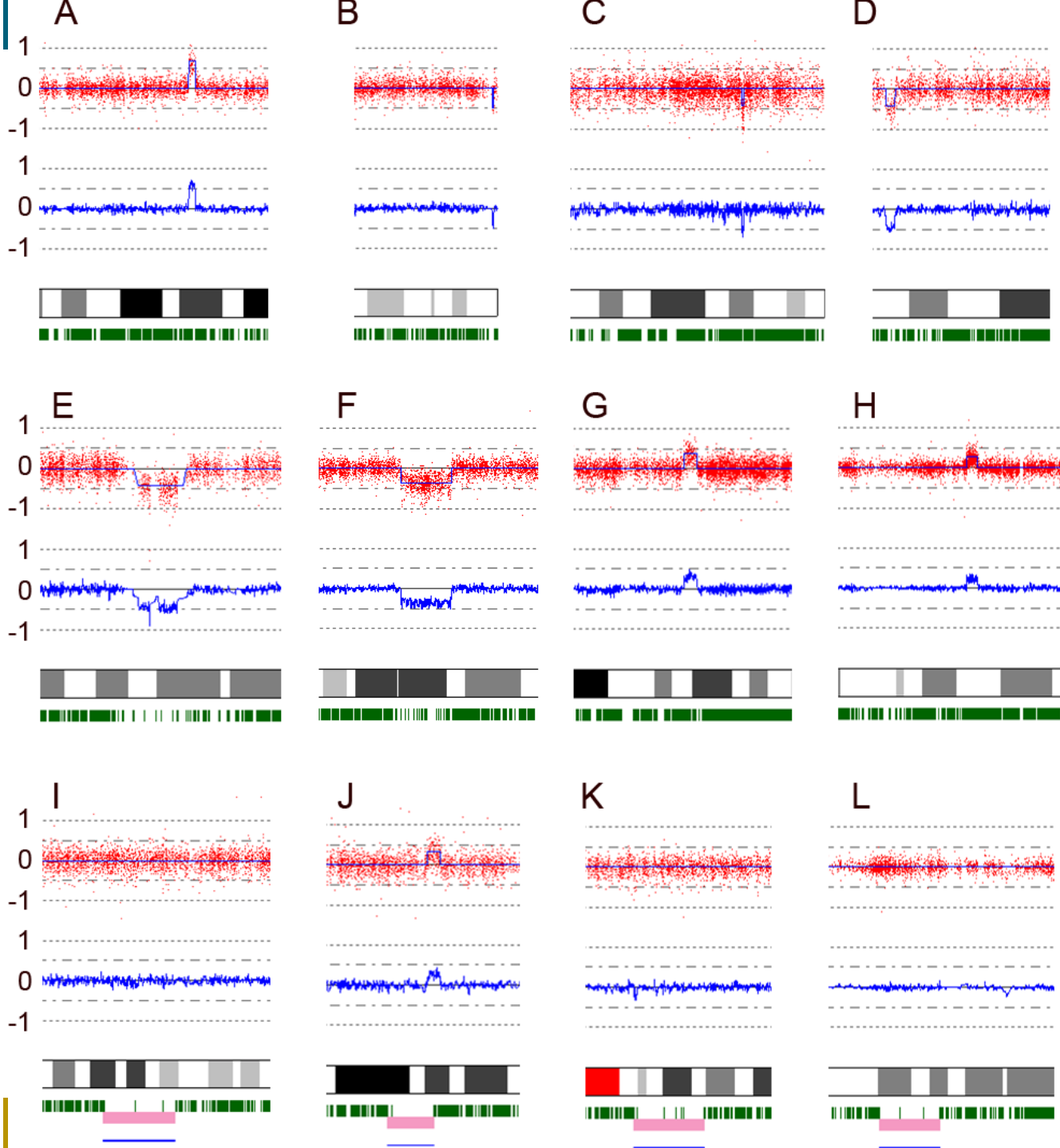
- POR analysis to determine the parent of origin
- Mendelian analysis to determine
  - -sample correctness
  - -paternity
  - -exclude UPD's

Examples of

Rare de novo CNVs < 1 Mb

Rare inherited CNVs > 1 Mb

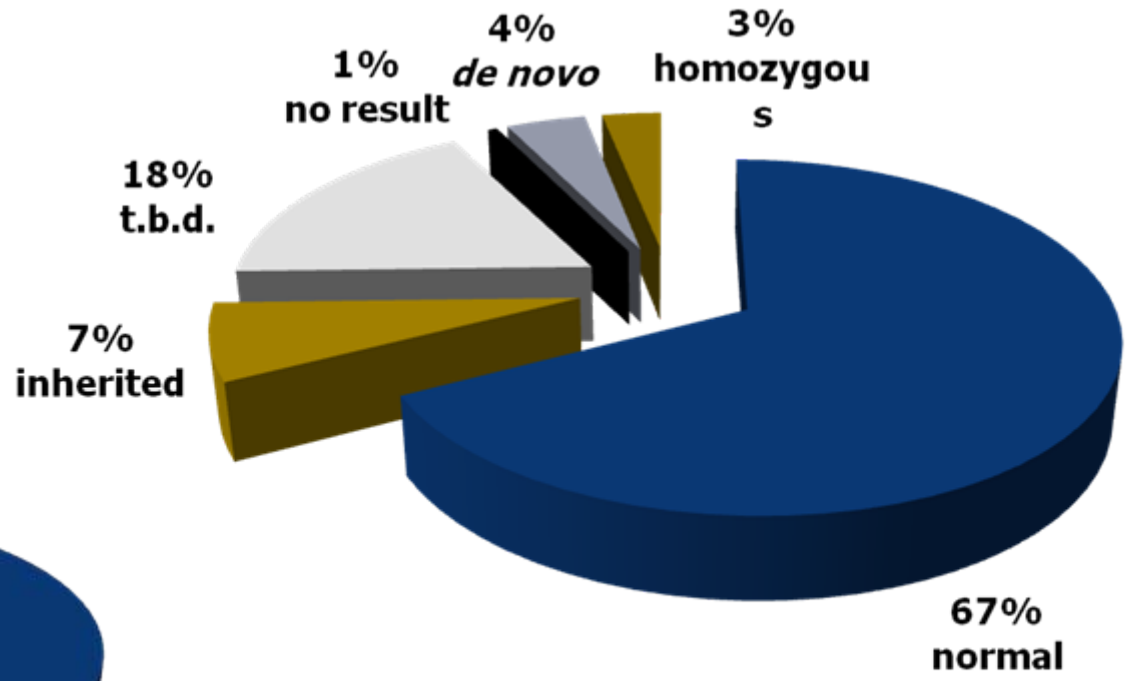
Rare homozygous region > 1 Mb

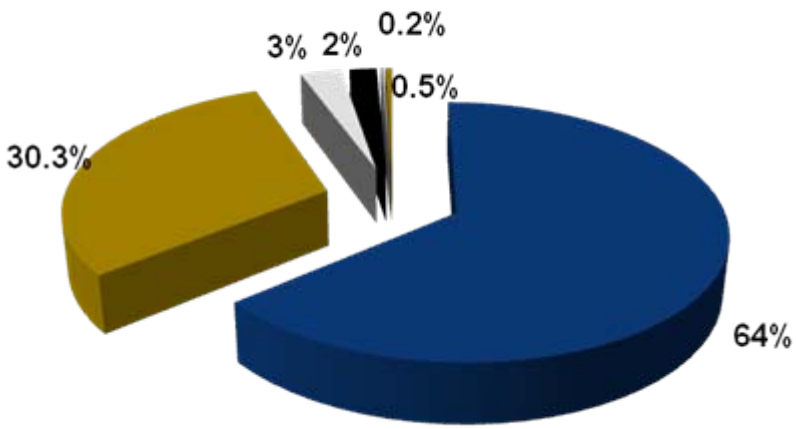


McMullan *et al.*
Hum Mutation 2009

Genome wide SNP array analysis in mental retardation (MR) and / or multiple congenital anomalies (MCA)

SNP array in MR / MCA (n = 1,707)





SNP array in parents (n = 421)

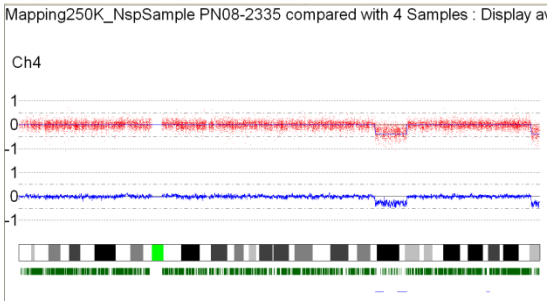
MR diagnosis in Nijmegen

Approach as of 2009

~~Karyotyping~~

~~Subtelomeric MLPA~~

~~FISH~~



Direct Microarray

Targeted DNA mutation analysis (when indicated)

Abnormal (25%)

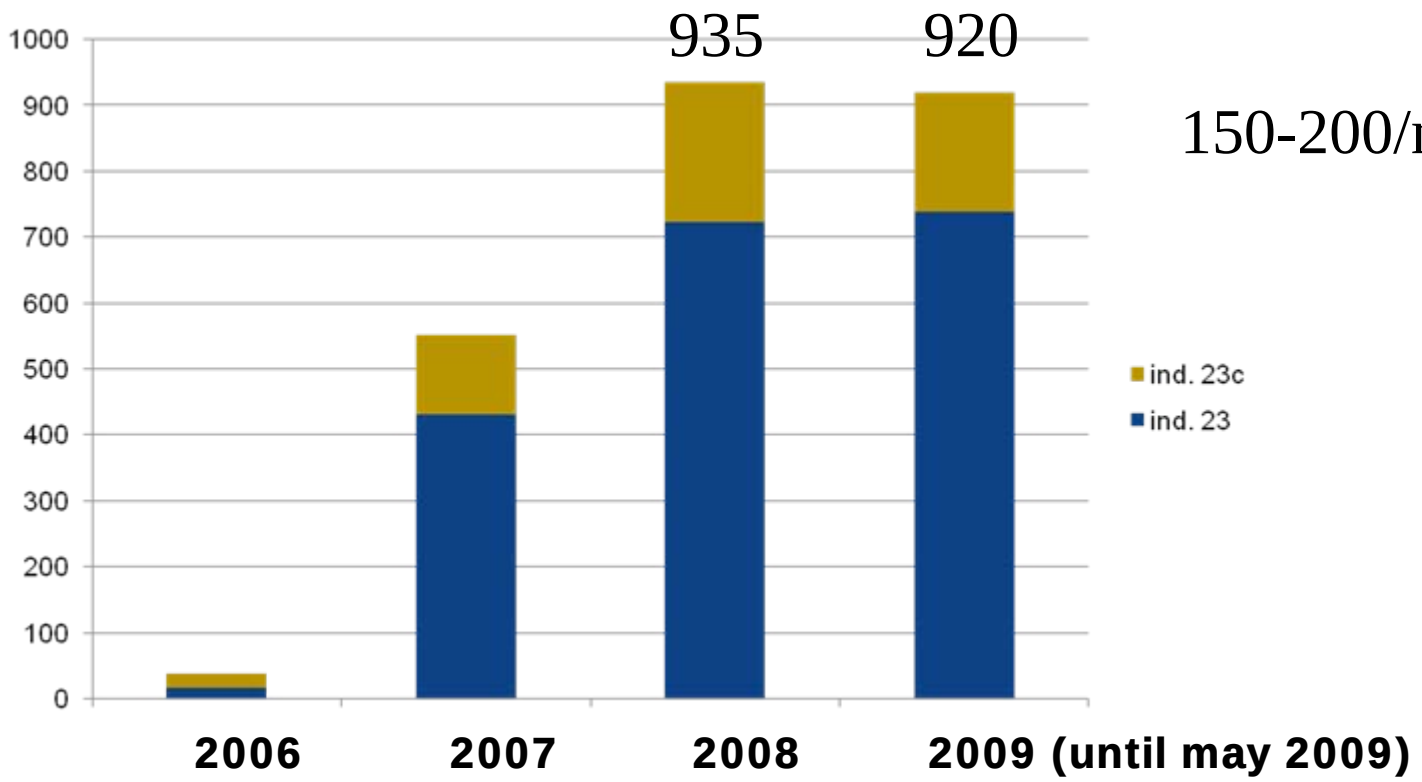
Normal (75%)

Pros of single test:
- fast diagnosis, no culturing required
- no duplicate analyses
- cheaper

Cons:
- misses balanced translocations/inversions

Array diagnostics 2009 - 250k SNP array

MR referrals in Nijmegen 1200/year????

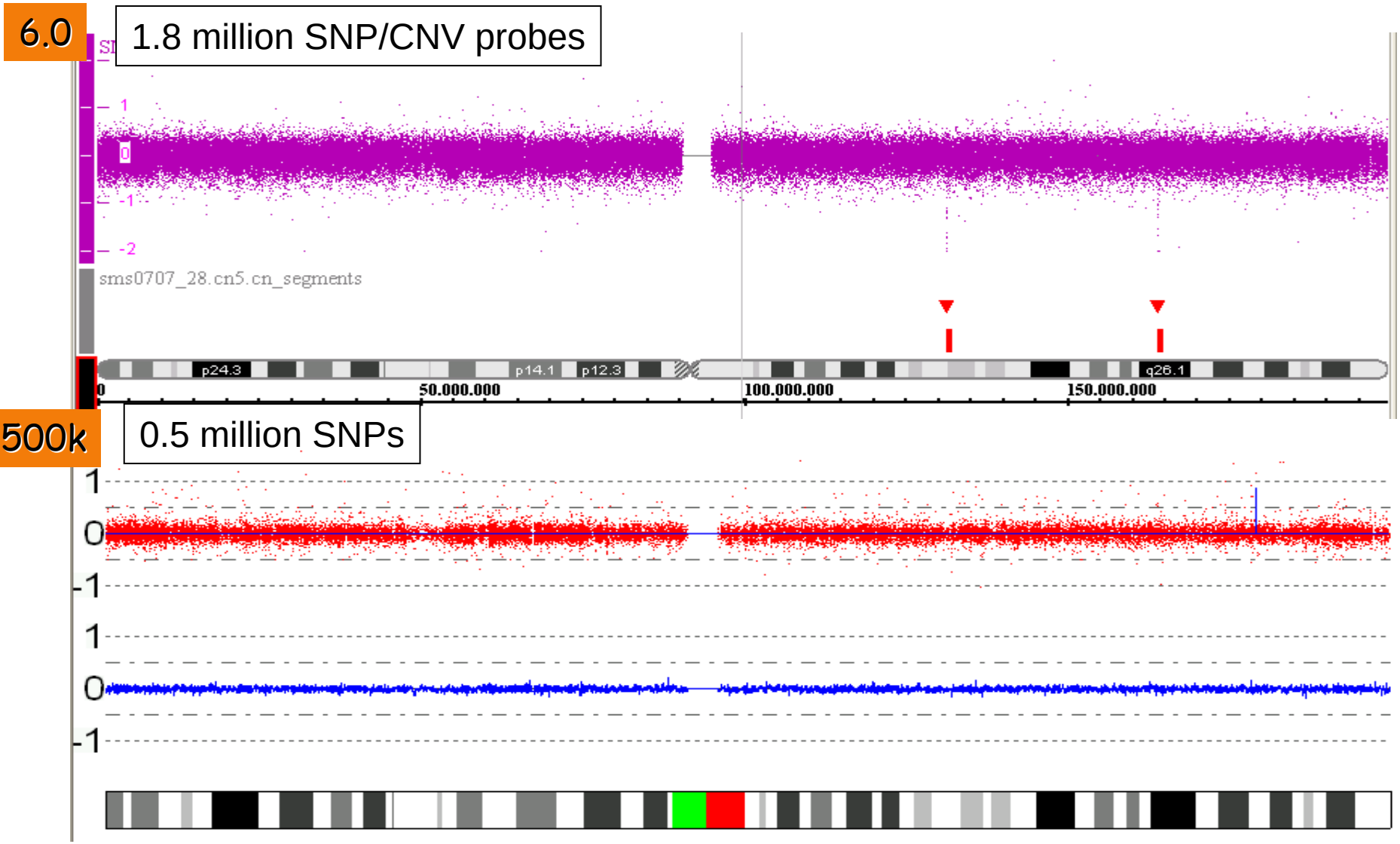


150-200/month

From the moment array became the first pass diagnostic test the number of array requests exploded

Developments

Latest generation SNP arrays detects even smaller CNVs



Currently validating and implementing the new 2.7M array

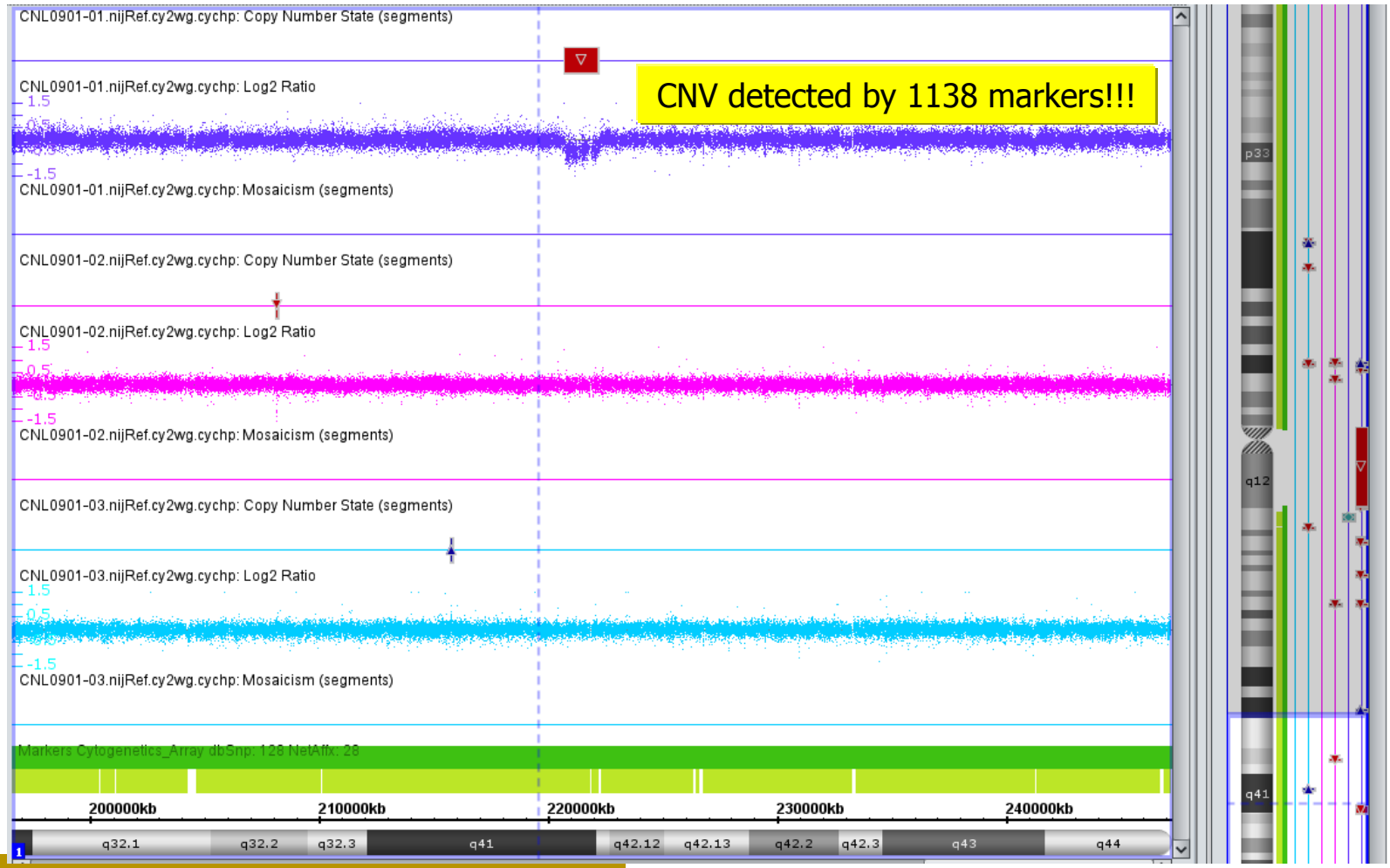
2,300,000 CNP/ 400,000 SNP

Example trio: 1.4 Mb *de novo* 1q41 deletion

Patient

Mother

Father



General Conclusions

- Rare *de novo* CNVs explain a significant proportion of mental retardation
- Microarray-based CNV profiling will soon be the first and is most cases the only diagnostic genetic test in mental retardation and or MCA
- Many very good platforms available, with/without genotype info
- A large control dataset is required for clinical interpretation!

Acknowledgements

UMC St Radboud

Nico Leijsten
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Marloes Steehouwer
Joris Veltman

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