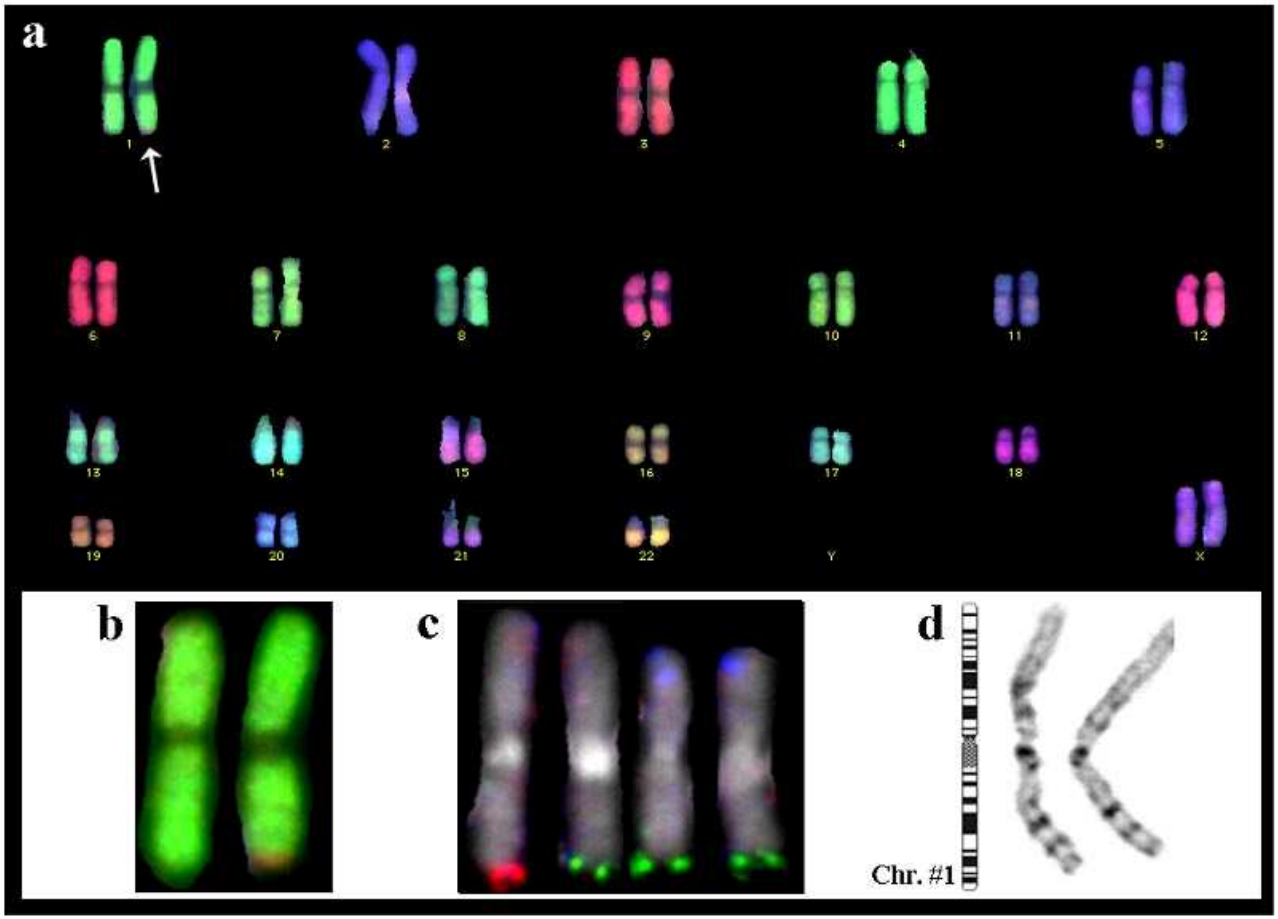
Array-CGH - 5 years in Brazil

2004-2009

Cytogenetics in 2000

- ~ half of patients with MR had no diagnosis
  - No precise risk of recurrence
  - No carrier detection
  - No prenatal diagnosis

del 1qter / dup 3qter



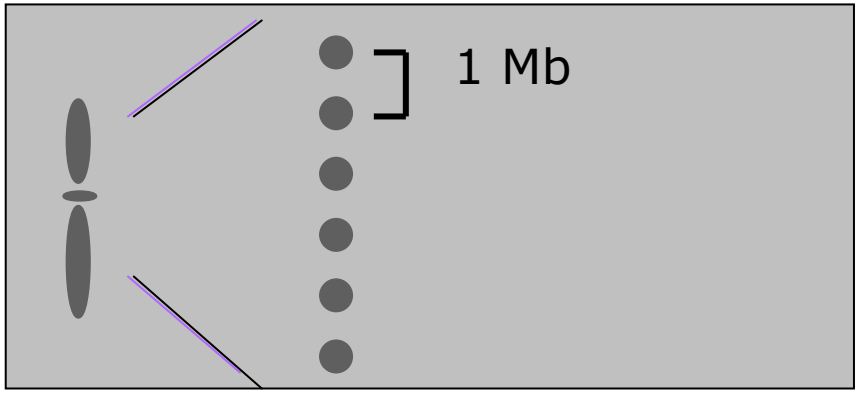
Since beginning of 90's, it was known that submicroscopic alterations accounted for some of the MR (Flint/ subtelomeres)

- FISH – high-resolution but limited number the sequences per time

- How to investigate a large number of target simultaneously:

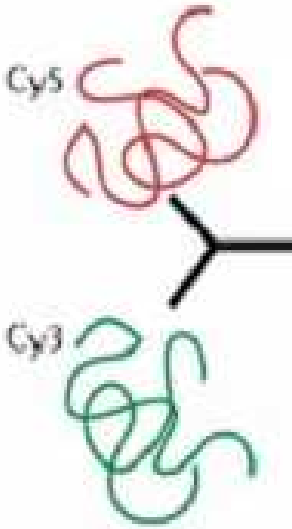
  - Array-CGH conceived by Pinkel and published by Lichter.

Array-CGH – 2000
Array-based Comparative Genomic Hybridization

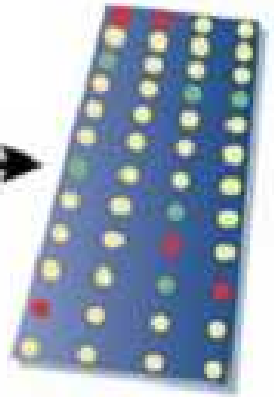


Markers (DNA sequences ordered on the chromosomes)

Reference DNA



cot1

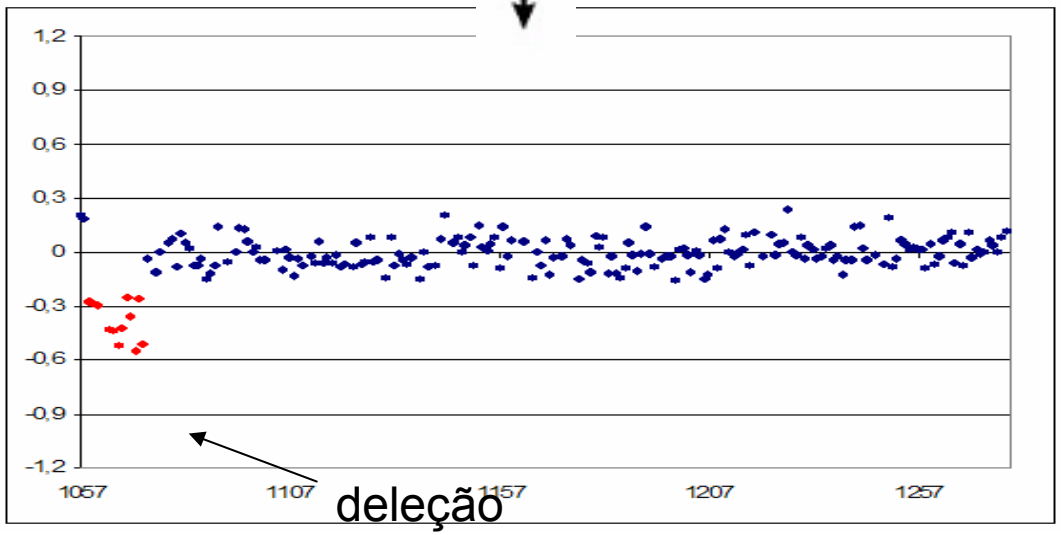


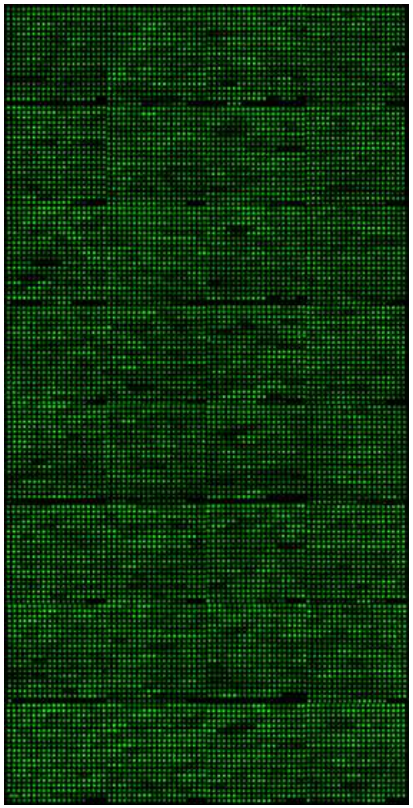
Array hybridization

- deleted
- Normal
- amplified

Test DNA

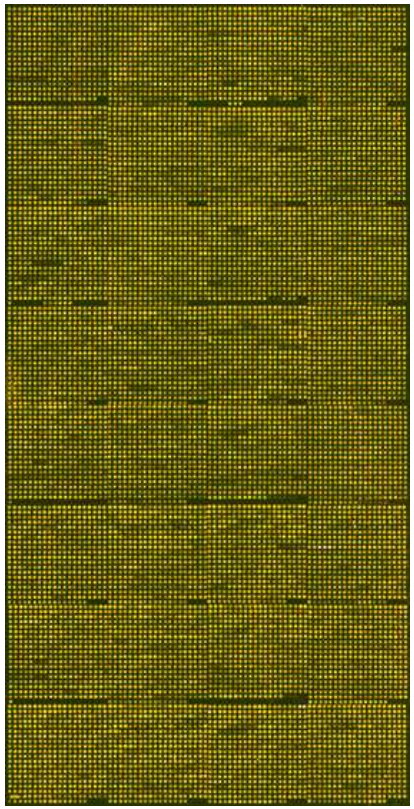
Detect and measure intensities

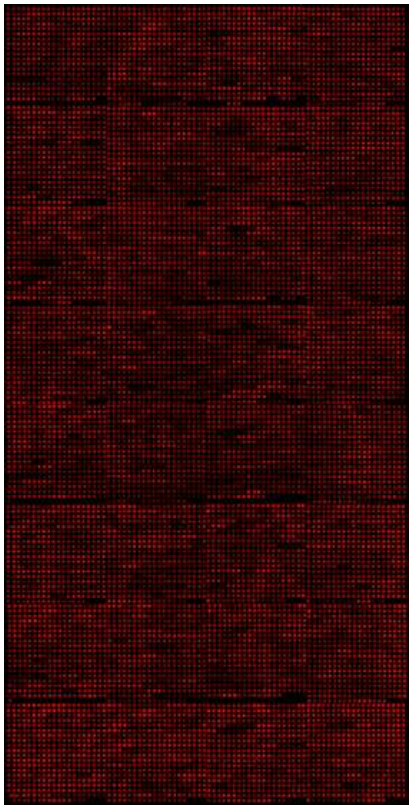




Test DNA

Superposed images

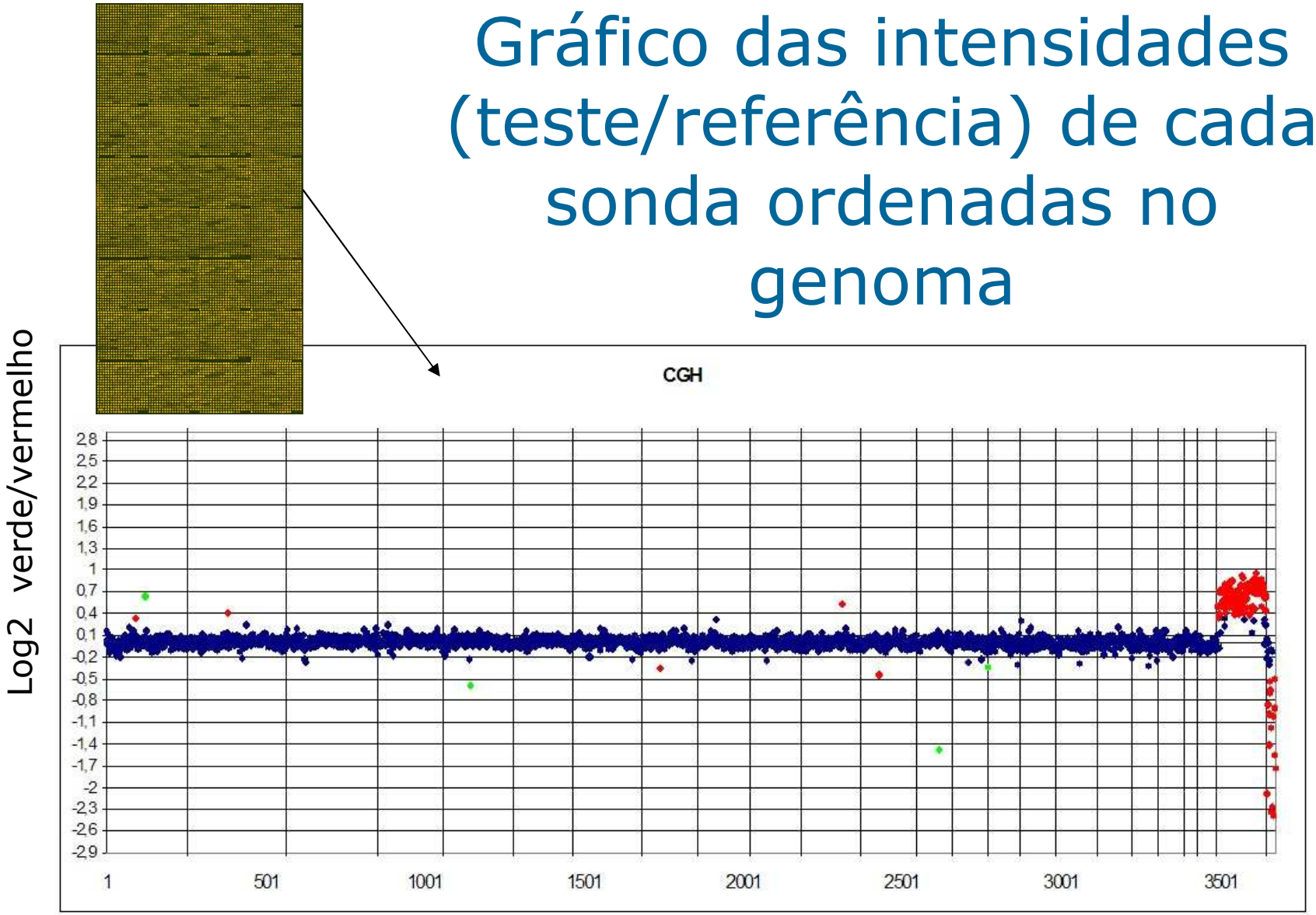




Reference DNA

•3500 BACs at ~1 Mb distribution in the genome

Gráfico das intensidades (teste/referência) de cada sonda ordenadas no genoma



sondas

1p → Yq

5 year USP

Projeto “Doelmatigheid” (“cust benefit”)

- 2002-2003
- Project at LUMC
  - “Improved prevention of mental retardation by the use of genome micro-array analysis as a complementary tool to chromosome diagnosis”.
- Built up array CGH.
- Investigation of idiophatic mental retardation.

Genomic arrays:

- 3500 BACs/PACs:
  - Markers spaced at ~ 1 Mb whole genome.
  - Subtelomeric probes (Flint)
- Set of probes from the Sanger Center (based on the human genome sequence)
  - BAC-end sequences verified
  - Only 20% probes FISH verified
  - Constantly verified by users (freely distributed among academic institutions)

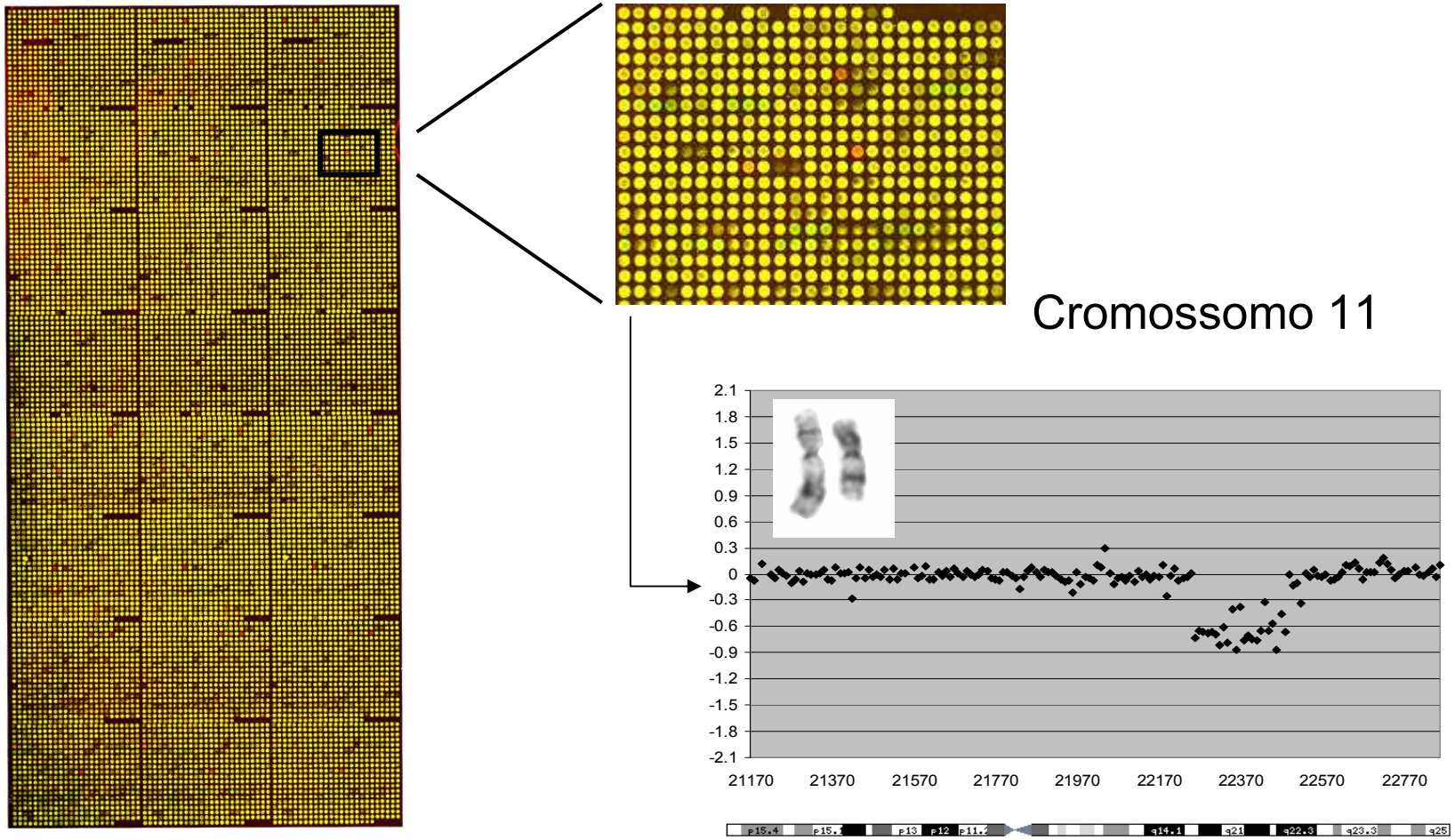
Protocols: Workshop Sanger Center

- Workshop 2002 (Welcome Trust):
  - Carter et al:*Cytometry* 2002;49(2):43-48
- Publication:
  - Fiegler et al:*Genes Chromosomes Cancer* 2003;36(4):361-74
  - New protocols used about 10% of the DNA used in previous arrays and obtained much better quality.

Array Production (LUMC)

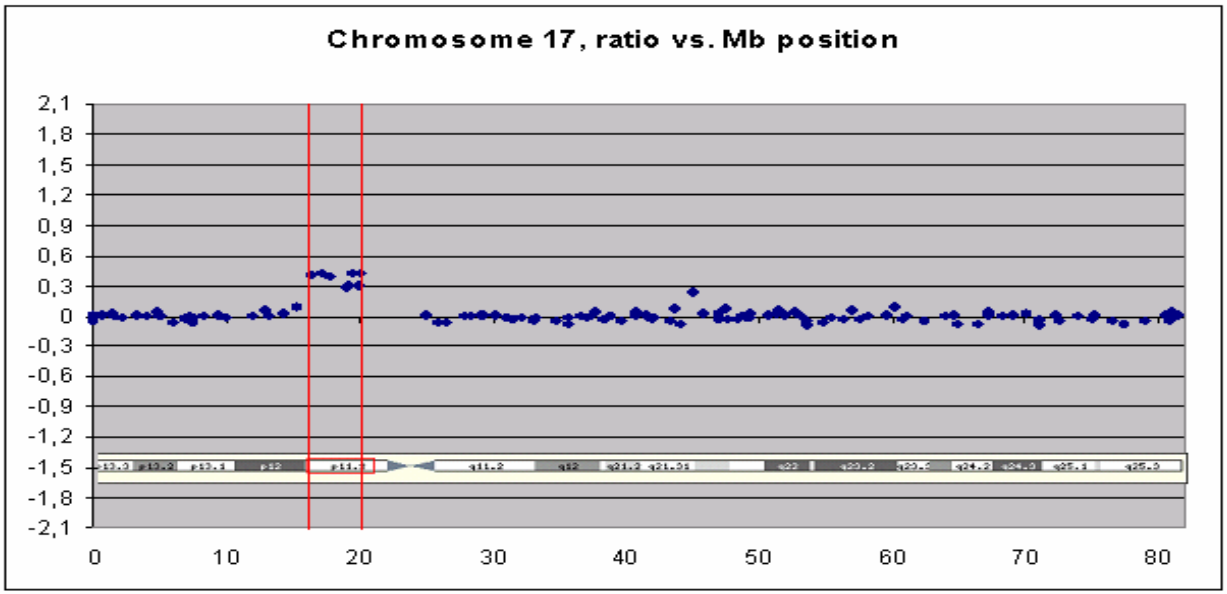
- Grow 3500 Bacterias
- Isolate 3.500 BAC DNAs
- BAC DNA amplification:
  - = ~ 20.000 PCRs

1 Mb arrays: Tested in cell lines



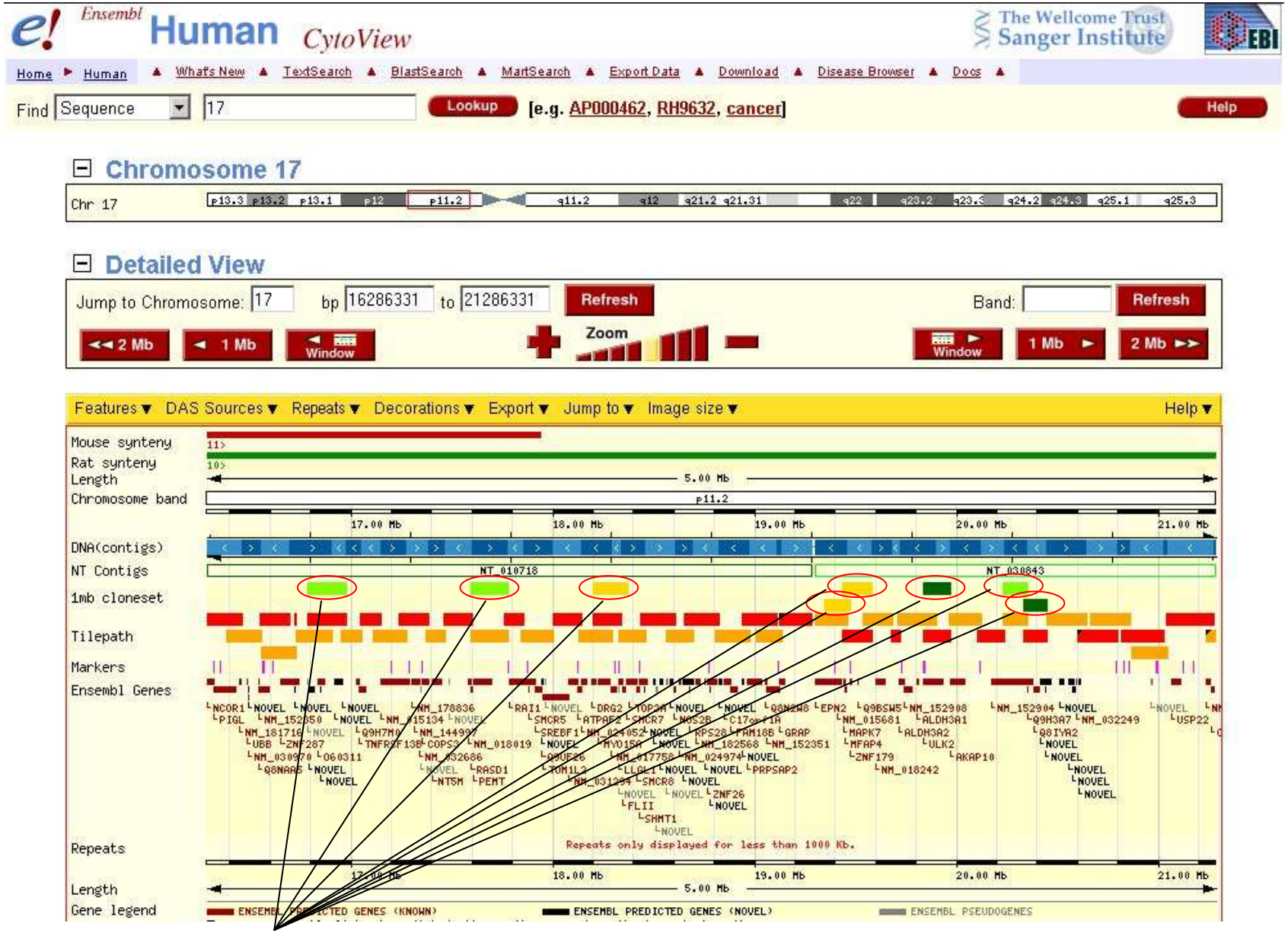
Amer. J. Med. Genet, in press

Amplification on 17p



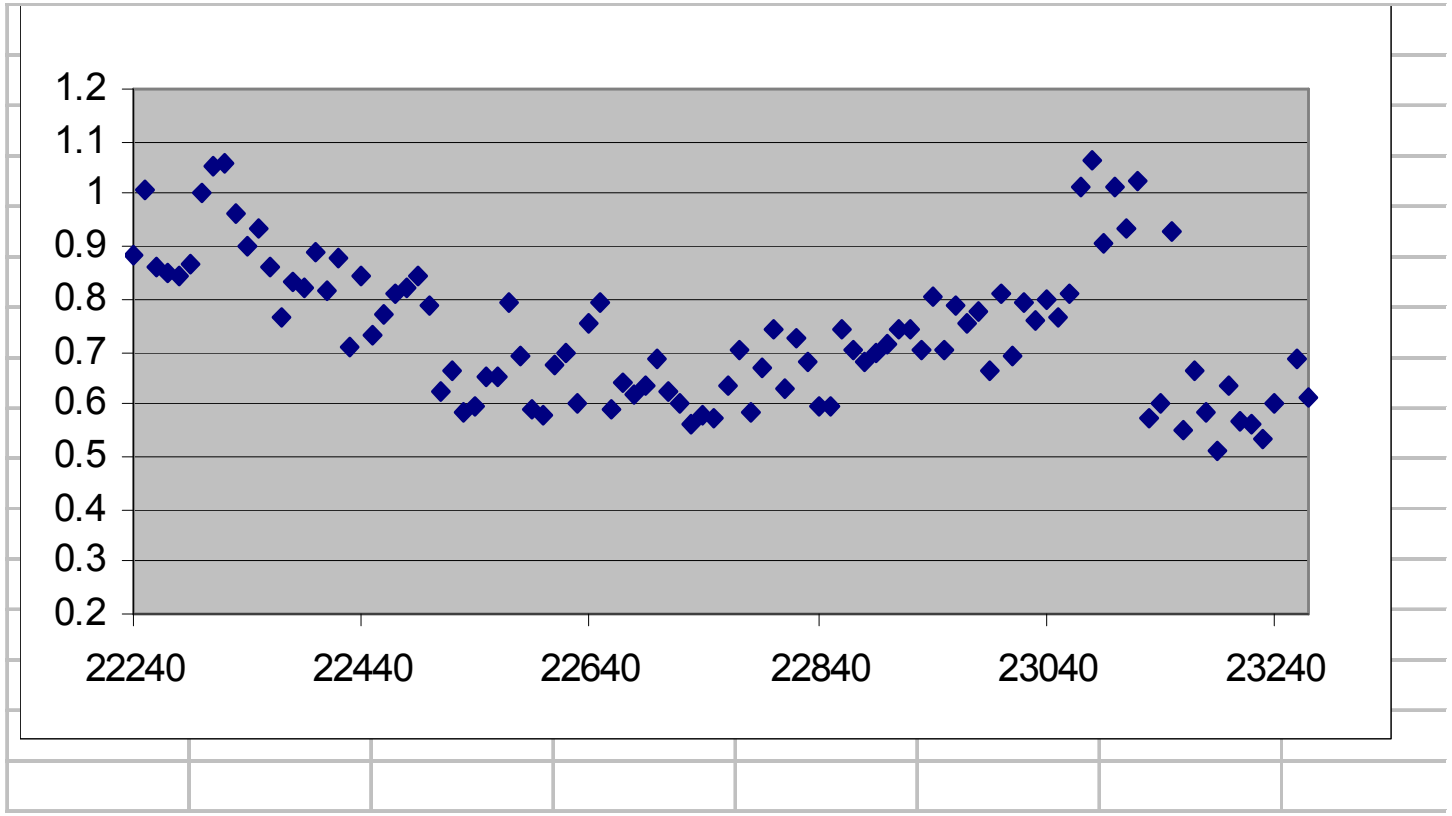
Unique position number	Clone name	Chromosome	Mb position
29230	RP11-219A15	17	16528821
29240	RP11-524F11	17	17340469
29250	RP11-189D22	17	17942724
29260	RP1-162E17	17	19090463
29270	CTB-1187M2	17	19175621
29280	RP11-78O7	17	19577928
29290	RP5-836L9	17	19977261
29300	RP11-121A13	17	20075917

3.5 Mb amplification



Amplified clones

http://www.ensembl.org/Homo_sapiens/cytoview?chr=17&vc_start=16286331&vc_end=21286331



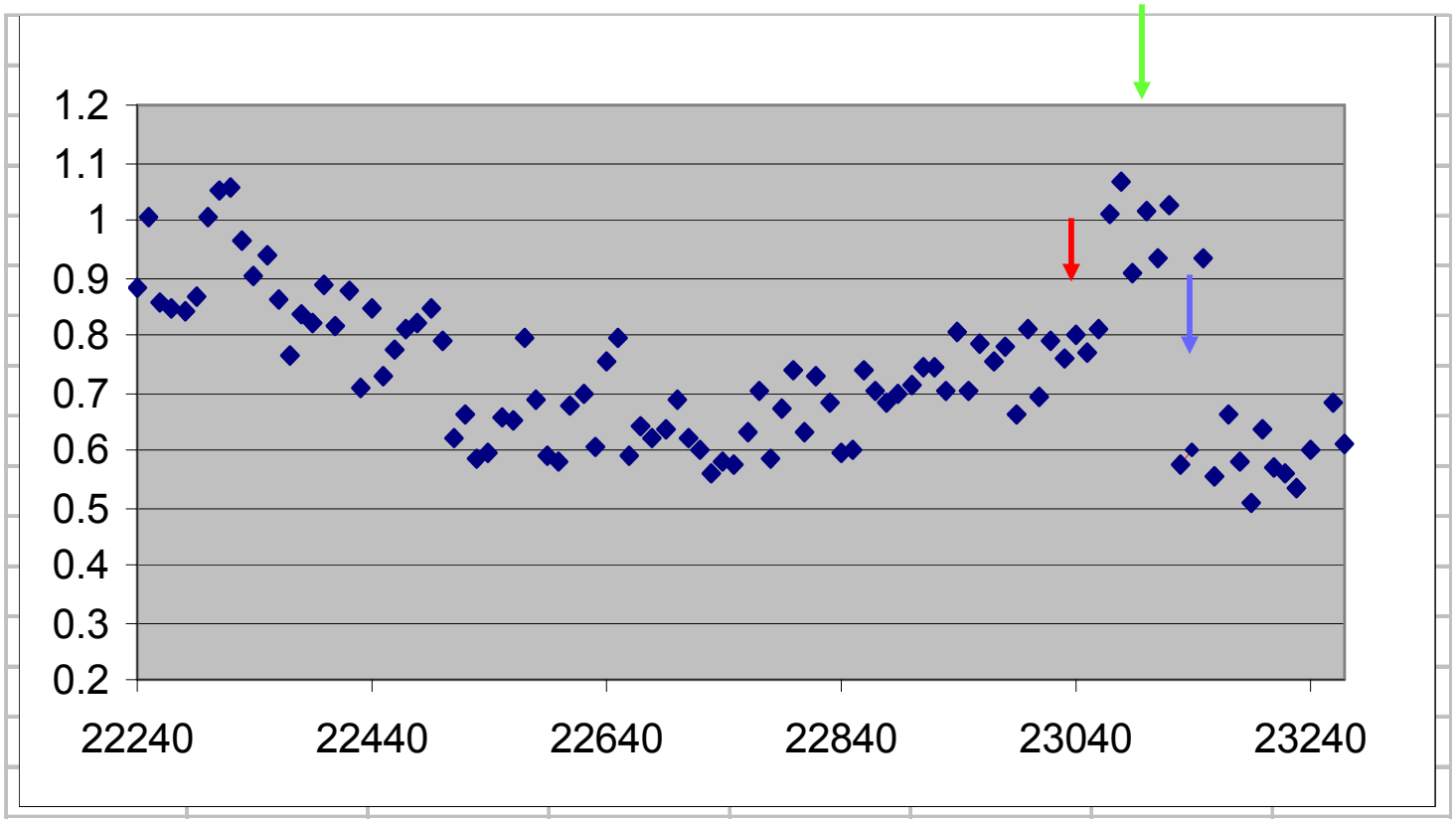
q?-q12.3

q12.3-q33.2

pter-q?

q12.3-q32.1

q31.1-qter


1.2
1.1
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0.9
0.8
0.7
0.6
0.5
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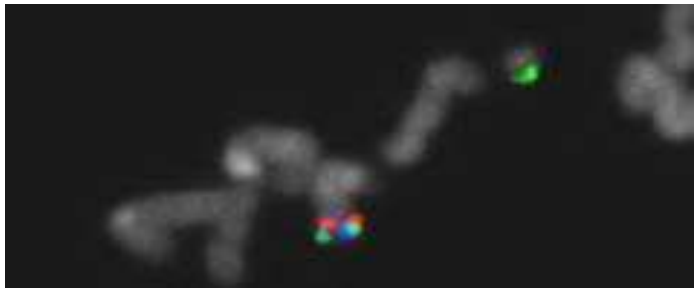
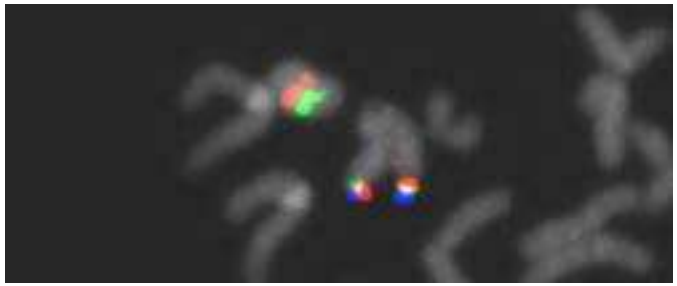
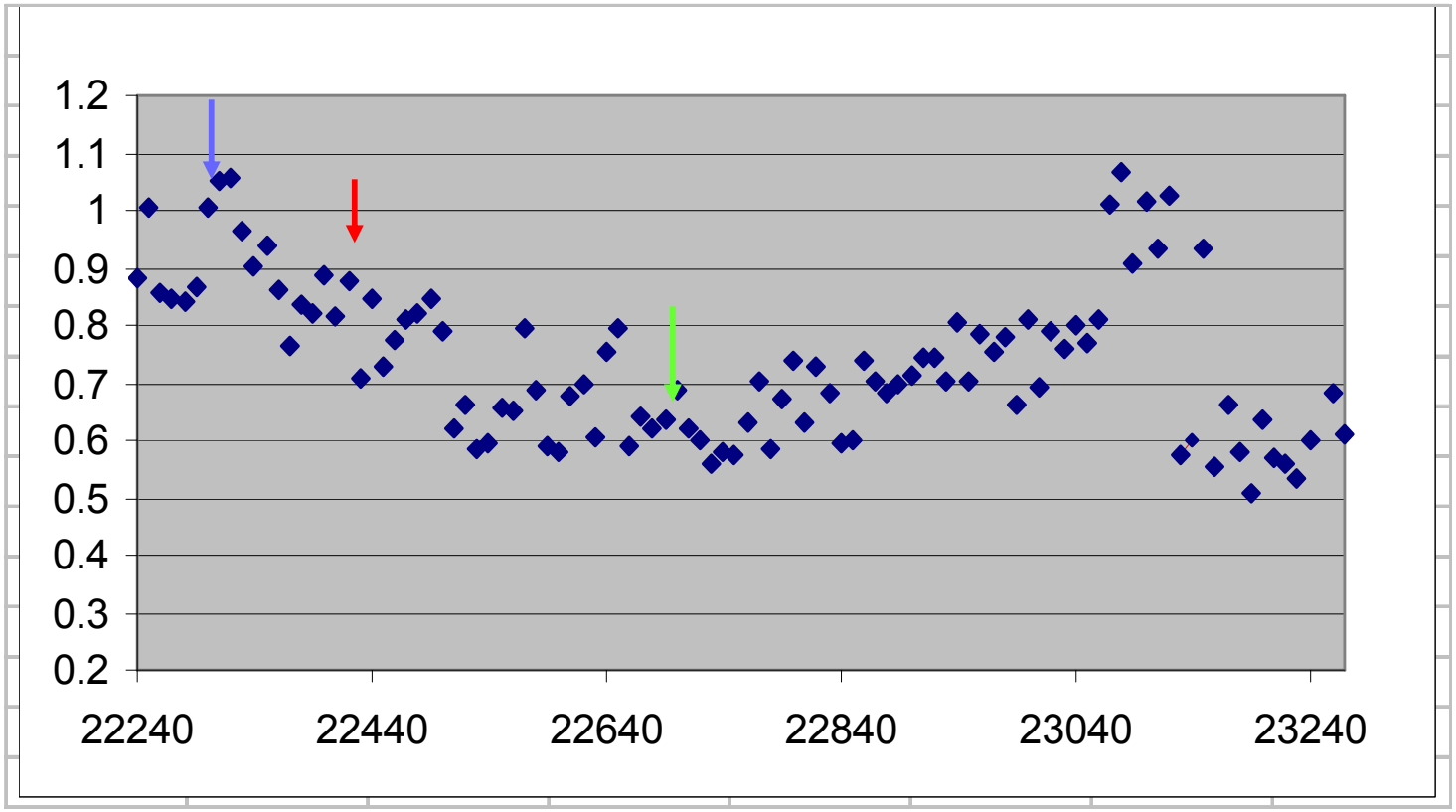
22640
22840
23040
23240



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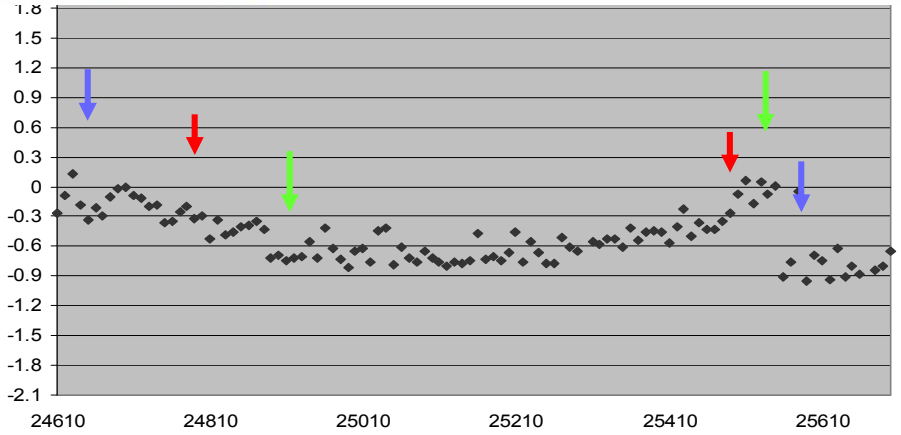
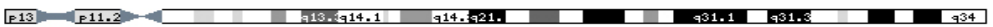
0.5
0.4
0.3
0.2
22240
22440
22640
22840
23040
23240

American Journal of Medical Genetics 132A:36–40 (2005)

Insights From Genomic Microarrays Into Structural Chromosome Rearrangements

Jeroen Knijnenburg,[1] Károly Szuhai,[1] Jacques Giltay,[3] Lia Molenaar,[2] Willem Sloos,[1] Martin Poot,[3] Hans J. Tanke,[1] and Carla Rosenberg[1*]





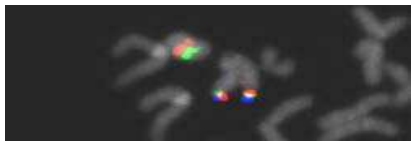
pter-q?

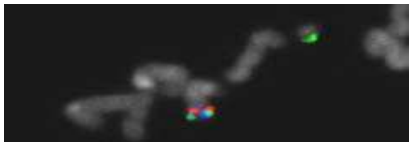
q12.3-q32.1

q31.1-qter

Secondary deletion

Primary deletion





Brazil – 2004_2008

- Arrays produced in Leiden.
- Genetic counseling at University of São Paulo
  - Service started >40 years ago.
  - Insufficiente to provide genetic counseling to all in need.
  - It has no financing
  - Faculties see patients connected to their own projects using research money
    - i.e., craniofacial anomalies, obesity, XLMR, etc.

Brazil – 2004_2008

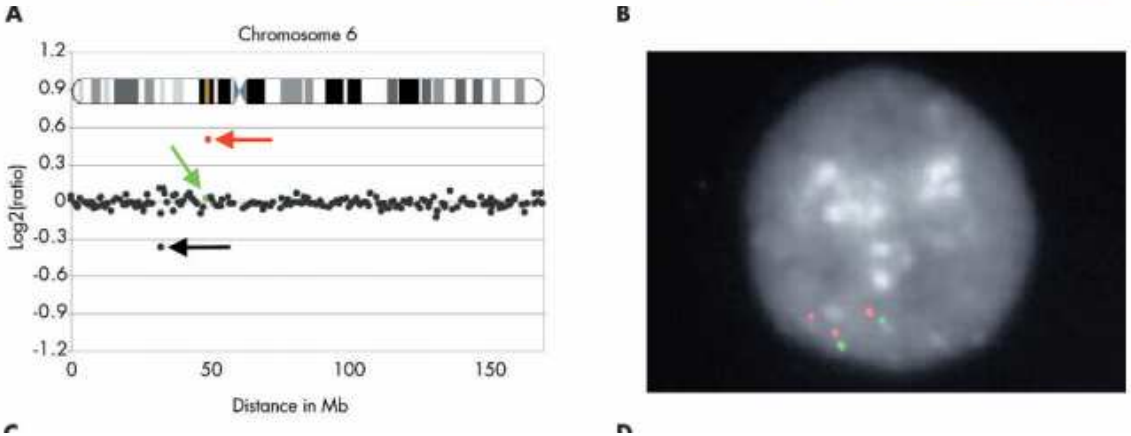
- Arrays produced in Leiden were provided free of charge.
- ~ 400 families with unexplained MR and/or congenital abnormalities.

LETTER TO JMG

Array-CGH detection of micro rearrangements in mentally retarded individuals: clinical significance of imbalances present both in affected children and normal parents

C Rosenberg*, J Knijnenburg*, E Bakker, A M Vianna-Morgante, W Sloos, P A Otto, M Kriek, K Hansson, A C V Krepischi-Santos, H Fiegler, N P Carter, E K Bijlsma, A van Haeringen, K Szuhai, H J Tanke

J Med Genet 2006;43:180–186. doi: 10.1136/jmg.2005.032268



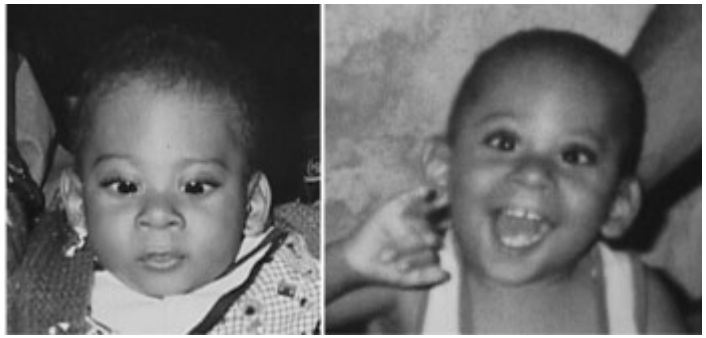
Submicroscopic chromosome imbalances in 16% of the patients (*de novo* or inherited from a balanced parent)

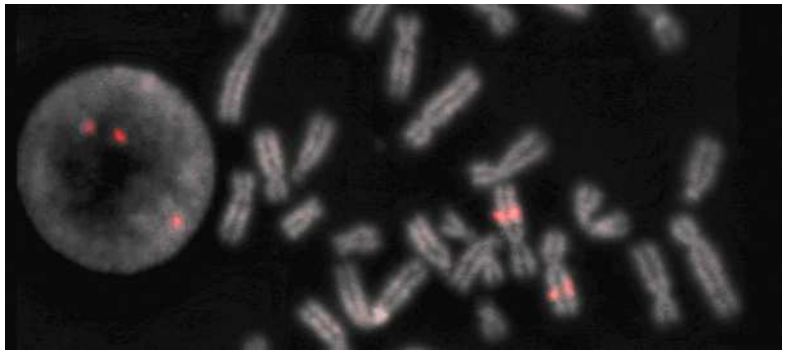
American Journal of Medical Genetics 139A:221–226 (2005)

Clinical Report

An Xq22.3 Duplication Detected by Comparative Genomic Hybridization Microarray (*Array-CGH*) Defines a New Locus (*FGS5*) for FG Syndrome

Fernanda Sarquis Jehee,[1] Carla Rosenberg,[1] Ana Cristina Krepischi-Santos,[1] Fernando Kok,[2] Jeroen Knijnenburg,[3] Guy Froyen,[4] Angela M. Vianna-Morgante,[1] John M. Opitz,[5] and Maria Rita Passos-Bueno[1]*





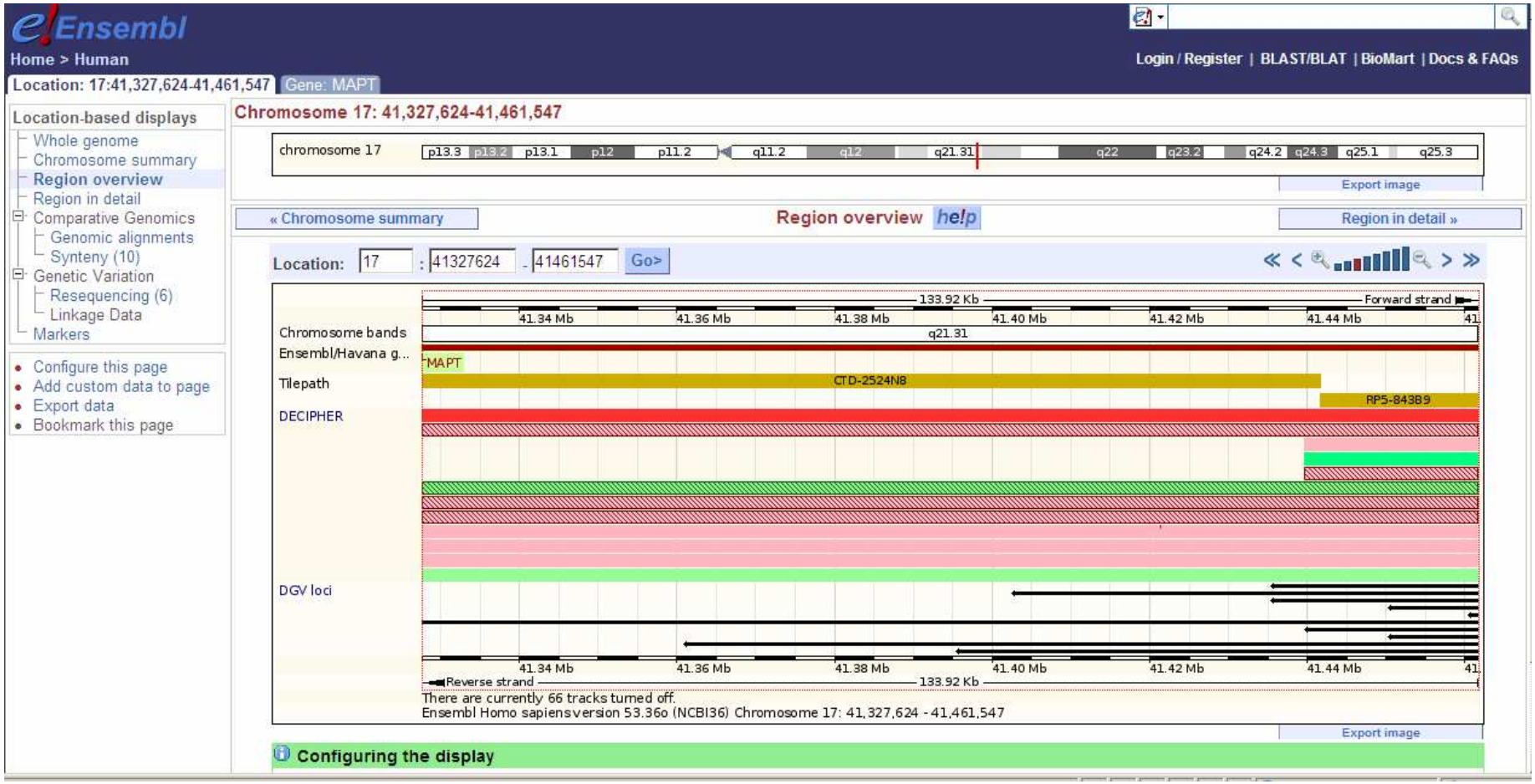
Carrier mother

28% idiophatic craniosynostosis showed submicroscopic chromosome alterations.

Frequency of submicroscopic chromosome alterations

- ~ 16 among patients ascertained because of MR
- But up to 30% if patients ascertained by a criteria other than MR.

DECIPHER


Ensembl
Home > Human
Login / Register | BLAST/BLAT | BioMart | Docs & FAQs
Location: 17:41,327,624-41,461,547
Gene: MAPT
Location-based displays
Whole genome
Chromosome summary
Region overview
Region in detail
Comparative Genomics
Genomic alignments
Synteny (10)
Genetic Variation
Resequencing (6)
Linkage Data
Markers
Configure this page
Add custom data to page
Export data
Bookmark this page
Chromosome 17: 41,327,624-41,461,547
chromosome 17
Export image
« Chromosome summary
Region overview
help
Region in detail »
Location: 17 : 41327624 - 41461547 Go>
133.92 Kb
Forward strand
41.34 Mb
41.36 Mb
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41.40 Mb
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Chromosome bands
q21.31
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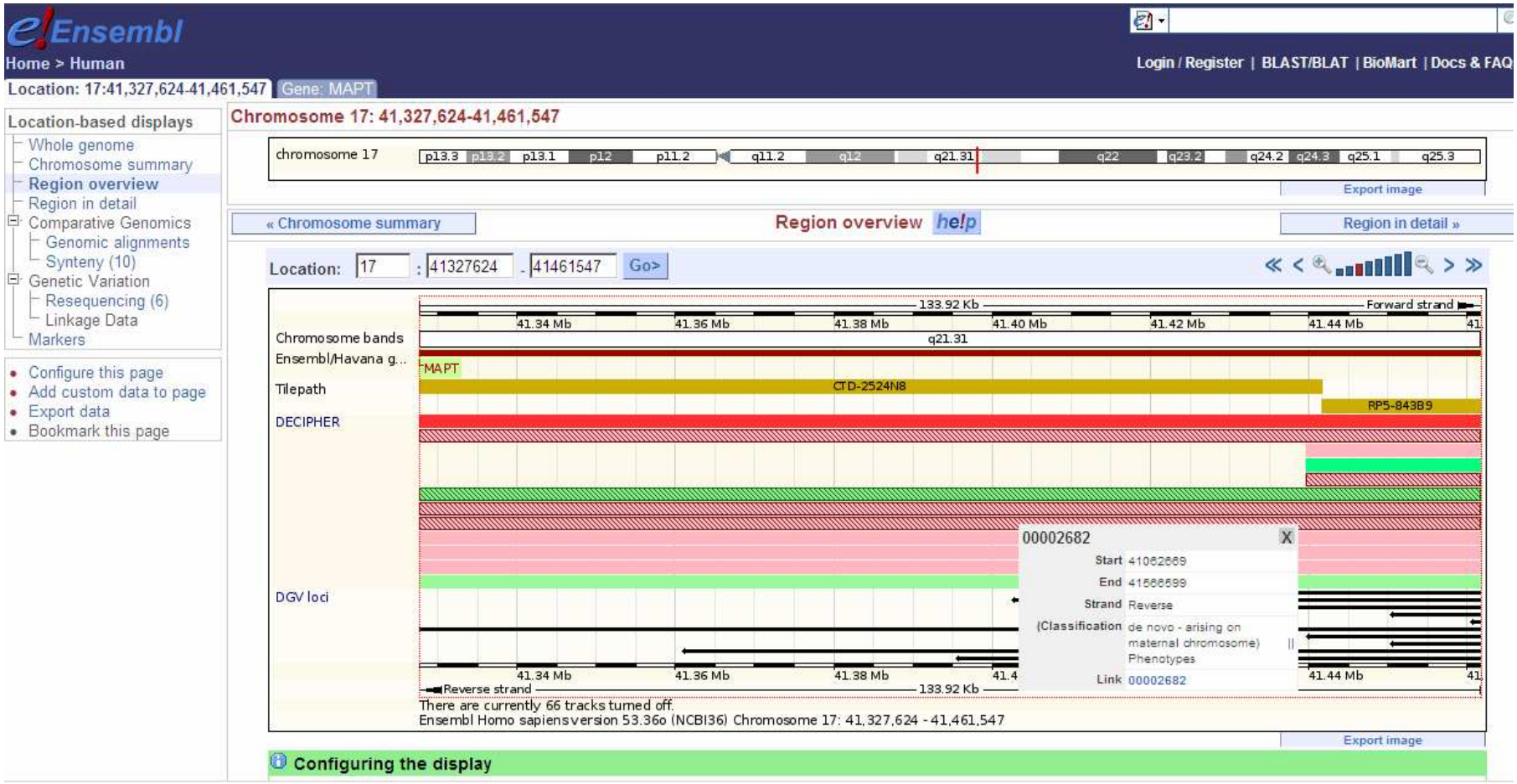
MAPT
Tilepath
CTD-2524N8
RP5-843B9
DECIPHER
DGV loci
Reverse strand
There are currently 66 tracks turned off.
Ensembl Homo sapiens version 53.36o (NCBI36) Chromosome 17: 41,327,624 - 41,461,547
Configuring the display


e!Ensembl
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Location: 17:41,327,624-41,461,547
Gene: MAPT
Location-based displays
Whole genome
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Region overview
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Comparative Genomics
Genomic alignments
Synteny (10)
Genetic Variation
Resequencing (6)
Linkage Data
Markers
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Add custom data to page
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Bookmark this page
Chromosome 17: 41,327,624-41,461,547
chromosome 17
p13.3 p13.2 p13.1 p12 p11.2 q11.2 q12 q21.31 q22 q23.2 q24.2 q24.3 q25.1 q25.3
Export image
« Chromosome summary
Region overview
help
Region in detail »
Location: 17 : 41327624 - 41461547 Go>
133.92 Kb
Forward strand
41.34 Mb 41.36 Mb 41.38 Mb 41.40 Mb 41.42 Mb 41.44 Mb
Chromosome bands
q21.31
Ensembl/Havana g...
MAPT
Tilepath
CTD-2524N8
RP5-843B9
DECIPHER
DGV loci
00002682
Start 41062669
End 41566599
Strand Reverse
(Classification de novo - arising on maternal chromosome) Phenotypes
Link 00002682
Reverse strand
There are currently 66 tracks turned off.
Ensembl Homo sapiens version 53.36o (NCBI36) Chromosome 17: 41,327,624 - 41,461,547
Configuring the display

nature genetics

published online 13 August 2006; doi:10.1038/ng1858

Microdeletion encompassing *MAPT* at chromosome 17q21.3 is associated with developmental delay and learning disability

homologous recombination.

making the deletions identical in size at this level of resolution

In clinical cytogenetics, the phenotypic recognition of microdeletion syndromes has usually preceded the elucidation of the underlying causative cytogenetic imbalance. Velocardiofacial syndrome, Prader-Willi syndrome and Williams syndrome were all described clinically before the cytogenetic imbalances responsible for these syndromes were identified[7]. In contrast, the introduction of FISH-based screening for subtelomeric chromosomal imbalances[8,9] has led to the cytogenetic characterization of new syn-

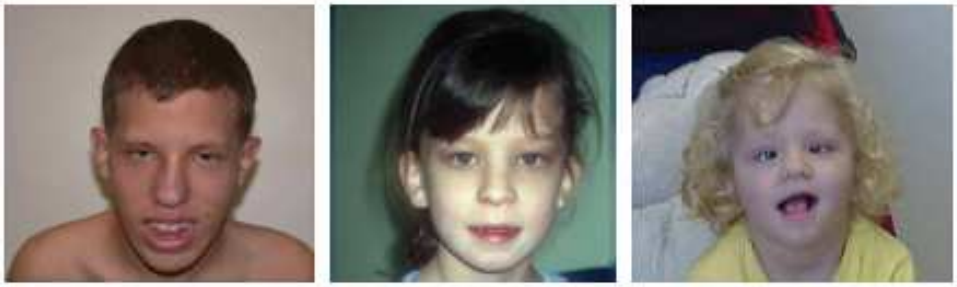

Figure 1 Clinical photographs of affected individuals. Craniofacial dysmorphic features are presented for each case in **Table 1**. We obtained informed consent to publish the photographs above.

Journal of
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Published Online First: 15 July 2008. doi:10.1136/jmg.2008.058701
Journal of Medical Genetics 2008;**45**:710-720

ORIGINAL ARTICLES

Clinical and molecular delineation of the 17q21.31 microdeletion syndrome

D A Koolen[1], A J Sharp[2,3], J A Hurst[4], H V Firth[5], S J L Knight[6], A Goldenberg[7], P Saugier-Veber[7], R Pfundt[1], L E L M Vissers[1], A Destrée[8], B Grisart[8], L Rooms[9], N Van der Aa[10], M Field[11], A Hackett[11], K Bell[12], M J M Nowaczyk[13], G M S Mancini[14], P J Poddighe[14], C E Schwartz[15], E Rossi[16], M De Gregori[16], L L Antonacci-Fulton[18], M D McLellan II[18], J M Garrett[18], M A Wiechert[18], T L Miner[18], S Crosby[18], R Ciccone[16], L Willatt[5], A Rauch[19], M Zenker[19], S Aradhya[20], M A Manning[21], T M Strom[22], J Wagenstaller[22], A C Krepischi-Santos[23], A M Vianna-Morgante[23], C Rosenberg[23], S M Price[4], H Stewart[4], C Shaw-Smith[5], H G Brunner[1], A O M Wilkie[24], J A Veltman[1], O Zuffardi[16,17], E E Eichler[2,25] and B B A de Vries[1]

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  jmg.2008.058701v2
  jmg.2008.058701v3
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Services

17q21.31 deletion = ~ 0.7% of all mental retardation

nature genetics published online 13 August 2006; doi:10.1038/ng1858

Copy number variation associated to specific syndromes

Microdeletion encompassing *MAPT* at chromosome 17q21.3 is associated with developmental delay and learning disability

homologous recombination.

making the deletions identical in size at this level of resolution

In clinical cytogenetics, the phenotypic recognition of microdeletion syndromes has usually preceded the elucidation of the underlying causative cytogenetic imbalance. Velocardiofacial syndrome, Prader-Willi syndrome and Williams syndrome were all described clinically before the cytogenetic imbalances responsible for these syndromes were identified[7]. In contrast, the introduction of FISH-based screening for subtelomeric chromosomal imbalances[8,9] has led to the cytogenetic characterization of new syn-

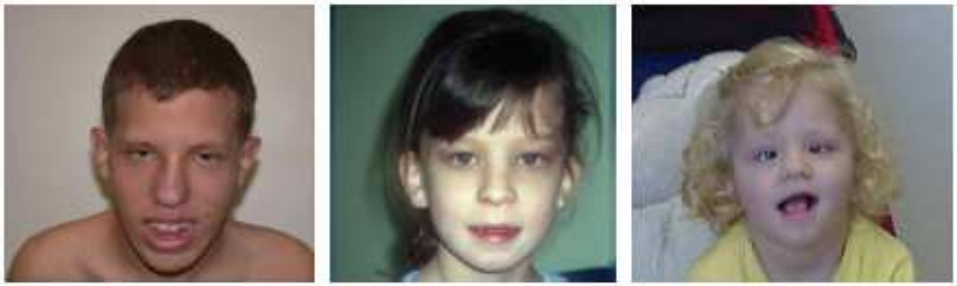

Figure 1 Clinical photographs of affected individuals. Craniofacial dysmorphic features are presented for each case in **Table 1**. We obtained informed consent to publish the photographs above.

But…

Many CNVs not obviously associated to anything

Large scale variation of DNA segments(50 kb – 2 Mb) em larga escala.

Detection of large-scale variation in the human genome

A John Iafrate[1,2], Lars Feuk[3], Miguel N Rivera[1,2], Marc L Listewnik[1], Patricia K Donahoe[2,4], Ying Qi[3], Stephen W Scherer[3,5] & Charles Lee[1,2,5]

Large-Scale Copy Number Polymorphism in the Human Genome

Jonathan Sebat,[1] B. Lakshmi,[1] Jennifer Troge,[1] Joan Alexander,[1] Janet Young,[2] Pär Lundin,[3] Susanne Månér,[3] Hillary Massa,[2] Megan Walker,[2] Maoyen Chi,[1] Nicholas Navin,[1] Robert Lucito,[1] John Healy,[1] James Hicks,[1] Kenny Ye,[4] Andrew Reiner,[1] T. Conrad Gilliam,[5] Barbara Trask,[2] Nick Patterson,[6] Anders Zetterberg,[3] Michael Wigler[1]*

Fine-scale structural variation of the human genome

Eray Tuzun[1,5], Andrew J Sharp[1,5], Jeffrey A Bailey[2,5], Rajinder Kaul[3], V Anne Morrison[1], Lisa M Pertz[2], Eric Haugen[3], Hillary Hayden[3], Donna Albertson[4], Daniel Pinkel[4], Maynard V Olson[3] & Evan E Eichler[1]

Inherited alterations

Array CGH Identifies Reciprocal 16p13.1 Duplications and Deletions That Predispose to Autism and/or Mental Retardation

Reinhard Ullmann,[1*] Gillian Turner,[2] Maria Kirchhoff,[3] Wei Chen,[1] Bruce Tonge,[4] Carla Rosenberg,[5] Michael Field,[2] Angela M. Vianna-Morgante,[5] Louise Christie,[2] Ana C. Krepischi-Santos,[5] Lynn Banna,[6] Avril V. Brereton,[4] Alyssa Hill,[2] Anne-Marie Bisgaard,[3] Ines Müller,[1] Claus Hultschig,[1] Fikret Erdogan,[1] Georg Wieczorek,[1] and H. Hilger Ropers[1]

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tractable, and easy to manage. Both are of normal physical

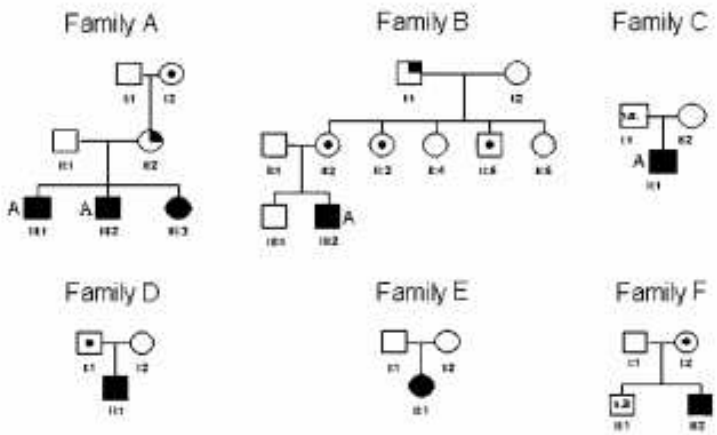

FIGURE 1. Pedigrees of all families analyzed in this study. Duplications were found in Families A to C, while deletions occurred in Families D to F. All affected patients were carriers of the duplication or deletion, respectively. Autistic patients are indicated by an "A" beside the symbol. ■, affected; □, unaffected; a dot in the symbol highlights unaffected carriers; n.a., not analyzed. Partially filled symbols indicate mildly affected patients.

Human Mutation DOI 10.1002/humu

Inherited alterations

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ScienceDirect

European Journal of Medical Genetics 51 (2008) 409–416

EUROPEAN JOURNAL OF MEDICAL GENETICS

http://www.elsevier.com/locate/ejmg

Original article

Private inherited microdeletion/microduplications: Implications in clinical practice

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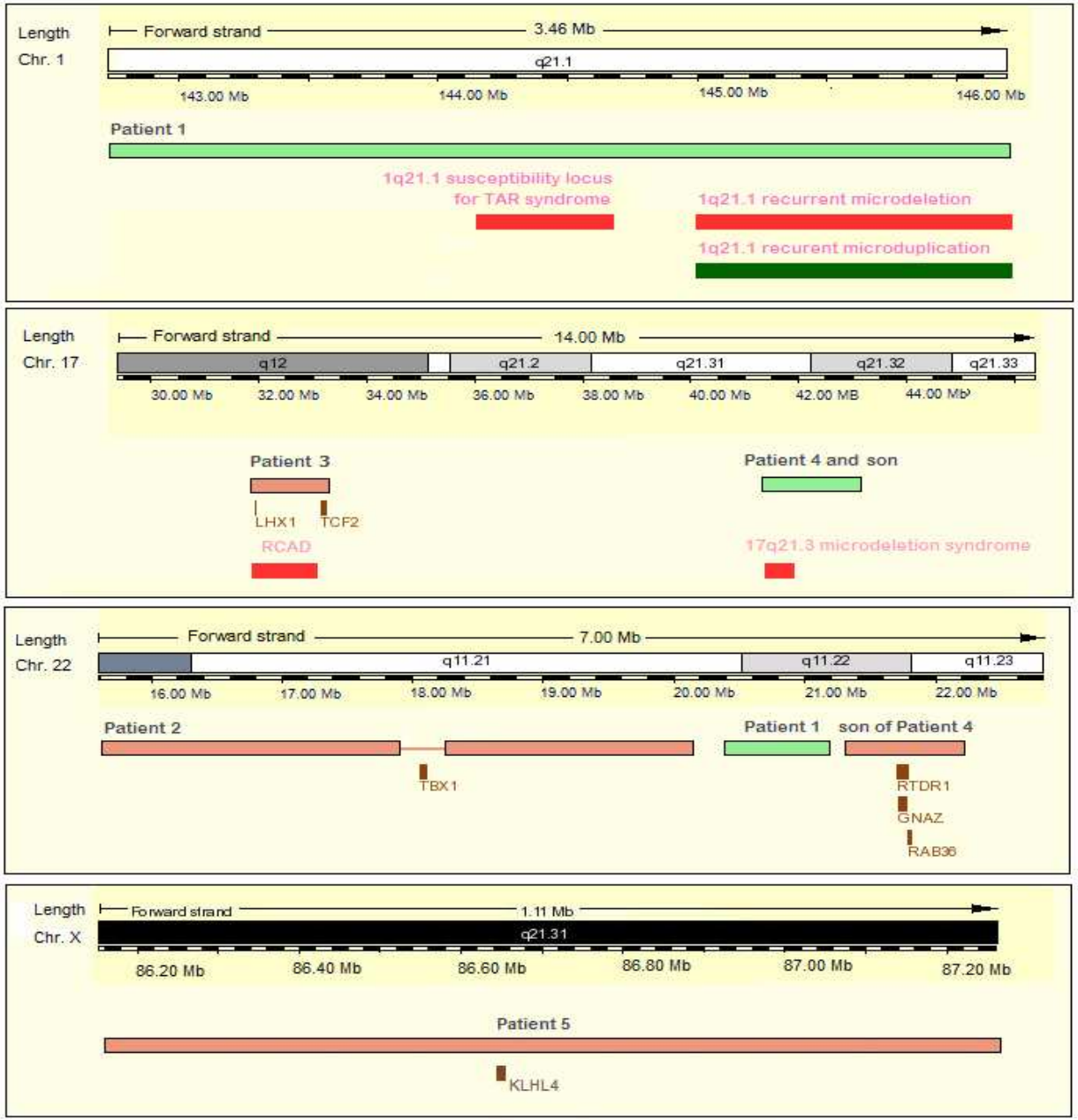
Genomic imbalances associated with müllerian aplasia

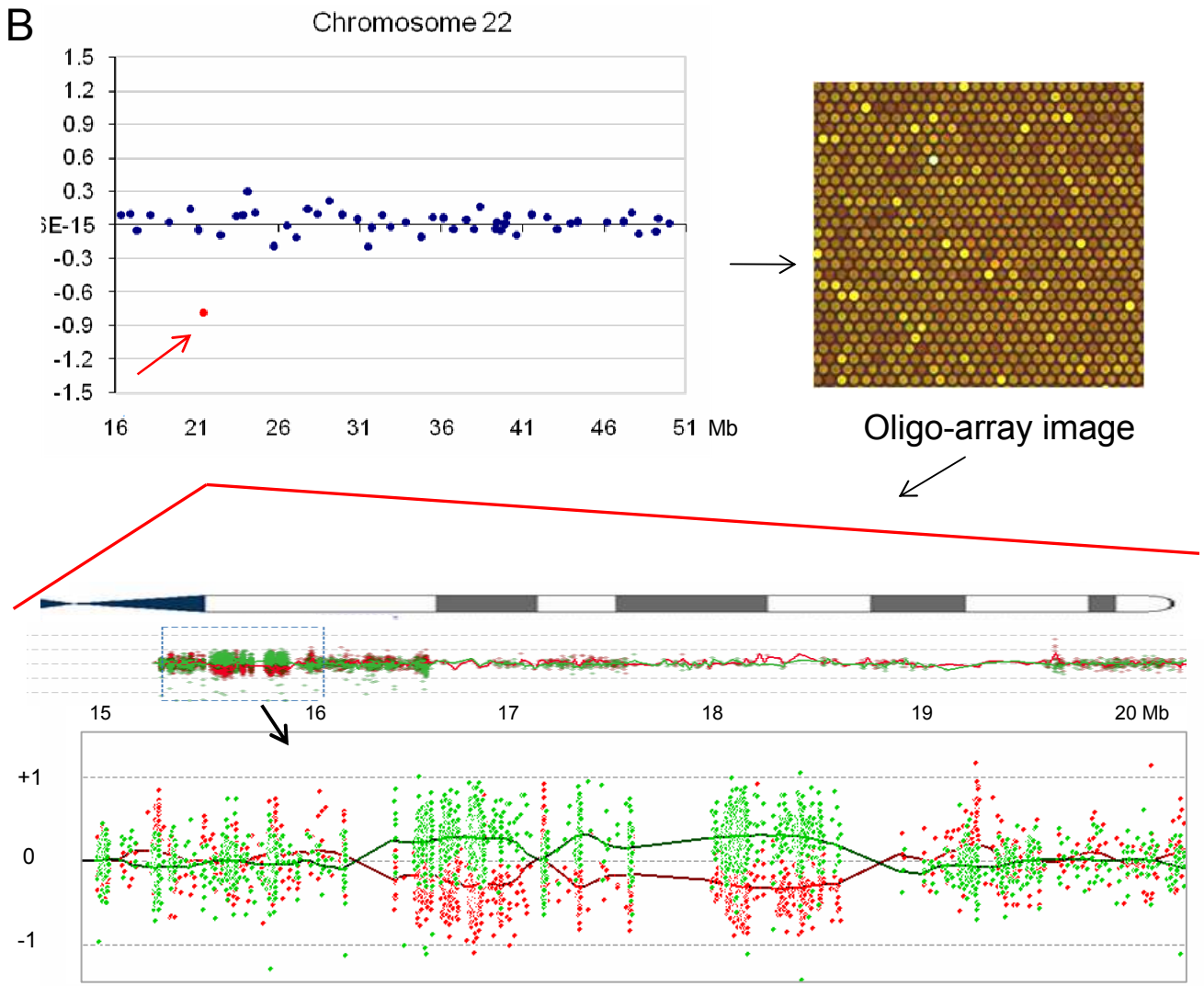
Carola Cheroki, Ana C V Krepischi-Santos, Károly Szuhai, Volker Brenner, Chong AE Kim, Paulo A Otto and Carla Rosenberg

J. Med. Genet. published online 26 Nov 2007;
doi:10.1136/jmg.2007.051839

-29% of patients (4/14)


Length
Chr. 1
Forward strand
3.46 Mb
q21.1
143.00 Mb
144.00 Mb
145.00 Mb
146.00 Mb
Patient 1
1q21.1 susceptibility locus
for TAR syndrome
1q21.1 recurrent microdeletion
1q21.1 recurent microduplication
Length
Chr. 17
Forward strand
14.00 Mb
q12
q21.2
q21.31
q21.32
q21.33
30.00 Mb
32.00 Mb
34.00 Mb
36.00 Mb
38.00 Mb
40.00 Mb
42.00 MB
44.00 Mb
Patient 3
LHX1
TCF2
RCAD
Patient 4 and son
17q21.3 microdeletion syndrome
Length
Chr. 22
Forward strand
7.00 Mb
q11.21
q11.22
q11.23
16.00 Mb
17.00 Mb
18.00 Mb
19.00 Mb
20.00 Mb
21.00 Mb
22.00 Mb
Patient 2
TBX1
Patient 1
son of Patient 4
RTDR1
GNAZ
RAB36
Length
Chr. X
Forward strand
1.11 Mb
q21.31
86.20 Mb
86.40 Mb
86.60 Mb
86.80 Mb
87.00 Mb
87.20 Mb
Patient 5
KLHL4

5 year USP



Wolters Kluwer Health | Lippincott Williams & Wilkins

Clin Dysmorphol. 2008 Jan;17(1):13-7

Links

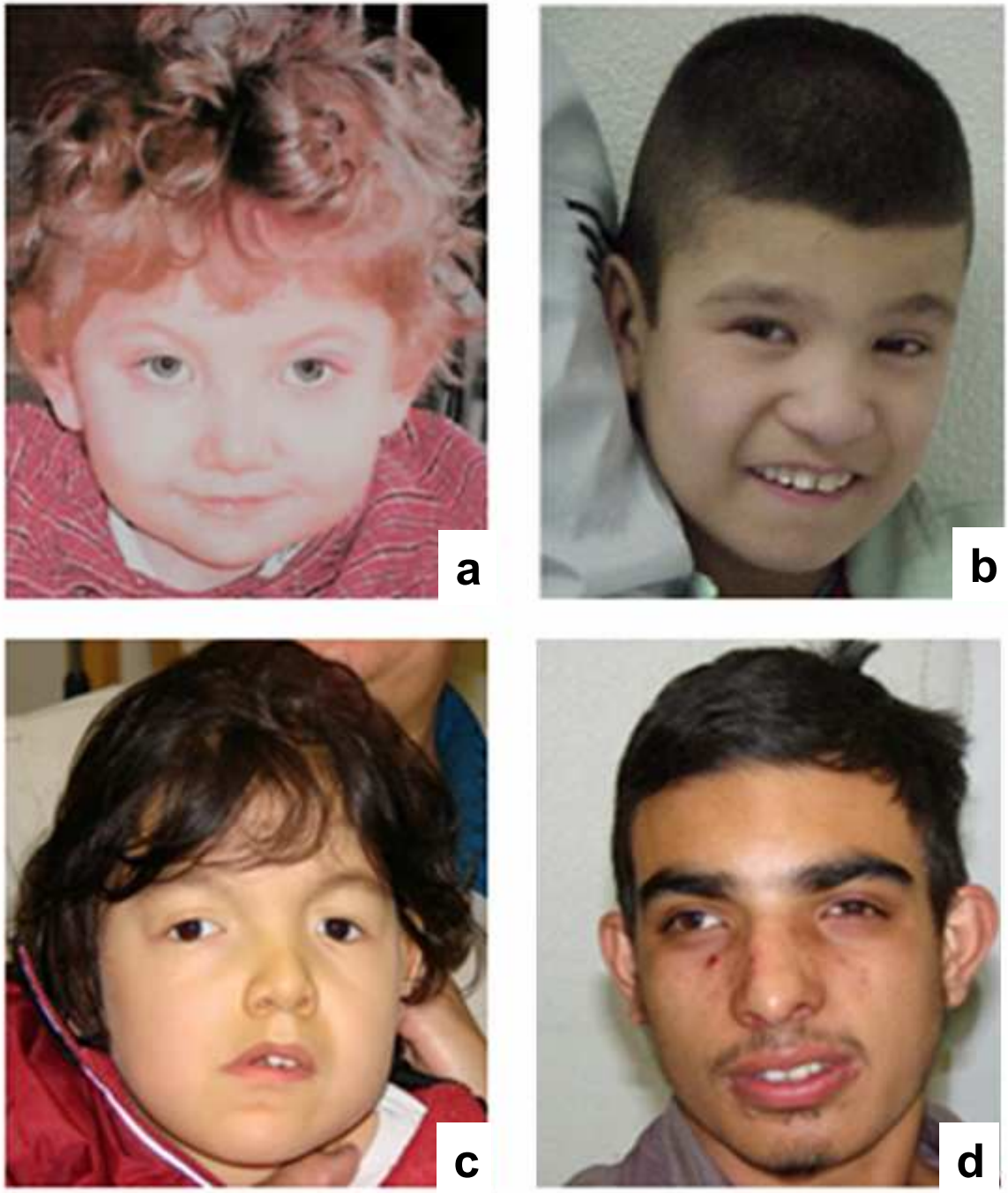
Expanding the phenotype of 22q11 deletion syndrome: the MURCS association.

Uliana V, Giordano N, Caselli R, Papa FT, Ariani F, Marcocci C, Gianetti E, Martini G, Papakostas P, Rollo F, Meloni I, Mari F, Priolo M, Renieri A, Nuti R.

After 2006

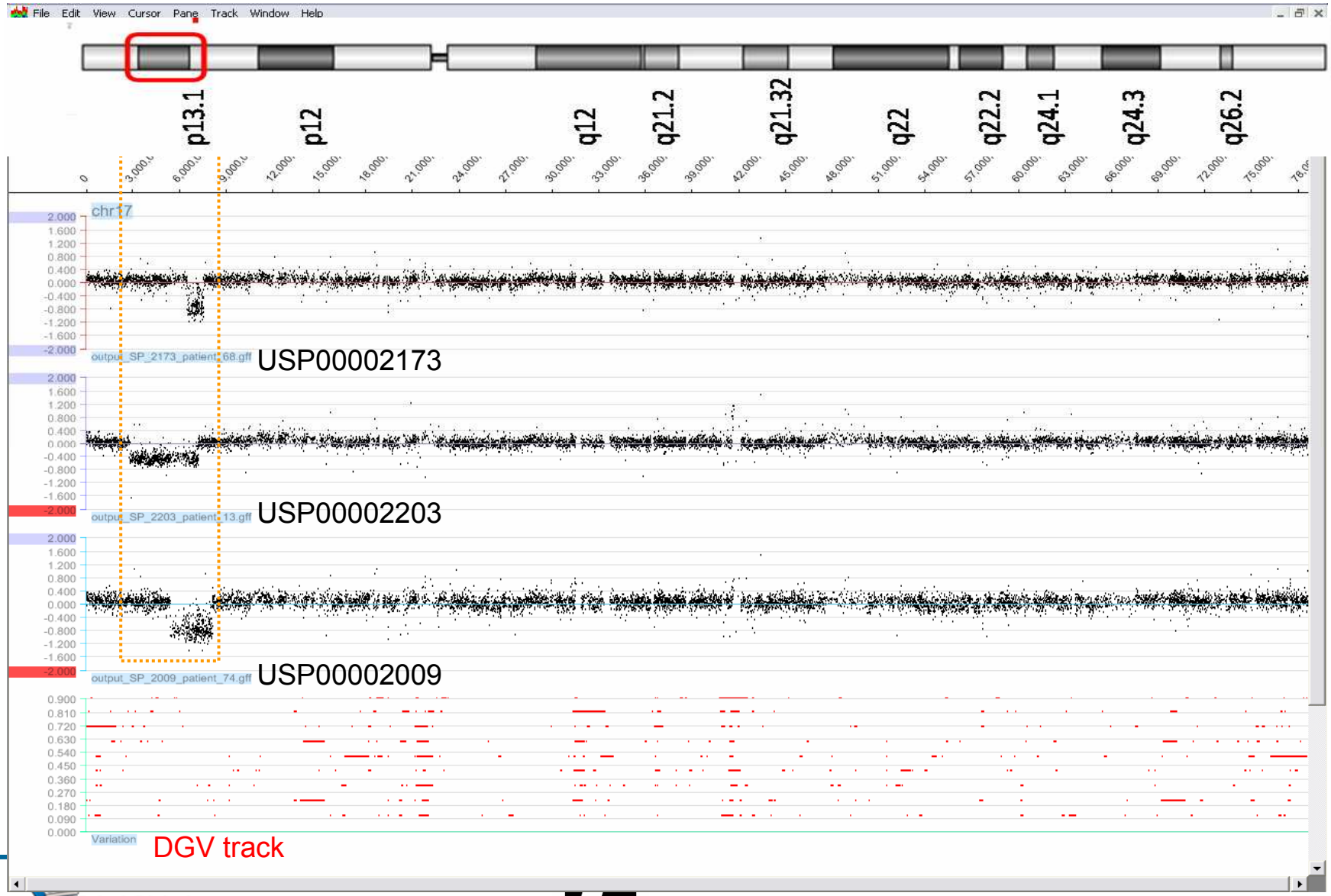
- 1 Mb arrays: “low-resolution”
- BAC tile-path arrays lower quality.

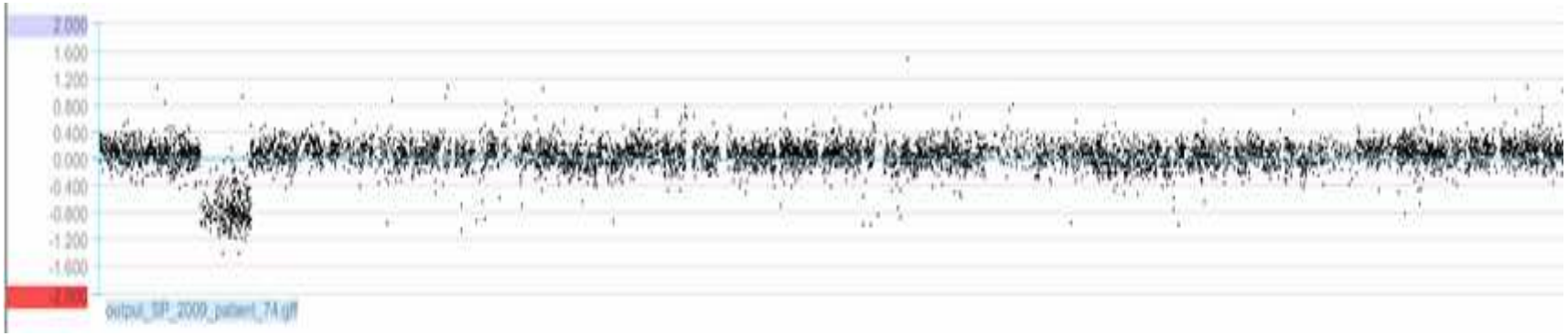
- Production BAC arrays expansive and labour intensive.
- Use of comercial oligo-arrays.

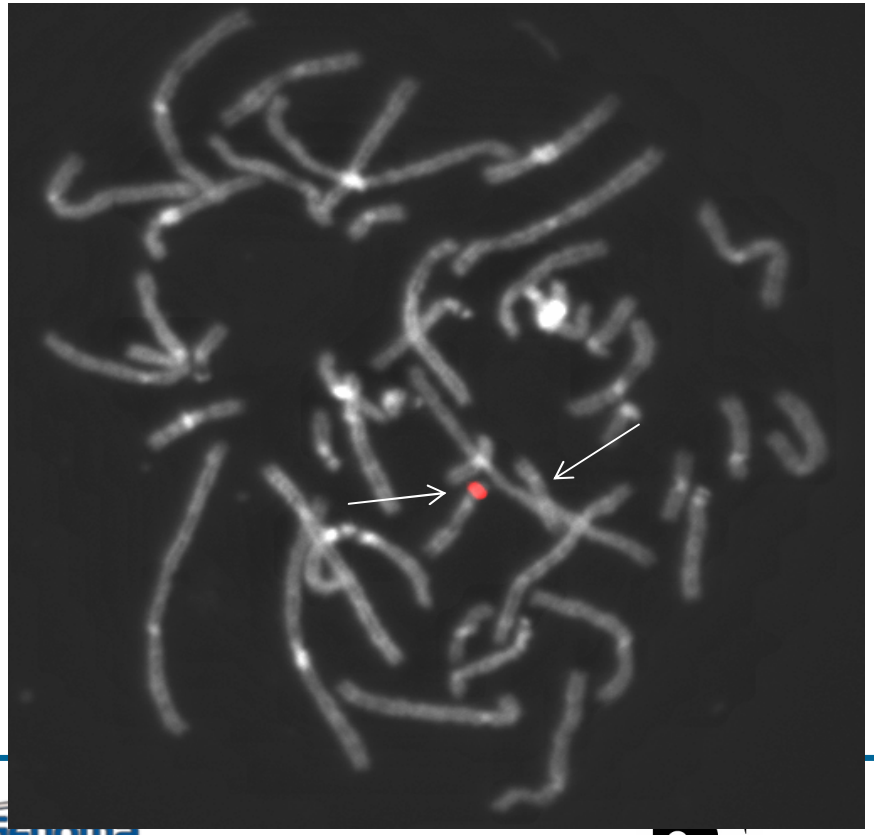

a
b
c
d

Chromosome 17 profiles of the 3 patients as seen in SignalMap – *ARRAY 244K*

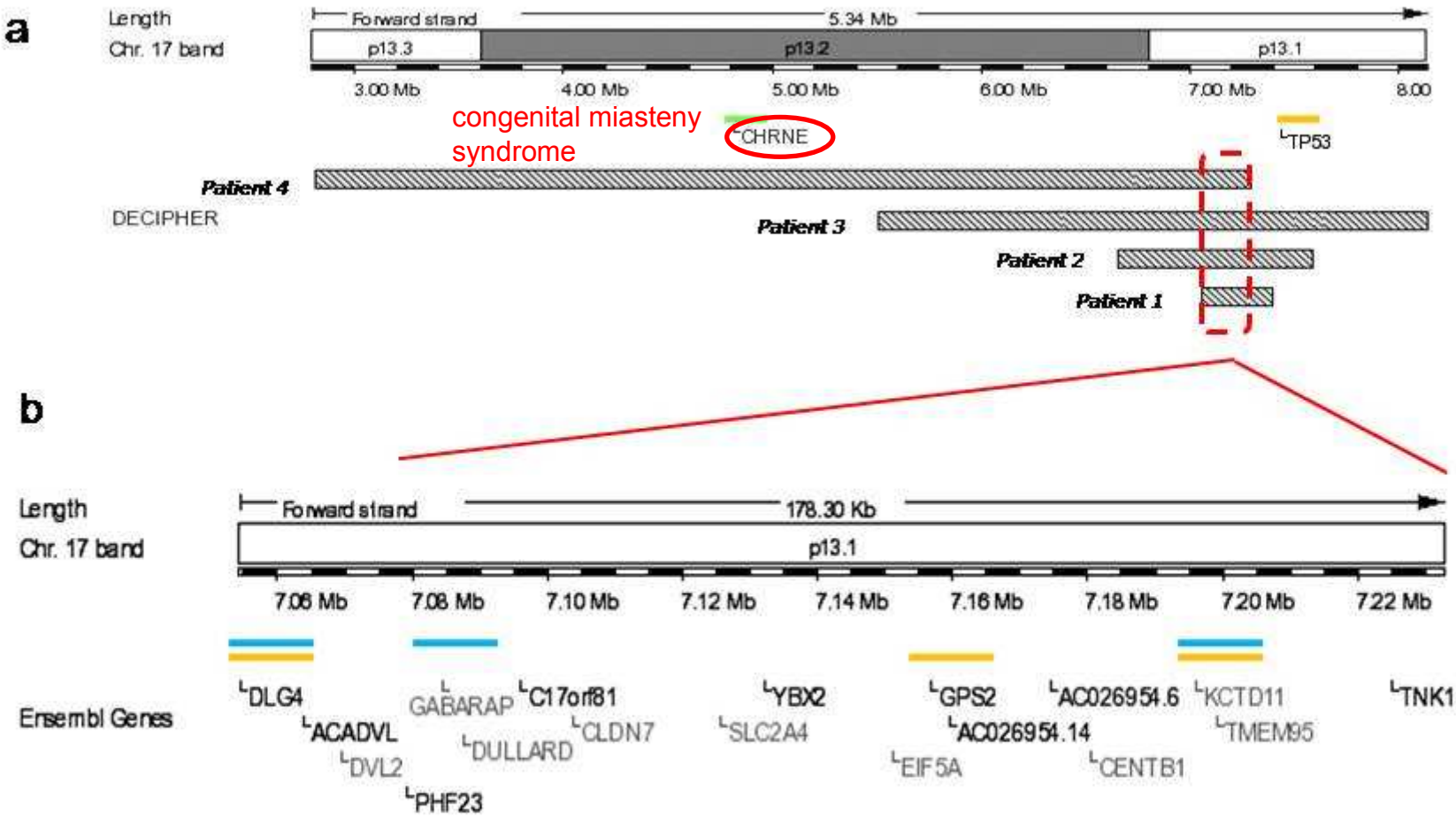
Probe log 2 ratios plotted along y-axis







- Confirmado por FISH
- *De novo* em todos os casos

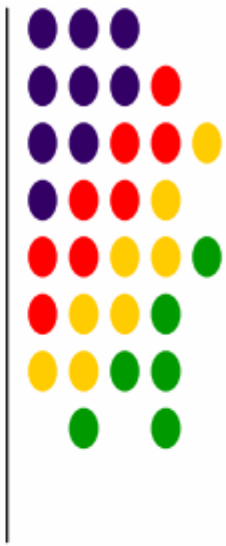

a
Length
Chr. 17 band
Forward strand
5.34 Mb
p13.3
p13.2
p13.1
3.00 Mb
4.00 Mb
5.00 Mb
6.00 Mb
7.00 Mb
8.00
congenital miasteny syndrome
CHRNE
TP53
Patient 4
DECIPHER
Patient 3
Patient 2
Patient 1
b
Length
Chr. 17 band
Forward strand
178.30 Kb
p13.1
7.06 Mb
7.08 Mb
7.10 Mb
7.12 Mb
7.14 Mb
7.16 Mb
7.18 Mb
7.20 Mb
7.22 Mb
Ensembl Genes
DLG4
ACADVL
DVL2
PHF23
GABARAP
DULLARD
C17orf81
CLDN7
YBX2
SLC2A4
GPS2
EIF5A
AC026954.6
AC026954.14
CENTB1
KCTD11
TMEM95
TNK1

Which array platform?

- Agilent platform?

- **ISCA Design Update**
  Agilent, OGT, and BlueGnome will all soon be selling the ISCA Consortium design. Currently the 4x44K and 2x105K arrays are available. The 8x60K and 4x180K arrays are in validation.

International Standard Cytogenomic Array (ISCA) Consortium
March 2009 Newsletter



The Consortium has a new name,
a new Steering Committee,
and a new website! . . .

We're now the International Standard Cytogenomic Array (ISCA) Consortium.

- **The ISCA Steering Committee was elected from a group of representatives from molecular, cytogenetic and clinical backgrounds and includes:**

Leslie Biesecker (NHGRI/NIH)
Nigel Carter (Sanger Institute, UK)
John Crolla (Salisbury, UK)
Evan Eichler (University of Washington)
Ada Hamosh (Johns Hopkins/OMIM)
David Ledbetter (Emory University)
Charles Lee (Harvard-Brigham & Women's)
Christa Martin (Emory University)
David Miller (Harvard-Boston Children's)
Nancy Spinner (Children's Hospital of Philadelphia)
Joris Vermeesch (Universiteit Leuven, Belgium)

ISCA Laboratories

Alberta Children's Hospital
ARUP/University of Utah
Baylor College of Medicine
Beth Israel Deaconess Medical Center
Children's Hospital of Philadelphia
Children's Memorial Hospital, Chicago
Cincinnati Children's Hospital
Credit Valley Hospital
Duke University Health System Clinical Labs
Emory University, Emory Genetics Laboratory
GeneDx
Hamad Medical Corporation, Qatar
Henry Ford Hospital, Michigan
Hospital for Sick Children, Toronto
Kaiser Regional Cytogenetics Lab
London Health Sciences Centre
Mayo Clinic
Mission Health and Hospitals, Fullerton Genetics Laboratory
Montefiore Hospital
Mount Sinai School of Medicine
Northwestern Reproductive Genetics, Chicago
Stanford Hospital and Clinics
Sudbury Regional Hospital
Texas Tech University
UMass Memorial Memorial Center
UMCG, Groningen, Netherlands
University of Alabama, Birmingham
University of Florida
University of Michigan
University of Nebraska
University of Oxford
University of Rochester
University of Sao Paolo
University of Wisconsin
University Medical Center Ljubljana
Wessex Regional Genetics Lab

Frequency of alterations with increased resolution

- Submicroscopic copy number imbalances is associated to 15-30% of unexplained MR and congenital abnormalities.
- This frequency does not seem to vary a lot between 3500 BAC arrays, 35000 BAC arrays or 44.000 oligos.

Diagnosis – Which size alterations should we look at?

- Larger alterations are more likely to cause a phenotype.
- Larger alterations are less likely to be inherited from normal parents.
- Alterations below certain size (400Kb? 250Kb?) are not investigated – cost/benefit.

- Geneticistas Clínicos
  - Fernando Kok
  - Paulo Otto

Angela M. Vianna-Morgante

Ana C. Krepischi-Santos

- Colaboradores:
  - Rafaella M.P. Nascimento
  - Regina C. Mingroni-Netto
  - Maria Rita Passos-Bueno
  - Celia Koiffmann