Introduction to Chromosomal Microarray Analysis

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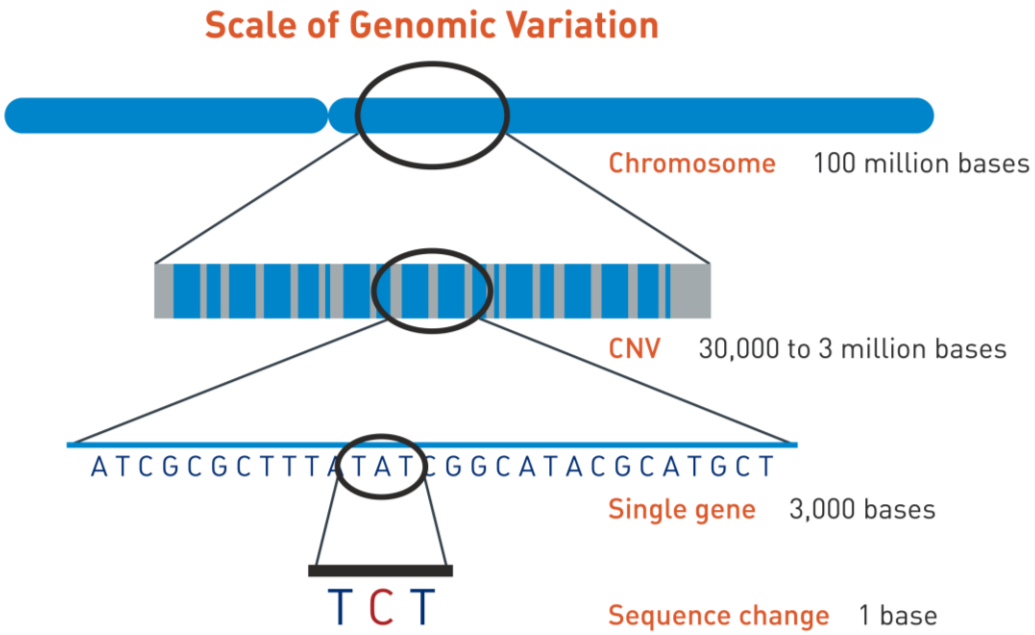
CANCER GENOMICS CONSORTIUM

Chromosomal microarray analysis (CMA)

Test that identifies copy number variants (CNV)



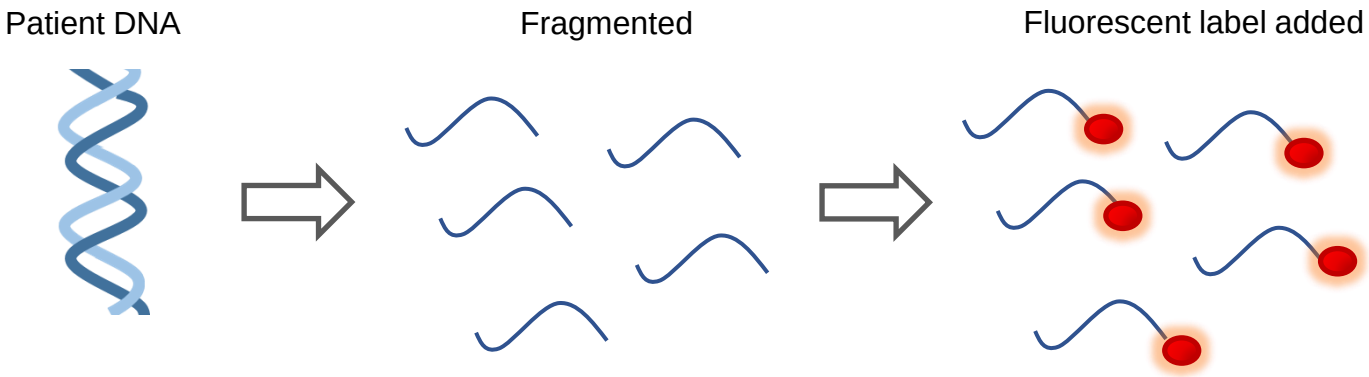
Genome-wide assay*

*There are certain parts of the genome (e.g. repetitive regions, centromeres, short arms of acrocentric chromosomes) that are not covered by microarray

*Depending on the array design, probes can be targeted for specific regions e.g. exon arrays

Image from jax.org

Overview of the CMA procedure

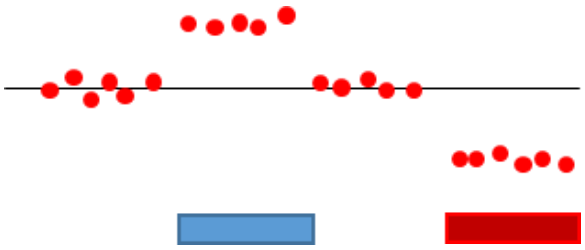


Microarray consists of DNA probes attached to a solid surface (hundreds of thousands to millions of probes, targeting different locations in the genome)

Fragments of patient DNA hybridize to complementary probes on the array

Array is scanned and fluorescence intensities measured

Each dot represents a unique probe, going along the chromosome



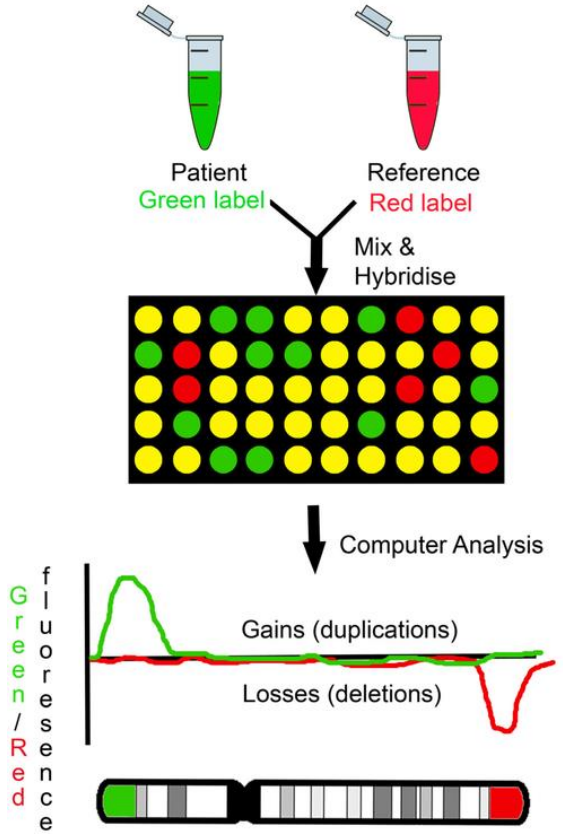
Region of copy number gain

Region of copy number loss

After a normalization process, regions of increased or decreased signal are detected.

Different types of array technology

Comparative genomic hybridization (CGH)



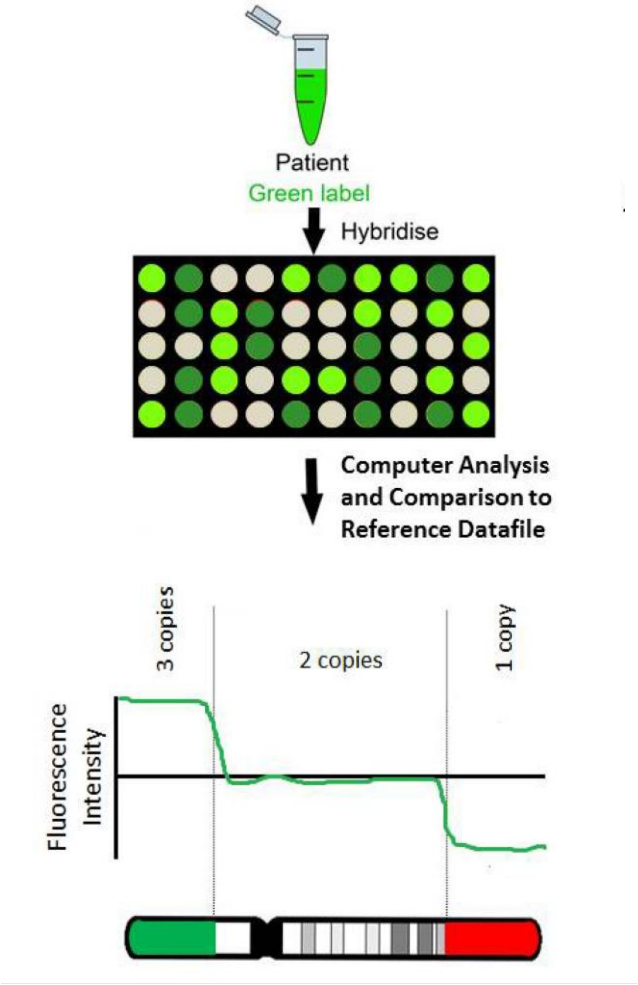
Spot	Patient	Control
	2 copies	2 copies
	3 copies	2 copies
	1 copy	2 copies

Karampetsou et al., *Journal of Clinical Medicine*. 2014; 3(2):663-678.

Different types of array technology

Single nucleotide polymorphism (SNP) array

Usually include copy number probes in addition to SNP probes

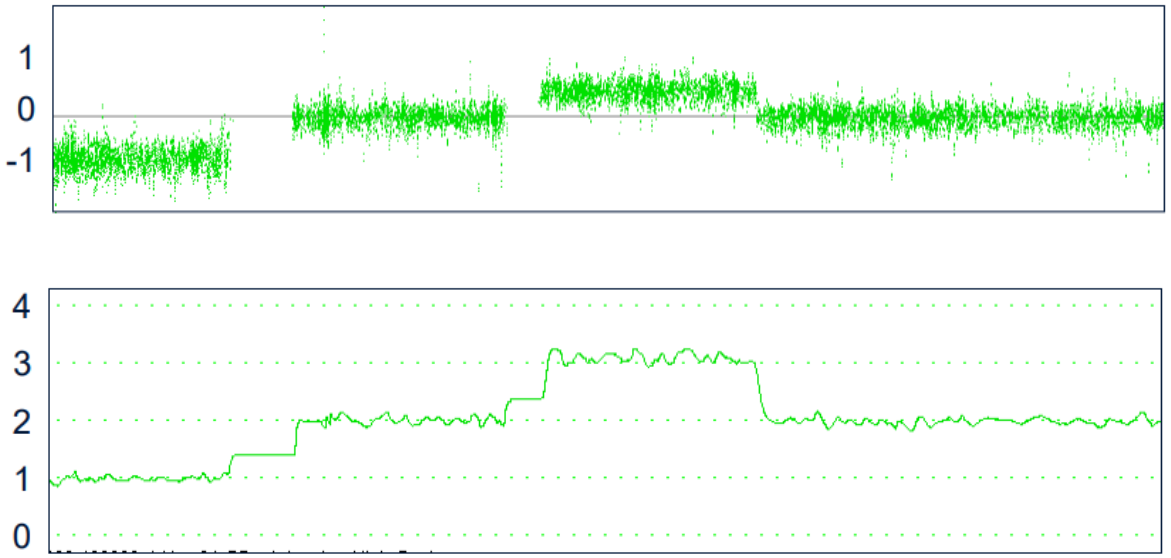


Karampetsou et al., *Journal of Clinical Medicine*. 2014; 3(2):663-678.

Different types of probes

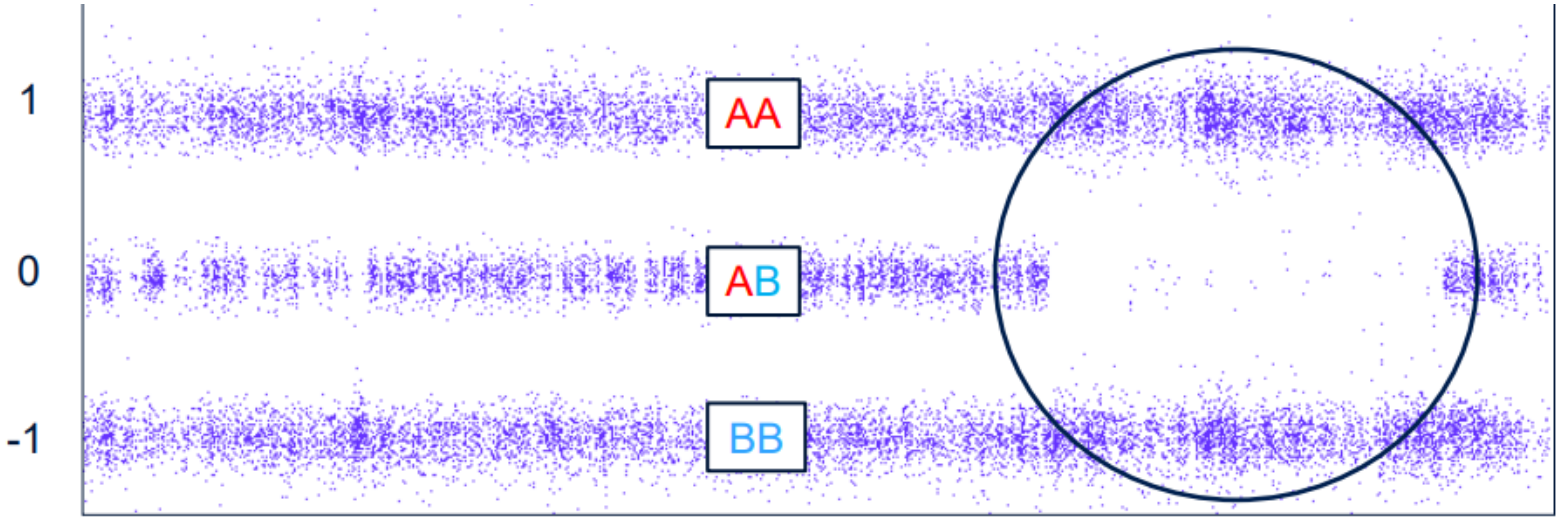
- BAC probes (longer) vs. oligonucleotide probes (shorter)
- Copy number probes (target conserved regions) vs. SNP probes (target known polymorphisms)

Microarrays can detect copy number variation



Slide courtesy of Dr. Celeste Eno

SNP microarrays can detect regions of homozygosity (ROH)

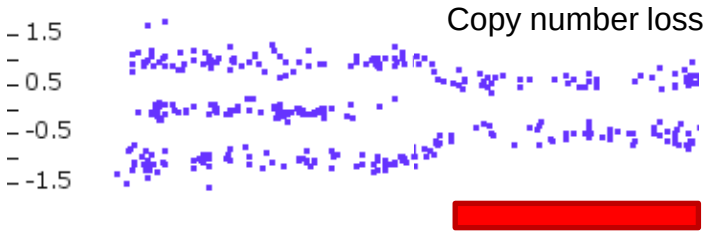


Sometimes also referred to as LOH (loss of heterozygosity), AOH (absence of heterozygosity)

Slide courtesy of Dr. Celeste Eno

Interpreting SNP probe results

Each dot represents a SNP, going along the chromosome from left to right.

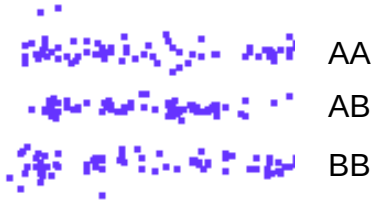


Each SNP has two possible alleles, referred to as “A” or “B”

Copy number state = 2

1) Homozygous for reference allele (AA)
2) Heterozygous (AB)
3) Homozygous for alternate allele (BB)

Each individual SNP has three possible states
Viewing all the SNPs across the region → three lines



Copy number state = 1

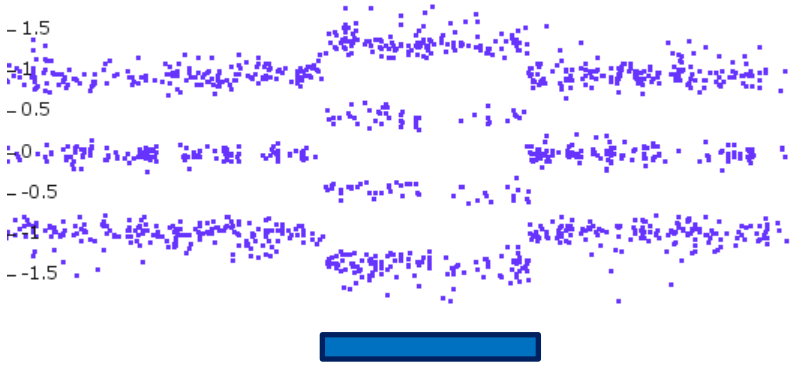
1) Reference allele (A)
2) Alternate allele (B)

Each individual SNP has two possible states
Viewing all the SNPs across the region → two lines

Interpreting SNP probe results

Each dot represents a SNP, going along the chromosome from left to right.

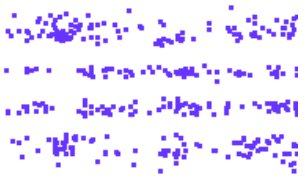
Copy number gain



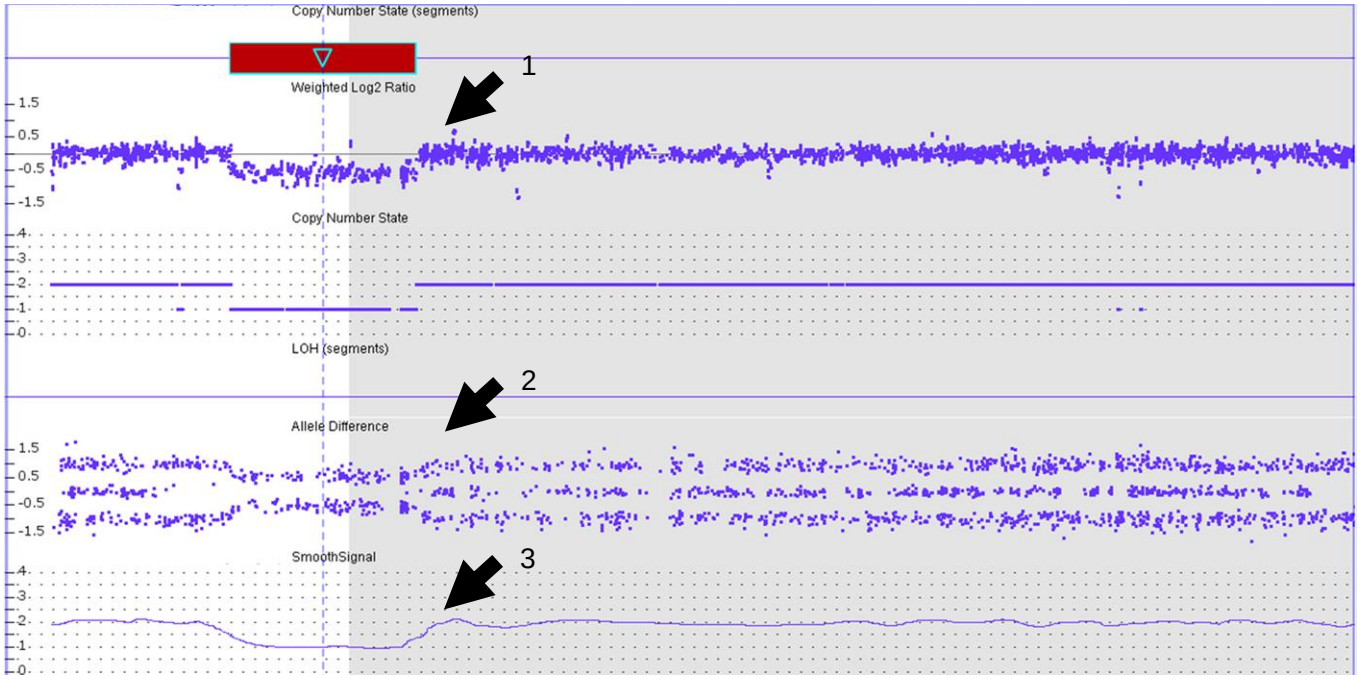
Copy number state = 3

1) Homozygous for the reference allele (AAA)
2) Heterozygous, with two copies of the reference allele and one of the variant allele (AAB)
3) Heterozygous, with one copy of the reference allele and two of the variant allele (ABB)
4) Homozygous for the variant allele (BBB)

Each individual SNP has four possible states
Viewing all the SNPs across the region → four lines



Example of a copy number loss



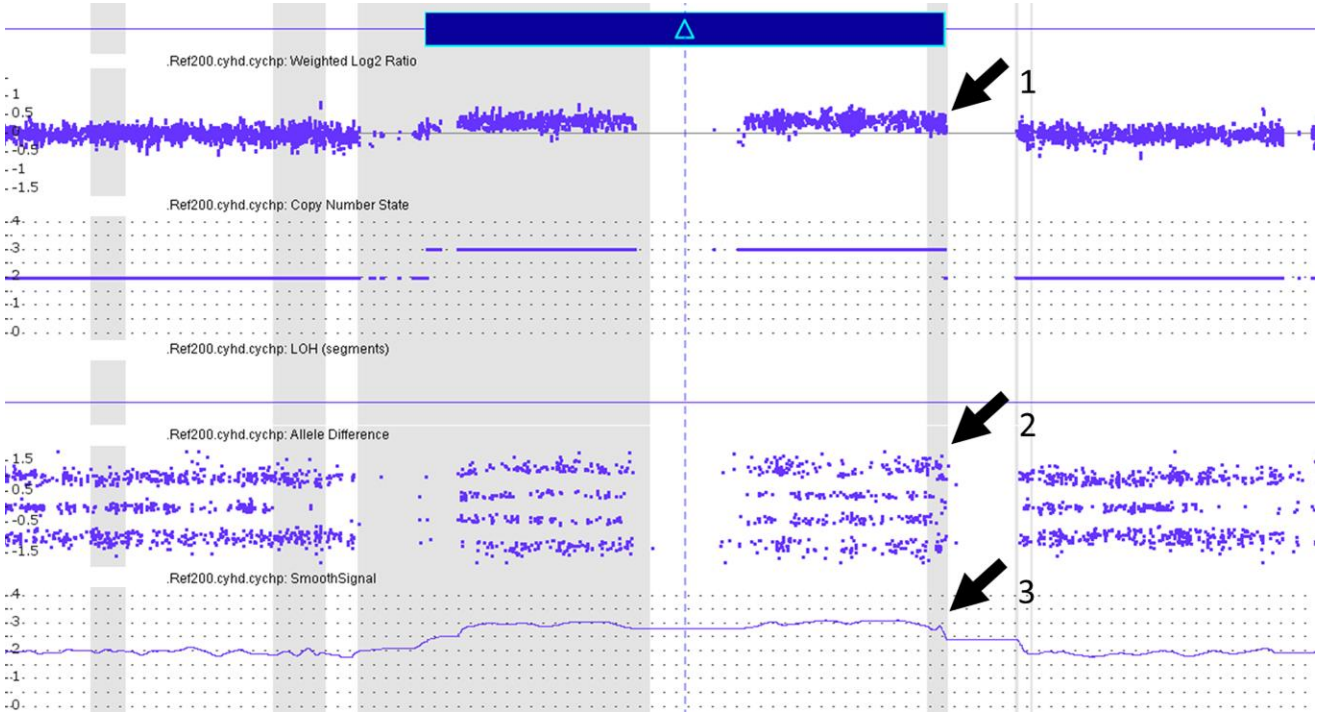
1. Dip in weighted Log2 ratio indicates decrease in copy number for this region

2. Reduction in number of possible SNP combinations also consistent with loss of one homolog

3. Dip in smooth signal graph also indicates copy number loss

Image from Affymetrix Chromosome Analysis Suite (ChAS)

Example of a copy number gain



1. Increase in weighted Log2 ratio indicates increase in copy number for this region
2. Increase in number of possible SNP combinations consistent with gain of one homolog
3. Peak in smooth signal graph also indicates copy number gain

Image from Affymetrix Chromosome Analysis Suite (ChAS)

What types of abnormalities can be detected by CMA?

- Abnormalities of chromosome number (trisomy, monosomy, etc.)
- Unbalanced abnormalities of chromosome structure (e.g. unbalanced translocation)
- Microdeletions/microduplications (too small to be observed by karyotype, often contain multiple genes)
- Other copy number variants (including benign polymorphisms as well as VUS)

Microarrays which include SNP probes:

- Regions of homozygosity: UPD, identity by descent (consanguinity), etc.
- Triploidy

Strengths and limitations of CMA

Advantages of microarray:

- Microarray can detect abnormalities too small to be seen by karyotype (resolution depends on the array platform and the lab's reporting criteria; ~50-100 kb)
- Uses extracted DNA; can be performed on a variety of tissue types (cells do not need to be cultured)
- Genome-wide approach: does not require prior knowledge of the abnormality you are looking for

Limitations of microarray:

- CMA cannot detect balanced abnormalities, single nucleotide variants, or indels below the resolution of CMA
- There are certain regions of the genome with poor probe coverage (near centromeres, telomeres, and other repetitive regions)
- Does not provide information about the underlying mechanism
  - Example: CMA can tell you if there are three copies of chromosome 21 material, but it doesn't tell you where that material is (i.e. trisomy 21, or unbalanced Robertsonian translocation)
  - A gain seen by CMA could be a tandem duplication or an insertion at a different location
  - Seeing a loss of one chromosome region and gain of another could be independent events, or it could be an unbalanced translocation
- Limited sensitivity for mosaicism (may miss low-level mosaic abnormalities)
- SNP arrays can detect AOH, but still cannot rule out all forms of polyploidy or UPD

Helpful References

Shao L, et al. Chromosomal microarray analysis, including constitutional and neoplastic disease applications, 2021 revision: a technical standard of the American College of Medical Genetics and Genomics (ACMG). Genet Med. 2021 Oct;23(10):1818-1829.

Miller DT, et al. Consensus statement: chromosomal microarray is a first-tier clinical diagnostic test for individuals with developmental disabilities or congenital anomalies. Am J Hum Genet. 2010 May 14;86(5):749-64.

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