Utility of Array Comparative Genomic Hybridization as a Primary Analysis for the Indication of Developmental Delay/Mental Retardation

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University of Utah CME Statement

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Speakers are also expected to openly disclose intent to discuss any off-label, experimental, or investigational use of drugs, devices, or equipment in their presentations.

This speaker has nothing to disclose.

What is cytogenetics

- The original whole genome analysis
  - Analysis of chromosomes from a tissue of interest to identify large scale genomic alterations
    - G-banded chromosome analysis (karyotype)
  - Molecular cytogenetics analyzes smaller regions for imbalances and rearrangements
    - FISH and Array CGH

Pediatric indications for a cytogenetic analysis

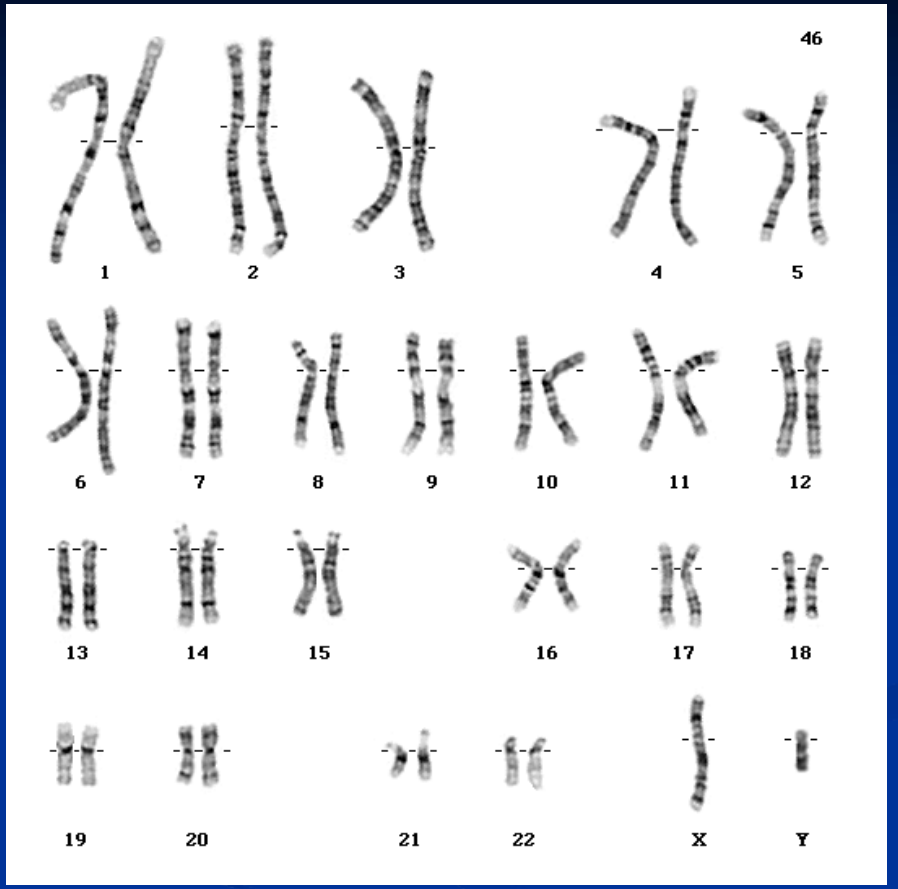
- Growth abnormality
  - Small/large for age
- Neurologic impairment
  - mental retardation / seizures / microcephaly / hypotonia / psycho-emotional dysfunction
- Dysmorphic features
- Cardiovascular malformations
- Other congenital anomalies

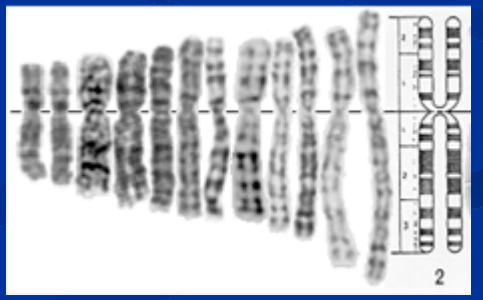
Chromosomal anomalies are responsible for birth defects in ~0.2% of live births

Most common tissue studied: peripheral blood

Standard Chromosome Analysis

- G-banding (Giemsa) chromosomes in metaphase
- Benefits:
  - Viewing entire genome
  - Can visualize individual cells and individual chromosomes
- Limits:
  - Limit of resolution around 5-10 Mb (depending on region of genome and length of chromosomes)
  - Need an actively growing source of cells

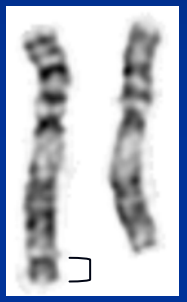




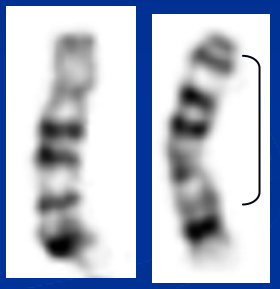
Common types of chromosome abnormalities detected with standard chromosome analysis:

- aneuploidies
- deletions, duplications
- inversions

Trisomy 21

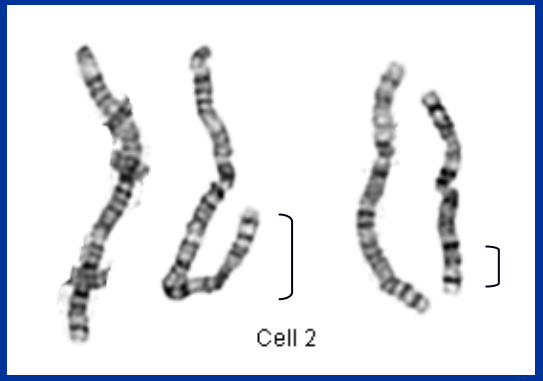


Terminal deletion of 11

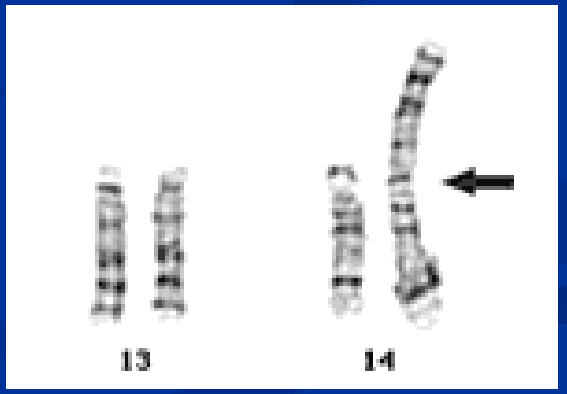


Pericentric inversion of 18

- Balanced and unbalanced translocations



Reciprocal translocation between 3 and 6



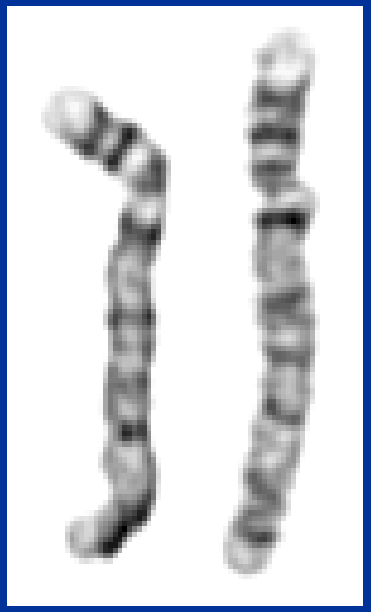
Unbalanced translocation between 13 and 14

Fluorescence *in situ* hybridization (FISH)

- First described by Pinkel, Straume, & Gray in 1986
- Label DNA with fluorescent molecule and hybridize to human chromosomes on a slide
- Benefits:
  - Can turn almost any DNA into a probe
  - For clinical use, most probes 100-500 kb
  - Much higher resolution as compared to G-banding for identifying deletions, insertions, and translocation breakpoints
  - Can use cells in any state of the cell cycle as well as archived tissue
  - Can analyze results on a cell-by-cell basis
  - Shorter TAT since tissue does not need to be cultured for metaphase cells
- Limits:
  - Only going to see the region of the genome complementary to your probe

Example of FISH to detect a small deletion

- Microdeletion of 4p detected by FISH using a probe for the Wolf-Hirschhorn syndrome (WHS) critical region (red) and chromosome 4 centromere (green)

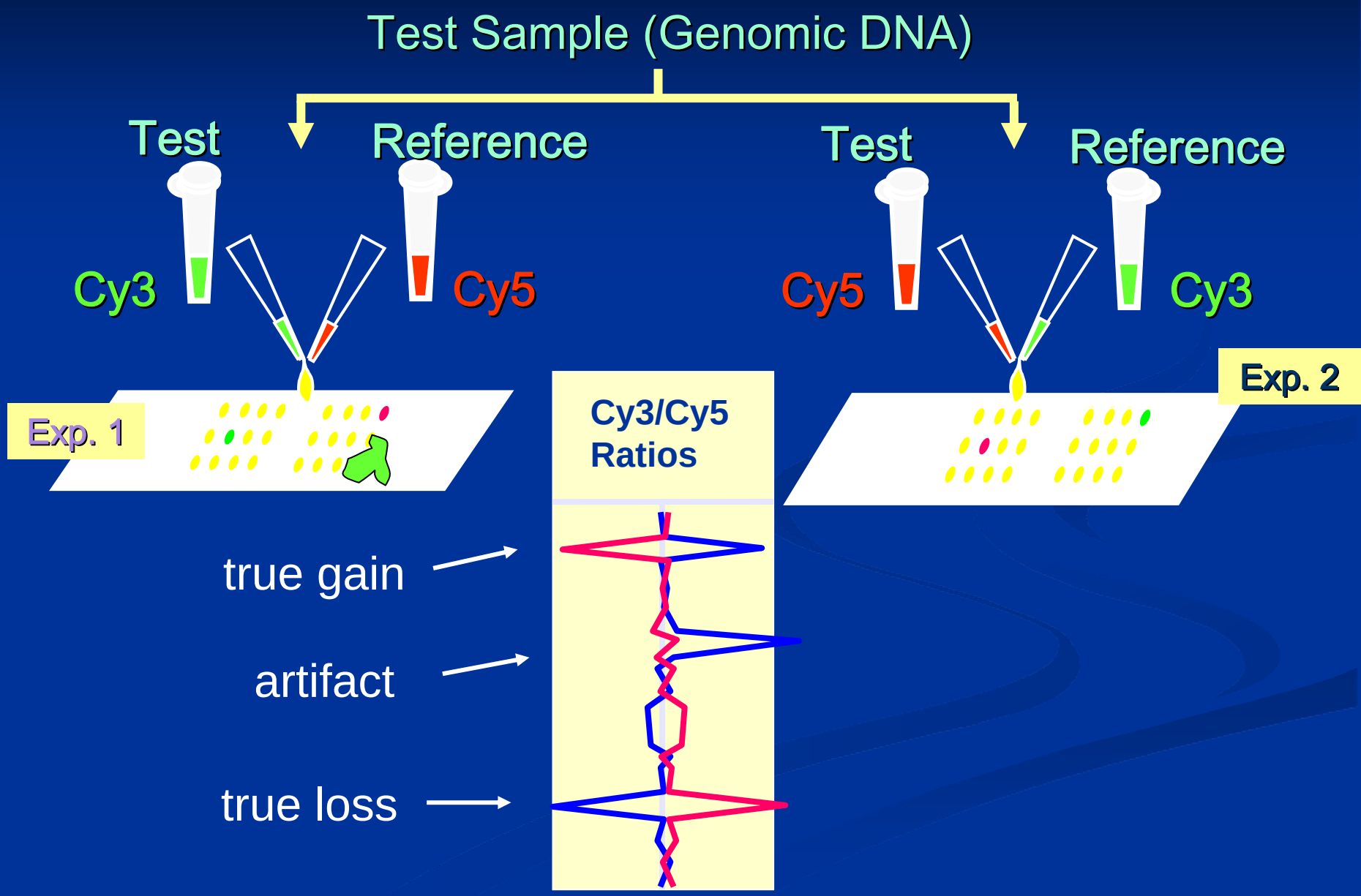


normal appearing 4s

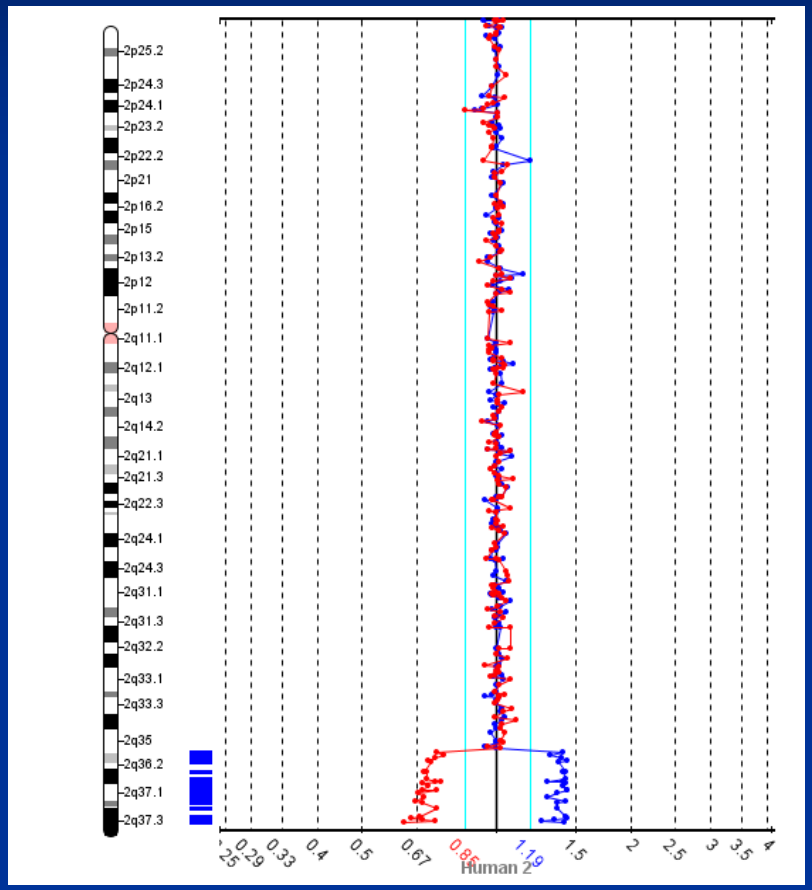
deletion between 2-4 Mb in 25-30% of patients with WHS

Must have suspicion of WHS to run this probe

Comparative Genomic Hybridization (CGH) Microarray

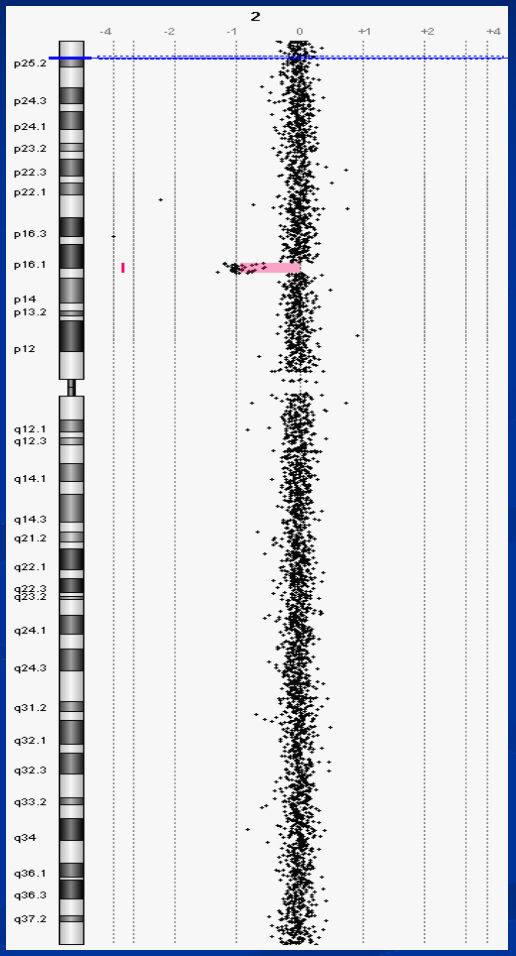


Array CGH data from a BAC-based chip with dye-swap experiment



Gain of terminal end of chromosome 2

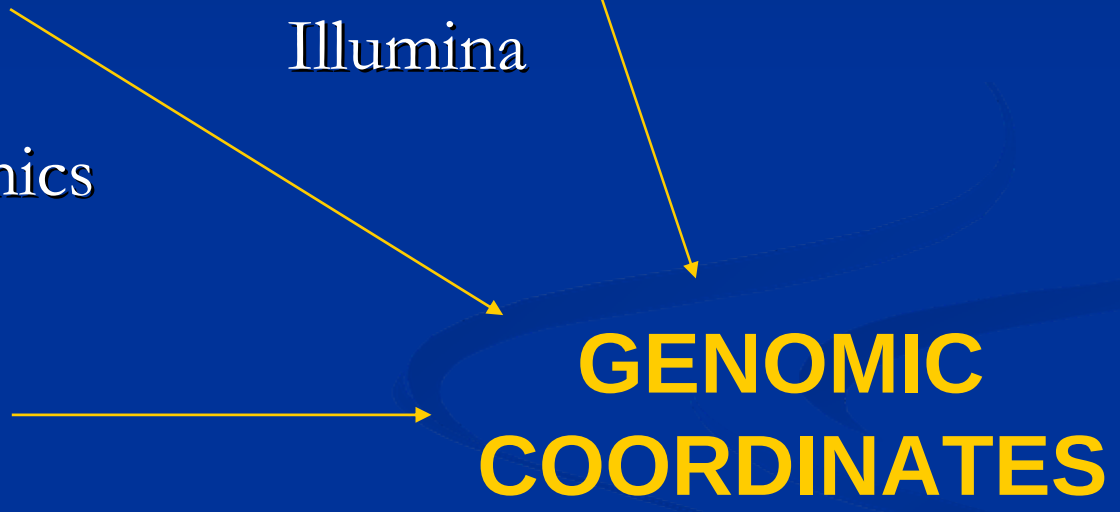
Array CGH data from an oligo-based chip without dye-swap



Loss of interstitial region in chromosome 2

Copy Number Array Platforms

- Oligo Arrays
  - Agilent
  - Nimblegen
  - Signature Genomics
- SNP Arrays
  - Affymetrix
  - Illumina
- BAC Arrays
  - BlueGnome
  - Signature Genomics
  - Spectral Genomics



CGH Microarray

- Benefits
  - Can customize array to concentrate clones in areas of interest (targeted regions) and/or spread clones throughout genome (backbone)
  - Resolution will depend on density of clones in region of interest, but can be as good as 5 kb
  - Detection of smaller abnormalities
  - Detection of cryptic abnormalities
  - Better definition of cytogenetic abnormalities
  - Interpretation usually less subjective than standard chromosome analysis
  - Can use on archived or non-growing tissue

CGH microarray

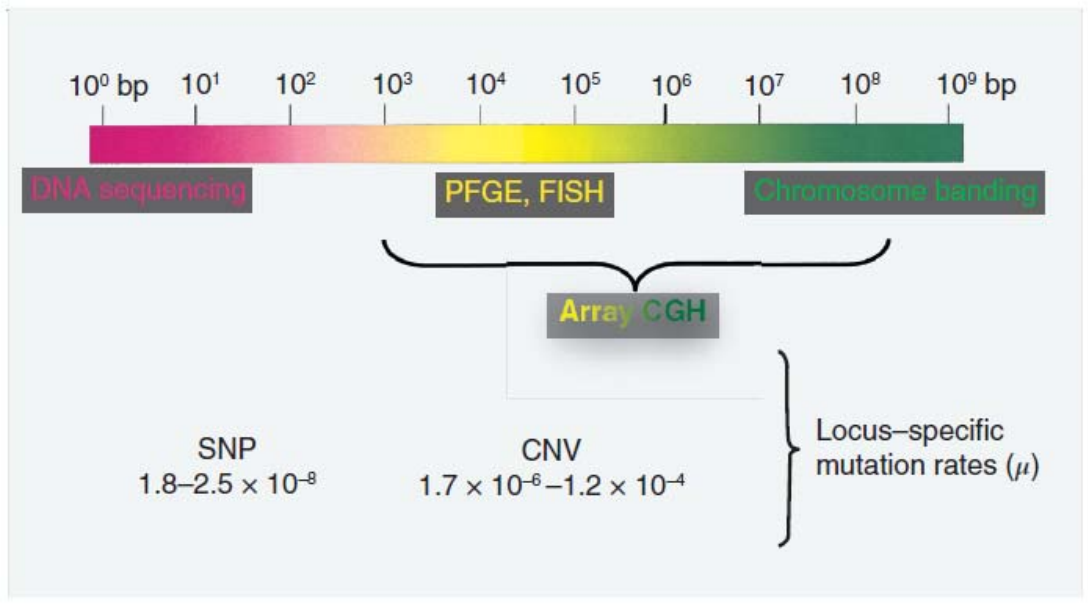
- Limits
  - Will not detect balanced rearrangements
  - May uncover copy number changes of unclear clinical significance
  - Will not detect copy number changes in regions of the genome that are not on the array platform (chip)

Detection rate for each technology

- Routine G-banded chromosome analysis
  - 5-10% (depending on severity of MR and MCA)
- Subtelomeric FISH (screening) *after* normal chromosomes
  - 2-3%
- Array CGH *after* normal chromosomes
  - 10-15%

Why the increased detection?

- The estimated per locus mutation rate for genomic rearrangements is approximately three to four orders of magnitude greater than that of single nucleotide substitution



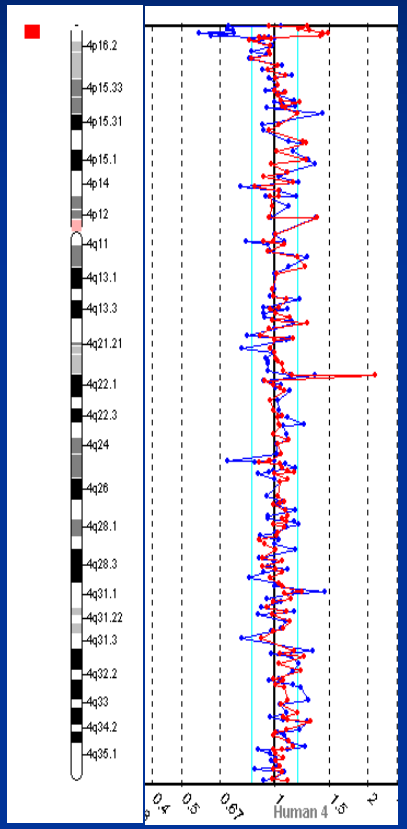
Genomic rearrangements and sporadic disease

James R Lupski

NATURE GENETICS SUPPLEMENT | VOLUME 39 | JULY 2007

Detection of small gains and losses: Microdeletion on 4p detected by CGH microarray

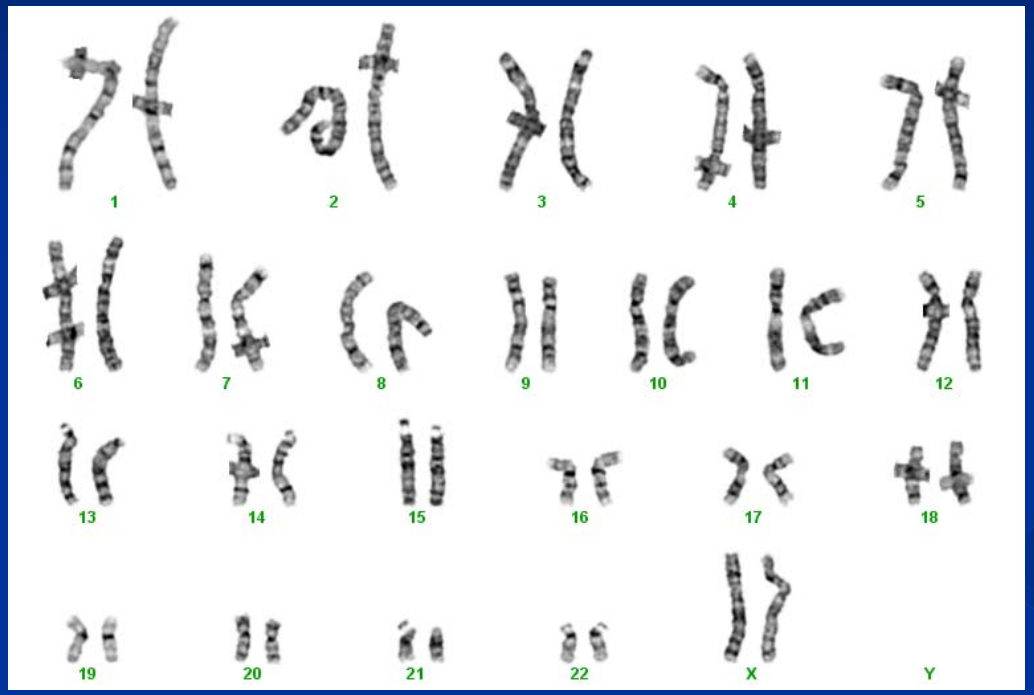
normal appearing chromosome 4s

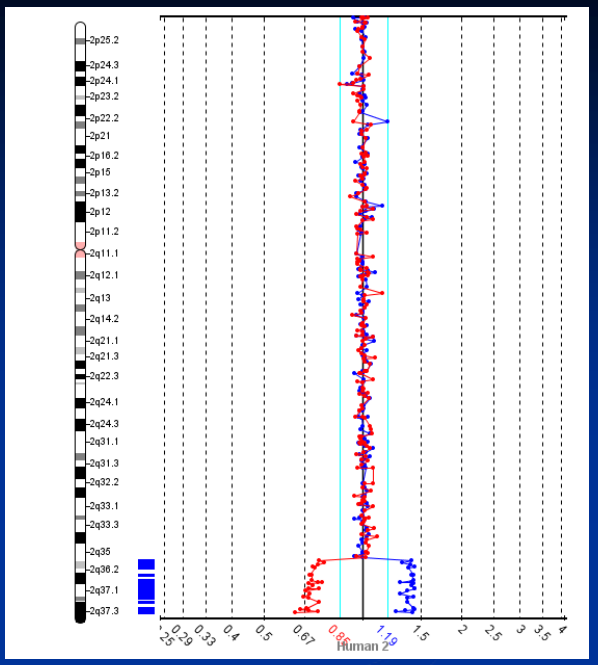


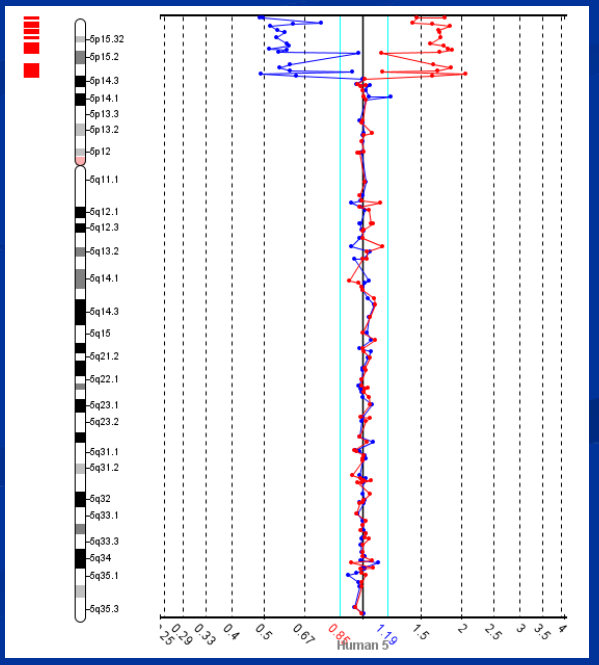
reciprocal deviation at distal end of 4p showing a loss

Array CGH results and patient's phenotype (growth retardation, distincitve facial features, seizures) consistent with WHS

Detection of large cryptic abnormalities







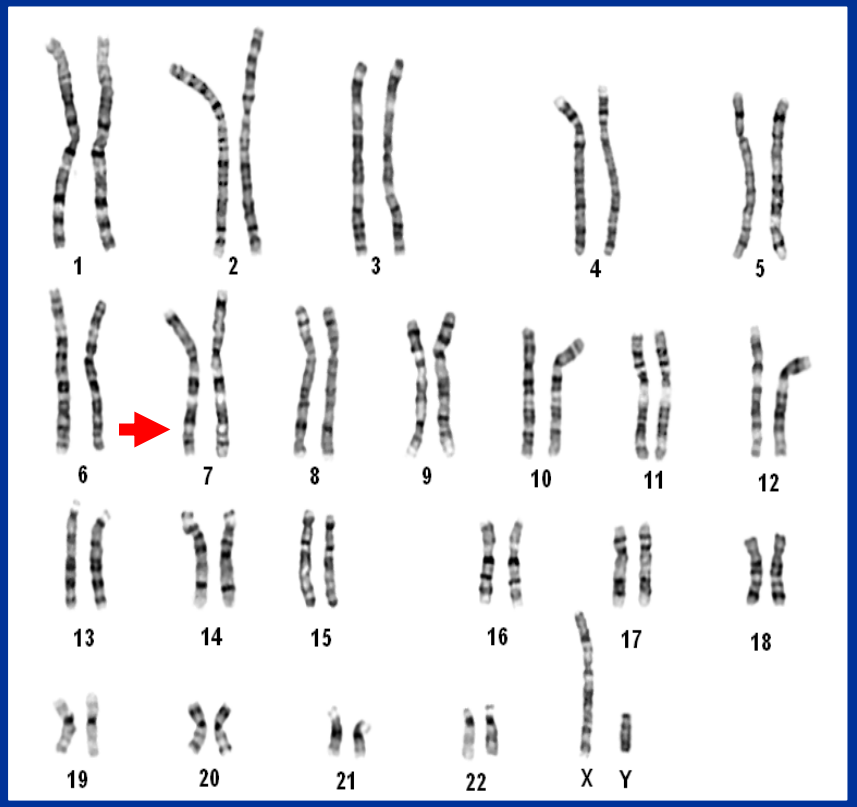
Chromosome analysis normal. Array CGH showed a 21 Mb duplication of 2qter and a 16 Mb deletion of 5pter – likely an unbalanced translocation with 2q "replacing" 5p.

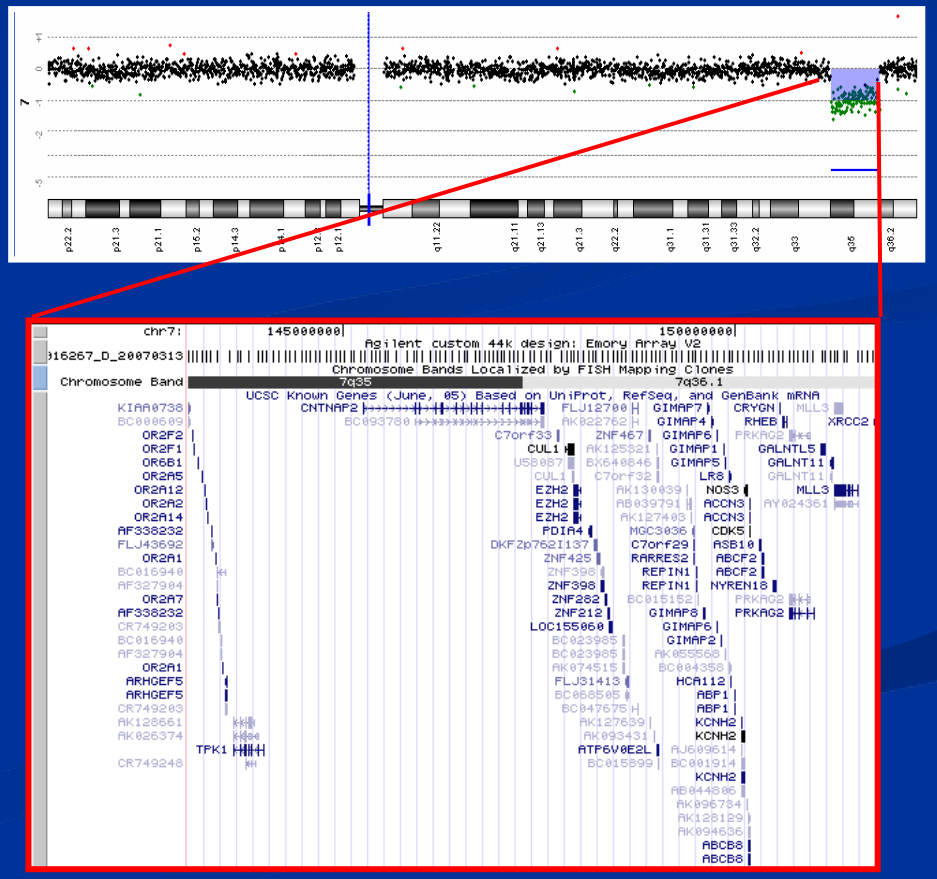
Better definition of cytogenetic abnormalities

G-band designation vs. **Array CGH and Database mapping**

7q34 (+/- a band = +/- 5 Mb)

7q35 – q36.1, size defined +/- 75 kb

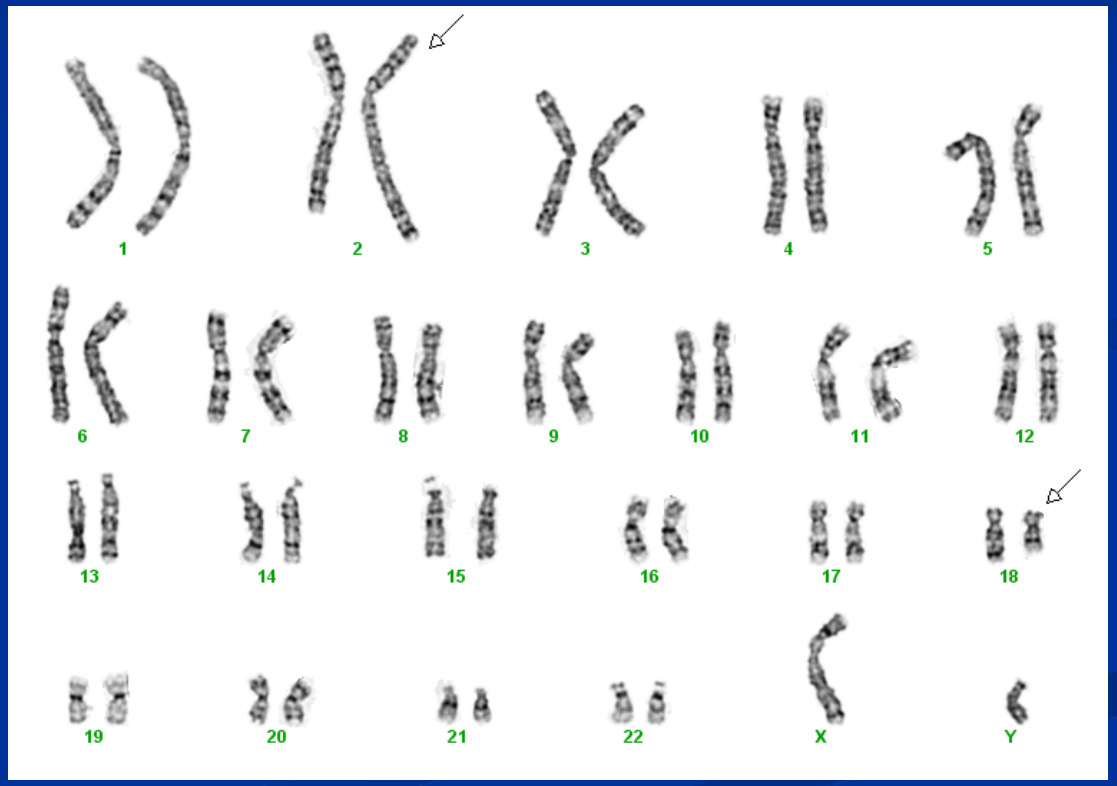




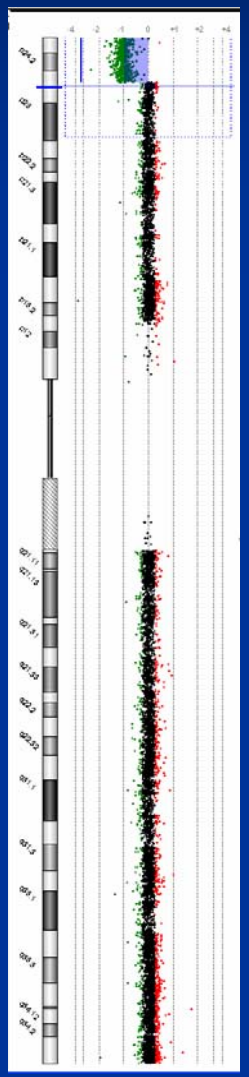
Slide courtesy of CL Martin

Combination of better definition of visible abnormality and identification of cryptic abnormalities in same patient

- 5 yo male
- developmental delay
- cytogenetic analysis showed a t(2;18) that looked balanced



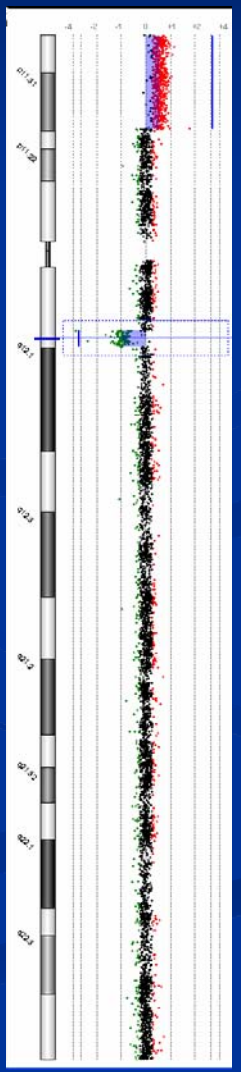
Microarray revealed three significant abnormalities



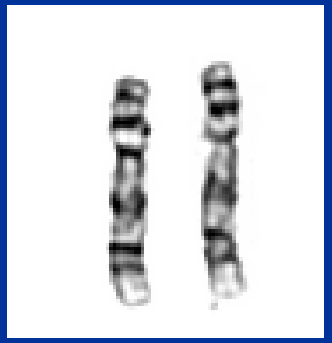
terminal deletion of 9p – 5.9 Mb

terminal duplication of 18p – 6.0 Mb

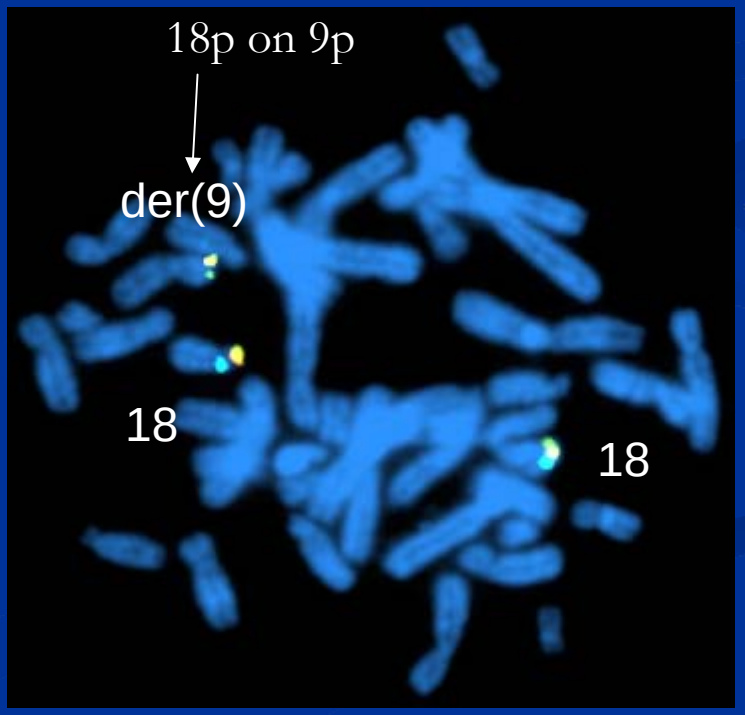
interstitial deletion of 18q – 1.2 Mb



- 18q11.2 LOSS
  - Suggests loss at the breakpoint of the t(2;18)
- 9p24 LOSS
- 18p11.3 GAIN
  - Suggests an unbalanced translocation with 18p gain on deleted 9p



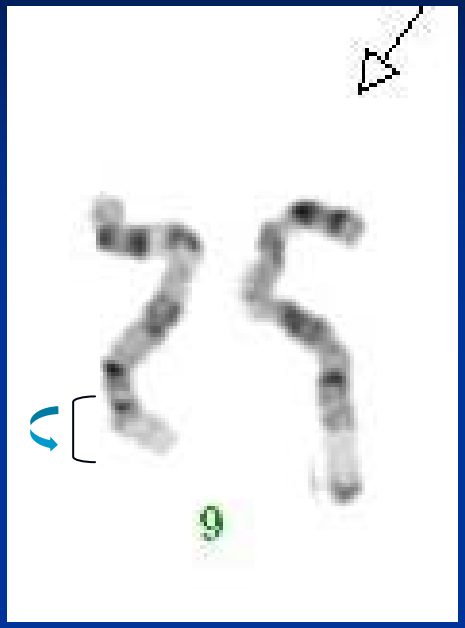
Normal appearing 9s



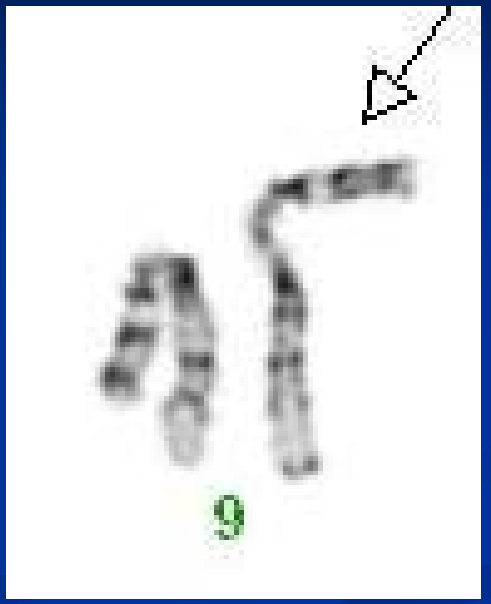
Less subjective analysis of chromosome rearrangements

- Prenatal and postnatal growth retardation
- Unusual facial features
- Hip dislocations
- Required G-tube for feeding
- At 3 years of age, functioning in the moderate range of mental retardation
- Both parents apparently phenotypically normal

Proband's 9s



Mother's 9s

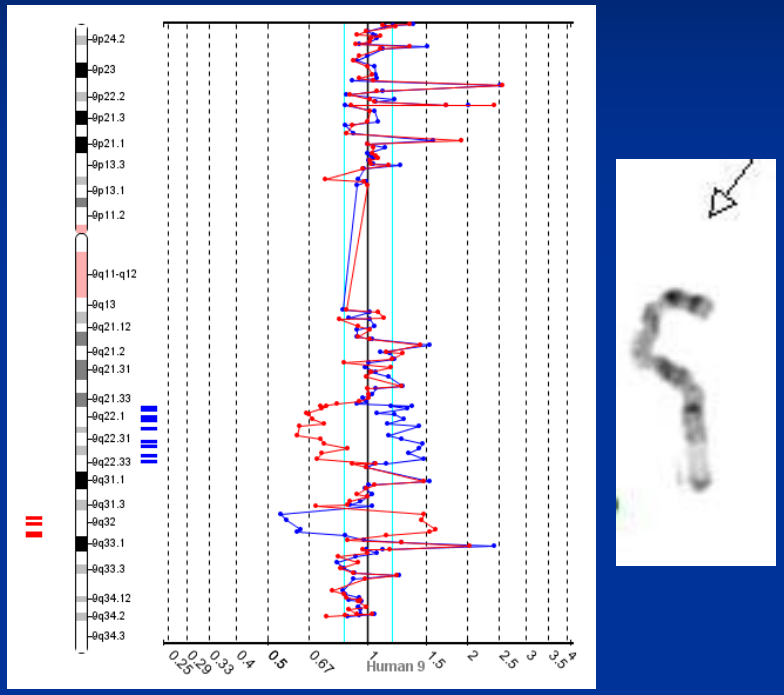


Interpretation:

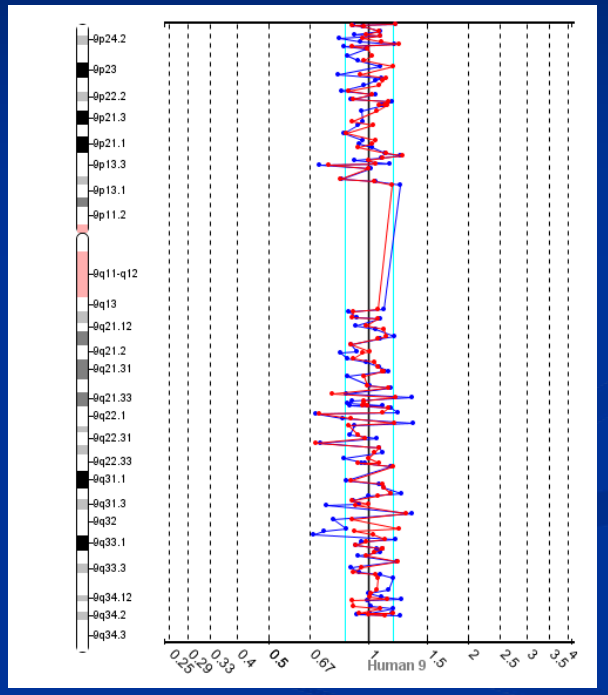
Both proband and mother have a paracentric inversion in the long arm of 9:
inv(9)(q32q34.3)

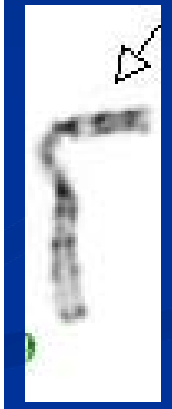
But this does not explain differing phenotypes

Differing array CGH results despite identical banding patterns



proband's complex unbalanced 9



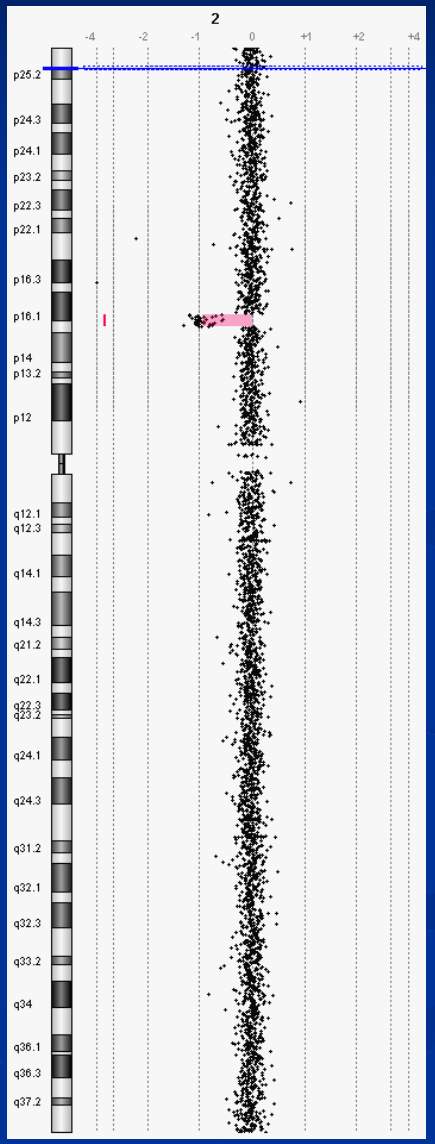


mother's balanced 9

Hypothesis – Mom's abnormal but balanced 9 underwent a complex recombination event during meiosis to become unbalanced, but coincidentally the banding pattern was retained

Detection of abnormalities of unknown clinical significance

- 3 y.o. female referred for microarray analysis
- Developmental delay
- Right polycystic kidney

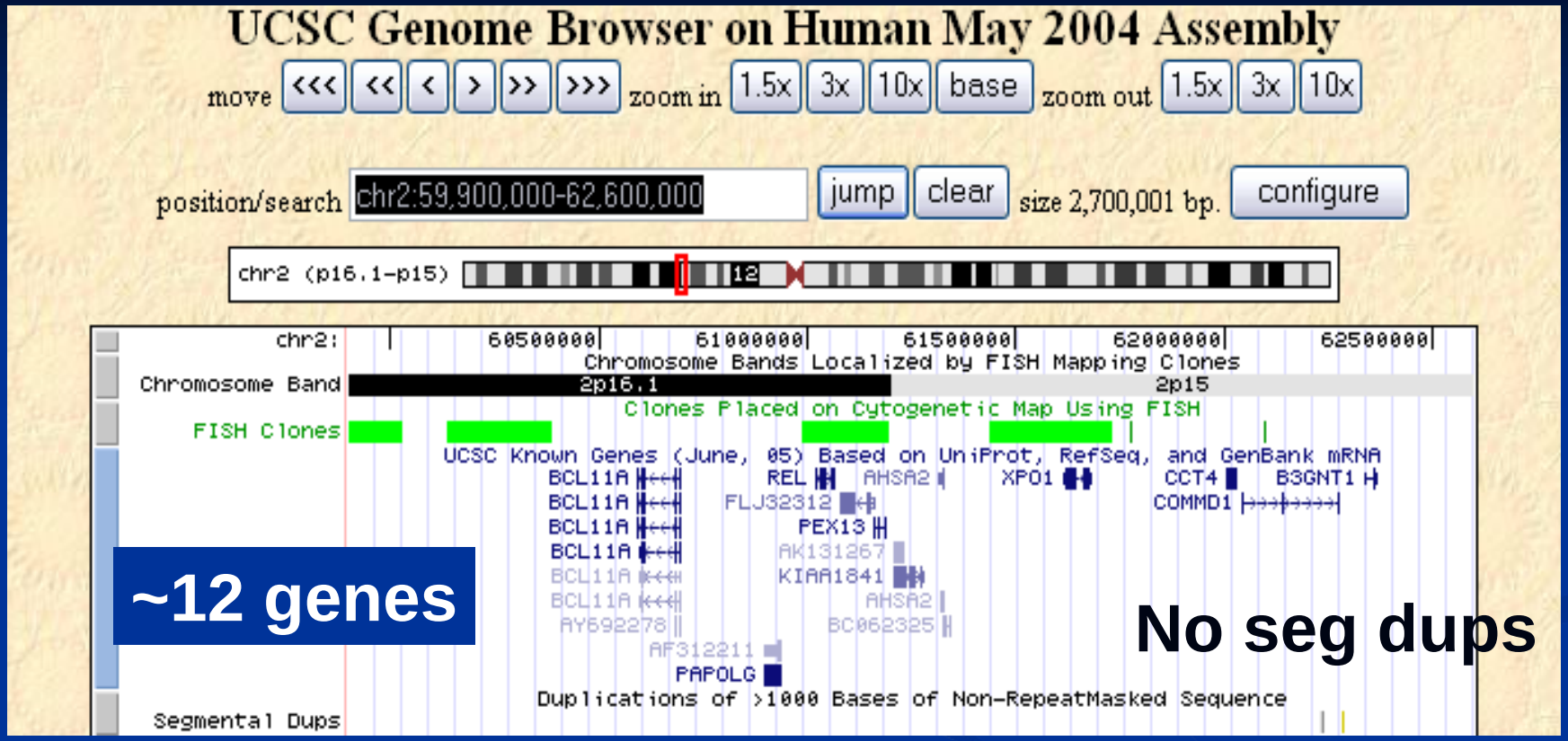


LOSS

chr2:59,900,000-62,600,000

Benign vs. Pathogenic

1. Size
2. Location in the Genome
3. Genomic Content
4. Comparison with other Cases
5. Inherited or *de novo*

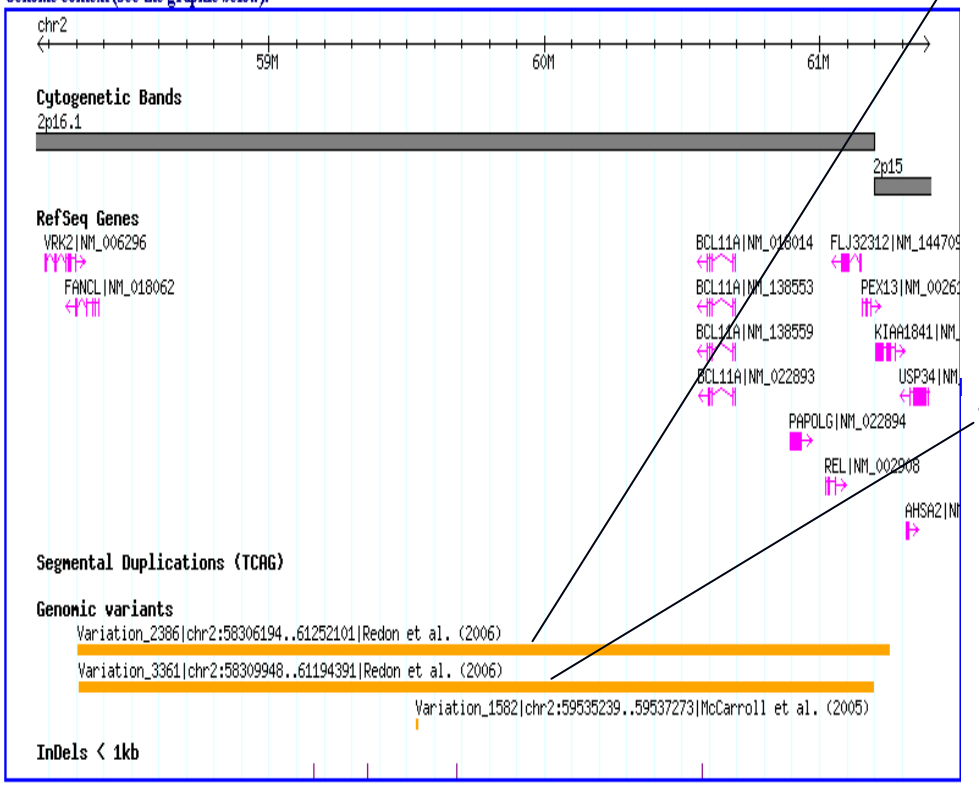

UCSC Genome Browser on Human May 2004 Assembly
move <<< << < > >> >>> zoom in 1.5x 3x 10x base zoom out 1.5x 3x 10x
position/search chr2:59,900,000-62,600,000 jump clear size 2,700,001 bp. configure
chr2 (p16.1-p15)
chr2: 60500000 61000000 61500000 62000000 62500000
Chromosome Bands Localized by FISH Mapping Clones
Chromosome Band 2p16.1 2p15
Clones Placed on Cytogenetic Map Using FISH
FISH Clones
UCSC Known Genes (June, 05) Based on UniProt, RefSeq, and GenBank mRNA
BCL11A REL AHSA2 XPO1 CCT4 B3GNT1
BCL11A FLJ32312 COMMD1
BCL11A PEX13
BCL11A AK131267
BCL11A KIAA1841
BCL11A AHSA2
AY692278 BC062325
AF312211
PAPOLG
Duplications of >1000 Bases of Non-RepeatMasked Sequence
Segmental Dups
~12 genes
No seg dups

Database of Genomic Variants on Human Genome Assembly Build 35 (hg17): Locus Summary

Back Home | Field Definitions

Locus: Locus0343

Genome context (see the graphic below):



Variation: Variation_2386
Landmark: chr2:58,306,194..61,252,101 (Genome Browsers: TCAG Segmental Duplication
Variation Type: CopyNumber
Overlap with TCAG Segmental Duplication: No
Gap within 100k: No
Known Genes: PAPOLG, KIAA1841, PEX13, REL, FANCL, BCL11A, FLJ32312
Method: Affymetrix 500K EA SNP Mapping Array
Reference: Redon et al. (2006)
Pub Med ID: 17122850
Frequency Information:
Subject Cohort: Control
Sample Size: 270 HapMap individuals
Normal Gain: 1
Normal Loss: 0
Total Gain/Loss: 1

GAIN

Variation: Variation_3361
Landmark: chr2:58,309,948..61,194,391 (Genome Browsers: TCAG Segmental Duplication
Variation Type: CopyNumber
Overlap with TCAG Segmental Duplication: No
Gap within 100k: No
Known Genes: PEX13, PAPOLG, REL, FANCL, BCL11A, FLJ32312
Method: WGTP CGH Array
Reference: Redon et al. (2006)
Pub Med ID: 17122850
Frequency Information:
Subject Cohort: Control
Sample Size: 270 HapMap individuals
Total Gain/Loss: 1

NOT SPECIFIED

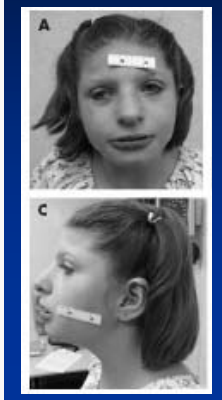
Comparison with other cases: del(2)(p15p16.1) – Literature Search

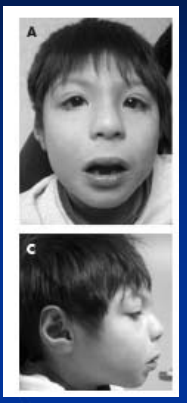
LETTER TO JMG

Clinical and molecular cytogenetic characterisation of a newly recognised microdeletion syndrome involving 2p15-16.1

E Rajcan-Separovic, C Harvard, X Liu, B McGillivray, J G Hall, Y Qiao, J Hurlburt, J Hildebrand, E C R Mickelson, J J A Holden, M E S Lewis

J Med Genet 2007;44:269–276. doi: 10.1136/jmg.2006.045013



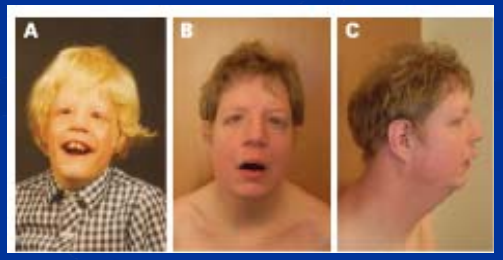


JMG ONLINE

A newly recognised microdeletion syndrome involving 2p15p16.1: narrowing down the critical region by adding another patient detected by genome wide tiling path array comparative genomic hybridisation analysis

N de Leeuw, R Pfundt, D A Koolen, I Neefs, I Scheltinga, H Mieloo, E A Sistermans, W Nillesen, D F Smeets, B B A de Vries and N V A M Knoers

J. Med. Genet. 2008;45:122-124
doi:10.1136/jmg.2007.054049



JMG ONLINE

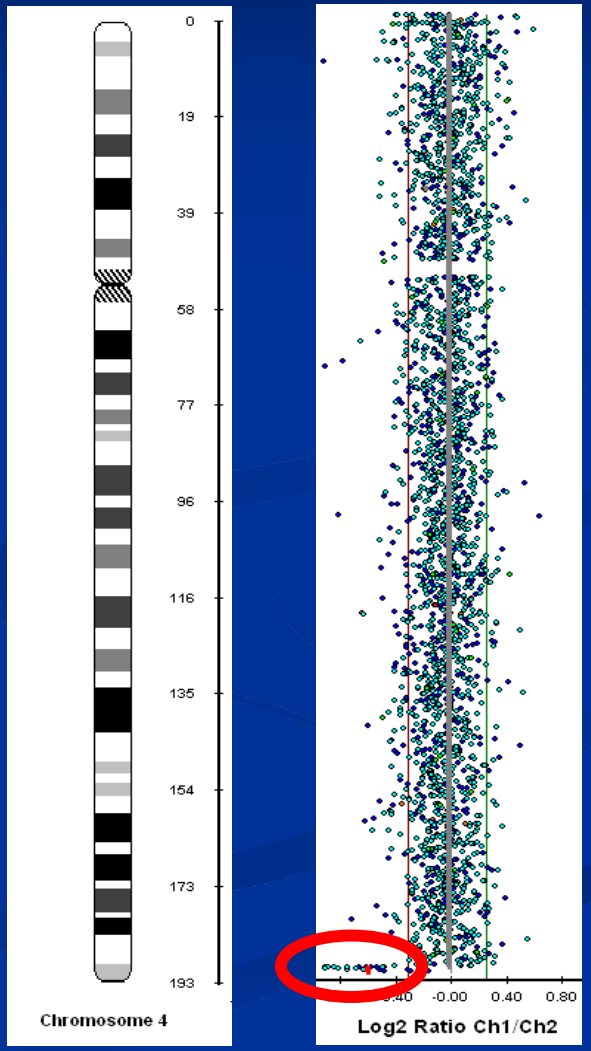
The facial dysmorphy in the newly recognised microdeletion 2p15 p16.1 refined to a 570 kb region in 2p15

E Chabchoub, J R Vermeesch, T de Ravel, P de Cock and J-P Fryns

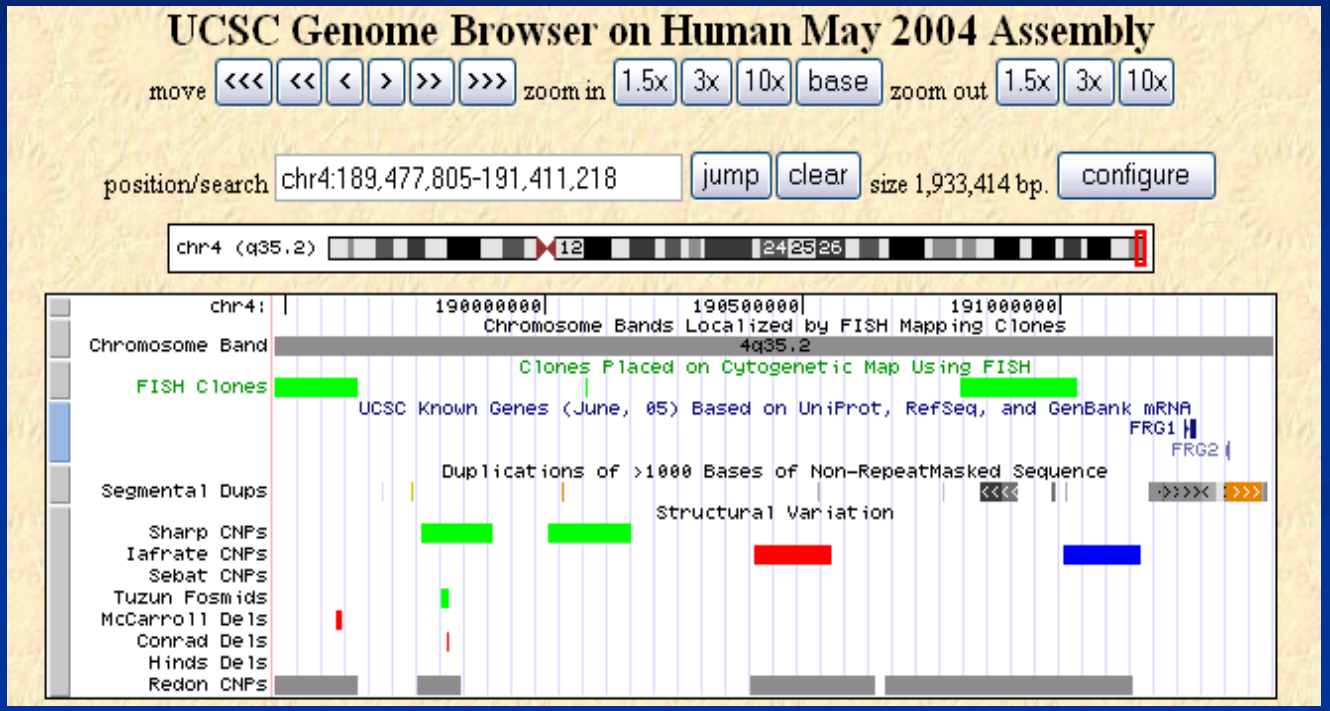
J. Med. Genet. 2008;45:189-192
doi:10.1136/jmg.2007.056176

2nd case with abnormality of unknown clinical significance

Referred for developmental delay and multiple congenital anomalies



Loss chr4:189,477,805-191,411,218



Low # genes

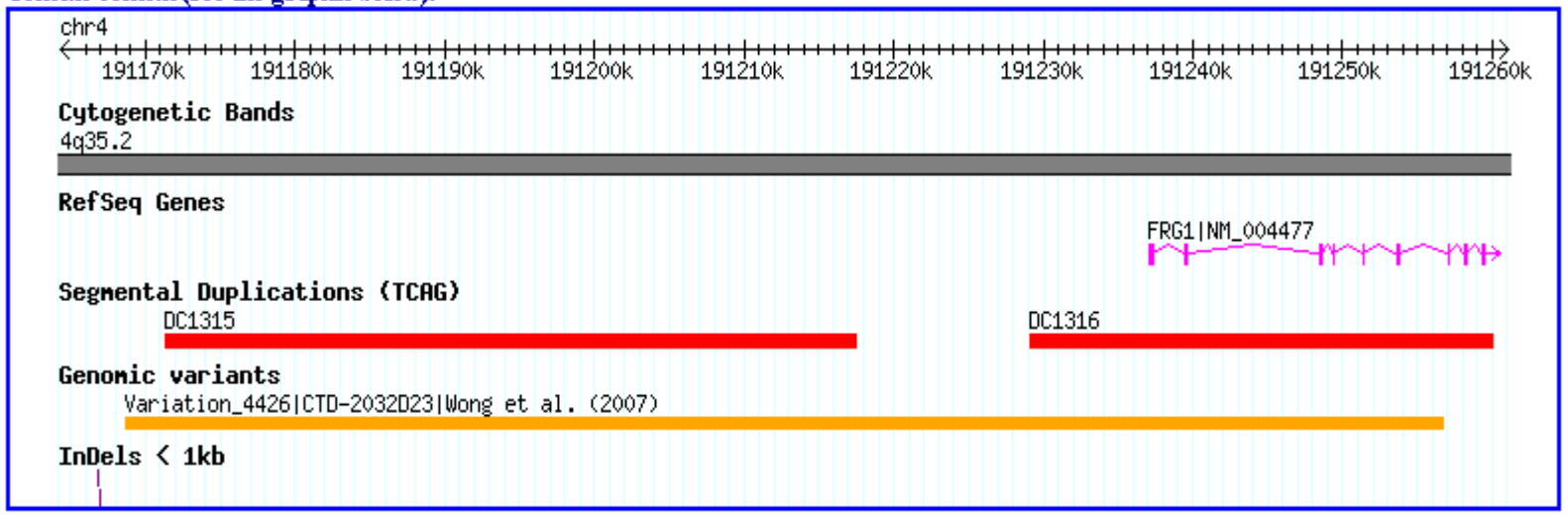
Some segmental duplications, Telomere associated repeats

Database of Genomic Variants on Human Genome Assembly Build 35 (hg17): Locus Summary

Back Home | Field Definitions

Locus: Locus1062

Genome context (see the graphic below):



Variation: Variation_4426

Landmark: CTD-2032D23 (Genome Browsers: TCAG Segmental Duplication , UCSC , Ensembl)
Variation Type: CopyNumber
Overlap with TCAG Segmental Duplication: Yes
Gap within 100k: No
Known Genes: FRG1
Method: Array CGH
Reference: Wong et al. (2007)
Pub Med ID: 17160897
Frequency Information:
Subject Cohort: Control
Sample Size: 95 Individuals
Normal Gain: 1
Normal Loss: 2
Total Gain/Loss: 3

Comparison with other Cases

ORIGINAL ARTICLE

Subtelomere FISH analysis of 11 688 cases: an evaluation of the frequency and pattern of subtelomere rearrangements in individuals with developmental disabilities

J B Ravnan, J H Tepperberg, P Papenhausen, A N Lamb, J Hedrick, D Eash, D H Ledbetter, C L Martin

J Med Genet 2006;43:478–489. doi: 10.1136/jmg.2005.036350

60	del(4)(qter)	4q	Father same	Yes	DD, MR, obese, upper palbebral fissures, 5th finger clinodactyly, chrorea movements	9⅝12	F
61	del(4)(qter)	4q	Father same	Yes	DD, MR	2	M

These cases were detected by FISH; Size not determined

- The same size deletion was subsequently identified in the proband's phenotypically normal father.
- Is the 4q deletion pathogenic or a benign familial variant?
  - Imprinting
  - Penetrance
  - Genetic background

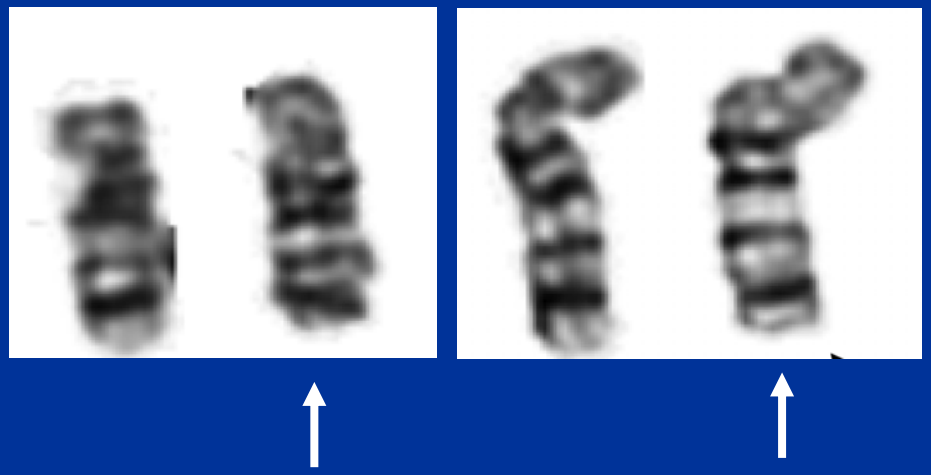
As with many new technologies, array CGH has provided data that challenges old paradigms

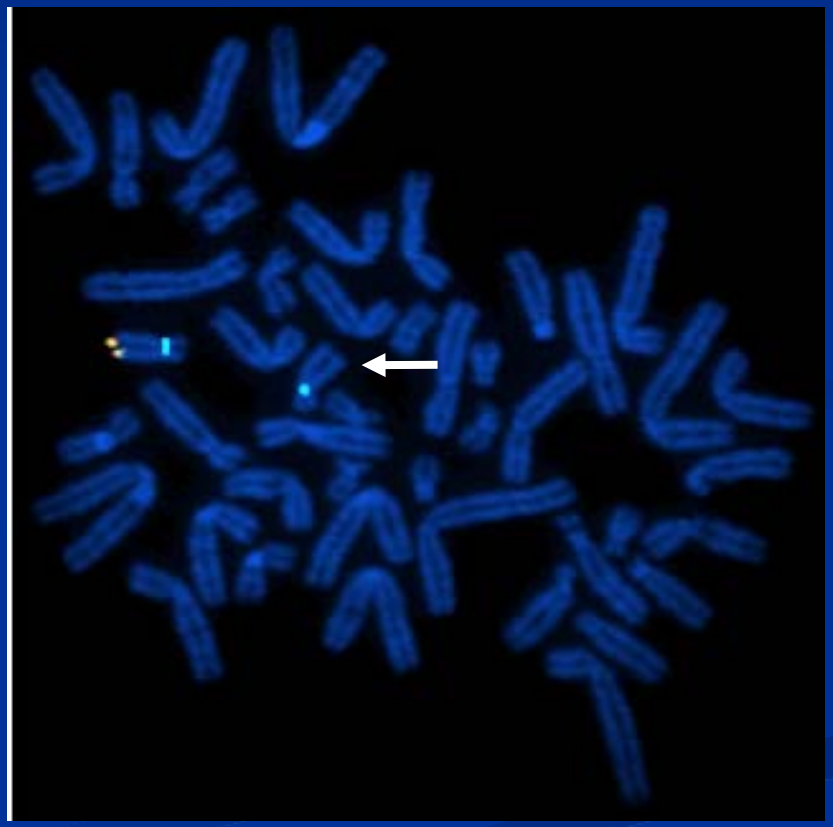
Expansion in Size of a Terminal Deletion: a Paradigm Shift for Parental Follow-up Studies

Clinical Presentation of Proband

- RB came to the clinic as a 3½-year-old female with hypomyelination, ataxia, anal stenosis and a history growth retardation (first noticed at 6 months), and mild developmental delay.
- No other birth defects were recognized.
- For family history, the mother reported having anal stenosis which required rectal dilatation as a child, two previous miscarriages, and a nephew with cleft lip and palate, but mother was phenotypically normal.

- Proband was found to have a terminal deletion of chromosome 18q on a 550 band karyogram, confirmed by the 18q subtelomere probe

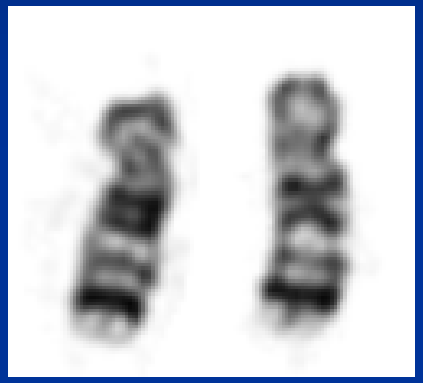


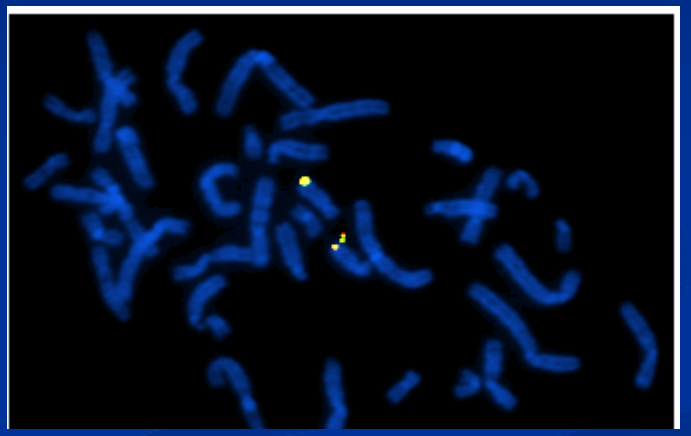


Aqua – 18 centromere
Red/Green Fusion – 18q

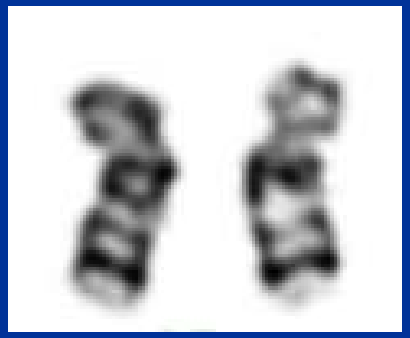
- Parental chromosomes were normal, but the mother was surprisingly found to have an 18q subtelomere deletion

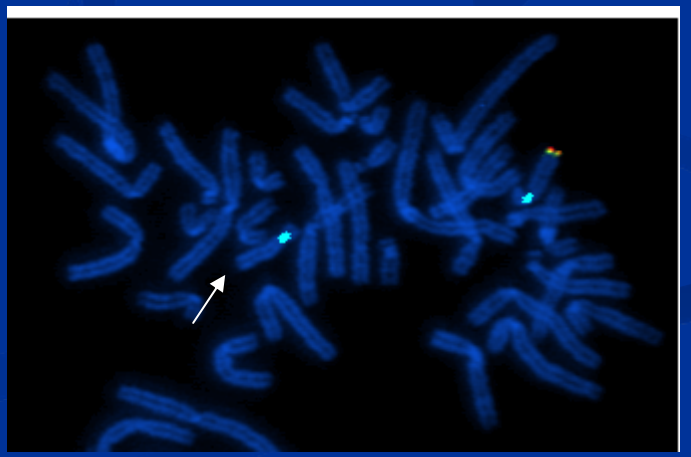
Dad





Mom



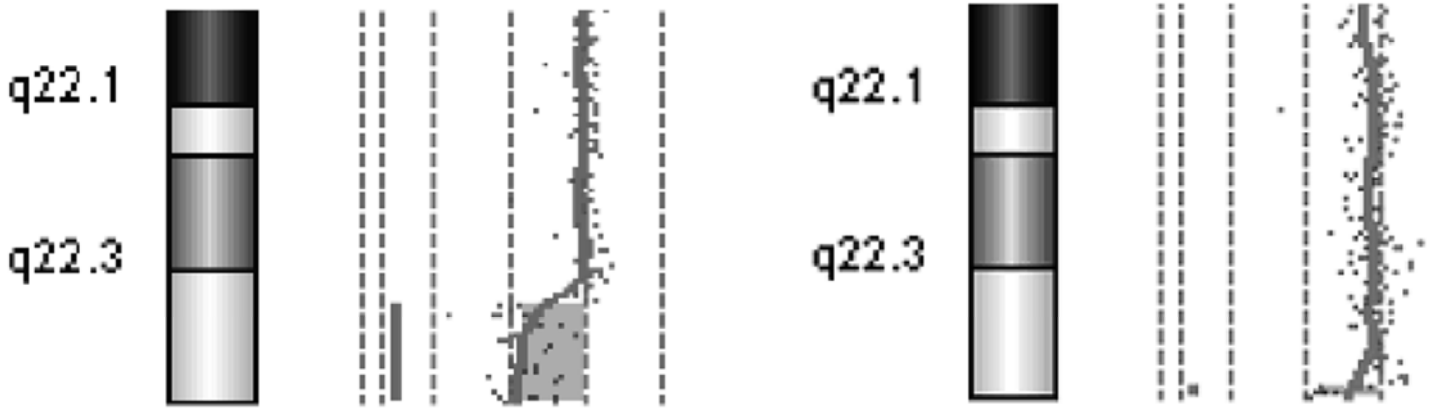


Comparison of proband and mother with array CGH shows expansion of terminal deletion

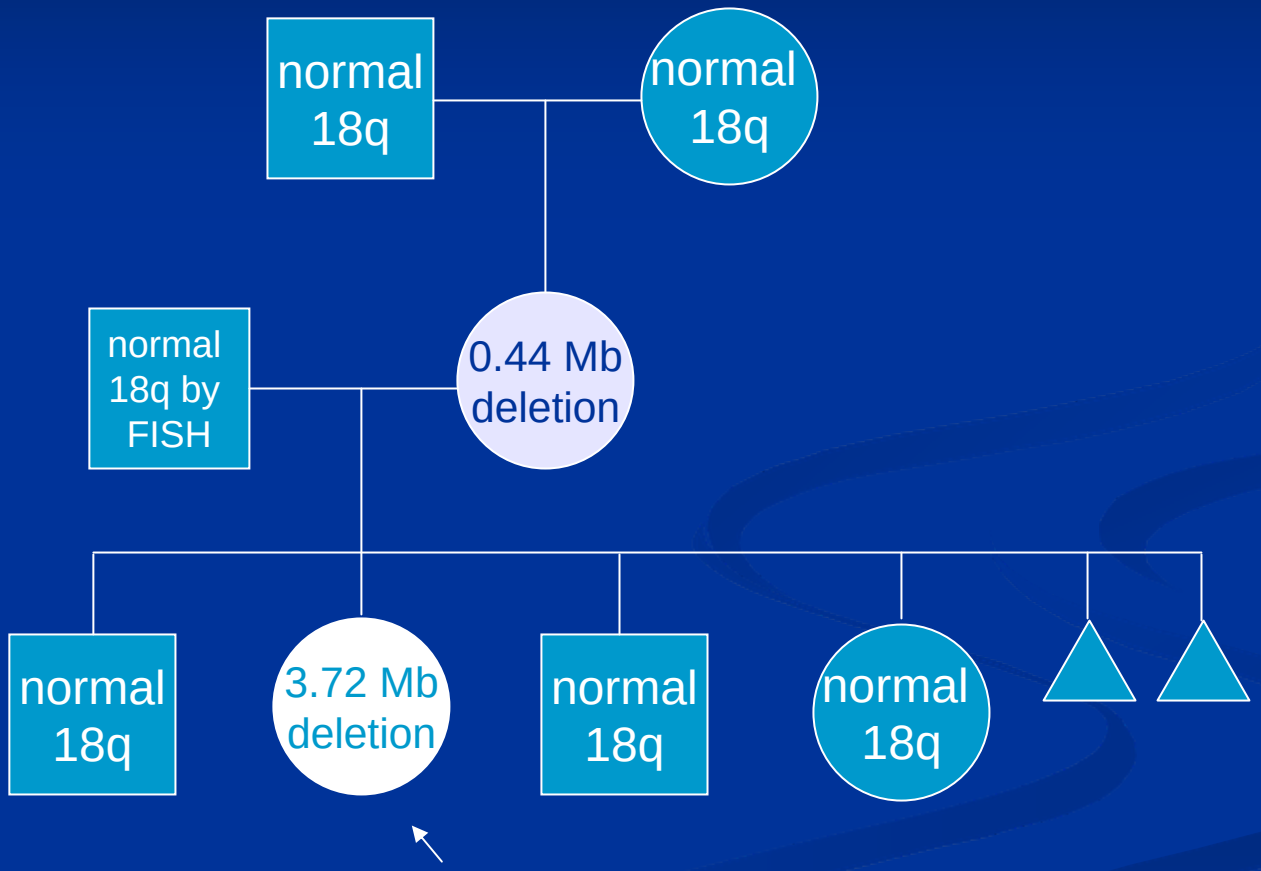
A.

	Chr	Cyto band	Location of most distal normal probe (bp)	Location of most proximal deleted probe (bp)	Location of most distal deleted probe (bp)	# of deleted probes	Approximate size of deletion (megabases)
proband	chr18	q23	72,268,375	72,366,480	76,083,258	42	3.7-3.8 Mb
mother	chr18	q23	75,544,270	75,641,908	76,083,258	9	0.44-0.54 Mb

B.



Other family members had normal array CGH results



STR markers confirm deletion in proband expanded from smaller deletion in mother

Marker	Location	Proband's alleles	Mother's alleles	Normal sibling's alleles
D18S1161	Proximal to proband deletion	231, 231	231, 231	231, 231
D18S462	Proximal to mother's deletion, within proband's deletion	306	304, 306	304, 304
D18S70	Within both mother's and proband's deletion	113	114	112, 114

Old Paradigm

- If parents are normal, then pure terminal deletions very likely de novo and parental studies not necessary
- Deletions are stable in size through generations; therefore, family studies can use a marker within the abnormality

New Possibility

- Parental studies should always be done
- Deletion size can expand between generations

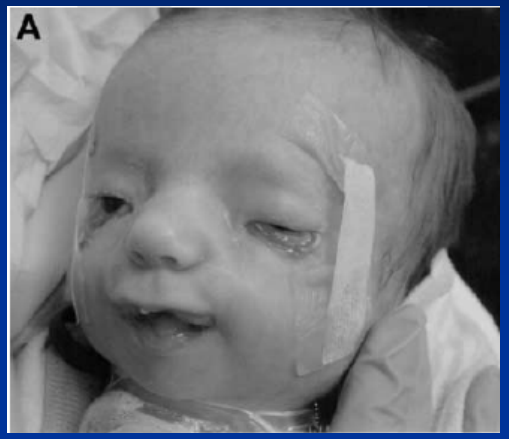
Old Paradigm

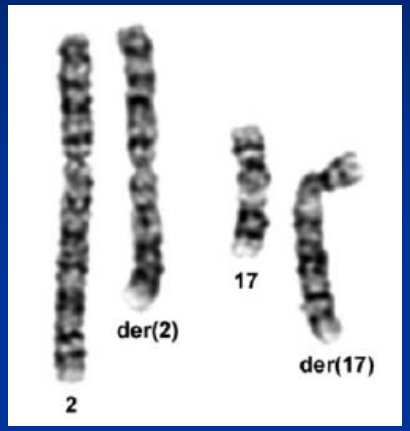
- Chromosome studies are sufficient for parental follow-up of a visible terminal deletion
- Differences in phenotype between a parent and offspring with a known, but unsized, deletion is likely due to differences in environment, genetic background, penetrance, epigenetic differences, or deletion unrelated to proband phenotype

New Possibility

- Chromosome studies may not be sufficient for parental studies since they may not recognize smaller deletions
  - Array CGH of parents may be needed
- Differences in phenotype between a parent and a child with a deletion may be due to alterations in the genetic content (size) of the deletion

Array CGH will not detect balanced rearrangements that may be clinically important





Chromosome analysis detected a balanced translocation

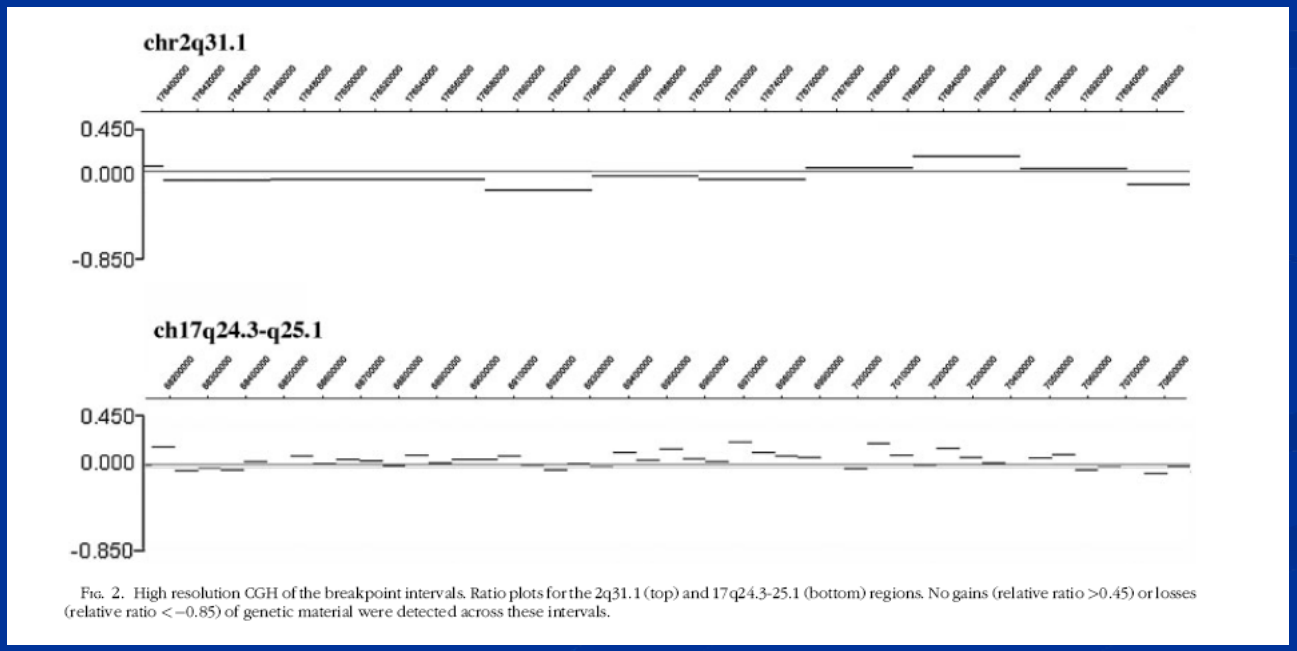
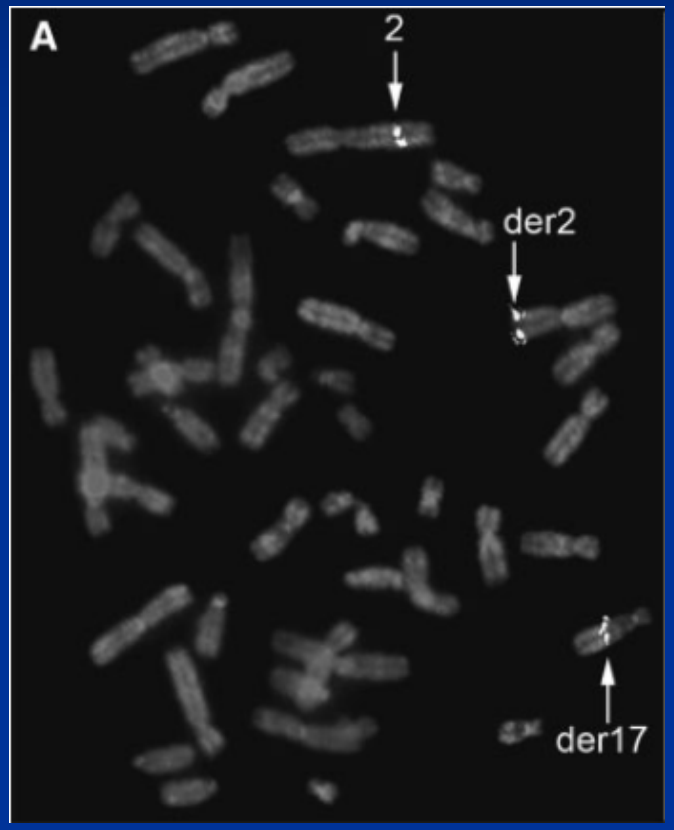
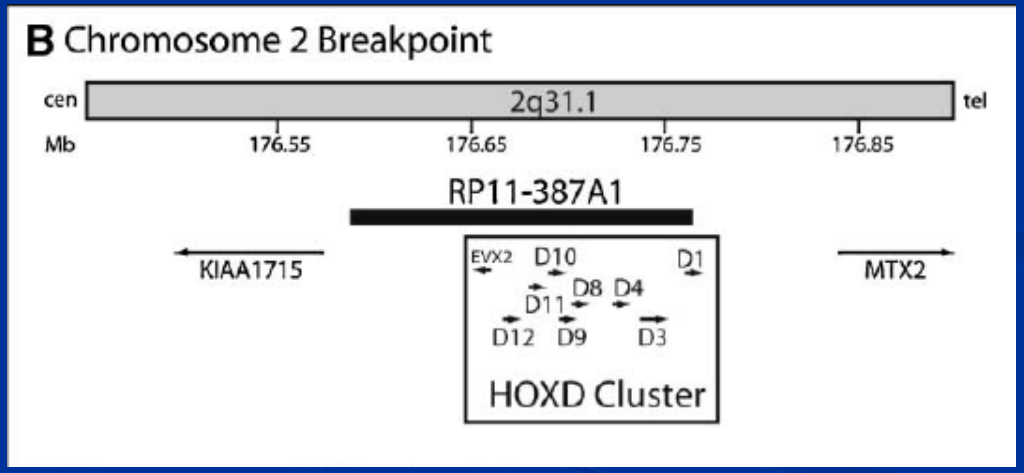


FIG. 2. High resolution CGH of the breakpoint intervals. Ratio plots for the 2q31.1 (top) and 17q24.3-25.1 (bottom) regions. No gains (relative ratio >0.45) or losses (relative ratio <−0.85) of genetic material were detected across these intervals.

High resolution array CGH analysis was normal (no loss or gain at breakpoints)

Characterization of breakpoints by FISH revealed likely genetic etiology





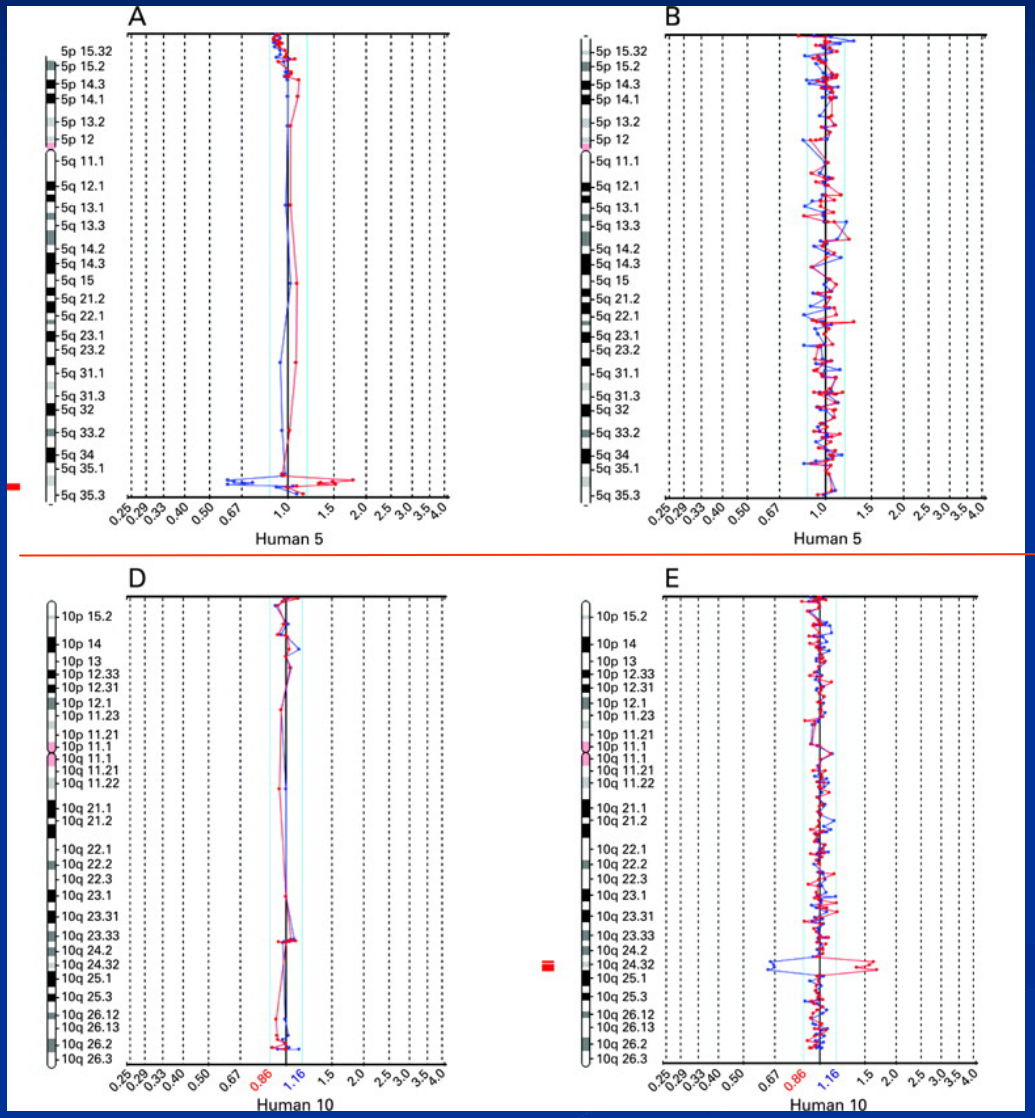
Translocation disrupted HOXD gene cluster

But, not all de novo balanced translocations are responsible for the observed phenotype:

Baptista et al. Am J Hum Genet 82, 927-936, 2008

- 31 phenotypically normal carriers of reciprocal translocation
  - No genomic imbalances at the breakpoints or elsewhere in the genome detected by array
  - 16/31 (52%) cases the breakpoint did disrupt a gene
- 14 abnormal carriers of reciprocal translocations
  - 4/14 (27%) cases showed disease causing imbalances by array
  - 5/14 (36%) cases the breakpoint did disrupt a gene

Abnormalities of regions of the genome not represented on the array platform will be missed



Targeted array detected a deletion of the region around the Sotos syndrome gene, but it was missed on the "1 Mb" chip

Targeted array missed a deletion within chromosome 10 (backbone too sparse) but it was detected on the "1 Mb" chip

Conclusions

- Microarray technology is a powerful tool for the detection of the etiology of developmental delay and multiple congenital anomalies
- The detection rate for these indications using microarray alone is 15-20%
  - ~1% of clinically significant alterations can be detected by a chromosome analysis and not a microarray analysis (example: balanced translocations and perhaps some cases of mosaicism)
- Microarray provides a more detailed, automatable and less subjective analysis of abnormal DNA copy number compared to standard chromosome analysis
- Proper counseling and follow-up is extremely important as a copy number change of unknown clinical significance can be identified (~ 5-10%)

Acknowledgments

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