Not all chromosomal microarrays for cytogenetics are created equal

Chromosomal microarray analysis (CMA) has significantly altered the predictive power of genetics in clinical research over the last 20 years. The development of CMA has greatly expanded the capability of clinical researchers to detect genetic disorders. Since microarrays first appeared in the early 2000s, **CMA has been evolving continually; therefore, not all arrays perform the same.** Single-nucleotide polymorphism (SNP) arrays have significant advantages over other array types in clinical research, as many congenital disorders are caused by SNPs. This infographic introduces you to the different types of CMA, the benefits of hybrid-SNP arrays, and selected case studies.

Evolution of chromosomal microarrays

Bacterial artificial chromosome (BAC) arrays

BACs are genomic probes of at least 150 kb in length. They were mainly used in the early days of DNA microarrays, since their large size limits the available space on an array and thus lowers resolution. An array containing between 2,000 to 30,000 probes offers a maximum resolution of approximately 1 million base pairs (bp).

- Probe size: **~150 kb**
- Density: **2,000–30,000 probes**
- Resolution: **~1 Mb**
- Samples required: **test**
- Variations detected: **CNV**

Oligonucleotide arrays

For higher-resolution arrays, large artificial chromosomes were replaced by much shorter oligonucleotide probes, usually between 25 to 60 bp in length, with up to 7 million probes on the surface of the array. This allows for resolution of the same order of magnitude— down to 25 bp or 1 bp for arrays with probe coverage designed to detect specifc SNPs.

Array-based comparative genomic hybridization (aCGH, aCGH-SNP arrays)

aCGH compares a test sample and a reference sample. Genomic DNA (gDNA) of a test sample is labeled with one fluorescent dye, and gDNA of a reference sample is labeled with a different fluorescent dye, and these samples are applied to the array. A difference in fluorescence intensity on the array shows either an excess or a lack of sequences in the test sample. Most aCGH arrays contain probes for copy number variation (CNV). aCGH-SNP arrays are newer versions of the original aCGH array with the addition of low-density, usually ≤60K, SNP probes.

- Probe size: **60 bp** for standard arrays; **25–60 bp** for custom print arrays
- Density: **up to 1 million probes**
- Resolution: **>6 kb, 1 bp***
- Samples required: **reference and test**
- Variations detected: **CNV, SNP****

* For arrays that have probe coverage designed to detect specific SNPs.
** Only aCGH-SNP arrays can detect SNPs.

SNP arrays and hybrid-SNP arrays

SNP arrays contain oligonucleotide DNA probes with sequences from regions in the genome that show SNPs among individuals. After hybridization of the fluorescently labeled test sample DNA, the absolute fluorescence at each spot on the array is measured. Based on their intensity, the prevalence of respective sequences in the DNA is calculated. No reference sample DNA is required. **Hybrid-SNP arrays are SNP arrays that also include high-resolution probes for genome-wide analysis of CNVs, which is also implicated in genetic disorders.**

- Probe size: **25 bp**
- Density: **up to 7 million probes**
- Resolution: **25 bp, 1 bp***
- Samples required: **test**
- Variations detected: **CNV, SNP**

* For SNP arrays and hybrid-SNP arrays that have probe coverage designed to detect specific SNPs.

Workflow of aCGH and hybrid-SNP arrays

In general, the workflow for CMA consists of the following steps:

1. Obtain the test gDNA and denature it into single-stranded DNA.
2. Amplify the sequences to a sufficiently high concentration for analysis and label with a fluorescent dye.
3. Hybridize the labeled DNA to the array, where the strands bind to their complementary sequences, then wash off unhybridized DNA.
4. Measure fluorescence intensity and perform chromosomal karyotyping analysis.

Not all chromosomal microarrays are the same

There are numerous aCGH and hybrid-SNP arrays on the market. The performance of each depends on several features such as design strategy, content coverage, density, and resolution, which may **impact the outcome of the analysis based on the ability to detect certain chromosomal aberrations.**

Design strategy and content coverage

Most advanced arrays used for clinical research purposes are **hybrid-SNP arrays, containing a combination of a large number of SNP and CNV probes**. The selection, size, placement, and spacing of these probes along the genome can have a big impact on performance.

> "In general, more powerful arrays can be designed using more probes. But **array design strategy proved to be at least as important a feature as probe number for CNV detection**. Deviating from the simplest design strategy of even probe spacing along the genome can yield both beneficial and detrimental consequences. Increasing probe densities in known CNV regions of the genome, in combination with a sufficient genome-wide backbone of probes, generally leads to more detection power. However, if the backbone coverage is not sufficient or regions such as gene deserts are devoid of probes, the design may not detect even some relatively large CNVs."
>
> Haraksingh et al.[1]

Number of probes

The density of the probes on an array is also a factor determining the resolution of the assay. For example, although medium-density aCGH-SNP arrays with >100,000 CNV probes and 30,000 or fewer unique SNP probes were suitable for detecting uniparental disomies (UPD), a study by Mason-Suares et al.[2] found that these arrays miscall absence of heterozygosity (AOH) regions arising from identity by descent. **They found that higher-density arrays give greater confidence in the detection and positive identification of copy-neutral abnormalities.**

Pereira et al.[3] published a study using high-density hybrid-SNP arrays on 15 intellectually disabled (ID) patients with normal karyotypes and negative X-fragile tests. They reported the rate of pathogenic CNV as 26.7%. Wang et al.[4] reported a similar percentage (25%) using the same high-density hybrid-SNP array in a group of 480 ID samples.

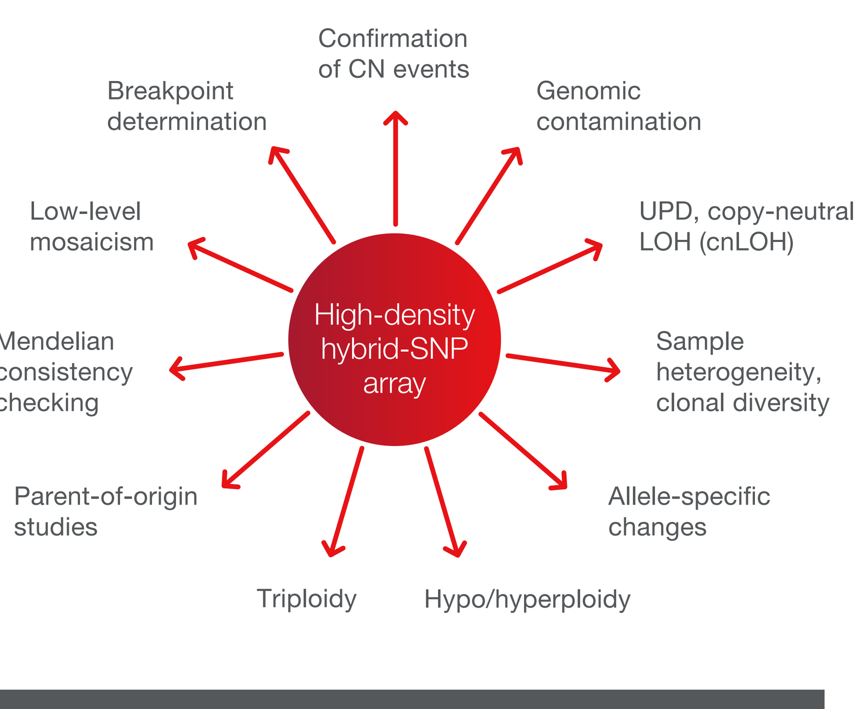
Benefits of hybrid-SNP arrays

High-density hybrid-SNP arrays are able to detect chromosomal anomalies that aCGH would miss. For example, hybrid-SNP arrays can provide answers to questions such as:

- Is loss of heterozygosity (LOH) arising from deletions or from long contiguous stretches of homozygosity (LCSH) due to consanguineous parents, or are other segregation defects present?
- Do both copies of a chromosome stem from one parent (uniparental disomy, or UPD) or not?
- Is the individual homo- or heterozygous for a certain allele?
- Is genomic mosaicism present in the individual?

> "High-resolution CMAs have improved the ability to identify CNVs undetectable by other technologies such as karyotyping, FISH, and targeted or lower-resolution array platforms due to lower resolution and/or coverage. Additionally, **the information yield of this platform is enhanced by the detection of LCSH**, which are accessed by genotyping of thousands of SNPs, and are suggestive of either UPD or increased risk of recessive conditions."
>
> Scionti et al.[5]

High-resolution hybrid-SNP arrays improve information yield, and thus are more cost-effective for a wide range of pre- and postnatal analyses.



Case studies
demonstrating the power of hybrid-SNP arrays

Liao et al.[6] presented 5 years of clinical experience using whole-genome, high-resolution hybrid-SNP arrays to investigate 446 fetuses that had structural malformations detected with ultrasound, but for which **standard karyotyping analysis showed normal karyotypes. Whole-genome, high-resolution hybrid-SNP arrays showed a clinically relevant genomic imbalance** in 51 fetuses. Pathogenic CNVs in 16 fetuses were less than 1 Mb in size, meaning they would not have been detected by other methods.

Liu et al.[7] published a study focusing on the analysis of a prenatal case with a high risk of Down syndrome. **Karyotype testing via G-banding and aCGH did not uncover any known pathogenic CNVs.** However, **the hybrid-SNP array analysis indicated a complete uniparental isodisomy (isoUPD)** of chromosome 4 based on the loss of heterozygosity across the entire chromosome.

Conventional oligonucleotide CGH arrays cannot measure changes in ploidy. Because SNP arrays can resolve allelic differences, changes in ploidy are detectable and can be used for prenatal applications. In a clinical research study by Edwards et al.[8] a four-year-old boy with small stature, moderate developmental disability, and other symptoms **showed a normal subtelomere profile by fluorescence *in situ* hybridization (FISH). However, when analyzed with a SNP array, an abnormal genotype was identifiable** over the entire chromosome 9, with 11% mosaicism.

A study from 2016 by Sinkey et al.[9] found that CMA testing was the preferred strategy for analyzing sonographically detected fetal anomalies based on its high cost-effectiveness, which increased the information yield by 17 per 1,000 fetuses. Although **CMA might initially cost more than traditional karyotyping methods, the benefit of increased diagnostic utility has been shown to make them cost-effective.**

Conclusion

The high cost-effectiveness of high-resolution hybrid-SNP arrays makes them powerful and widely applied clinical research tools for detecting genomic anomalies. Hybrid-SNP arrays offer significant advantages over traditional karyotyping techniques as well as other types of CMAs.

For details and additional case studies, **read our white paper** "The power of high-resolution SNP arrays for detecting inherited chromosomal anomalies".

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