Genomic Microarray Testing in Constitutional and Oncology Settings

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Learning Objectives

- Provide an overview of the technical details and basic interpretation of results from genomic microarray analysis
- Compare the utility of genomic microarray analysis to other genomic analysis techniques and understand the advantages, disadvantages and limitations of these tests
- Understand the utility of genomic microarray for different clinical indications including diagnosis of heritable genetic conditions in children, adults, pregnancy and fetal loss and for diagnosis and monitoring in cancer

Genomic Composition

- Subdivided into 23 chromosome pairs, mt DNA
- Total Size = 3.1 Gb (haploid), 6.2 Gb (diploid)
  - 3,100 Mb or 3,100,000 kb or 3.1 billion bp
  - Chromosome size range: chr. 1 = 249 Mb (8%) → chr. 21 = 48 Mb (1.5%)
- Gene content = 1.5% exonic, 26% intronic, ~8% regulatory sequences (~20,000 genes)
  - Distribution of genes is uneven across and within chrs.
- Gene size = avg. 10-20 kb; range 0.8 kb-2.2 Mb

➢ Influences technical capabilities and interpretation

What is Genomic Microarray Analysis (GMA)?

Definition:

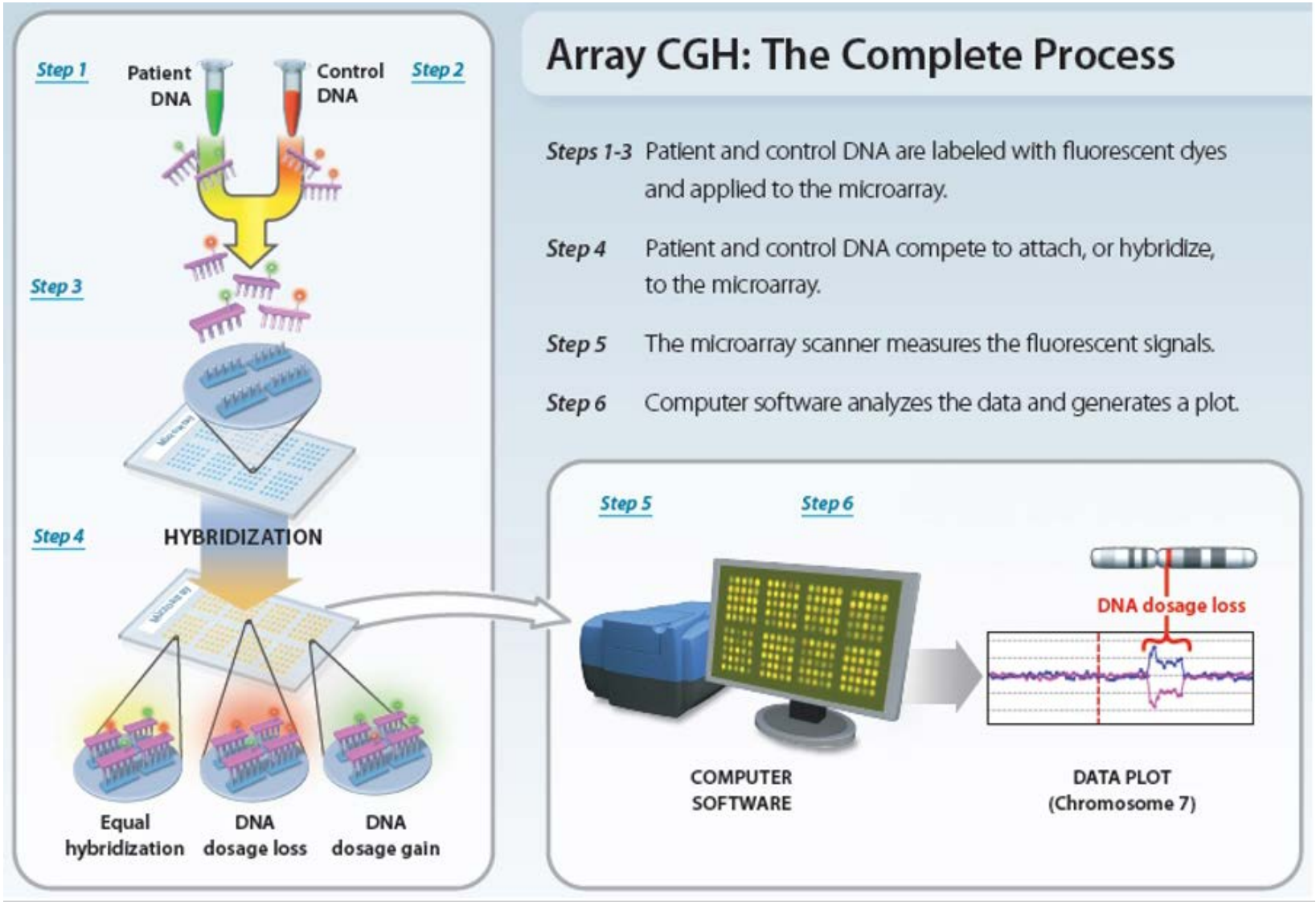
- A genome-wide analysis technology used to assess DNA copy number, and in some cases genotype, in a sample

Provides detection of genomic alterations:

- Copy number variants (CNVs)
  - Losses and gains, deletions and duplications
- Copy-neutral changes (SNP-based platforms only)
  - Long contiguous stretches of homozygosity (LCSH)
  - Absence- or loss-of-heterozygosity (AOH/LOH)
    - NOTE: “AOH” is preferred in the constitutional setting

Synonyms

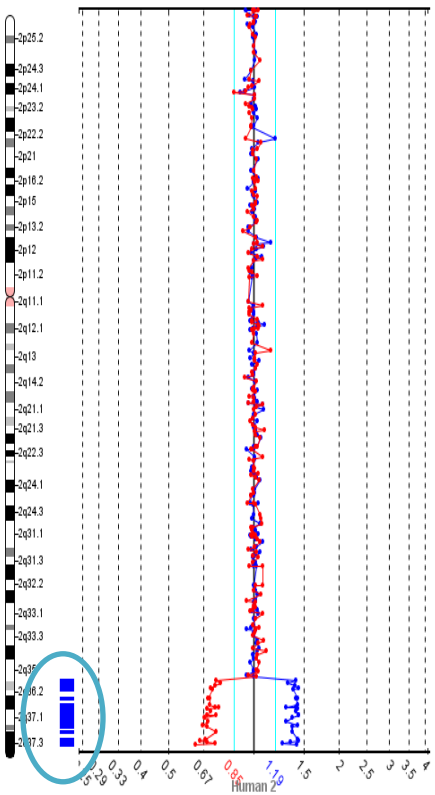
- Genomic microarray
- Cytogenomic microarray
- Chromosomal microarray
- Cytogenetic microarray
- SNP array
  - Interrogation of genotype information = copy neutral alterations
- Array comparative genomic hybridization (CGH)
  - Unlikely to interrogate genotype
- DNA microarray
  - Detect DNA (CGH) or RNA (cDNA after RT, expression profiling)
- Microarray
- Array



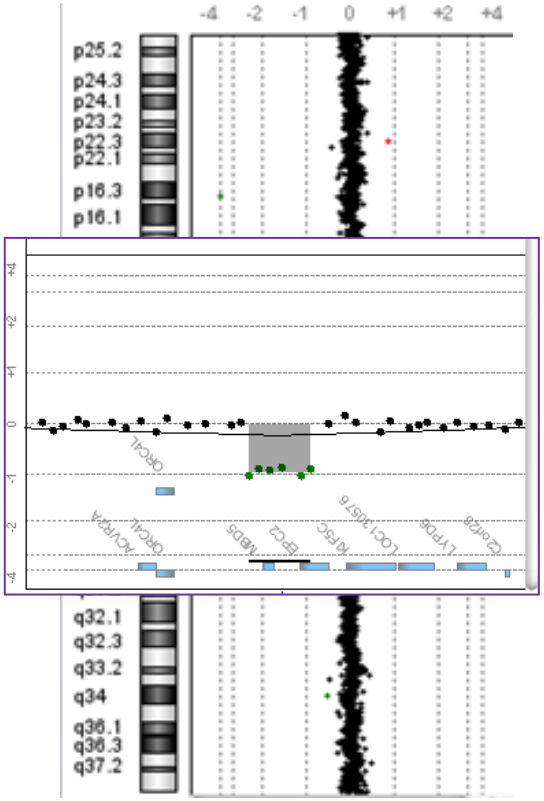
Theisen, A. (2008) Nature Education 1(1):45

Evolution of Genomic Microarrays

BAC-based chip, dye-swap experiment

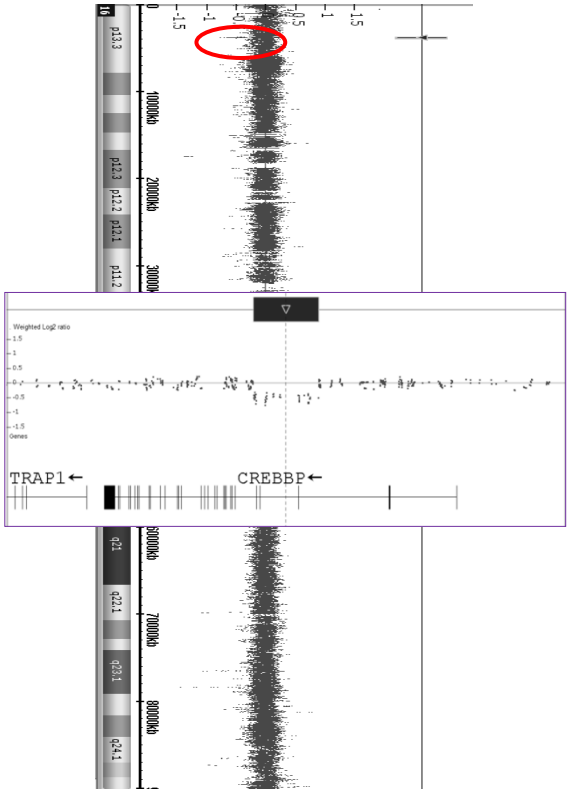


Oligo-based chip (without dye-swap)



Oligos +/- SNP-based chip

+Targeting for disease genes

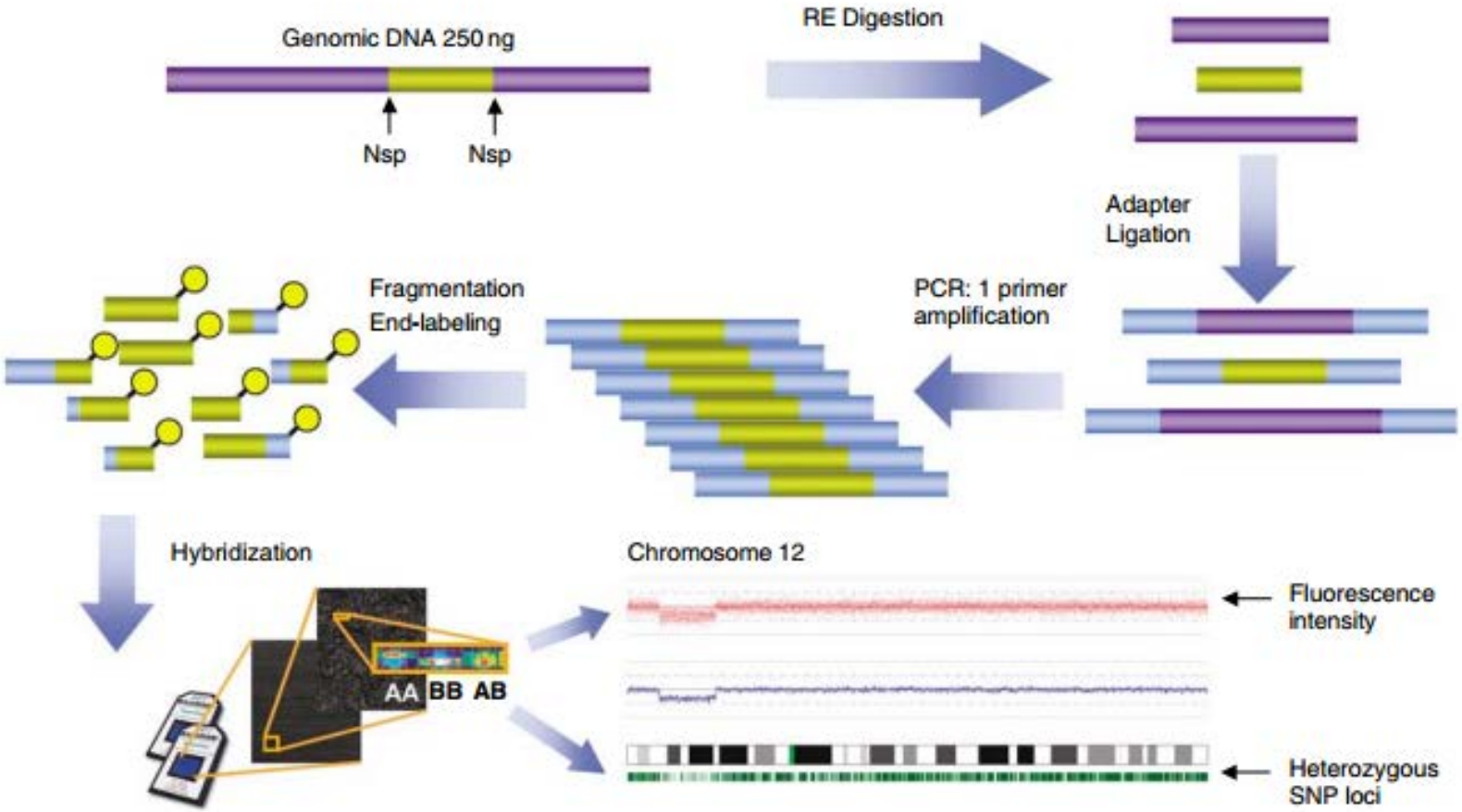


Resolution: 1 Mb → 50-100's kb → 10's kb

Strategies for Improved GMA Design

- Probe design
  - Smaller: BACs to oligos (higher density)
  - Incorporate SNPs (genotyping)
- Procedural modifications
  - Adapter ligation
  - PCR
  - Barcoding
- Array modifications
  - Replicate sampling
  - Incorporated hybridization parameters
  - Reference sample sets
- Sophisticated data processing and QC measurement algorithms

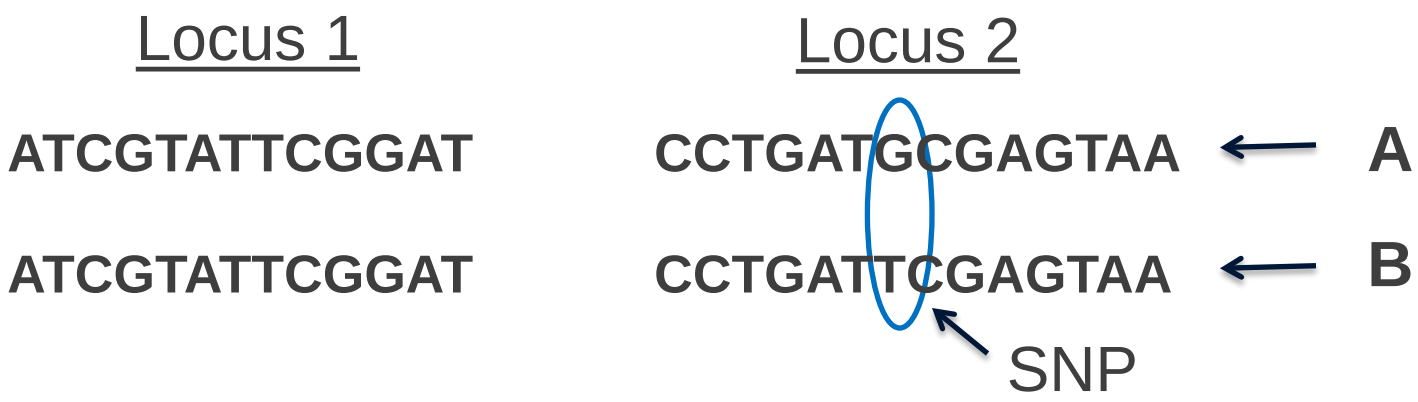
Cytogenomic SNP Microarray Process



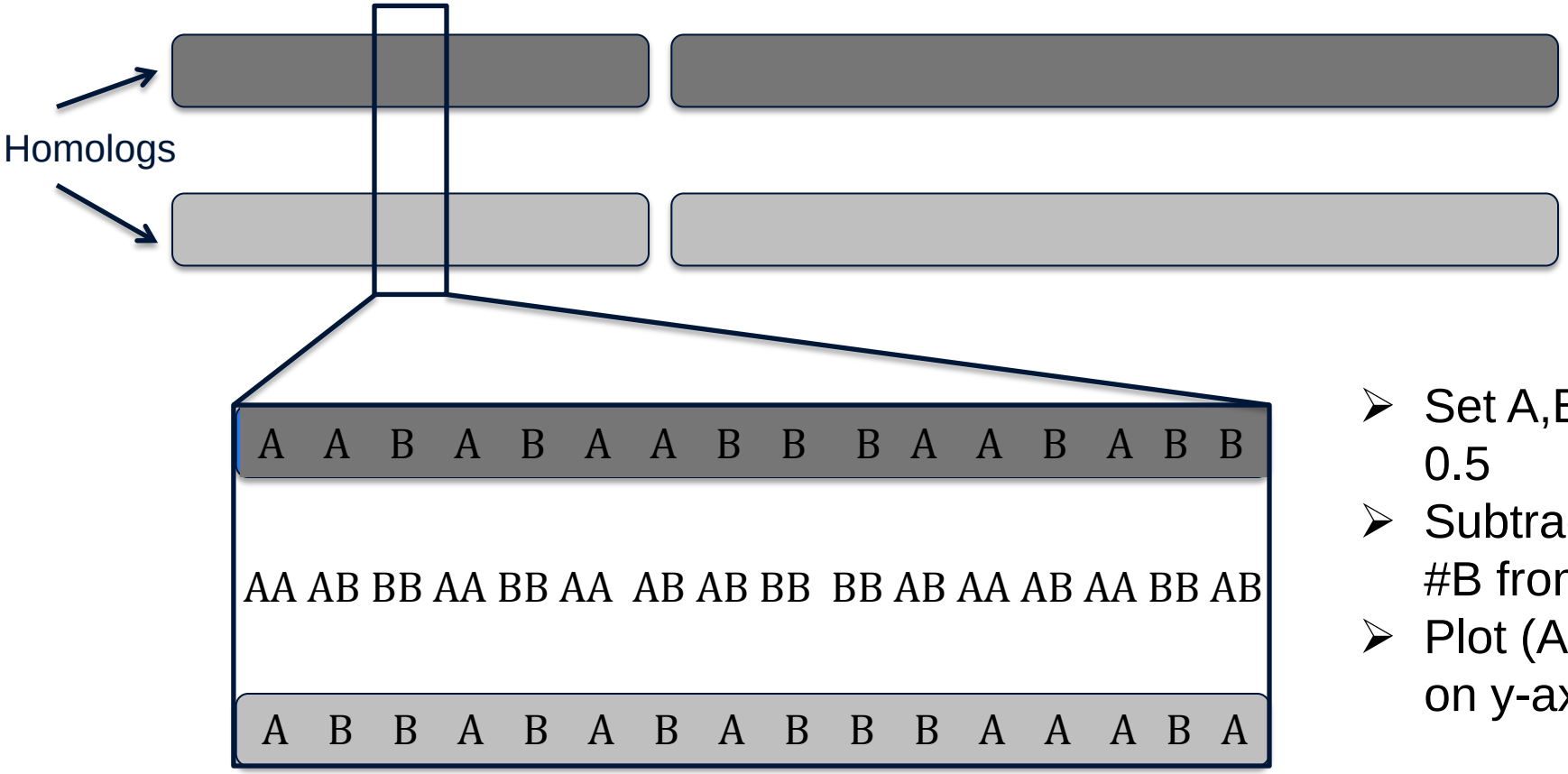
Tiu et al., Leukemia, 2007

SNP array design

- Polymorphic probes (contain SNPs)
  - Detect copy number and genotype
  - Used to interrogate genotype (alleles, A or B) at select loci across the genome
  - SNP probes are not evenly distributed and are lower in density
- Copy number probes
  - Used to increase density of coverage genome-wide, within genes

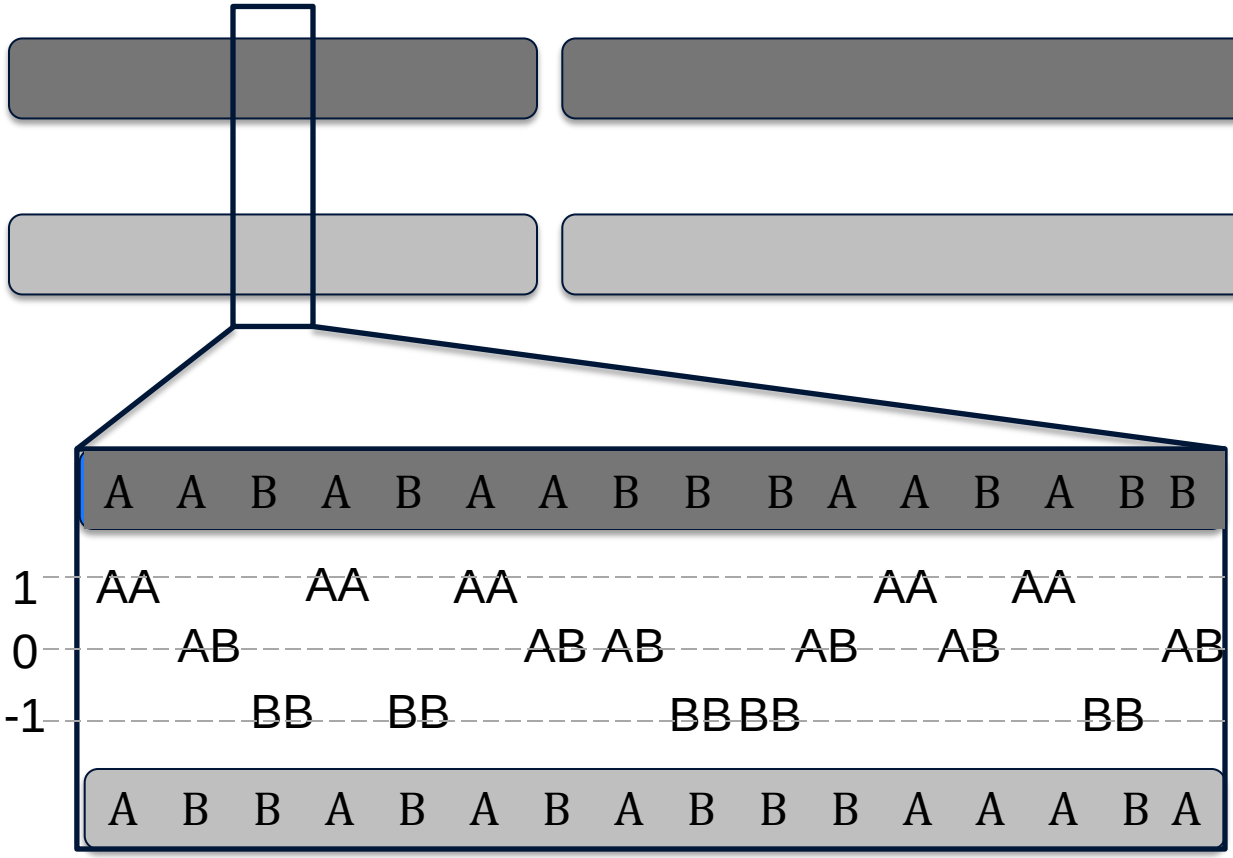


Converting SNP genotype to SNP pattern



- Set A,B = 0.5
- Subtract #B from #A
- Plot (A-B) on y-axis

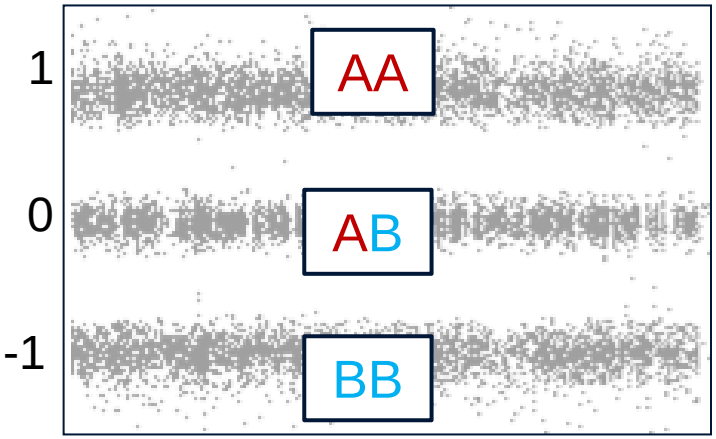
Converting SNP genotype to SNP pattern



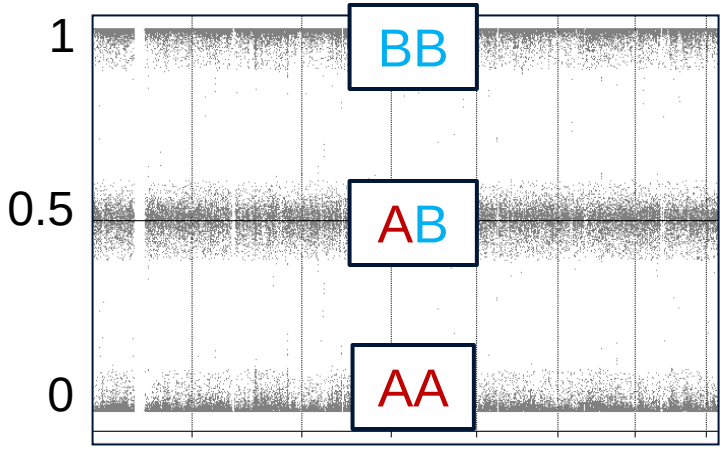
- Set A,B = 0.5
- Subtract #B from #A
- Plot (A-B) on y-axis

SNP Patterns by Allele Difference or Allele Frequency

Allele Difference



(B) Allele Frequency



The A-B calculation

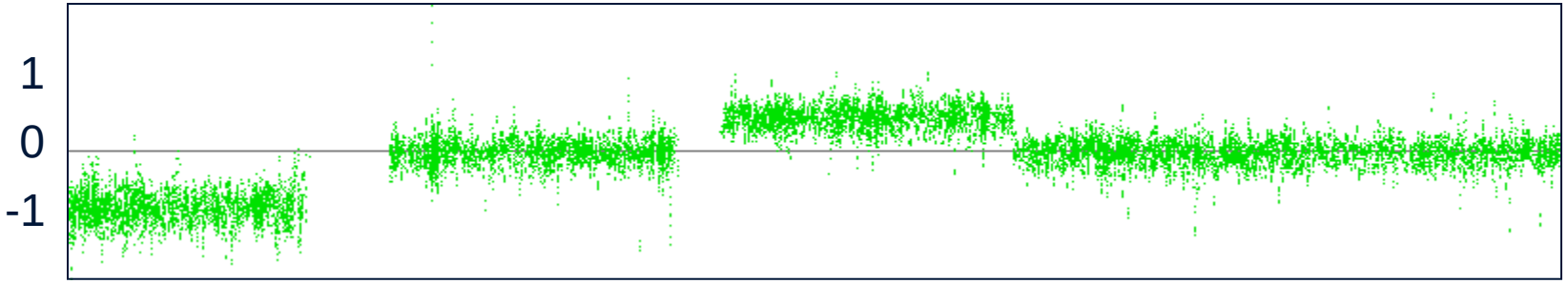
AA: (0.5+0.5) – 0 = 1
AB: 0.5 – 0.5 = 0
BB: 0 – (0.5+0.5) = -1

The B/(A+B) calculation

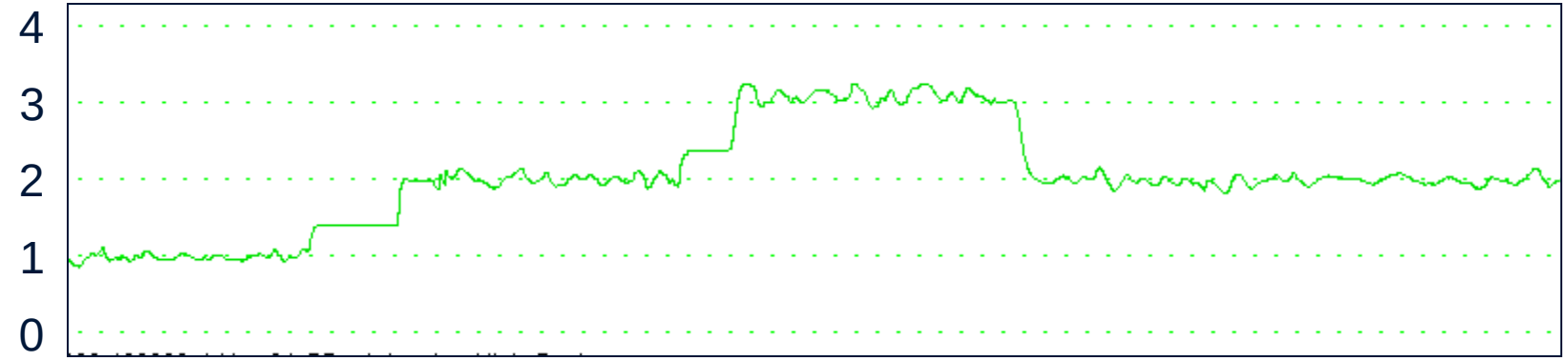
BB :(0.5+0.5)/(0+0.5+0.5)= 1
AB: 0.5/(0.5+0.5) = 0.5
AA: 0/(0.5+0.5+0) = 0

Copy number information using the Log2 ratio

Log2 ratio: relative measurement of fluorescence intensity (for each marker) in the sample compared to a reference signal profile



Copy number indicator: conversion of log2 to absolute CN

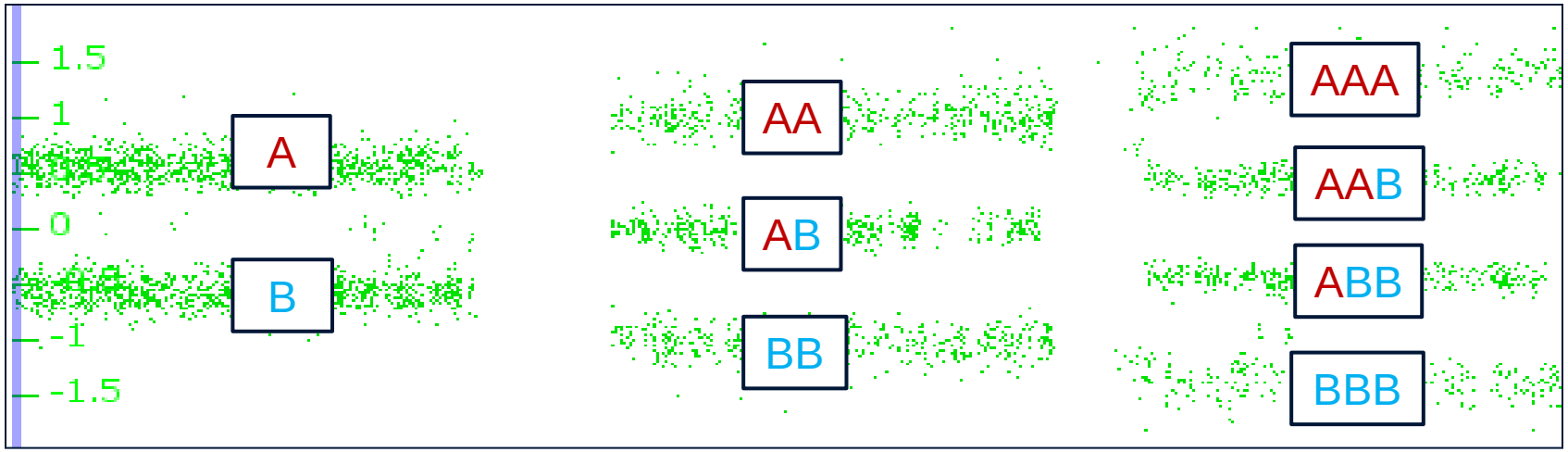


Copy number information by allele difference

Deletion
(1 allele, 2 tracks)

Normal Diploid
(2 alleles, 3 tracks)

Duplication
(3 alleles, 4 tracks)



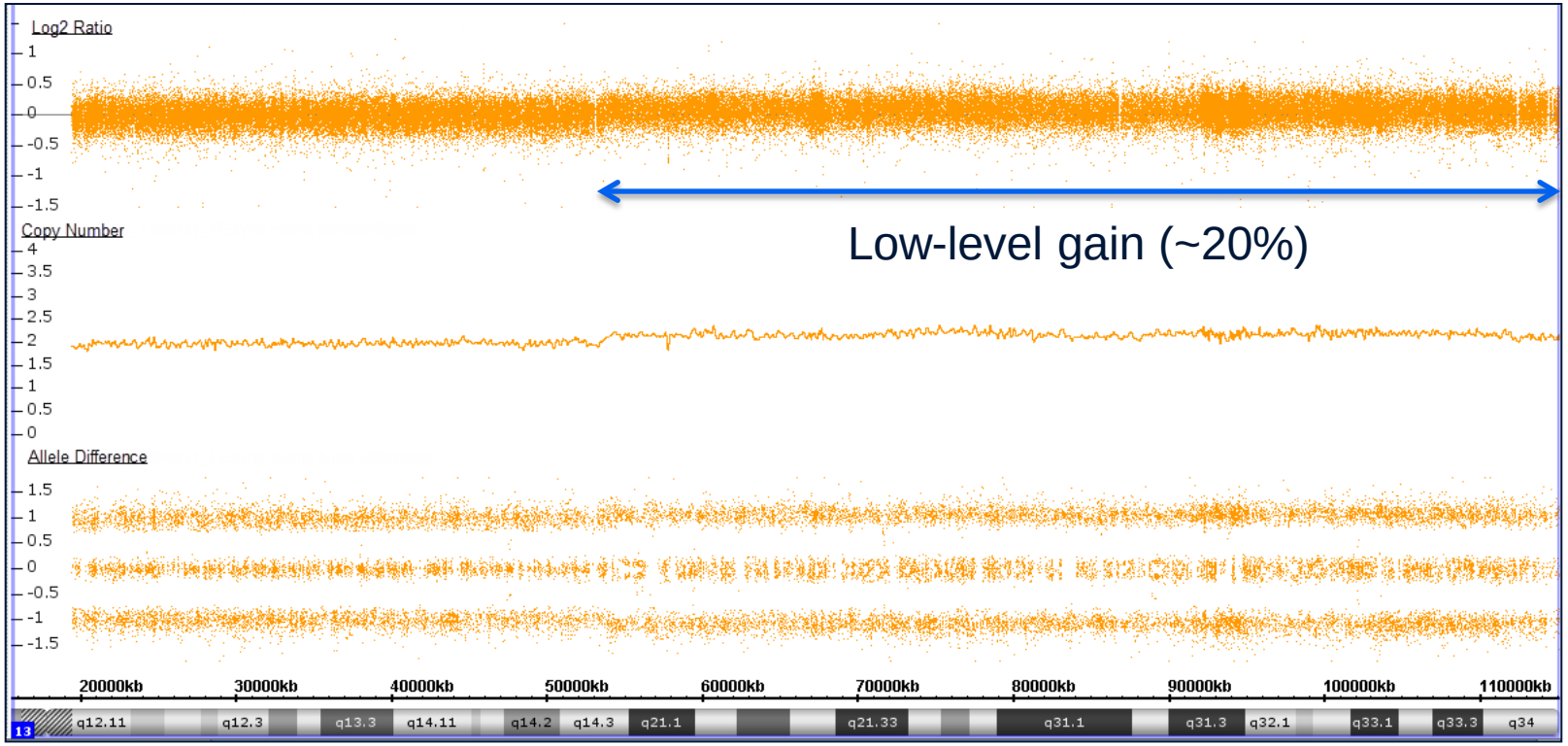
A: 0.5 – 0 = 0.5
B: 0 – 0.5 = -0.5

AA: (0.5+0.5) – 0 = 1
AB: 0.5 – 0.5 = 0
BB: 0 – (0.5+0.5) = -1

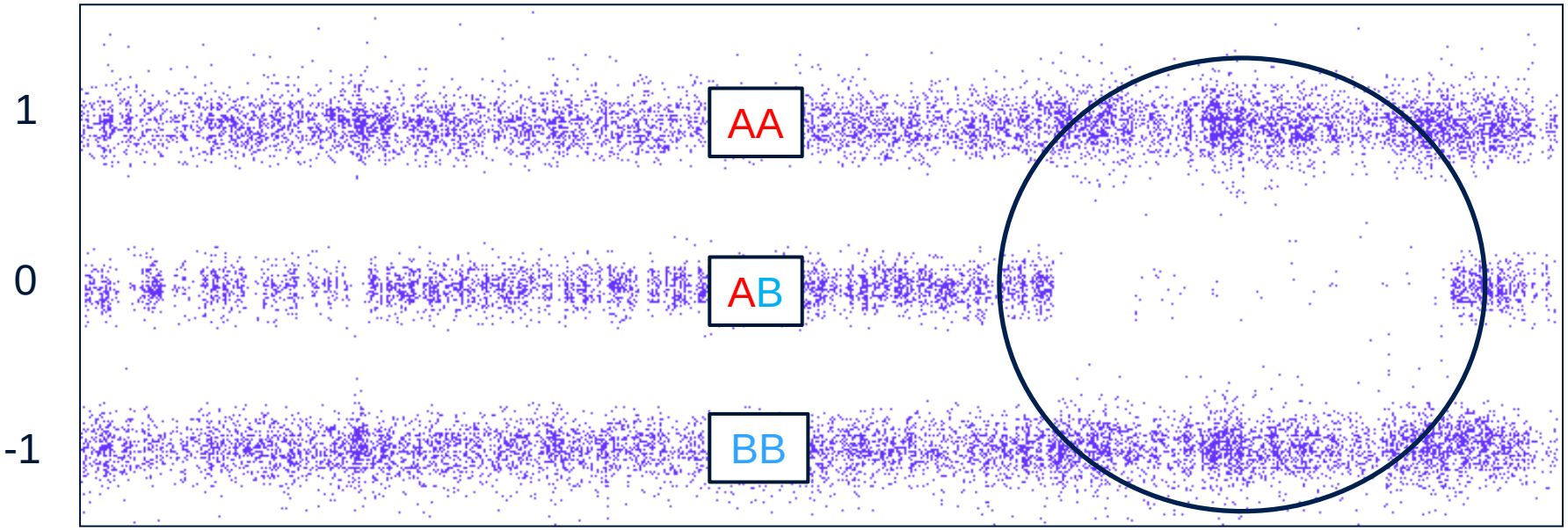
AAA: (0.5+0.5+0.5)–0 = 1.5
AAB: (0.5+0.5)–0.5 = 0.5
ABB: 0.5–(0.5+0.5) = -0.5
BBB: 0–(0.5+0.5+0.5) = -1.5

SNP probe confirmation of low-level alterations

Chr. 13



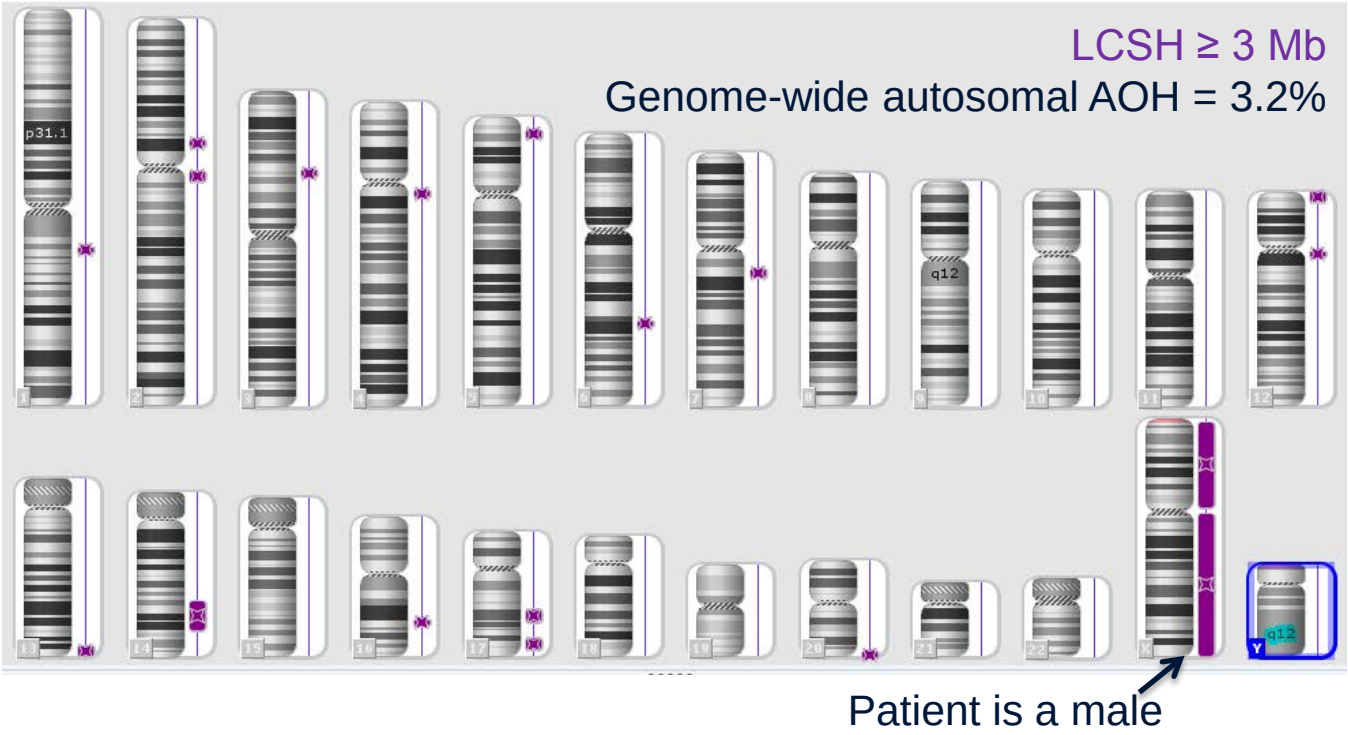
SNP probe detection of changes that are copy-neutral



- There are still 2 alleles present, but they are homozygous at every locus
- There is an absence of heterozygosity (AOH)
- This region is a long contiguous stretch of homozygosity (LCSH)

Copy-neutral absence of heterozygosity (AOH) may indicate…

- Inheritance of identical alleles from each parent
  - Common ancestry



➢ Confers recessive disease risk, test is NOT diagnostic for an AR conditions

Copy-neutral absence of heterozygosity (AOH) may indicate…

- Inheritance of identical alleles from each parent (closer degree of relationship between parents)
  - ≥10% genome-wide AOH raises suspicion for 1st or 2nd degree relatives

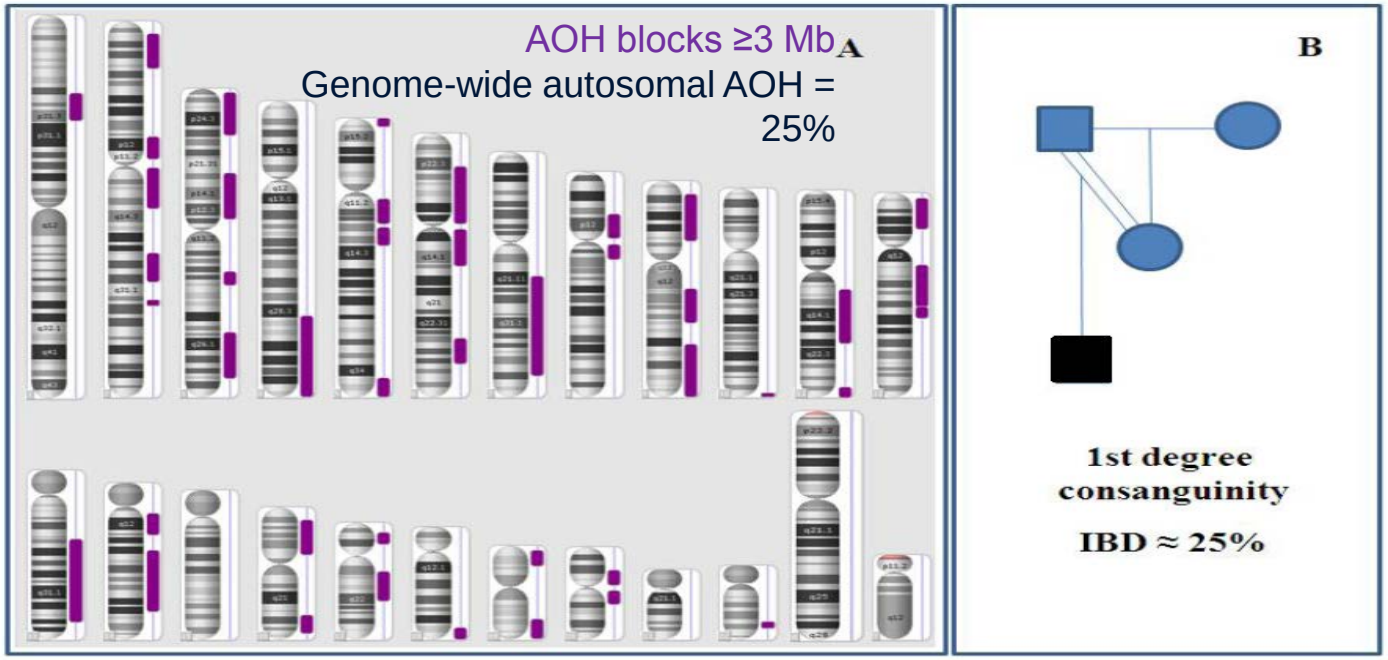


Figure: Kearney et al., Clin Lab Med 31 (2011)

➢ Exercise caution when reporting genome-wide AOH larger than 10%

Genetics in Medicine | ACMG STANDARDS AND GUIDELINES

American College of Medical Genetics and Genomics: standards and guidelines for documenting suspected consanguinity as an incidental finding of genomic testing

Catherine W. Rehder, PhD[1], Karen L. David, MD, MS[2,3], Betsy Hirsch, PhD[4], Helga V. Toriello, PhD[5], Carolyn M. Wilson, MS[6] and Hutton M. Kearney, PhD[6]

Genomic testing, including single-nucleotide polymorphism–based microarrays and whole-genome sequencing, can detect long stretches of the genome that display homozygosity. The presence of these segments, when distributed across multiple chromosomes, can indicate a familial relationship between the proband's parents. This article describes the detection of possible consanguinity by genomic testing and the factors confounding the inference of a specific parental relationship. It is designed to guide the documentation of suspected consanguinity by clinical laboratory professionals and to alert laboratories to the need to establish a reporting policy in conjunction with their ethics review committee and legal counsel.

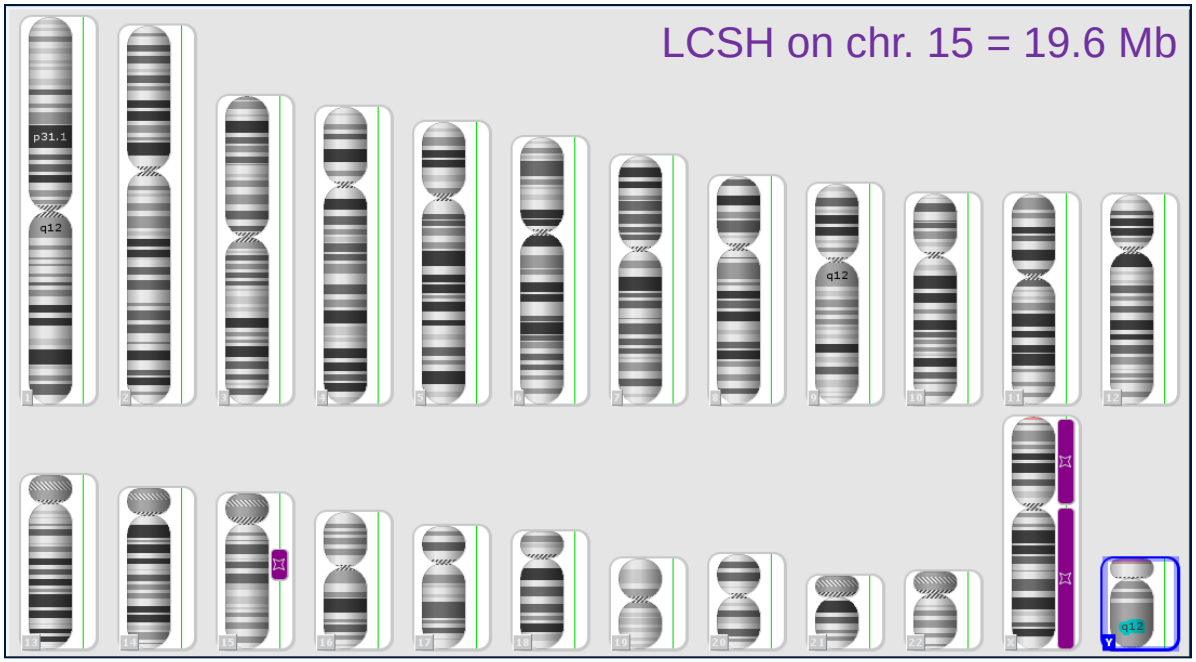
Genet Med 2013:15(2):150–152

Key Words: consanguinity; homozygosity; laboratory guideline

- There is clinical utility in the detection of genomic AOH, even when the % is quite low (<3%)
- Cases with >10% genomic AOH have the potential of uncovering a situation of familial abuse
- Laboratories are encouraged to develop a reporting policy in conjunction with their ethics review committee and legal counsel

Copy-neutral absence of heterozygosity (AOH) may indicate…

- Inheritance of both alleles from one parent (UPD)
  - Uniparental disomy

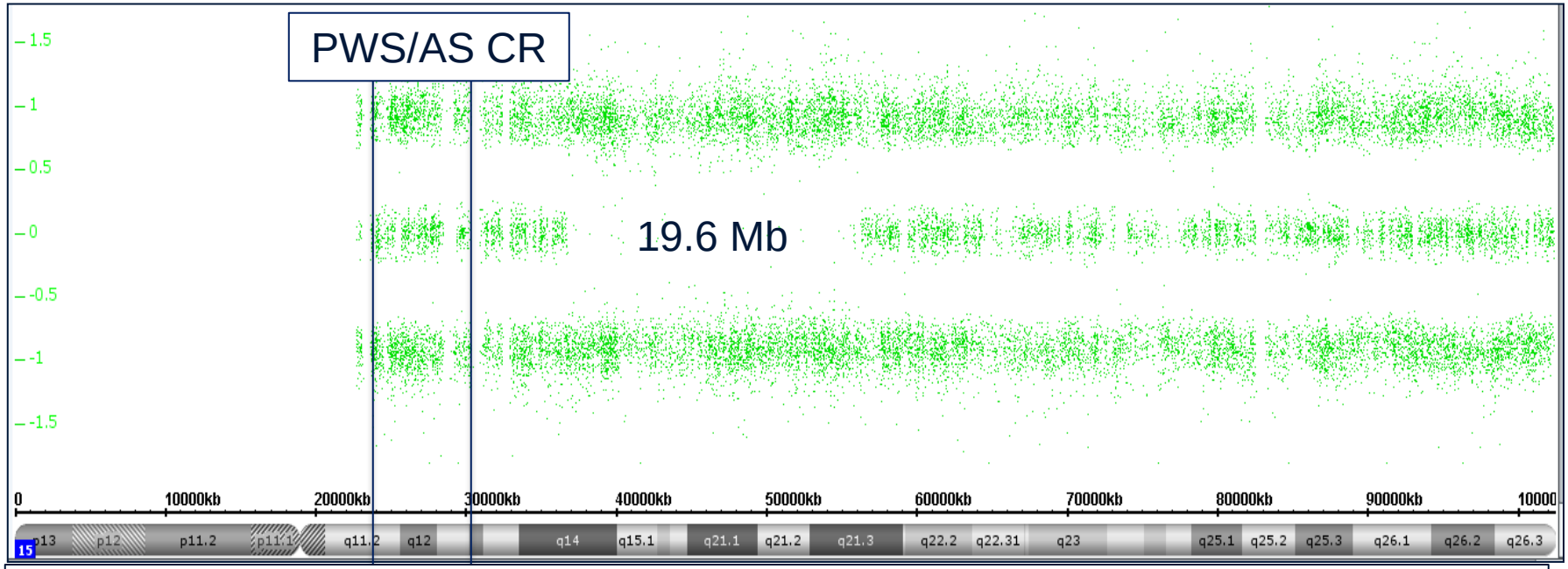


- Usually results from aberrant segregation event during meiosis or mitosis
- Usual observation is LCSH on a single chromosome

Copy-neutral absence of heterozygosity (AOH) may indicate…

Case: methylation testing consistent with a diagnosis of PWS

Chromosome 15



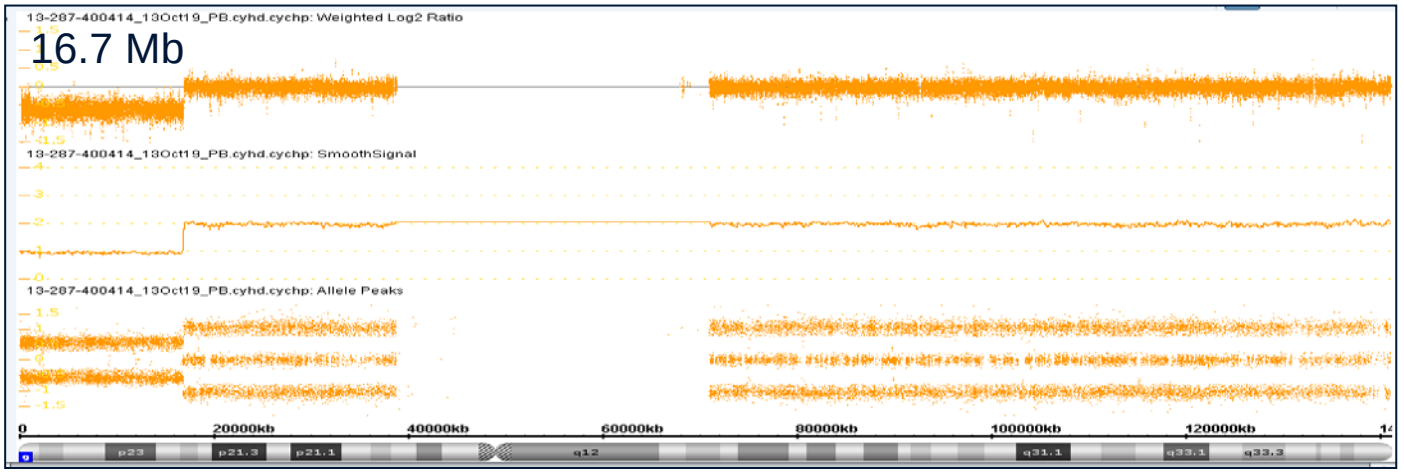
- Risk for imprinting disorder if involving certain chromosomes (6, 7, 11, 14, 15, 20)
  - The LCSH does not have to overlap the imprinted genes
- Not all UPD will be detectable by GMA (i.e. complete heterodisomy)
- Risk for recessive disease for genes within LCSH region

Comparison of Constitutional Cytogenetic Tests

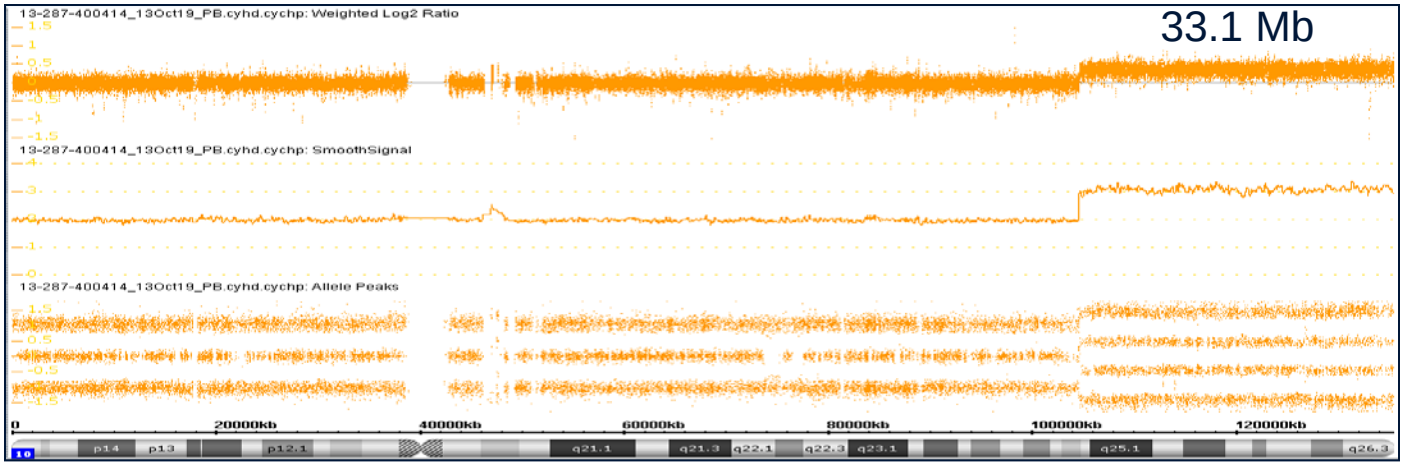
Technique	Resolution	Sensitivity (mosaicism)	Culturing required?	Global ?	Unbalanced abs?	Balanced abs? Structural info?	AOH?
G-banded chromosomes	3-5 Mb (550 bands)	10-15%	Yes	Yes	Yes	Yes	No
Metaphase FISH	100's kb	n/a	Yes	No	Yes	Yes	No
Interphase FISH	100's kb	1-5%	No	No	Yes	Yes	No
SNP-A	10-100's kb	10-20%	No	Yes	Yes	No	Yes

Case: 1 m/o female with cleft palate, congenital micrognathia

Chr. 9

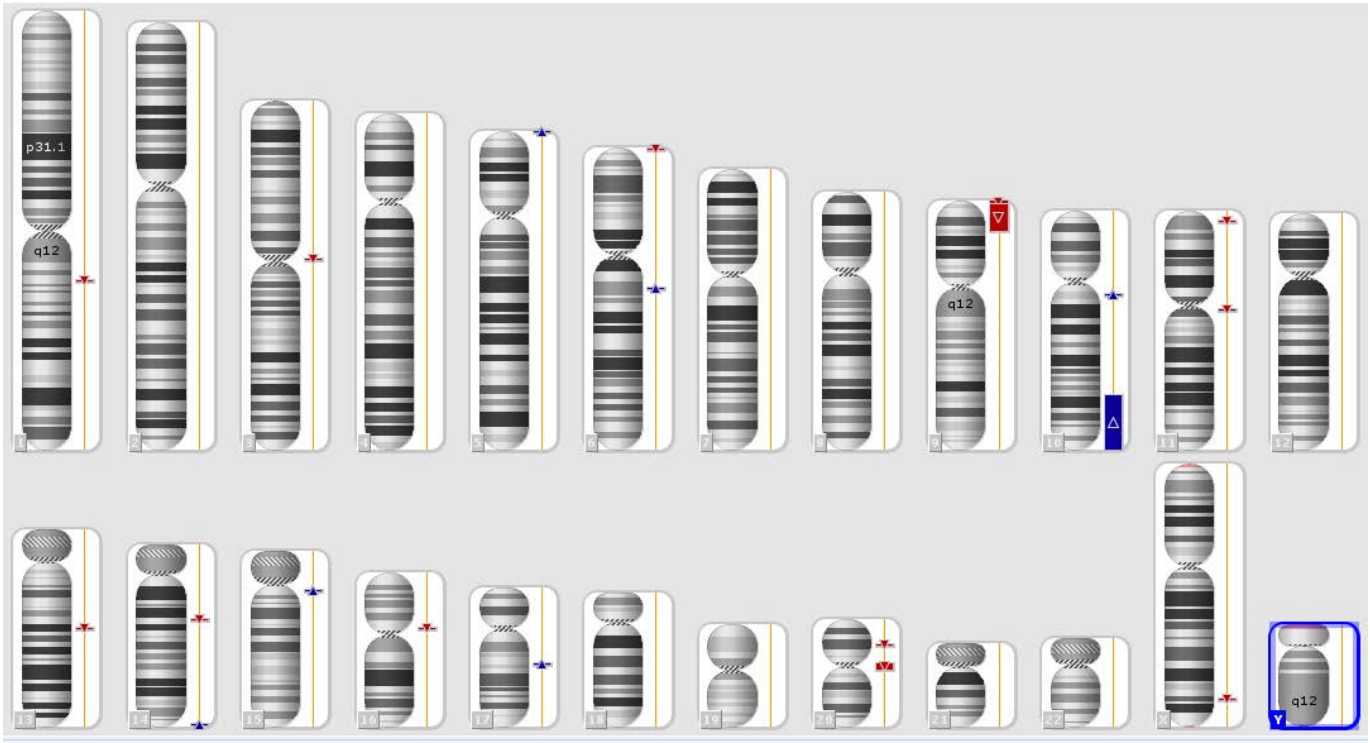


Chr. 10



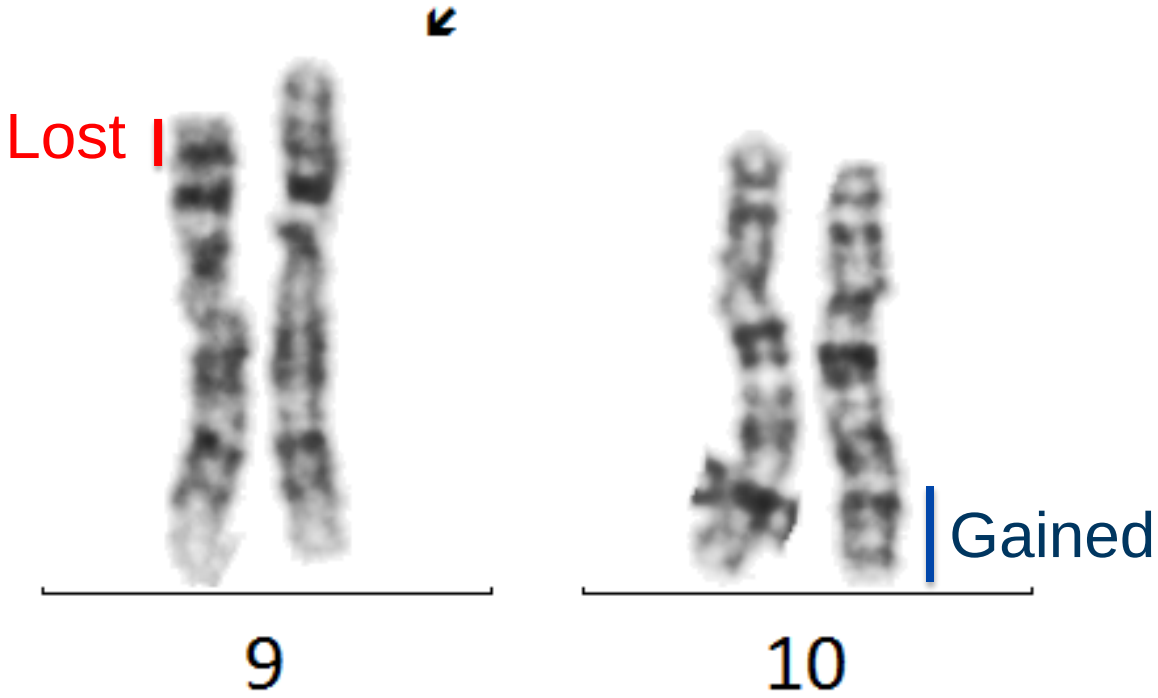
Case: 1 m/o female with cleft palate, congenital micrognathia

Genome view



- Pattern of terminal loss and gain affecting two different chromosomes is suggestive of an unbalanced translocation

Limited chromosome study showed: 46,XX,der(9)t(9;10)(p22;q24.3)



Clinical Utility of GMA

- Constitutional genetics: diagnosis of heritable genomic abnormalities (variants) in children, adults, pregnancy, and fetal loss
  - Abnormalities may be inherited or *de novo*
- Cancer genetics: detection of acquired or somatic (versus germline/constitutional) genomic abnormalities for the diagnosis, prognosis, therapy, and/or monitoring of many types of cancer, esp. hematologic

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

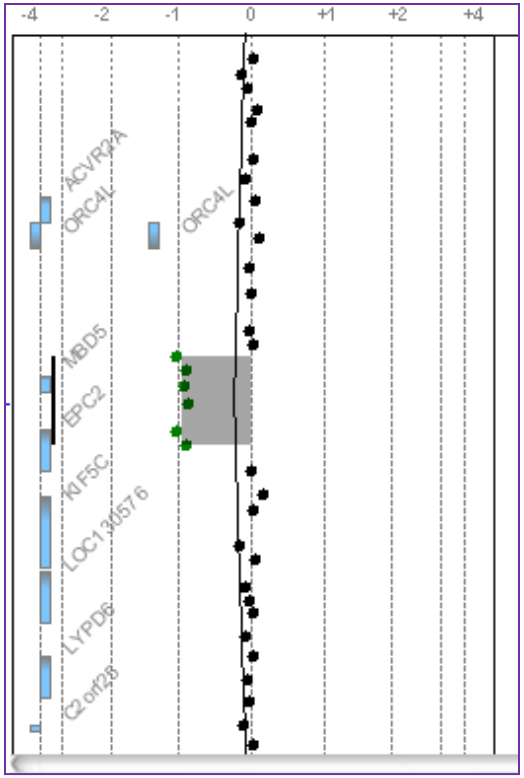
David T. Miller,[1,*] Margaret P. Adam,[2,3] Swaroop Aradhya,[4] Leslie G. Biesecker,[5] Arthur R. Brothman,[6] Nigel P. Carter,[7] Deanna M. Church,[8] John A. Crolla,[9] Evan E. Eichler,[10] Charles J. Epstein,[11] W. Andrew Faucett,[2] Lars Feuk,[12] Jan M. Friedman,[13] Ada Hamosh,[14] Laird Jackson,[15]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

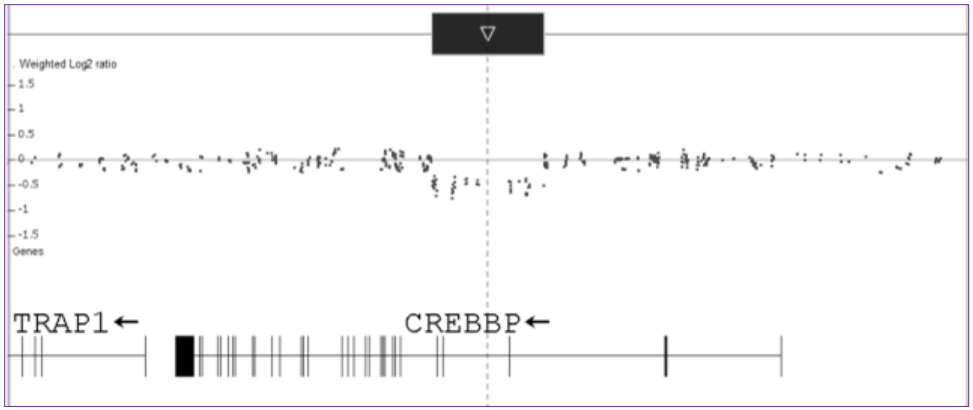
Chromosomal microarray (CMA) is increasingly utilized for genetic testing of individuals with unexplained developmental delay/intellectual disability (DD/ID), autism spectrum disorders (ASD), or multiple congenital anomalies (MCA). Performing CMA and G-banded karyotyping on every patient substantially increases the total cost of genetic testing. The International Standard Cytogenomic Array (ISCA) Consortium held two international workshops and conducted a literature review of 33 studies, including 21,698 patients tested by CMA. We provide an evidence-based summary of clinical cytogenetic testing comparing CMA to G-banded karyotyping with respect to technical advantages and limitations, diagnostic yield for various types of chromosomal aberrations, and issues that affect test interpretation. CMA offers a much higher diagnostic yield (15%–20%) for genetic testing of individuals with unexplained DD/ID, ASD, or MCA than a G-banded karyotype (~3%, excluding Down syndrome and other recognizable chromosomal syndromes), primarily because of its higher sensitivity for submicroscopic deletions and duplications. Truly balanced rearrangements and low-level mosaicism are generally not detectable by arrays, but these are relatively infrequent causes of abnormal phenotypes in this population (<1%). Available evidence strongly supports the use of CMA in place of G-banded karyotyping as the first-tier cytogenetic diagnostic test for patients with DD/ID, ASD, or MCA. G-banded karyotype analysis should be reserved for patients with obvious chromosomal syndromes (e.g., Down syndrome), a family history of chromosomal rearrangement, or a history of multiple miscarriages.

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

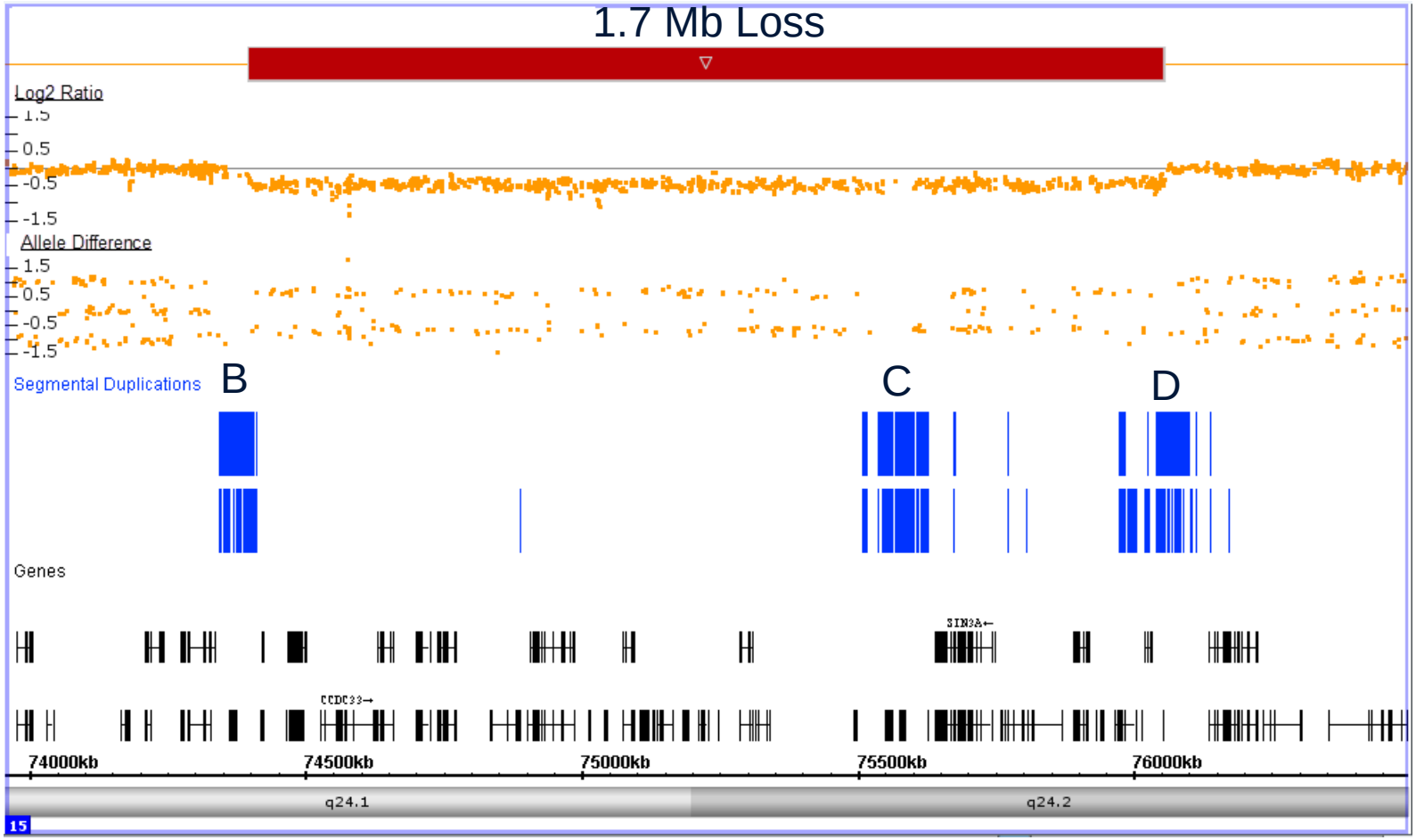
Detection of gene-level pathogenic alterations

Cases: MBD5 whole gene deletion, CREBBP intragenic deletion

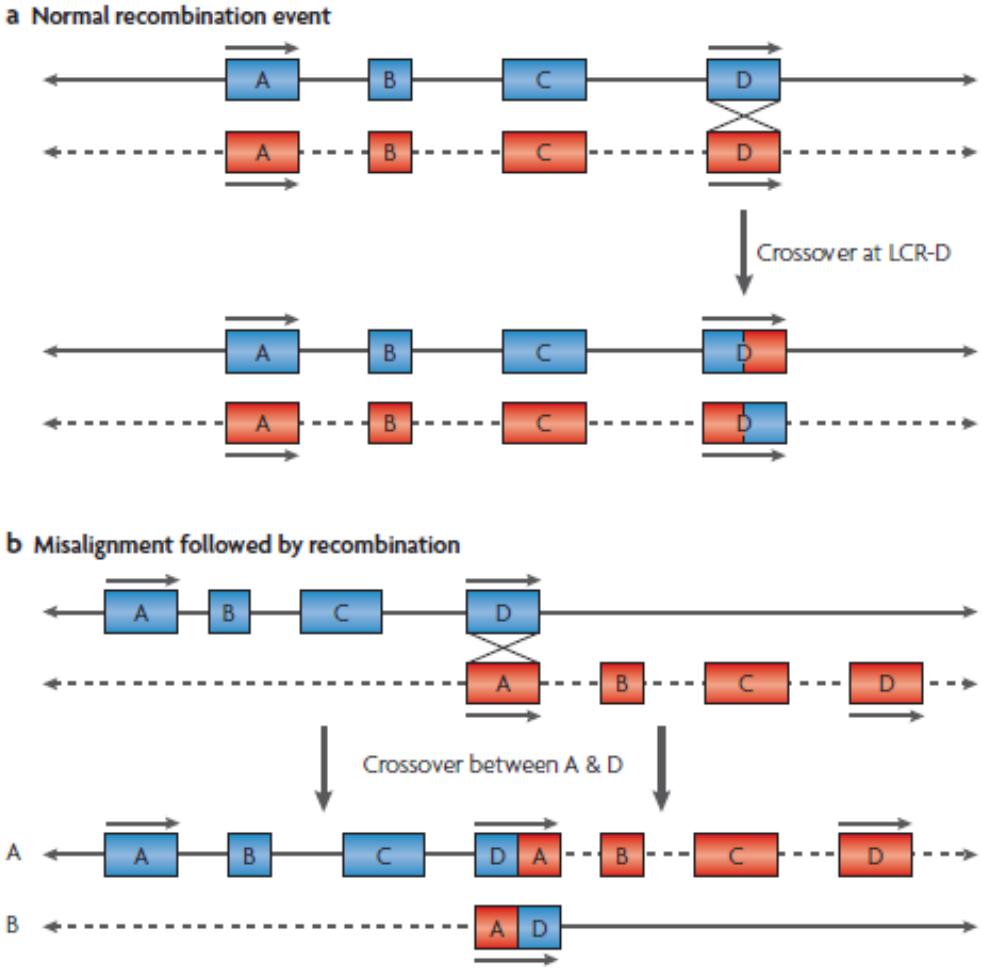




Discovery of new recurrent pathogenic CNVs
Case: 15q24 microdeletion syndrome



Mechanisms of recurrent structural change: Non-allelic homologous recombination



Emanuel and Saitta, Nat Rev Genet 2007

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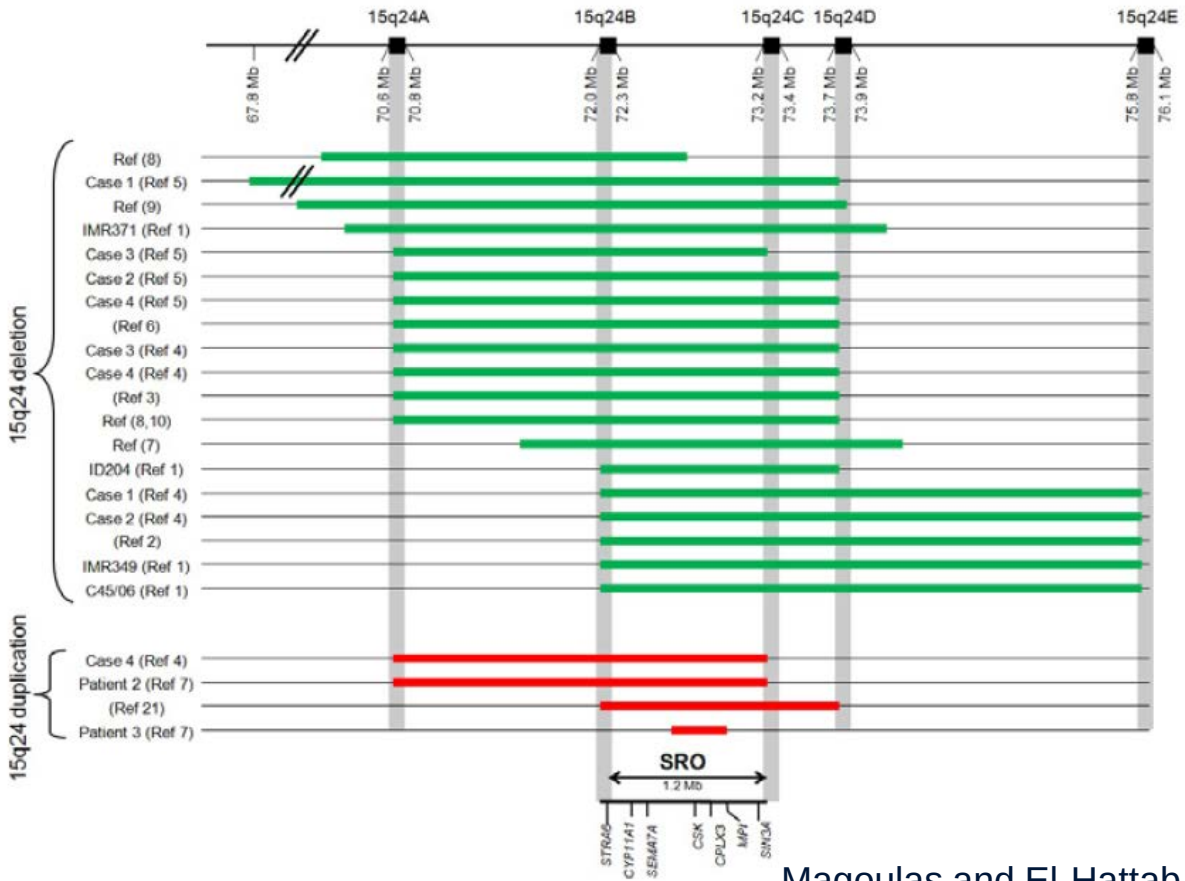


Magoulas and El-Hattab, OJRD 2012, 7:2

The American College of Obstetricians and Gynecologists
WOMEN'S HEALTH CARE PHYSICIANS

Society for Maternal-Fetal Medicine

COMMITTEE OPINION

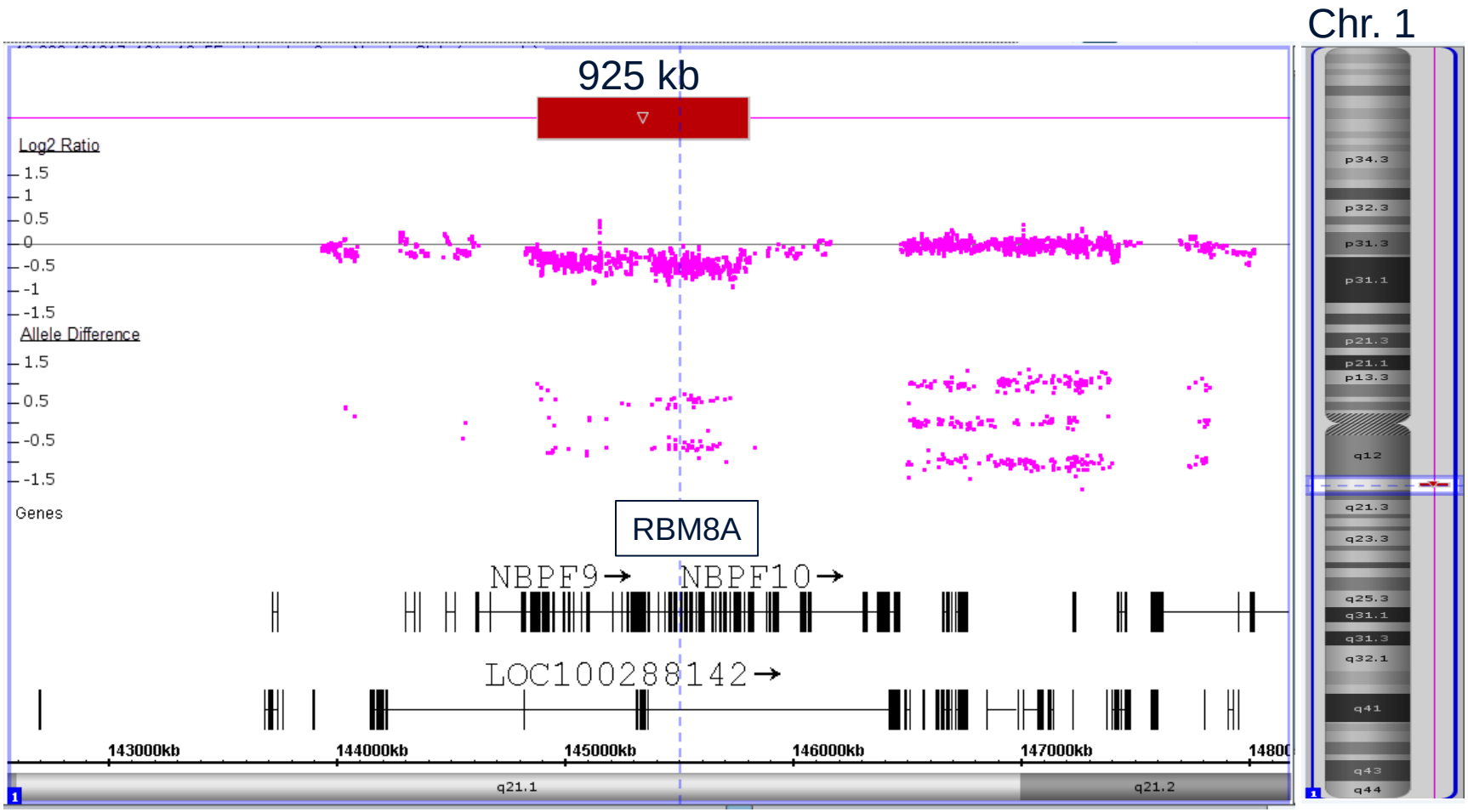
Number 581 • December 2013 *(Replaces No. 446, November 2009. Reaffirmed 2015)*
(See also Practice Bulletin No. 88)

The American College of Obstetricians and Gynecologists Committee on Genetics
Society for Maternal-Fetal Medicine

The Use of Chromosomal Microarray Analysis in Prenatal Diagnosis

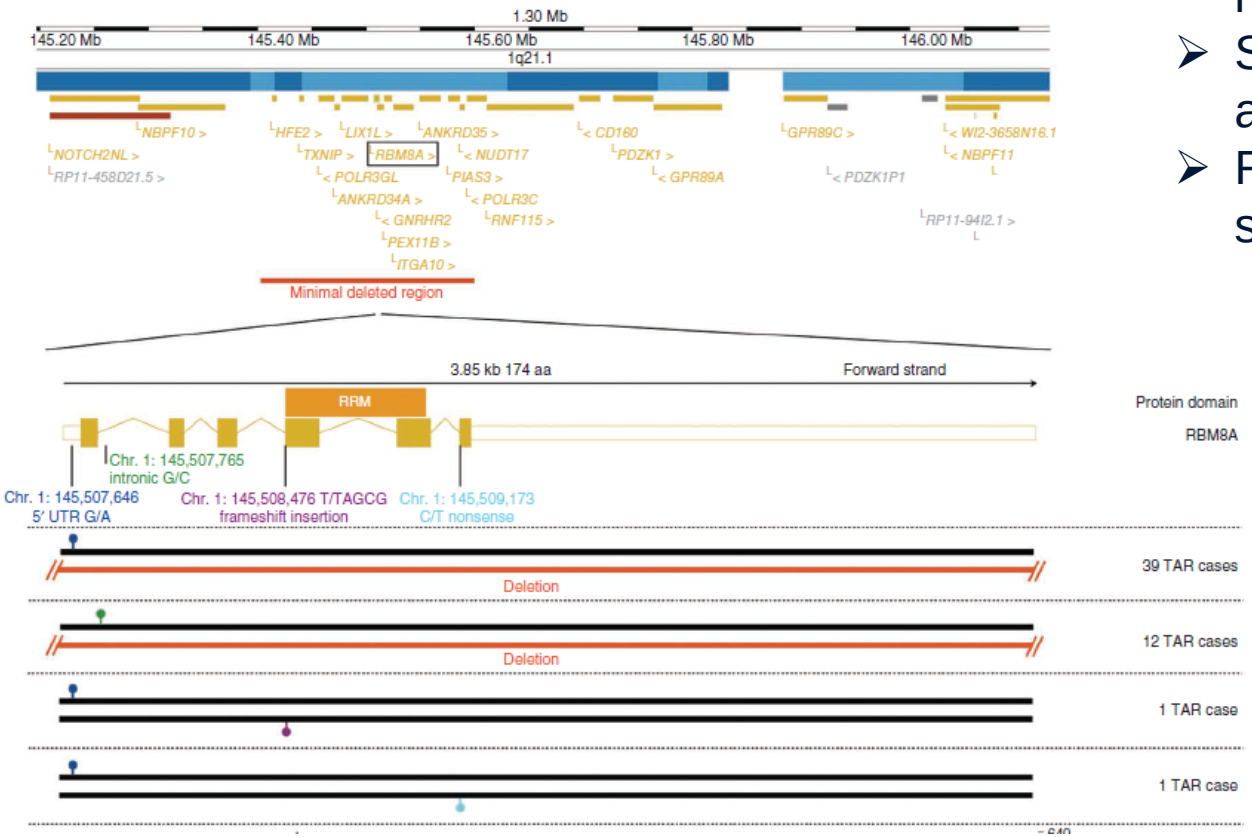
- Use in prenatal diagnosis: in patients with a fetus with one or more structural abnormalities identified on ultrasound, patients undergoing invasive prenatal diagnostic testing, not restricted to women aged 35+
- Use in intrauterine fetal demise or stillbirth: when further cytogenetic analysis is desired, not recommended for first or second trimester losses due to limited data on utility

Case: GA 21w, Advanced maternal age, US findings: skeletal anomalies, rocker bottom feet, abnormal arms



nature genetics

Compound inheritance of a low-frequency regulatory SNP and a rare null mutation in exon-junction complex subunit *RBM8A* causes TAR syndrome



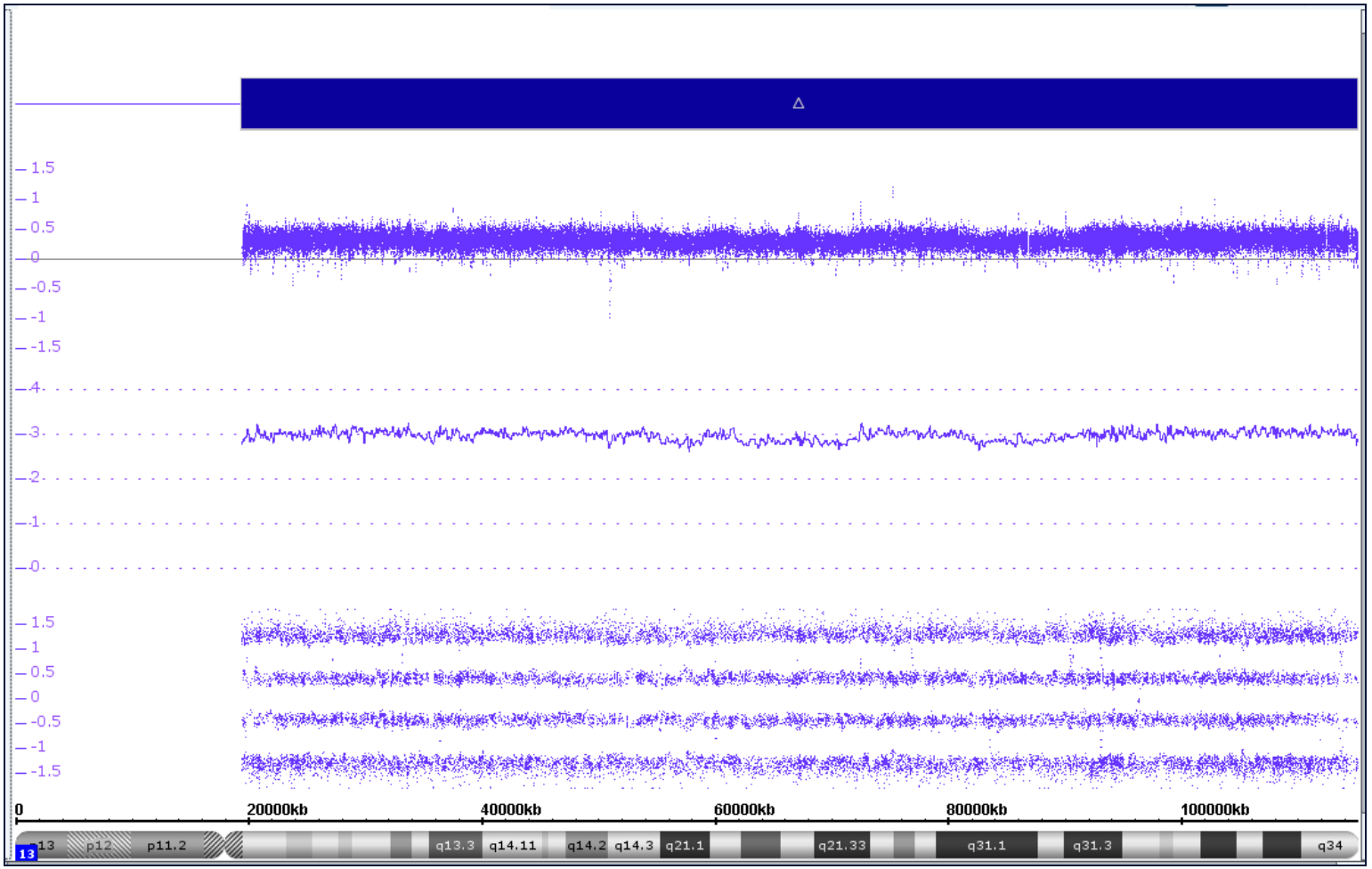
Albers et al, 2012

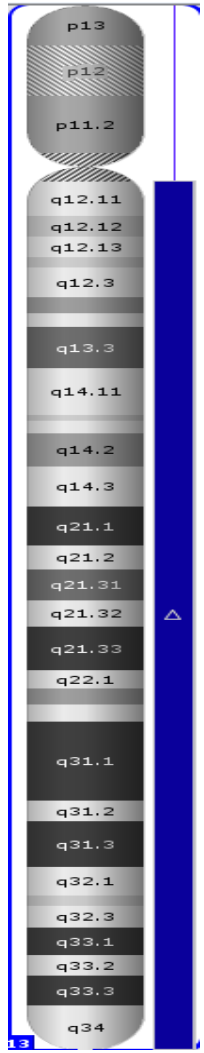
Results Summary

- Deletion conferring risk for recessive disease: thrombocytopenia-absent radius (TAR) syndrome
- Significance of deletion alone is uncertain
- RBM8A sequence analysis should be considered

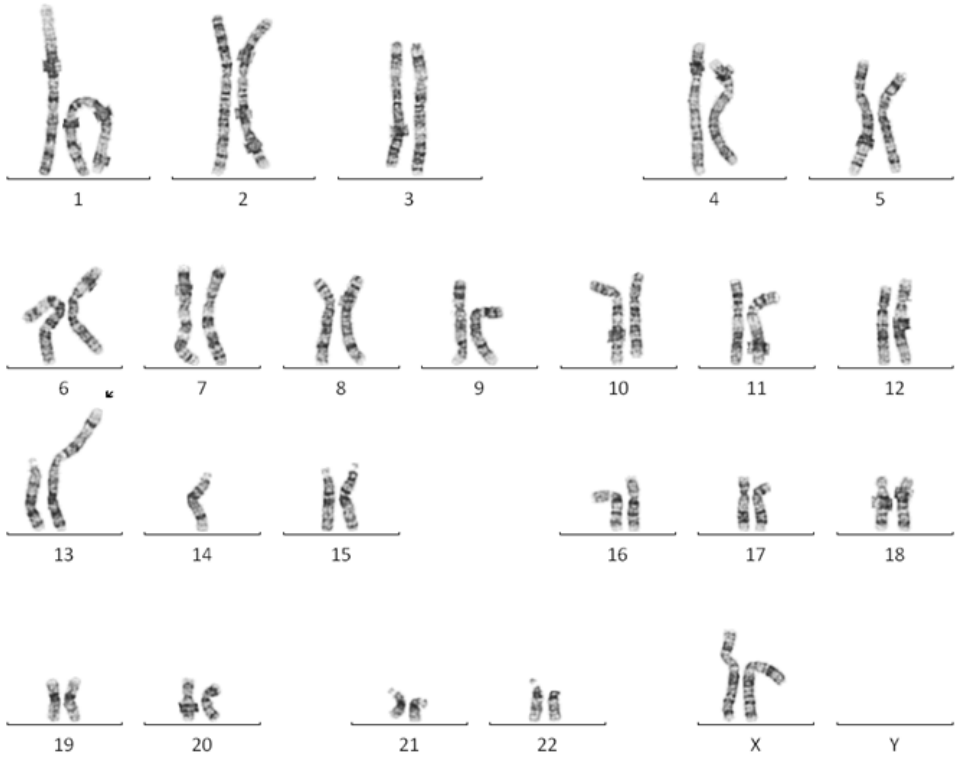
Case: IUFD 24 weeks, fetal tissue, CHR: no grow

Chromosome 13



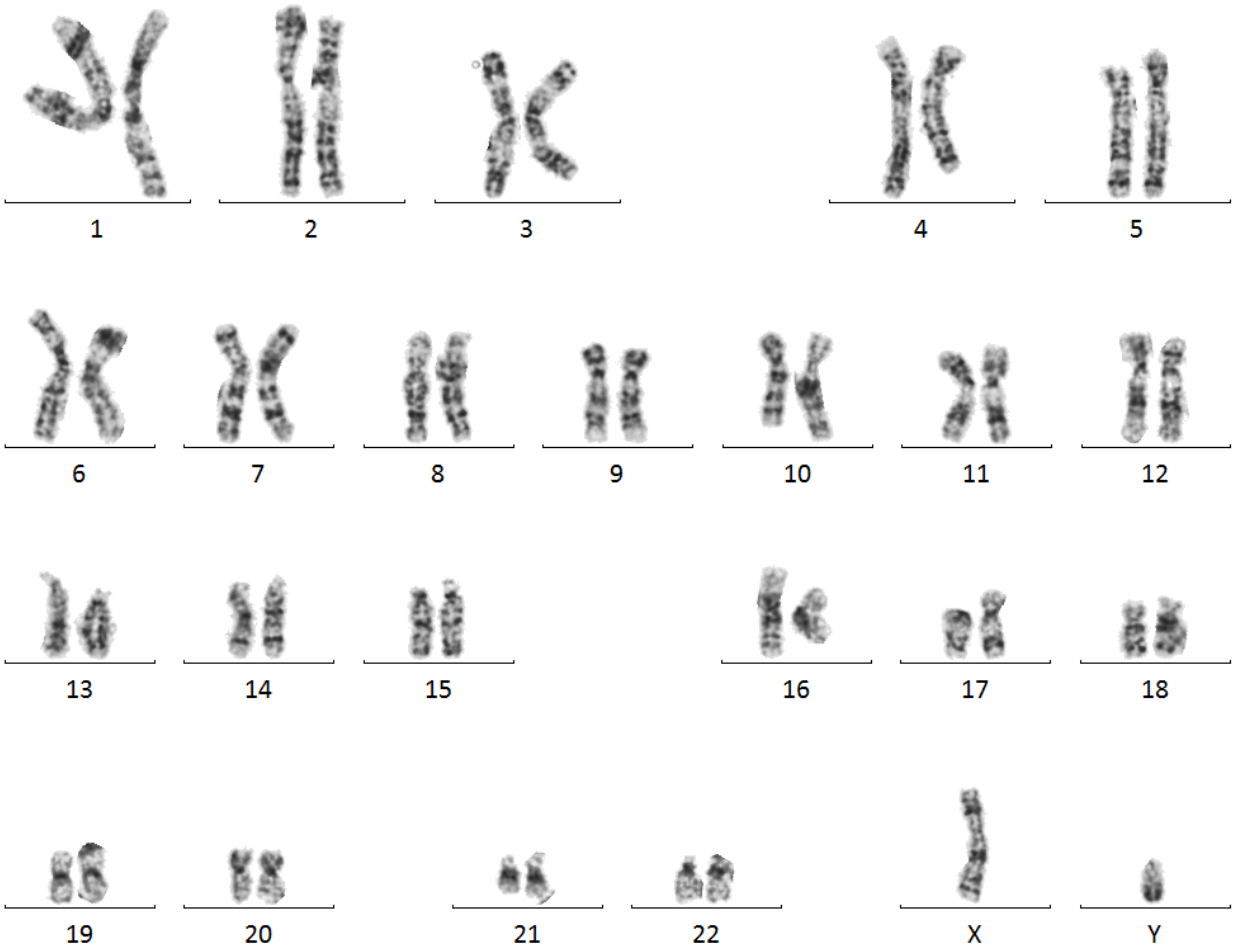


Maternal chromosome analysis: 45,XX,der(13;14)(q10;q10)

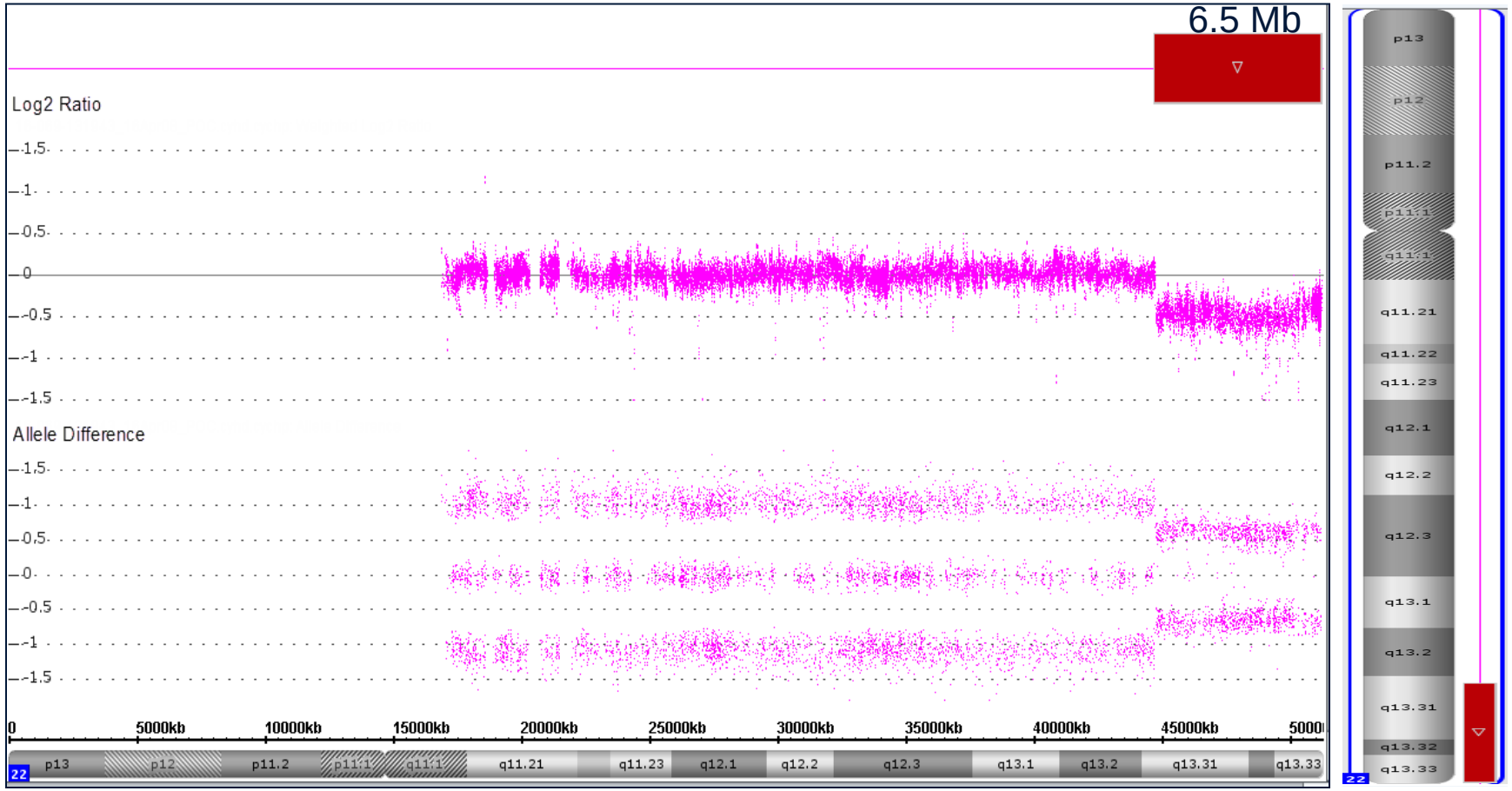


- GMA cannot characterize the structure of copy number changes
- Consideration for recurrence risk should be incorporated into interpretation

Case: MAB, 46,XY on villi



Case: MAB, 46,XY on villi



Clinical Utility of GMA

- Constitutional genetics: diagnosis of heritable genomic abnormalities (variants) in children, adults, pregnancy, and fetal loss
  - Abnormalities may be inherited or *de novo*
- Cancer genetics: detection of acquired or somatic (versus germline/constitutional) genomic abnormalities for the diagnosis, prognosis, therapy, and/or monitoring of many types of cancer, esp. hematologic

Recurrent cytogenetic findings in MDS

Schanz et al., 2012 J Clin Oncol (Table 2)

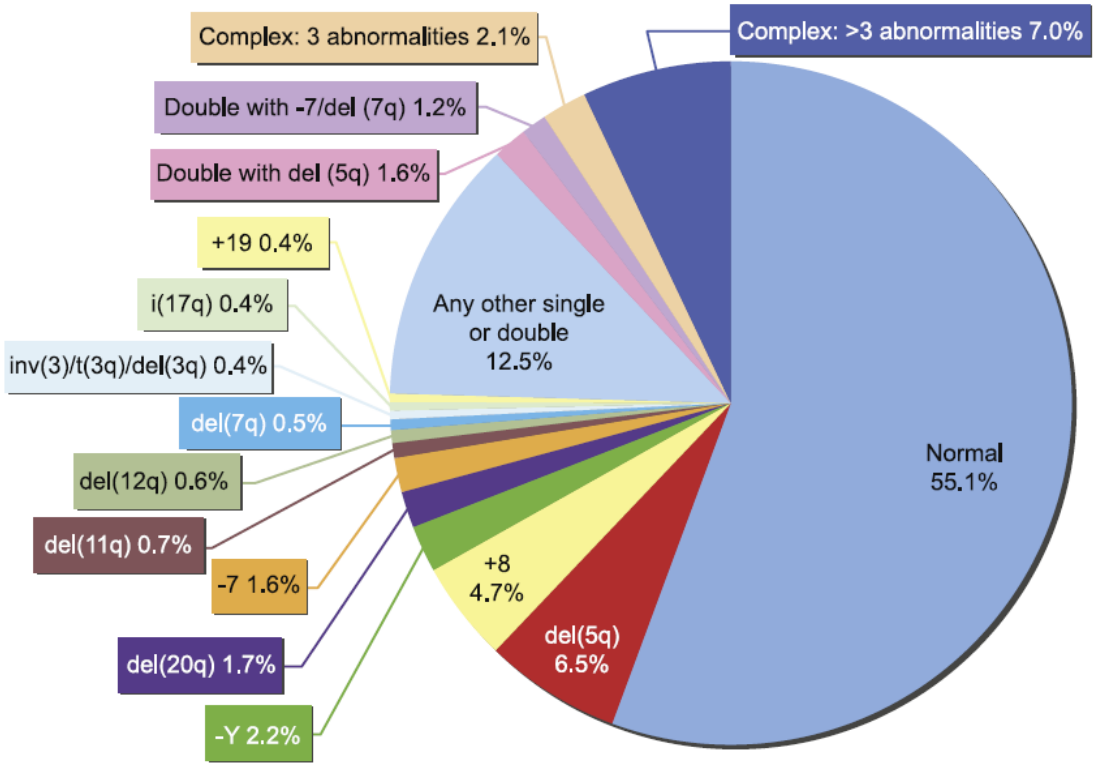


Image source: Nybakken and Bagg, JMD 2014

Cytogenetic Prognostic Stratification (IPSS-R)

Greenberg et al., 2012, Blood; Schanz et al., 2012 J Clin Oncol

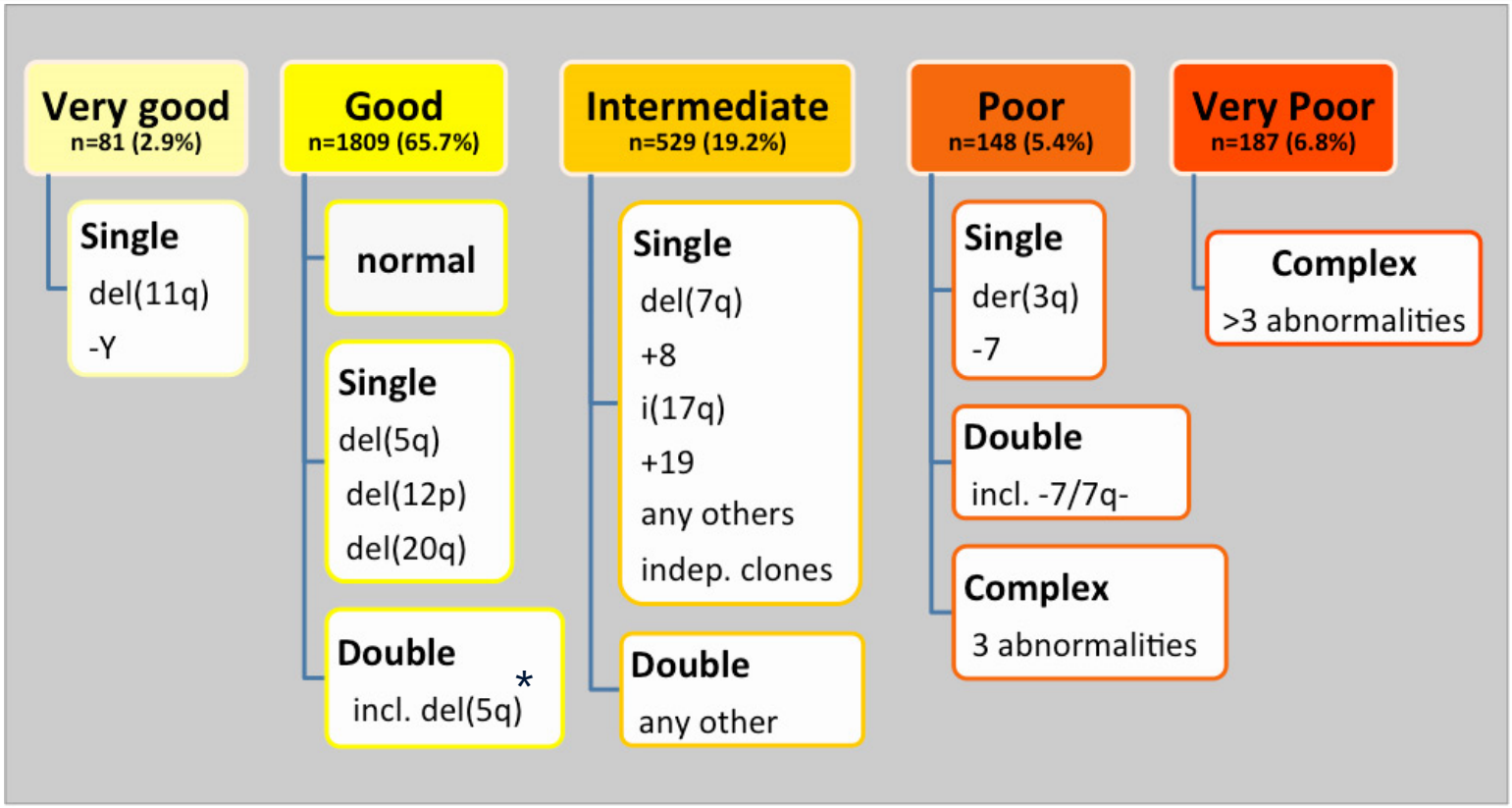


Image source: EMSCO

*WHO 2016 revision: excluding -7

SNP-A increases the diagnostic yield in MDS from 50% to 70-80%

Normal karyotype (n=296, composite of multiple studies)

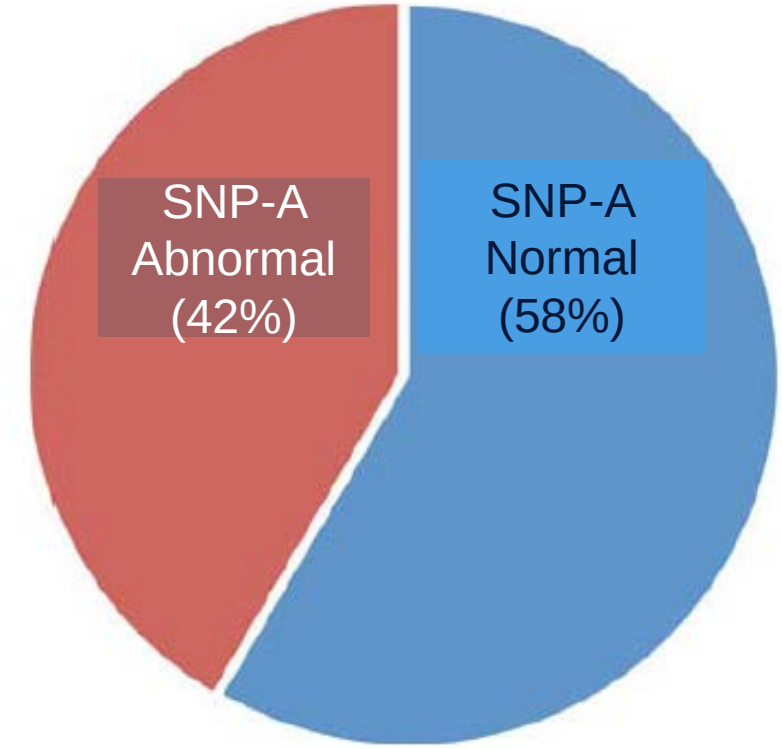
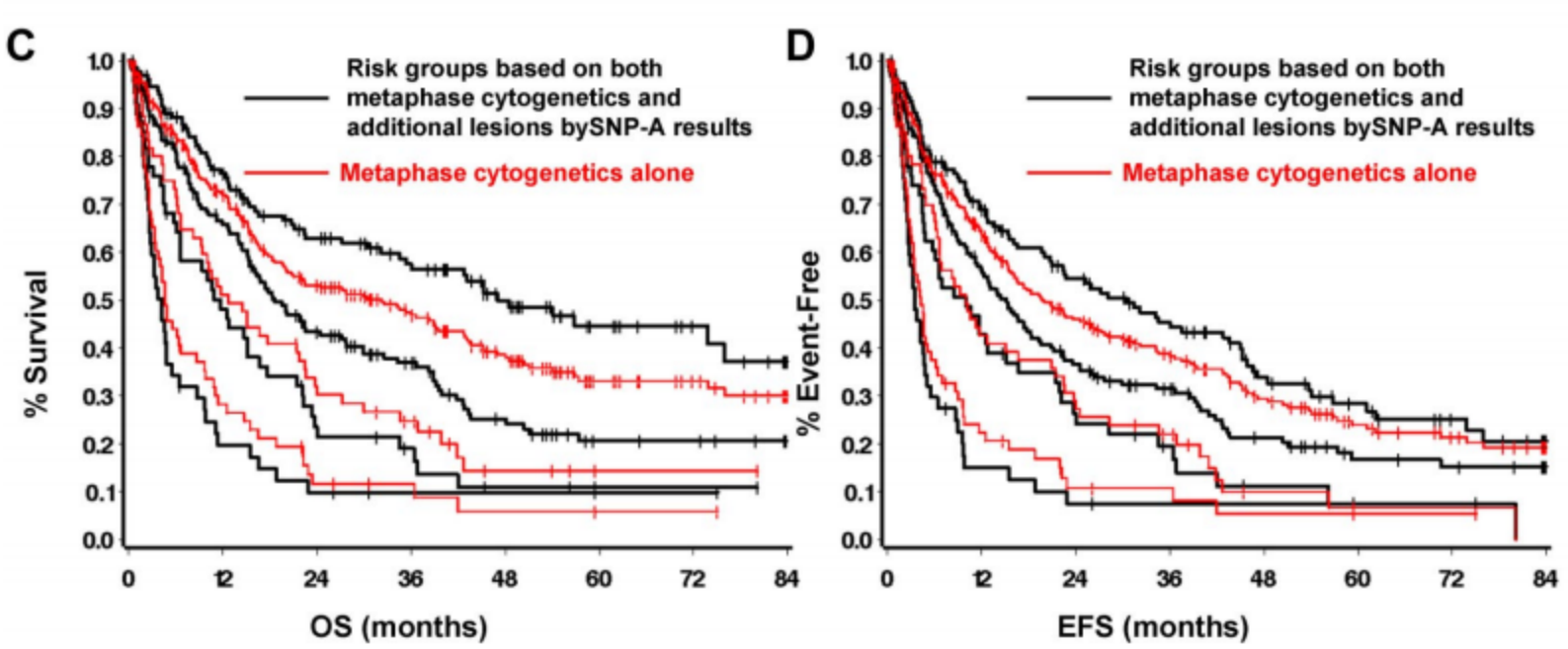


Image source: modified from Kulasekararaj, Br J Haematol 2013

See references: Gondek et al., 2008; Heinrichs et al., 2009; Tiu et al., 2011; others

SNP-A findings can enhance disease classification and prognostic stratification



Tiu et al., Blood, 2011

Comparison of cytogenetic tests for MDS

Technique (resolution)	Sensitivity	Culturing required?	Global?	Unbalanced abs?	Balanced abs?	LOH?	Utility	PB?
MC (5-10's Mb)	5-15%	Yes	Yes	Yes	Yes	No	Dx, Monitor	No
FISH (100's kb)	1-5%	No	No	Yes	Yes	No	Dx*, Monitor	No*
SNP-A (10-100's kb)	10-20%	No	Yes	Yes	No	Yes	Dx*, Monitor*	Yes

*Used conditionally

Brief Report

MYELOID NEOPLASIA

Utility of peripheral blood for cytogenetic and mutation analysis in myelodysplastic syndrome

Azim M. Mohamedali, Heba Alkhatabi, Austin Kulasekararaj, Sneha Shinde, Syed Mian, Farooq Malik, Alexander E. Smith, Joop Gäken, and Ghulam J. Mufti

Department of Haematological Medicine, King's College London, Rayne Institute, London, United Kingdom

Key Points

- There is 100% concordance in the cytogenetic and mutation profile between PB and BM in myelodysplastic syndrome.

Recent studies have shown that more than 80% of bone marrow (BM) samples from patients with myelodysplastic syndrome (MDS) harbor somatic mutations and/or genomic aberrations, which are of diagnostic and prognostic importance. We investigated the potential use of peripheral blood (PB) and serum to identify and monitor BM-derived genetic markers using high-resolution single nucleotide polymorphism array (SNP-A) karyotyping and parallel sequencing of 22 genes frequently mutated in MDS. This pilot study showed a 100% SNP-A karyotype concordance and a 97% mutation concordance between the BM and PB. In contrast, mutation analysis using Sanger sequencing of PB and serum-derived DNA showed only 65% and 42% concordance to BM, respectively. Our results show the potential utility of PB as a surrogate for BM for MDS patients, thus avoiding the need for repeated BM aspirates particularly in elderly patients and those with fibrotic or hypocellular marrows. (*Blood*. 2013;122(4):567-570)

- SNP-A has advantage of utility for analysis on peripheral blood, which may avoid the need for repeated bone marrow procedures, particularly for elderly patients and those with fibrotic or hypocellular marrows

Incidental or secondary findings from GMA testing

- Constitutional
  - Genome-wide AOH, suggestive of consanguinity
  - Alteration (usually deletion) of dosage sensitive gene/region associated with adult-onset or hereditary cancer predisposition
    - May or may not be associated with indication for testing
  - Mosaicism associated with hematologic disease (rare)
- Oncology
  - Genome-wide AOH, suggestive of consanguinity
  - Constitutional pathogenic/likely pathogenic CNVs

➢ Genetic counseling is recommended prior to this test to inform persons being tested about the advantages and limitations of the test

American College of Medical Genetics standards and guidelines for interpretation and reporting of postnatal constitutional copy number variants

Hutton M. Kearney, PhD[1], Erik C. Thorland, PhD[2], Kerry K. Brown, PhD[3], Fabiola Quintero-Rivera, MD[4], and Sarah T. South, PhD[5], A Working Group of the American College of Medical Genetics (ACMG) Laboratory Quality Assurance Committee

Genetics in Medicine • Volume 13, Number 7, July 2011

ACMG Standards and Guidelines for constitutional cytogenomic microarray analysis, including postnatal and prenatal applications: revision 2013

Sarah T. South, PhD[1,2], Charles Lee, PhD[3], Allen N. Lamb, PhD[1,2], Anne W. Higgins, PhD[4] and Hutton M. Kearney, PhD[5]; for the Working Group for the American College of Medical Genetics and Genomics (ACMG) Laboratory Quality Assurance Committee

Genetics in Medicine • Volume 15, Number 11, November 2013

Genetics inMedicine | ACMG STANDARDS AND GUIDELINES

American College of Medical Genetics and Genomics technical standards and guidelines: microarray analysis for chromosome abnormalities in neoplastic disorders

Linda D. Cooley, MD, MBA[1], Matthew Lebo, PhD[2], Marilyn M. Li, PhD[3], Marilyn L. Slovak, PhD[4] and Daynna J. Wolff, PhD[5]; A Working Group of the American College of Medical Genetics and Genomics (ACMG) Laboratory Quality Assurance Committee

Genetics in Medicine • Volume 15, Number 6, June 2013

Conclusions

- GMA is a genome-wide analysis technology with clinical utility for diagnosis of hereditary diseases and conditions in children, adults, pregnancy, and fetal loss and for diagnosis and monitoring in cancer
- Compared to other genomic analysis technologies, GMA has the advantages of providing high resolution, genome-wide coverage for gene-level detection of CNVs, as well as CN-AOH/LOH, which may signify recessive allele inheritance or imprinting disorders in hereditary disease, or bi-allelic mutations in cancer
- Challenges with GMA testing include: standardization of interpretation and reporting (esp. for VUS), detection and communication of incidental findings, and in some cases, reimbursement for testing

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Questions?

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