The power of high-resolution SNP arrays for detecting inherited chromosomal anomalies

In this white paper, we describe:

- The development and use of SNP arrays for analyzing chromosomal anomalies
- The benefits of using SNP arrays over other types of arrays
- Case studies of instances where SNP arrays detected anomalies that gave rise to developmental defects

Introduction

The Human Genome Project (HGP) opened up tremendous vistas for understanding human variation. In clinical sciences, understanding variations in the human genome provides an opportunity to analyze how these differences in sequences contribute to inherited disorders. Before the HGP, analysis of chromosomal anomalies, such as aneuploidies, deletions, and duplications, and their contributions to birth defects or inherited syndromes was performed by karyotyping using G-band analysis. However, chromosomal DNA microarrays (CMAs), which combined microscale manufacturing techniques with well-understood nucleic acid hybridization chemistries, significantly altered the landscape of medical genetics research in the mid- to late 2000s. These microarrays were optimized for detecting genetic variations, including variations in the number of times a specific sequence was present (copy number variations, or CNVs) and variations in sequence at a single base pair (single-nucleotide polymorphisms, or SNPs). Further advancements in microarray technology led to the introduction of SNP arrays, which contain both CNV and SNP probes. Since thousands to millions of sequences can be queried at a time, a single microarray experiment has the potential to give novel information about a subject's genome, highlighting the nucleotide variants present at each of the many loci assayed by the array.

Types of DNA microarrays

Copy number variation analyzed on chromosomal microarrays historically has made use of many different kinds of immobilized nucleotide probes, including bacterial artificial chromosomes (BACs), cDNAs, and oligonucleotides. Screening on microarrays in clinical contexts was first done using BAC arrays around 2003. These microarrays consisted of between 2,000 to 30,000 BAC probes and provided a rough idea of chromosomal microdeletions in mental retardation and developmental dysmorphisms [1]. BAC arrays were relatively easy to manufacture at the time and were useful because they could identify CNVs in discrete regions of the human genome known to play roles in specific genetic diseases. However, resolution was limited due to the large size of the probes (Figure 1).

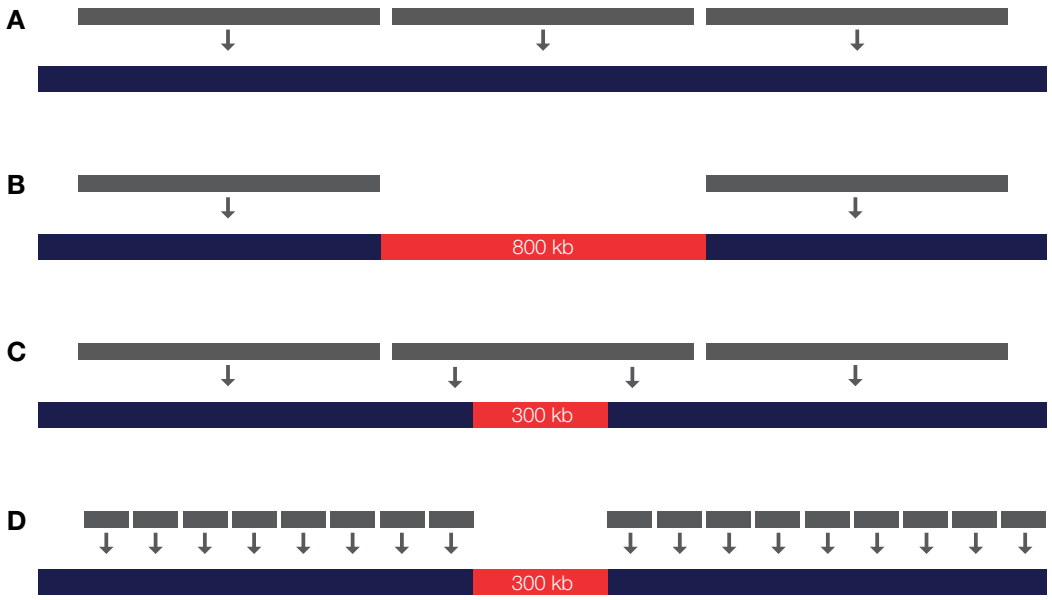

Figure 1. Smaller probe size improves microarray resolution. (A) DNA probes (gray) hybridize normally to sample DNA (blue), indicating a normal copy number in this region. **(B)** The middle probe does not hybridize to an 800 kb deletion (red), resulting in accurate detection of the deletion during data analysis. **(C)** The middle probe has sufficient homology to hybridize on each side of a 300 kb deletion. This deletion is likely to be missed during data analysis. **(D)** Smaller probes have sufficient resolution to hybridize on each side of the 300 kb deletion but not span it, resulting in accurate detection.

Once oligonucleotide deposition techniques were perfected, oligo arrays became the platform of choice for CNV analysis due to increased resolution. Oligonucleotide arrays consist of up to 1 million or more single-stranded 25–85 bp oligonucleotides immobilized on discrete areas of the array. Many studies have shown that oligonucleotide arrays offer higher resolution for the detection of smaller CNVs compared to BAC arrays, from about 1 Mb resolution with BAC arrays to around 100 kb with oligonucleotide arrays [2,3]. Because they can accommodate a very large number of sequences, a subset of oligonucleotide arrays are produced that contain large numbers of SNP probes. Each SNP is assayed by a set of partially overlapping probes that can distinguish single-nucleotide differences. These SNPs are scattered throughout the genome, and therefore a single microarray experiment can yield a profile of a donor's genome, including CNVs and SNPs at defined loci. Furthermore, because of their high density, oligonucleotide and SNP arrays can query regions of the genome with resolution down to 100 nucelotides.

There are two basic strategies for using oligonucleotide-based arrays for CNV measurements (Figure 2). One of these, array comparative genomic hybridization (aCGH), measures the amount of each sequence present in test and normal samples. For this method, the genomic DNA of a test sample is labeled with one fluorescent dye, and genomic DNA of a normal sample is labeled with a different fluorescent dye (Figure 2A). The labeled DNAs are mixed together in equal proportions and hybridized to the array. The test and control DNA competitively hybridize to the complementary sequences on the array. The amount of fluorescence is directly proportional to the abundance of sequence in the sample. The fluorescence signal of both colors is measured at every position on the array. Various manipulations of the signal measurements are used to convert the fluorescent signals to a ratio of test to control intensities, and thus into a sequence copy number. A typical clinical CGH microarray contains a few hundred thousand probes while the number of probes on research CGH microarrays may reach into the millions.

The other strategy makes use of SNP arrays (Figure 2B). In these arrays, oligonucleotide-based DNA probes, based on regions in the genome that show single-nucleotide diversity among individuals, are immobilized on the array. Unlike aCGH analyses, genomic DNA from only a test sample is needed; no normal control is necessary. The genomic DNA is labeled with a fluorescent dye, and after hybridization and washing, the absolute fluorescence at each immobilized oligonucleotide position is measured. To convert the measured signal to a CNV, the intensities are compared to numerous normal control samples that were run independently and combined to create a reference data set. The resulting ratio between the test and normal reference is calculated *in silico*. As with aCGH, the ratio of the test to reference signals is converted to a CNV measurement.

Advantages of high-density SNP arrays over aCGH

Although both types of arrays can be used to detect copy number variants, analyzing CNVs by SNP arrays provides several advantages over aCGH assays. As described earlier, copy number differences between the test and normal samples can be detected in aCGH analysis. However, because only a single sequence is queried on each oligonucleotide, certain types of chromosomal anomalies will be missed. In contrast, having multiple SNPs present at each locus refines and provides more information at each site, allowing these potentially pathogenic anomalies to be detected. These anomalies can only be detected by having knowledge of the sequence present at a site, instead of just the number of copies. For example, long contiguous stretches of homozygosity (LCSH), arising from consanguineous parents or other segregation defects, can be present. In an aCHG assay, these regions would show as normal diploid. However, these LCSH can uncover recessive alleles and give information about the pathology. Thus, when a candidate recessive gene disorder that either matches a proband phenotype or is relevant to the family history is localized within one of the LCSH regions, targeted investigation such as fine-scale genotyping or direct sequencing may uncover the causative mutation [4].

Another type of anomaly missed by aCGH is uniparental disomy (UPD). UPD arises when two copies of a chromosome, or part of a chromosome, are received from one parent and no copy from the other parent. UPD can arise due to a meiosis I error, where a pair of nonidentical chromosomes are inherited from one parent (heterodisomy) or a meiosis II error, where a duplicated chromosome from only one parent is inherited (isodisomy). UPD gives rise to very large blocks of LCSH, and thus may have clinical relevance as previously described. Additionally, UPD can uncover parent-specific genomic imprinting, giving rise to imprinting disorders. For detecting UPD, an assay must be able to distinguish between allelic differences or haplotypes in the query sample across large stretches of the genome. SNPs are most commonly used to differentiate between alleles or haplotypes. Traditional aCGH analyses are performed using probes that are designed to avoid SNPs (or at least, SNPs are not taken into consideration during design). Therefore, it is not possible to discriminate between the two alleles that are present. Because they cannot discriminate between different alleles, aCGH assays cannot determine whether a locus is homozygous or heterozygous—they can merely confirm the number of copies present.

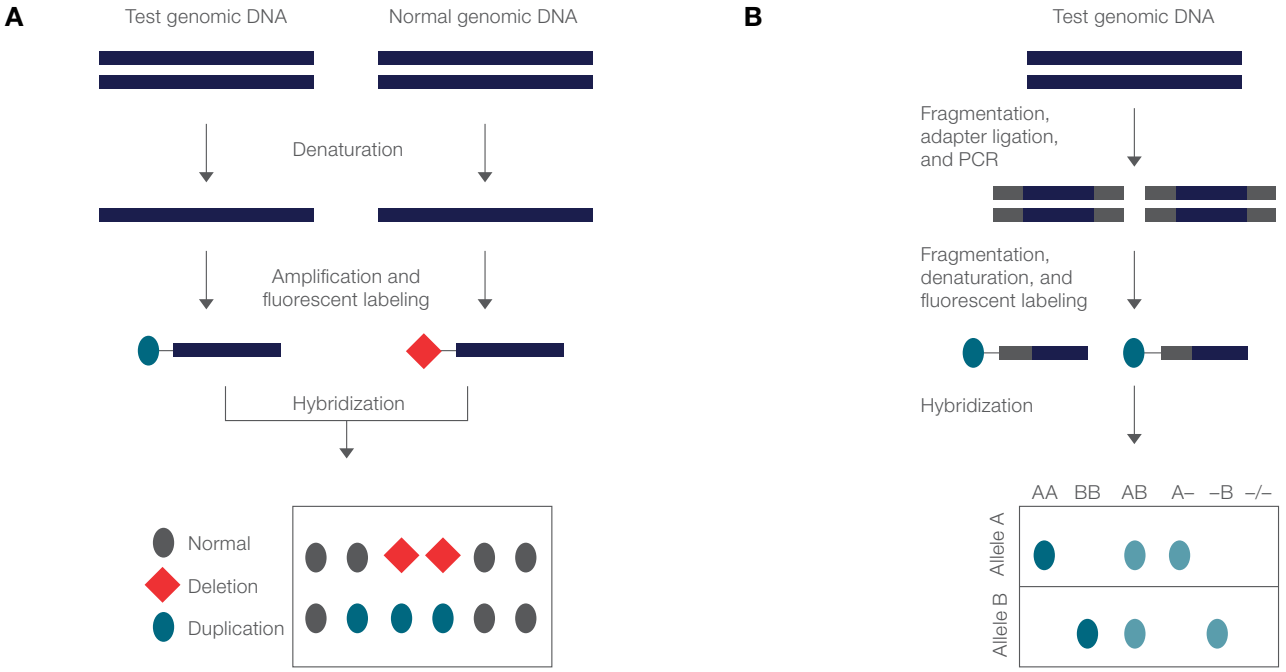

Figure 2. Example workflows for oligonucleotide-based arrays. (A) In aCGH, test and normal genomic DNA samples are labeled with different fluorophores and hybridized to the same array. Deletions and duplications are indicated by an abundance of one signal over the other. **(B)** In SNP arrays, the test genomic DNA sample alone is hybridized to the array. Allele frequency and copy number are determined by comparing the signal intensity to a reference data set. If the sample is homozygous for an allele (AA or BB), it will only bind to the "Allele A" or "Allele B" position on the array. If the sample is heterozygous for an allele (AB), it will bind to both positions on the array but with reduced intensity. If the sample has a deletion (A– or –B), it will bind to the "Allele A" or "Allele B" position on the array but with reduced intensity. Deletions of both alleles (–/–) will not produce signals above background. Duplications (not shown) will have an increase in signal intensity relative to the reference.

Because aCGH measures the normalized signal intensity relative to a control, differences in ploidy (where the entire chromosomal complement is present at more than 2x) are also missed by aCGH analysis. SNP arrays, however, can easily detect whether the sample is polyploid because they enumerate the SNPs that are present in a sample. This fact makes SNP analysis very useful in cancer research (for example, see references 5 and 6), but it is also very useful for understanding causes of reproductive anomalies [7].

Human chimeras, although rare, are usually discovered in newborns that display disorders of sex development (DSD). These usually arise from the fusion of two separate zygotes by several different possible mechanisms. Because SNP arrays can distinguish between the parental haplotypes, they can be used to uncover the mechanism that produced a chimera. For example, Shin et al. [8] used SNP arrays to uncover dispermic fertilization of two identical haploid ova, formed by parthenogenetic activation in absence of any recombination event, as the underlying mechanism that gave rise to a human chimera. Since aCGH analyses lack haplotype information, such a diagnosis would not be possible by traditional aCGH methods.

A hydatidiform mole (HM) is the abnormal growth of a fertilized egg or an overgrowth of tissue from the placenta, and may produce persistant trophoblastic disease. HMs are classified as either complete or partial based on their chromosomal complement; these subclassifications are important for clinical practice and investigative studies. Their ability to distinguish and enumerate haplotypes mean SNP arrays can be useful to determine the etiology of partial and complete hydatidiform moles. In a 2016 study, Xie et al. [9] used SNP arrays to analyze samples not initially diagnosed as HM. They found nine cases with abnormal karyotypes, including three with complete UPD that would have been missed by other techniques.

Most contemporary arrays used for clinical purposes are hybrid arrays, containing a combination of a large number of probes for SNPs and copy number probes that lack SNP information. The density of the probes on the array is also a factor in determining the resolution of the assay. Although low-density arrays with 30,000 or fewer unique copy number and SNP probes were suitable for detecting UPD, a study by Mason-Suares et al. [10] found that these arrays miscall absence of heterozygosity (AOH) regions arising by identity by descent. Higher-density arrays give greater confidence in the detection and positive identification of copy-neutral abnormalities. Figure 3 summarizes the range of applications for SNP arrays.

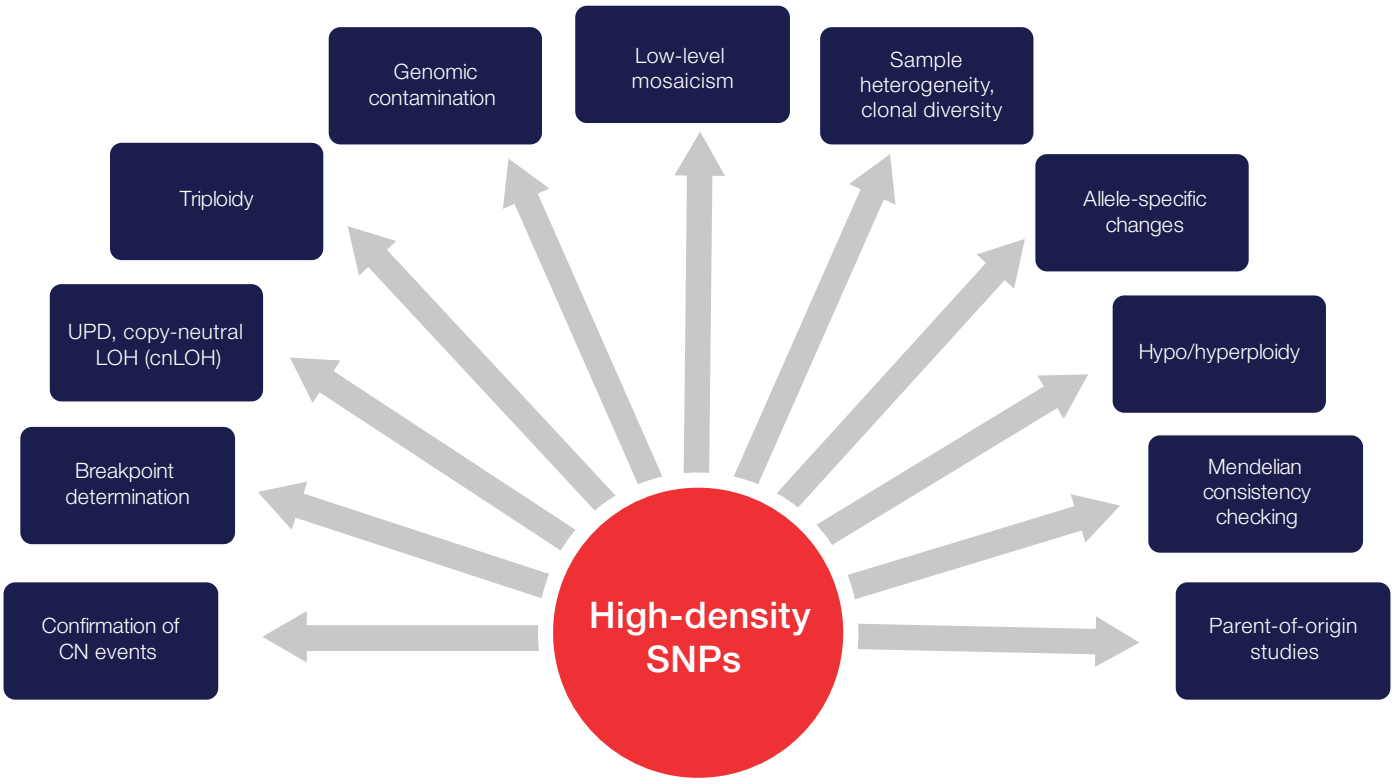

Figure 3. The power of SNP arrays. High-density SNP arrays with high genotyping accuracy enable a range of applications, including confident breakpoint determination and copy number change confirmation.

The higher resolution of SNP arrays can result in finding more variants of unknown significance (VOUS). Examination of the VOUS inheritance pattern in a family can provide "living proof" that the variant is nonpathogenic. For example, if a child with a congenital abnormality carries a VOUS that is also present in the phenotypically normal parents, the VOUS is not likely to be the causative variant. The need for parental testing in the future could decrease with the sharing of the variant findings across labs and would improve the interpretation of VOUS results. In the meantime, many providers will omit VOUS in their reports, since it may lead to higher anxiety for the parents.

It is important to note that neither aCGH nor SNP arrays can easily detect balanced chromosomal rearrangements, such as translocations with no change in copy number. Making these determinations still requires conventional karyotyping or FISH analysis. Since no single platform is able to fulfil all the needs of prenatal testing, complementary approaches, such as SNP array and molecular karyotyping followed by next-generation sequencing to identify pathogenic variants, might be necessary. Some examples of multiplatform use are described in the following sections. Therefore, choosing an appropriate platform or platforms for clinical analyses requires balancing many factors, including likelihood of successful outcomes, cost- or time-effectiveness, and minimizing uncertainty in the interpretation of results.

High-resolution SNP arrays improve information yields for pre- and postnatal analyses

Because of the increased density and superior resolution relative to traditional karyotypes, CMA analysis using SNP arrays has been an established method for diagnosing products of conception. Many studies have documented the benefits of analysis using SNP arrays. For example, in 2013 Liao et al. [11] summarized the results from their laboratory analysis of 446 fetuses with structural malformations. CMA was used to reveal a clinically relevant genomic imbalance in 51 of these fetuses (51/446; 11.4%). Of these, pathogenic CNVs in 16 fetuses were less than 1 Mb in size; these would have been missed by low-resolution BAC arrays or traditional karyotyping. There was little difference in the number of pathogenic CNVs and VOUS detected in this study. They concluded that because high-resolution SNP arrays identify a higher proportion of pathogenic CNVs that would otherwise be undetected by standard chromosome analysis (with an acceptable proportion of VOUS), whole-genome high-resolution SNP array analysis provides a high diagnostic yield in prenatal investigation of fetuses with structural malformations.

In January 2019, Daum et al. [12] published a study of a large-scale analysis of 6,995 prenatal CMA tests. Out of 6,803 prenatal CMAs in which ploidy was normal, they describe four cases in which results were pathogenic or likely pathogenic based on SNP array results. One case was found to have a 75 kb deletion encompassing the Bloom syndrome gene (*BLM*), one case had a 3.4 Mb deletion of 14q32.2q32.31, one case had a 6.2 Mb deletion in 15q11.2q13.1, and one was homozygous for most of chromosome 11. The increased diagnostic yield of the SNP array was demonstrated by showing that the first case involved deletion of the maternal locus and indicated a founder mutation in the *BLM* gene of the father; the second case was due to deletion of the maternal segment of chromosome 14, resulting in only the paternal segment being present and a diagnosis of Kagami-Ogata syndrome [13]; the third case was due to deletion of the Prader-Willi/Angelman syndrome locus; and the fourth case was uniparental disomic for most of maternal chromosome 11. In each case, the parents used this information to decide their course of action.

Consequences of LCSH

The effect of LCSH or regions of homozygosity (ROH) can not only reveal monogenic recessive disorders, but can also play a role in uncovering recessive variants that contribute to complex genetic disorders [14-16]. Therefore, it is important to determine how common LCSHs are. In 2015, Wang et al. [17] analyzed 14,574 cases by hybrid SNP arrays. They found that 9,759 cases (67%) were neutral for copy number, but 1,453 cases (10%) had clinically significant CNVs, and 3,362 cases (23%) had CNVs of unclear significance (VOUS). A total of 832 cases (6%) from 805 families had one or more significant ROHs. Of these, 651 (78%) cases had multiple ROHs from more than one chromosome; these were interpreted as indicative of inheritance by descent. This study demonstrates that ROHs are much more frequent than previously recognized and often reflect parental relatedness. This information can also be used to understand factors that could contribute to autosomal recessive diseases and may unravel UPD in many cases.

Similarly, in 2018 Chave et al. [18] published a study where the frequencies of LCSH were analyzed to gain pathogenic insight and to identify ancestral haplotype-derived LCSH in 407 individuals with developmental delay, intellectual disability, autism spectrum disorder, and other phenotypes. For his study, a region was defined as LCSH if it was homozygous for more than 1 Mb on an autosome. His team found that in 95% of these individuals, at least one LCSH was detected. Of these, 2.6% of the samples were suggestive of UPD. For about 8.5%, the LCSH results suggest some regions more likely to have a clinical impact came from consanguineous unions. They identified 11 regions that could represent ancestral haplotypes in the South Brazilian population. This work further illustrates the importance and usefulness of interpreting LCSH identified in CMA analysis that includes SNPs.

SNP arrays are useful for identifying etiologies of disorders even when consanguineous unions are known. De Noronha et al. [19] describe their diagnosis of deafness in a family of consanguineous individuals in a 2018 study. Using data from SNP arrays, they showed that the total proportion of the ROHs in the genome can be used as a rough assessment of degree of parental relationship, or inheritance by descent (IBD). All siblings in the family they studied had ROHs scattered on various chromosomes. They found that the brothers with deafness had an IBD factor of 4.6% and 3.1% and had a LCSH that covered the same region. The regions of LCSH found only in the affected siblings suggested five candidate recessive genes related to deafness: *ORC1* (1p32), *BSND* (1p32.1), *FREM2* (13q13.3), *SMARCA4* (19p13.2), and *MAN2B1* (19p13.2). Similarly, according to Edwards et al. [20] in 2016, accurate diagnosis of an individual that had breathing and sinus invertus from a consanguineous family was made possible using a SNP array. Although previous results using aCGH found no aberrations, the researchers found long regions of homozygosity, including a candidate locus (*LRRC6*). Sequencing the *LRRC6* gene in the affected individual and other family members identified the causative mutation and confirmed the suspected diagnosis of primary ciliary dyskinesia (PCD). Thus, identifying LCSH by analyzing data from the SNP array simplified the formation of hypotheses about causative mutations and reduced the cost of sequence confirmation to one gene.

Occasionally, SNP arrays can reveal chromosomal aberrations that are not anticipated. One study, published in 2015 by Liu et al. [21], focused on analysis of a prenatal case with a high risk of Down syndrome . Karyotype testing of the fetal amniotic fluid sample revealed a normal karyotype. Analysis by aCGH did not uncover any known pathogenic CNVs. However, the SNP array indicated a complete isodisomic UPD (isoUPD) of chromosome 4 in the fetus, based on the loss of heterozygosity across the entire chromosome 4. These results were confirmed by subsequent whole-exome sequencing (WES). WES was also used to identify potential pathogenic variants, and none were found. The team of researchers concluded that this was the first prenatal report of complete maternal isoUPD of chromosome 4 that lacked any clinical findings. Although no phenotypic abnormalities were observed in this child after his first year of birth, the SNP array was able to provide investigators comprehensive genetic information for prenatal diagnosis of a rare UPD event, and provide a baseline for phenotypic monitoring as the child grows.

UPD and imprinting disorders

The expression of a small set of genes is controlled by genomic imprinting, an epigenetic phenomenon where the allele of only one parent is active. Chromosomal deletions, duplications, UPD, and other aberrations that cover imprinted loci can result in disorders such as Beckwith-Wiedemann syndrome (BWS), Prader-Willi syndrome (PWS), and Angelman syndrome (AS). Molecular testing of aberrant regions, typically using techniques that analyze methylated DNA, is very useful for diagnosing these imprinting disorders. However, SNP-based CMA provides extra diagnostic utility, since it can detect precise breakpoints and the sizes of aberrations and isoUPD, as well as determine the ratio of mosaicism [22].

To illustrate how SNP arrays can be used to uncover defects in individuals suspected of BWS, PWS, or AS, Liu et al. [23] collected 492 pre- and postnatal samples, including four from individuals suspected of having an imprinting disorder [23]. A SNP-based microarray was used to identify two individuals that lost either paternal or maternal genomic material. The results clearly illustrated the deletion size (5.25 Mb) and the breakpoints of the aberrations (15q11q13), uncovering the critical PWS/AS loci. Knowing the breakpoints can distinguish penetrance of the different phenotypes [24,25]; thus, the determination of precise breakpoints is helpful for predicting the prognosis and management of affected individuals.

Temple syndrome is another imprinting disorder, usually presented as UPD of maternal chromosome 14 (upd(14)mat). Because it is usually associated with a relatively mild phenotype and the typical clinical findings are not present at birth or early childhood, clinical diagnosis of upd(14)mat is often difficult. Additionally, the UPD of paternal chromosome 14 results in a more severe syndrome (Kagami-Ogata syndrome [13]). Thus, identifying the underlying molecular defects in individuals suspected of having Temple or Kagami-Ogata syndrome can lead to appropriate interventions. In a study published by Bertini et al. [26] in 2017, researchers analyzed an individual suspected to be afflicted with Temple syndrome. Conventional karyotyping showed a Robertsonian translocation of chromosome 13 and 14 but no other structural defects. Data from an array that could detect CNVs and SNPs did not reveal any deletion or duplication, but did show a loss of heterozygosity of about 13.6 Mb in the distal portion of chromosome 14 (14q11.2q12). Using the SNPs on the array and microsatellite analysis, it was determined that this individual displayed UPD for the maternal chromosome, leading to a Temple syndrome diagnosis. It was speculated that the Robertsonian translocation resulted in the formation of a trisomic 14 zygote, which upon trisomic rescue resulted in the UPD.

In conclusion, although analysis of methylated DNA can contribute to the understanding of imprinting anomalies, SNP-based CMA is an efficient method for precisely estimating the sizes and mosaicism rates of most types of chromosomal aberrations, and can quickly identify imprinting disorders that arise from UPD.

Trisomy

Conventional oligonucleotide CGH arrays cannot measure changes in ploidy. However, because SNP arrays can resolve allelic differences, changes in ploidy are detectable and can be used for prenatal diagnoses. For example, a 4-year old boy with small stature, moderate developmental disability with severe speech dyspraxia, and pectus excavatum was examined in a 2016 study published by Edwards et al. [20]. Prior testing showed a normal karyotype in 100 cells, normal subtelomere profile by fluorescence *in situ* hybridization (FISH), and no anomalies detected using multiplex ligation-dependent probe amplification (MLPA) tests for autosomal deletions. However, when extracted blood DNA was analyzed with a SNP array, an abnormal genotype was identifiable over the entire chromosome 9. Further analysis suggested trisomy 9 mosaicism in approximately 11% of the afflicted individual's cells. Importantly, no mosaicism was detectable in initial tests, nor even in subsequent conventional cultured karyotypes of 150 cells. These results suggest that the defective cells were lost from the sample in the culturing process. This potential loss of abnormal cells by culturing and resulting missed calls illustrates one of the limitations of conventional karyotyping methods.

SNP arrays and neurodevelopmental disorders

In addition to identifying causes for physical developmental anomalies in pre- and postnatal testing, hybrid SNP arrays can also be useful for understanding the underlying mechanisms of neurodevelopmental disorders. These disorders could include developmental delays (DD), intellectual disabilities (ID), multiple congenital abnormalities (MCA), and autism spectrum disorder (ASD). The use of SNP arrays in large-scale studies, both as a unique tool and in combination with other methods, was reviewed by Scionti et al. [27] in 2018. Briefly, they describe research showing that CNVs were commonly seen in patients that had a normal karyotype and fragile X test, making up 25% of the patients with this phenotype. Some of the studies described in the review were able to uncover potential causative loci in the CNVs, suggesting possible intervention strategies. The reliability of this technology to identify gains and losses in patients with DD, ID, MCA, or ASD has therefore been well documented. As a result, the American College of Medical Genetics and Genomics (ACMG) [28], the International Collaboration for Clinical Genomics (ICCG) [29], and the American Academy of Neurology (AAN) [30] have recommended chromosomal microarray analysis as the first-line test in patients with unexplained DD, ID, MCA, or ASD.

Cost effectiveness and diagnostic yield

As the costs of health care increase, there is an increased emphasis on balancing health care quality with expenditures. It is therefore critical that any test utilized provide the maximum benefit to the clinicians and patients.

The diagnostic utility of SNP arrays relative to conventional karyotyping in analyzing products of conception (POC) has been repeatedly demonstrated. As shown in the case studies described above, SNP-based CMA can identify pathogenic aberrations where karyotyping fails. These anomalies include whole-chromosome aneuploidies, triploidies, segmental imbalances below the standard resolution of karyotyping, and whole-genome isoUPDs.

SNP-based arrays can overcome the difficulties and artefacts produced by culture failure and false negatives produced by maternal cell contamination, thus enabling reporting of accurate fetal-specific results [31]. Mosaicism also introduces challenges for traditional karyotyping methods. SNP microarrays are more sensitive for identifying mosaicism than traditional chromosome analysis, as they can detect mosaicism levels as low as 5% [32-35]. Since at least one percent of all clinical POC cases have some degree of mosaicism [35], the use of SNP arrays can provide diagnostic utility where traditional karyotyping might fail. Furthermore, SNP-based CMA has been highly successful when analyzing difficult samples, such as archived formalin-fixed, paraffin-embedded (FFPE) POC samples [31]. This is particularly beneficial for cases of recurrent pregnancy loss, and provides options for women who have experienced a pregnancy loss but did not have a fresh tissue sample analyzed at the time of the loss. SNP-based microarrays therefore present opportunities to increase the amount of diagnostic information obtained from POC analyses.

Although SNP-based CMA analysis might entail higher initial costs than traditional karyotyping methods, the benefit of increased diagnostic utility has been shown to make them cost effective. Analysis as recent as 2018 using models that incorporated actual reimbursement rates set by governmental agencies and the reliable estimates of test outcomes derived from studies with tens of thousands of patients clearly showed that prenatal CMA testing is cost effective [36]. In the same study, parental CMA testing that is used to aid in the interpretation of a VOUS detected in patients was explored and found to be cost effective; however, the authors noted that the need for parental testing in the future could decrease with the sharing of the variant findings in genetic databases, which would improve the interpretation of VOUS results. A separate analysis by Sinkey et al. [37] in 2016 found that CMA testing was the preferred strategy for analyzing sonographically detected fetal anomalies, based on the incremental cost effectiveness ratio between CMA and karyotype. They showed that CMA alone led to an additional 17 diagnoses per 1,000 fetuses, further illustrating the advantages of CMA. The authors conclude that in spite of the increased cost, CMA analysis is superior for providing diagnostic information, and thus is cost effective when used for prenatal diagnosis of an anomalous fetus.

High-density SNP arrays are subtypes of CMAs, and thus the cost benefits of CMA molecular testing described above also hold true for SNP arrays. However, because they can detect UPD and other regions of extensive homozygosity, SNP arrays can provide added value. In one study directly comparing the diagnostic yield of SNP arrays to aCGH in intellectual disabilities, it was found that the ability to uncover homozygous mutations increased the overall diagnostic yield from 14.3% for aCGH to 28.6% with SNP arrays [38]. Similar findings have been reported (for examples, see [39-43]). Thus, the increased diagnostic yield of high-density SNP platforms can further enhance the cost efficiency of molecular testing.

Conclusions

Establishing a causative diagnosis reduces or eliminates prolonged medical evaluation and testing, thus reducing both short- and long-term health care costs. The results of studies like those described can help guide health care providers, organizations, professional societies, and policymakers to determine how and to whom particular health care services are provided. Given its much higher diagnostic yield over conventional karyotyping, CMA is recommended by a number of medical societies as the first-tier test in the diagnosis of unexplained global developmental delay (GDD) or intellectual disability (ID [28-30,44] Taken together, these findings provide a strong cost-effectiveness rationale for the US health-care system to use CMA as the first-tier test for the genetic diagnosis of unexplained GDD or ID. About a third of positive CMA findings are clinically actionable, with some of the actions being specific pharmacological treatments [45-48]. Because they increase the diagnostic yield of CMA testing, high-density SNP platforms can further enhance the cost efficiency of these tests. A concrete diagnosis obtained by a high-density SNP array can end diagnostic uncertainty and thus has the potential to substantially modify the clinical management of patients.

Glossary

ASD: Autism spectrum disorder, a condition related to brain development that impacts how a person perceives and socializes with others, causing problems in social interaction and communication.

LOH: Loss of heterozygosity, referring to a locus that no longer has two different alleles. This can involve deletion of one of the normally diploid copies, leaving only one.

BAC: Bacterial artificial chromosome, an *E. coli* replication that can accommodate and maintain large inserts such as segments of human DNA.

CMA: Chromosomal microarray, a method to analyze genomic content by hybridization to hundreds of thousands to millions of immobilized probes.

cnLOH: Copy-neutral loss of heterozygosity, referring to when a locus that should be heterozygous based on inheritance is diploid but homozygous for a variant.

CNV: Copy number variant, a sequence that is present at more or less than the normal diploid number.

DD: Developmental delay, a delay in reaching developmental milestones.

FFPE: Formalin-fixed, paraffin-embedded, a method for preserving dissected tissue by infiltration with formalin and storage in wax blocks.

GDD: Global developmental delay, a significant delay in cognitive or physical development.

Haplotype: A set of DNA variations, or polymorphisms, that are contiguous and tend to be inherited together.

ID: Intellectual disability, significant limitations in both intellectual functioning and adaptive behavior.

LCSH: Long contiguous stretches of homozygosity, large stretches of the diploid genome that have identical sequence.

MCA: Multiple congenital abnormalities, two or more unrelated major structural malformations that cannot be explained by an underlying syndrome or sequence.

SNP: Single-nucleotide polymorphism, a difference in nucleotide sequence involving only a single base change.

UPD: Uniparental disomy, a chromosome or region that has the genotype of only one parent, but in two copies.

VOUS: Variant of unknown significance, a difference in nucleotide sequence that is not correlated with a known phenotype, disease, or change in protein function.

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