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Reviews

Microarray-Based Genomic DNA Profiling Technologies in Clinical Molecular Diagnostics

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BACKGROUND: Microarray-based genomic DNA profiling (MGDP) technologies are rapidly moving from translational research to clinical diagnostics and have revolutionized medical practices. Such technologies have shown great advantages in detecting genomic imbalances associated with genomic disorders and single-gene diseases.

CONTENT: We discuss the development and applications of the major array platforms that are being used in both academic and commercial laboratories. Although no standardized platform is expected to emerge soon, comprehensive oligonucleotide microarray platforms—both comparative genomic hybridization arrays and genotyping hybrid arrays—are rapidly becoming the methods of choice for their demonstrated analytical validity in detecting genomic imbalances, for their flexibility in incorporating customized designs and updates, and for the advantage of being easily manufactured. Copy number variants (CNVs), the form of genomic deletions/duplications detected through MGDP, are a common etiology for a variety of clinical phenotypes. The widespread distribution of CNVs poses great challenges in interpretation. A broad survey of CNVs in the healthy population, combined with the data accumulated from the patient population in clinical laboratories, will provide a better understanding of the nature of CNVs and enhance the power of identifying genetic risk factors for medical conditions.

SUMMARY: MGDP technologies for molecular diagnostics are still at an early stage but are rapidly evolving. We are in the process of extensive clinical validation and utility evaluation of different array designs and technical platforms. CNVs of currently unknown importance will be a rich source of novel discoveries.

Human DNA mutations range from single-nucleotide changes to whole-chromosome alterations. At the small-size end of the mutation spectrum, de novo changes in single base pairs occur at the rate of about 1.7×10^{-8} per base pair per generation *(1)*. It is estimated that there are 2 nonsilent point mutations in each newborn *(2)*. At the large-size end of the mutation spectrum, alterations in chromosome number causing aneuploidy affect about 0.3% of live births *(3)*. In between are microscopic and submicroscopic genomic rearrangements involving different segments of chromosomes that may affect many genes or exons of a gene. The de novo rate of mutation of copy number variants (CNVs)[5] in each newborn has been estimated as 1 in 8 for deletion and 1 in 50 for duplication *(2)*, which are between the rates for de novo point mutations and chromosomal aneuploidy. Recent studies have shown that the human genome contains many CNVs *(4–7)*, which are the result of a distant (inherited) or current (de novo) loss or gain of genomic sequences.

A portion of these alterations will produce a change in the human phenotype and in extreme cases will cause abnormal development and medical conditions. The purpose of clinical genetic diagnostics is to detect such mutations effectively and to correlate these changes with corresponding medical conditions. Various methods have different resolutions that reveal different sizes of genomic imbalance (Fig. 1).

Point mutations can be detected effectively with the Sanger sequencing method after specific amplification of target regions by the PCR. The detection of chromosomal aneuploidy and large rearrangements are within the repertoire of traditional cytogenetics.

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[5] Nonstandard abbreviations: CNV, copy number variant; FISH, fluorescence in situ hybridization; MLPA, multiplex ligation-dependent probe amplification; MGDP, microarray-based genomic DNA profiling; CGH, comparative genomic hybridization; SNP, single-nucleotide polymorphism; BAC, bacterial artificial chromosome; UPD, uniparental disomy; ASD, autism spectrum disorder; pCNV, pathogenic CNV; bCNV, benign CNV; uCNV, CNV of unknown importance.

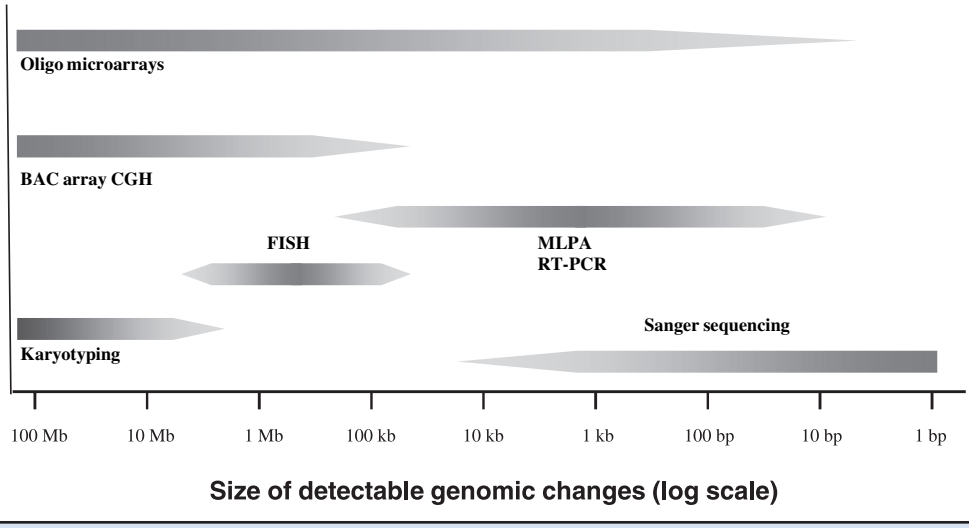

Fig. 1. Various methods with different resolutions detect different sizes of genomic changes.

Point mutations (changes in single or multiple base pairs up to kilobase pairs) can be detected with Sanger DNA sequencing. Karyotyping can detect large chromosomal rearrangements with low resolution (down to 5 Mb). FISH provides much higher resolution (down to 100 kb) for detecting submicroscopic genomic imbalances, but it interrogates only selected loci at one time. MLPA and real-time PCR (RT-PCR) complement FISH and DNA sequencing, but all of these methods suffer from a low information content, limited throughput, and the requirement of choosing candidate targets before the test. Microarrays [BAC and oligonucleotide (oligo)] are capable of detecting both microscopic and submicroscopic copy number changes for the whole genome in a single assay. The high-resolution microarrays (oligo CGH arrays and SNP hybrid arrays) provide the most efficient means to identify genomic imbalance widely (down to kilobase pairs and approaching base pair resolution).

Fluorescence in situ hybridization (FISH) represents a merging of cytogenetic techniques with molecular technology. Such a merger and its applications in the clinical setting were necessitated by the discovery of a set of submicroscopic genomic imbalances associated with specific clinical manifestations (collectively known as microdeletion and microduplication syndromes), but this method interrogates only selected loci at a time. Other techniques designed to detect copy number changes, such as multiplex ligation-dependent probe amplification (MLPA) and real-time PCR, are complementary to those of FISH and DNA sequencing, but all of these methods suffer from low information content, limited throughput, and the requirement of choosing candidate targets before the test.

Microarray-based genomic DNA profiling (MGDP) technologies are capable of detecting both microscopic and submicroscopic copy number changes for the whole genome in a single assay. They provide unprecedented sensitivity and cost-effectiveness for a large group of mutations that have evaded conventional approaches, and they are changing clinical practices. MGDP may be used as a first-tier tool in clinical genetics for many conditions previously evaluated via conventional cytogenetic approaches. We review the current status and some considerations regarding the clinical applications of MGDP technologies.

Development of Genomic DNA Profiling Microarrays

Microarrays have been widely used for gene expression analysis in the past decade; however, it is worth mentioning that DNA microarrays were initially designed for interrogating vast amounts of genomic sequence polymorphisms and variants. The intention to use microarrays for diagnostic purposes was deeply rooted from the very beginning. There are 2 major microarray platforms for genomic DNA profiling—comparative genomic hybridization (CGH) arrays and genotyping arrays—which were developed in parallel yet interactively. Regardless of the technical differences in chip manufacturing and probe types used with these platforms, they share the same principle and rely on the specific hybridization of target and probe sequences. CGH arrays use a 2-color scheme. The method infers the copy number changes in a test sample by comparing it with a reference sample. Genotyping arrays, on the other hand, do not use a control sample; rather, they use the intensity of the hybridization signal to in-

dicate the relative DNA copy number. In addition, genotyping arrays provide information on single-nucleotide polymorphism (SNP) genotypes.

TRANSFORMATION OF CGH MICROARRAYS FROM LOW-RESOLUTION TARGETED BAC ARRAYS TOWARD WHOLE-GENOME HIGH-DENSITY OLIGONUCLEOTIDE ARRAYS

CGH was first reported by Kallioniemi et al. in 1992 to interrogate cancer genomic DNA with metaphase chromosomes as probes *(8)*. The basic strategy of the technique is to differentially label the DNA from cancer cells and the DNA from healthy reference cells with different fluorochromes and to cohybridize the labeled samples to a metaphase spread from a healthy reference cell. The ratio of the intensities of the 2 fluorochromes reflects the copy number differences between cancer cells and healthy cells. Because this technique uses metaphase chromosomes as probes for hybridization, it is now also called "chromosome CGH." In essence, chromosome CGH is similar to FISH painting, which uses labeled DNA from the entire genome as the probe set. This technology permits a genome-wide survey and identifies the genomic imbalance at different regions of specific chromosomes. Although chromosome CGH has demonstrated its effectiveness in detecting larger genomic imbalances in the cancer genome, the detection power has ultimately been limited by the resolution of a metaphase chromosome.

The major breakthrough in CGH came from microarray technology about a decade ago. Instead of hybridizing labeled genomic targets to metaphase chromosomes, the new scheme uses cloned genomic DNA as probes in a microarray format. Because these probes contain sequence information that permits their specific localization in the human genome, the regions with a genomic imbalance can be delineated by data-visualization software that denotes all of the probes along the genome. The resolution of microarray-based CGH, which is determined by the density and size of the probe, is a substantial improvement over chromosome CGH.

The first CGH microarray, termed matrix CGH, was developed in 1997 by Solinas-Toldo et al. with cosmid and plasmid artificial chromosome clones as probes *(9)*. Subsequently, bacterial artificial chromosome (BAC) clones *(10)* and cDNA clones *(11)* were used to construct CGH microarrays. Subsequent improvements were focused on increasing the probe density and array coverage, as well as improving the signal-to-noise ratio.

The most recent advancement in CGH microarrays has been the use of oligonucleotide sequences as probes *(12)*. Compared with BAC arrays, oligonucleotide arrays have several advantages, including:

1. Reproducibility. As opposed to BAC arrays, in which the content of the probe (i.e., PCR product of a BAC clone) varies from batch to batch, the probe sequences in oligonucleotide arrays are uniformly defined and devoid of highly repetitive sequences. Consequently, oligonucleotide arrays are more reproducible.
2. Sensitivity and specificity. The smaller interprobe spacing of oligonucleotide arrays offers a much higher probe density for better detection of smaller genomic imbalances and more accurate breakpoint mapping, therefore providing much improved sensitivity over BAC arrays. The fact that oligonucleotide probes are selected from the reference human genome sequence allows all users to use any sequence of interest as a potential target, providing a specificity that is impossible with BAC arrays, in which clones can be selected only from existing libraries and need to be validated for their physical location.
3. Customization. Oligonucleotide probes are synthesized in situ on the arrays, allowing for easy customization of content. In addition, many commercial manufacturers offer a large number of preselected array CGH oligonucleotide probes and computer interfaces, making custom design and updating of CGH arrays quite feasible and fast for clinical laboratories, whereas BAC arrays are cumbersome to update and time-consuming for printing.
4. Robustness and reliability. The substantially increased capacity of oligonucleotide arrays enables multiprobe confirmation for a single event (the detection sensitivity and specificity are a function of consecutive probes), as well as increased robustness because the higher signal-to-noise ratios provide higher confidence in CNV diagnosis *(13–15)*.

IMPROVEMENT OF GENOTYPING ARRAYS FROM LOW-RESOLUTION SNP ARRAYS TO HIGH-RESOLUTION HYBRID ARRAYS THAT INTEGRATE SNP AND CNV PROBES

Whereas CGH arrays successfully combine microarray technology with CGH, genotyping arrays for CNVs arose from SNP arrays that were originally designed for sequencing *(16)*, genotyping *(17)*, and gene expression *(18)*. These SNP arrays with short oligonucleotides were developed by Affymetrix with their proprietary photolithographic technology *(19)*. Since then, the density of SNP arrays has been growing constantly. The DNA chips designed to genotype 10×10^3, 50×10^3, 100×10^3, 500×10^3, and even more SNPs have allowed improved detection resolution.

At about the same time that Agilent Technologies developed long oligonucleotide–based whole-genome CGH arrays with their ink-jet technology *(20)*, Affymetrix SNP arrays demonstrated their ability to detect changes in genomic copy number in addition to genotyping *(21–23)*. Similarly, Illumina used a

Table 1. Major commercial oligonucleotide array platforms and their current products.

Company	Array platform	Resolution (median probe spacing)	Probe number	Oligonucleotide probe type	Detection
Agilent Technologies, Santa Clara, CA	4x44K CGH array	43 kb	43 000+	60-mer	CNV
	8x60K CGH array	41.4 kb	55 000+		
	2x105K CGH array	21.7 kb	99 000+		
	4x180K CGH array	13 kb	170 000+		
	244K CGH array	8.9 kb	236 000+		
	2x400K CGH array	5.3 kb	411 000+		
	1 Million CGH array	2.1 kb	963 000+		
Affymetrix, Santa Clara, CA	Genome-Wide Human SNP Array 6.0	0.7 kb	906 600 SNP probes and 946 000 CNV probes	25-mer	CNV, genotype, and LOH[a]
Illumina, San Diego, CA	HumanCNV370-Quad DNA analysis BeadChip	4.9 kb	320 000 SNP probes and 60 000 non-SNP probes for CNVs	50-mer	CNV, genotype, and LOH
	Human610-Quad DNA analysis BeadChip	2.7 kb	550 000 SNP probes and 60 000 non-SNP probes for CNVs		
	Human1M-Duo BeadChip	1.5 kb	1.1×10^6 SNP and CNV probes targeting exons		
NimbleGen, Madison, WI	HG18 CGH 4x72K WG Tiling v2.0	40 kb	72 000	50- to 75-mer	CNV
	385K WG Tiling, single array	6.27 kb	385 000/array		
	385K WG Tiling, 4-set array	1.57 kb			
	385K WG Tiling, 8-set array	713 bp			

[a] LOH, loss of heterozygosity.

different manufacturing approach in commercializing BeadChips for genotyping and copy number analysis *(24)*.

Table 1 lists the currently available oligonucleotide-array platforms for genomic DNA profiling. Reduced manufacturing costs, unprecedented detection power, and the feasibility of custom design/updates have made these arrays attractive in basic and translational research.

The major advance in genotyping arrays for genomic profiling is the development of hybrid genotyping arrays, i.e., the Affymetrix Genome-Wide Human SNP Array 5.0 and the latest 6.0 version, which combines SNP probes for genotyping with CNV probes for detecting changes in copy number *(25)*. The SNP Array 5.0, which is the prototype of the hybrid array, contains 500 000 SNP probes for genotyping and 420 000 nonpolymorphic probes for CNV analysis. Among the nonpolymorphic probes, 320 000 probes were chosen to provide even spacing across the genome, concentrating on areas not represented by SNPs, and the remaining 100 000 probes covered 2000 known CNVs (50 probes per CNV). The SNP Array 6.0 has 906 600 SNP probes, 744 000 copy number probes evenly spaced along the genome, and another 202 000 probes that target 5700 previously reported CNV regions. Thus, a single 6.0 array offers a total of 1.8 million probes for simultaneous SNP genotyping, CNV analysis, and loss-of-heterozygosity detection.

Although the Affymetrix SNP Array 6.0 (hybrid arrays) is mainly a research tool at the moment, many diagnostics laboratories are actively validating this platform for clinical applications. Although long-oligonucleotide CGH arrays do not have as many probes as short-oligonucleotide genotyping arrays, the signal-to-background dynamic range is generally better with long-oligonucleotide CGH arrays; however, the rich genotyping information, in addition to the CNV data offered by the hybrid arrays, provides additional value to the platform. For instance, the genotyping data simultaneously provide information regarding

the parent of origin for the detected de novo CNV if parental samples have also been examined. The most important advantage of the hybrid arrays is their capability of detecting copy number–neutral genomic rearrangements, namely uniparental disomy (UPD), through loss-of-heterozygosity analysis. In the long run, the genotyping information can be used for SNP-association analysis.

Translational Research and Applications of MGDP Technologies for Patient Samples

MGDP technologies have been used extensively in clinical and translational research for interrogating patient samples. These studies provided crucial information about clinical utility before their diagnostic applications. A recent review by Stankiewicz and Beaudet *(26)* summarized the applications of microarrays for CGH, primarily dealing with low-resolution or targeted BAC arrays in the evaluation of patients with dysmorphic features, developmental delay, and/or idiopathic mental retardation. The published data produced with different array platforms and patient cohorts indicate an overall detection rate for pathogenic genomic imbalance of patients with multiple congenital anomalies and/or developmental delay/mental retardation of 12%–18%. Only 3%–5% of such patients can be detected by G-banded karyotyping, and an additional 9%–13% can be detected by microarray-based tests *(26)*. As expected, the detection rate is higher with whole-genome tiling arrays than with targeted arrays. A series of such studies overwhelmingly verified the superior sensitivity and detection power of array CGH compared with karyotyping and FISH analysis. As mentioned above, the most important advance in array CGH is replacing BACs with oligonucleotides. Similarly, short-oligonucleotide arrays have also been validated against and found superior to BAC arrays.

INTERROGATING SAMPLES FROM PATIENTS AND HEALTHY INDIVIDUALS WITH OLIGONUCLEOTIDE ARRAYS

Oligonucleotide CGH arrays became commercially available in 2004 and now include platforms from Agilent Technologies *(20)*, NimbleGen *(27)*, Affymetrix *(28)*, and Illumina *(24)* (Table 1). In-house spotted-oligonucleotide arrays *(29)* and custom-designed oligonucleotide CGH arrays *(13, 14, 30–32)* have also demonstrated high sensitivity and reproducible detection of genomic imbalance in testing with samples from patients and healthy individuals. Consequently, many laboratories have shifted from BAC arrays to oligonucleotide arrays *(15)*. Some diagnostic laboratories that provide large-scale services are now using oligonucleotide arrays for clinical diagnostics.

The initial implementation of oligonucleotide CGH arrays for clinical applications focused on targeted pathogenic genomic regions. Shen et al. developed targeted oligonucleotide arrays that used Agilent custom arrays to interrogate 179 clinically relevant genomic loci *(14)*. The multiplex arrays demonstrated results that were 100% concordant with the findings from BAC arrays, showing very high sensitivity. In addition, smaller genomic imbalances that were not detectable by BAC arrays were reliably detected with this oligonucleotide-array system *(14)*. Similarly, Ou et al. emulated BAC arrays with oligonucleotide probes and created an array of 44 000 oligonucleotides that verified an enhanced ability for detecting copy number changes compared with BAC arrays *(13)*. In addition to showing the superior analytical sensitivity of oligonucleotide arrays compared with BAC arrays, these studies also demonstrated the convenience of oligonucleotide-array design and manufacturing. Aradhya and Cherry used samples from 20 patients with dysmorphic features, developmental delay, or mental retardation to directly compare the performance of whole-genome BAC arrays of 1-Mb resolution (Spectral Chip 2600; PerkinElmer) with that of Agilent whole-genome oligonucleotide CGH arrays of 35-kb resolution *(33)*. All of these patients had a typical karyotype. Ten clinically important genomic-imbalance events were detected with the oligonucleotide arrays, whereas only 6 were detected with the BAC arrays. The study clearly demonstrated the superior effectiveness of the oligonucleotide arrays. Because of the large size of the BAC clones and the widespread nature of CNVs, BAC arrays provide very low specificity for detecting CNVs. Conversely, oligonucleotide arrays avoid repeat sequences via the selection of probes that avoid nonspecific regions.

With the intention of improving the capture of frequent subtelomeric imbalances associated with developmental delay/mental retardation, custom-designed oligonucleotide CGH arrays were developed with improved coverage in subtelomeric regions. Exploiting the design concept used earlier in BAC arrays *(34)*, Toruner et al. *(32)* randomly removed one third of the probes from an off-the-shelf Agilent whole-genome CGH array of 44 000 probes and replaced them with 14 000 subtelomeric probes. The resulting arrays provided 5-kb resolution in subtelomeres and 125-kb resolution in the remaining genome. The oligonucleotide CGH array–based molecular ruler *(31)*, another design that emerged at nearly the same time, provided 50-kb resolution for subtelomeric regions and 75-kb resolution for the rest of the genome. This array detected a subtelomeric genomic imbalance of 10.9% and a pathologic imbalance of 4.7% genome wide in clinical samples *(30)*. These custom designs provide

Table 2. Exon-level DNA-profiling arrays.

Reference	CGH array platform	Target gene(s)[a]	Specific probe no.	Resolution
Rouleau et al. *(39)*	Agilent 11K array	*BRCA1*	1679	NA[b]
del Gaudio et al. *(40)*	Agilent 44K array	*DMD*	8769	NA
Staaf et al. *(41)*	Agilent 4x44K array	*BRCA1*, *BRCA2*, *MSH2*, *MLH1*, *PTEN*, and *CDKN2A*	9612	<500 bp, partial exon
Saillour et al. *(42)*	NimbleGen tiling array	*CFTR*, *SGCA*, *SGCB*, *SGCD*, *SGCE*, *SGCZ*, and *DMD*	72 500	Single exon and mosaic
Hegde et al. *(43)*	NimbleGen tiling array	*DMD*	385 747 (mean spacing, 5 bp)	Single exon and mosaic
Wong et al. *(44)*	Agilent 44K array	130 Nuclear genes involved in metabolic and mitochondrial disorders	≅44 000 (mean spacing, 250–300 bp)	Single exon and mosaic

[a] *BRCA1*, breast cancer 1, early onset; *BRCA2*, breast cancer 2, early onset; *MSH2*, mutS homolog 2, colon cancer, nonpolyposis type 1 (E. coli); *MLH1*, mutL homolog 1, colon cancer, nonpolyposis type 2 (E. coli); *PTEN*, phosphatase and tensin homolog; *CDKN2A*, cyclin-dependent kinase inhibitor 2A (melanoma, p16, inhibits CDK4); *CFTR*, cystic fibrosis transmembrane conductance regulator (ATP-binding cassette sub-family C, member 7); *SGCA*, sarcoglycan, alpha (50kDa dystrophin-associated glycoprotein); *SGCB*, sarcoglycan, beta (43kDa dystrophin-associated glycoprotein); *SGCD*, sarcoglycan, delta (35kDa dystrophin-associated glycoprotein); *SGCE*, sarcoglycan, epsilon; *SGCZ*, sarcoglycan zeta.
[b] Data not available.

enhanced coverage for clinically relevant regions, as well as decent coverage for the rest of genome for better measurement of the sizes of imbalance events.

Compared with oligonucleotide CGH arrays, fewer clinical applications have been studied on genotyping arrays. Friedman et al. used the Affymetrix 100K SNP arrays to investigate genomic imbalance *(28)*. Eight control trios that included the unaffected parents and an affected child with a previously recognized chromosomal abnormality or UPD were studied. The Affymetrix array detected the known abnormalities in all control cases, including 4 cases of UPD. The array also detected de novo deletions as small as 178 kb among 100 idiopathic mental-retardation patients with a typical karyotype. In addition, Ming et al. used the 50K Xba chip from the Affymetrix 100K array set for a similar study of patients with multiple congenital anomalies *(35)*. Although both studies illustrated the sensitivity and detection power of Affymetrix arrays for investigating genomic imbalance, the analytical sensitivity and cutoff for detecting copy number–neutral UPD have yet to be firmly established.

Flexible probe selection permits easy design of oligonucleotide arrays that can detect deletions and duplications at the exon level of specific genes (Table 2). Although PCR products for *NF1*[6] (neurofibromin 1) *(36)*, *NF2* [neurofibromin 2 (merlin)] *(37)*, and other large, clinically important genes *(38)* have been used to develop exon-level microarrays, the current manufacturing technology for oligonucleotide arrays has simplified the process so that it is more accessible to ordinary laboratories. These custom-designed, target gene–specific arrays detect duplication as effectively as deletion at the single-exon level, with more precise breakpoint mapping *(39–44)*. Targeting panels of genes associated with one disease at the exon level is attractive for molecular diagnostics.

GENOMIC IMBALANCES IN AUTISM SPECTRUM DISORDERS

Autism spectrum disorders (ASDs) are complex, pervasive developmental-delay disorders characterized by impairments in communication, social interaction, and behavior. Studies of twins have suggested a strong genetic component to ASD. Thus far, few contributory genes have been identified, but cytogenetic changes have been one of the most consistent identifiable causes of autism. Changes detectable by high-resolution G-banding have been reported in 3%–5% of autism patients *(45)*, involving the chromosomal regions at 2q37, 5p15, 11q25, 16q22.3, 17p11.2, 18q21.1, 18q23, 22q11.2, 22q13.3, and Xp22.2p22.3 *(46)*. MGDP is a powerful new venue for exploring the complex nature of ASD genetics. Jacquemont et al. *(47)* used whole-genome 1-Mb BAC CGH arrays to interrogate 29 syndromic autism patients with typical karyotypes and found that more than a quarter of the patients showed spontaneous pathogenic copy number changes. The size of the imbalances ranged from 2 Mb to 17.3 Mb. This study highlighted the immense effectiveness of array CGH in genetic analysis of patients with autism. It

[6] Human genes: *NF1*, neurofibromin 1; *NF2*, neurofibromin 2 (merlin); *PARK2*, Parkinson disease (autosomal recessive, juvenile) 2, parkin; *DMD*, dystrophin.

Table 3. Detection rate of clinically relevant CNVs in ASDs by array technologies.

Reference	Array platforms	Actual resolution	Patient cohort	De novo CNV detection rate
Jacquemont et al. *(47)*	BAC CGH array	1 Mb	29 Syndromic autism	27.5% (8 of 29)
Sebat et al. *(48)*	Oligonucleotide CGH array	35 kb	118 Sporadic autism	10.2% (12 of 118)
			77 Multiplex[a] familial autism	3% (2 of 77)
Szatmari et al. *(49)*	Affymetrix 10K SNP array	476 kb	173 Families, simplex	5.8% (10 of 173)
Weiss et al. *(50)*	Affymetrix 5.0 array	30 kb	751 AGRE families	6.7% (50 of 751)
Christian et al. *(51)*	BAC CGH array	Tiling 19K set	397 AGRE samples	2.3% (9 of 397)
Marshall et al. *(52)*	Affymetrix 500K SNP array	75 kb	427 ASD families	6.3% mean (27 of 427); 7.1% (4 of 56) in simplex families; 2% (1 of 49) in multiplex families
Morrow et al. *(56)*	Affymetrix 500K SNP array and targeted BAC CGH array	75 kb	42 Consanguineous samples, multiplex	0
			52 Consanguineous samples, simplex	1.9% (1 of 52)

[a] Multiplex, family with ≥2 ASD patients; simplex, family with 1 ASD patient; AGRE, Autism Genetic Resource Exchange.

also demonstrated that extensive genomic imbalances are an important underlying cause of syndromic ASD.

In another study that used oligonucleotide arrays with much higher resolution, Sebat et al. *(48)* revealed the importance of de novo CNVs as genetic risk factors for sporadic ASD. Subsequent studies have provided further evidence to support the notion that copy number changes are strongly associated with idiopathic ASD *(49–55)*. Array technology has detected pathogenic CNVs in 5.8%–10.2% of sporadic ASD patients (Table 3), whereas the rate is consistently low in familial ASD patients (2%–3%). This finding suggests that there are 2 different ASD genetic mechanisms: sporadic ASDs are more likely to be caused by de novo deletions or duplications, whereas familial ASDs are more likely to be due to other types of inherited mutations. Array studies with consanguineous families further demonstrated the existence of recessive gene mutations that are largely not genomic deletions or duplications *(56)*.

Collectively, these studies clearly demonstrate the clinical value of genomic profiling in the evaluation of autism. It is strongly evident that the detection yield of MGDP is much more consistent and higher than traditional cytogenetic techniques *(57)*.

Clinical Validation and Diagnostic Applications of MGDP Technologies

CLINICAL VALIDATION—SENSITIVITY AND SPECIFICITY

As for any new technology, systematic validation is required before MGDP can be used for routine clinical applications *(14, 58, 59)*. The validation process should test all aspects of a new technology, but most importantly the sensitivity and specificity. Sensitivity and specificity are often tested by means of a positively testing cohort with known mutations that have been identified with a well-accepted technology. In the case of MGDP, results from karyotyping and FISH studies are often used. Typically, sensitivity testing examines the false-negative rate, and specificity testing inspects the false-positive rate; however, several aspects of MGDP validation are unique.

Microarray technology is analog in nature. The change in genomic copy number is reflected by a change in signal intensity or a color shift (red vs green) of the microarray features (probe spots). The first critical step is to define the analytical resolution, i.e., the size of CNV that can be detected reliably. Assuming that the feature-analysis software is well established for each array platform (not necessarily the case), CNV calling depends on the algorithm used. Owing to the different array platforms and probe densities, the analytical resolution of each array needs to be specifically defined. It is important to note that sensitivity and specificity vary, depending on the number of consecutive probes examined. Higher sensitivity and specificity can be achieved when more consecutive probes are used. In addition, the signal-to-background ratio for a deletion or duplication changes with the array platform, the labeling chemistry, and the hybridization protocol. Thus, it is necessary to define an appropriate threshold for each clinical-array design. For example,

Table 4. Clinical utility of diagnostic genomic-profiling microarrays.

Reference	Array platform	Sample size, n	GI[a]/CNV size	pCNV	bCNV	uCNV
Shaffer et al. *(65)*	Signature Genomic targeted BAC CGH array	8789	≥200 kb	6.90%	1.20%	3.90%
Lu et al. *(66)*	Baylor CMA V4 targeted BAC CGH array	775	≥1 BAC clone	7.60%	10%	0.60%
	Baylor CMA V5 targeted BAC CGH array	1738	≥1 BAC clone	8.90%	8.1%	1.70%
Pickering et al. *(67)*	Spectral Genomics Spectral Chip 2600 and Constitutional Chip	1176	≥1 BAC clone	9.86%	3.99%	
Aston et al. *(68)*	Spectral Genomics Spectral Chip 2600 and Constitutional Chip	669	≥2 BAC clones	10.8%	NA	
Baldwin et al. *(30)*	Targeted plus whole-genome oligo CGH array	211	>500 kb	15.64% (10.90%, targeted; 4.74%, backbone)	8.53% (6.16%, complex; 2.36% familial)	1.90%
Shen et al. *(14)*	Agilent 2x11K focused oligo CGH array	211	≥3 Consecutive probes, smallest 23 kb	11.9%	4.26%	
Fan et al. *(69)*	Agilent 44K whole-genome oligo CGH array	100	≥3 Consecutive probes, smallest 17 kb	15%	0.72/case	
Xiang et al. *(70)*	Agilent 44K whole-genome oligo CGH array	50	>500 kb	6.00%	12.00%	

[a] GI, genomic imbalance; CMA, chromosomal microarray analysis; NA, data not available; oligo, oligonucleotide.

chromosomal mosaicism is not so rare in patients with birth defects and mental retardation. BAC arrays have found 8% of abnormal findings to be mosaic mutations; however, different array platforms have different sensitivities for detecting mosaicism *(60–62)*.

Because microarray technology has much higher resolution than karyotyping and FISH, its analytical resolution cannot be assessed by karyotyping or FISH. In many cases, additional CNVs not detectable by karyotyping or FISH were identified with array technologies. Therefore, other technologies covering smaller-scale mutations, such as MLPA, real-time PCR, or even PCR/sequencing, should be used for validation purposes *(14)*.

CLINICAL VALIDATION—PLATFORM PITFALLS

It is also necessary to check for missing spots on the array that may be clinically important. For example, some commercial arrays do not cover the pseudochromosomal regions of chromosomes X and Y. On the other hand, poor-performing probes (mismapped or poorly hybridizing probes) should be taken out of the array *(59)*. The latter action is particularly necessary in BAC arrays because one mismapped or poorly hybridizing clone will appreciably affect detection sensitivity and specificity. Most of these poorly hybridizing BAC clones may contain low-copy repeats. Thus, the presence of low-copy repeats is more of a problem in the BAC probe than the oligonucleotide probe, whereas oligonucleotide probes that do not perform well may contain secondary structure. Identifying such bad probes and removing them will improve array performance considerably *(14)*. Although such probes cannot be removed from commercial chips, posthybridization data analysis (data filtering) can provide an alternative solution for amending this problem.

It has been suggested that a minimum of 30 abnormal samples be used for clinical validation *(58)*; however, the region and size of the genomic imbalance that the abnormal cases cover are more important than the actual number. Ideally, every probe should be evaluated with abnormal samples. For this purpose, aneuploidy cell lines, as well as samples with well-defined genomic imbalances of different sizes, are very valuable.

UNDERSTANDING CNVs

The most important issue for array interpretation in clinical genetic testing is to define the nature of the identified CNVs *(63)*. In general, CNVs can be categorized into 3 major groups: CNVs with established pathogenicity (pCNVs), i.e., deletions/duplications that overlap with re-

gions associated with defined genomic disorders or disrupt known disease genes; CNVs of a benign nature (bCNVs), i.e., deletions/duplications repeatedly observed in nonpathologic individuals and with no evidence of an association with any particular clinical phenotype; and CNVs with unknown importance (uCNVs), i.e., deletions/duplications without an established association with any genomic disorder and involving genes of unclear clinical importance [*(14*, *30*, *65–70)*, Table 4]. Even when bCNVs and uCNVs are intentionally avoided during array design, a large percentage of the identified imbalances often belong to bCNVs or uCNVs, because a substantial number of novel CNVs are still being discovered (Table 4).

We anticipate that many of CNVs of currently unknown importance (uCNVs) will eventually be confirmed as bCNVs as more precise knowledge about their locations, distributions, and frequencies in healthy populations becomes available. Meanwhile, the accumulating evidence from clinical (phenotyping), genetic (inheritance and segregation), and functional (gene content and expression) studies may reveal a portion of these uCNVs to be pathogenic. We believe that uCNVs are a rich source for identifying novel genetic risk factors for a variety of medical conditions.

Our understanding of CNVs will certainly improve with systematic CNV-discovery efforts *(64)*. At the same time, continued studies of genotype–phenotype association are also essential. Public databases such as DECIPHER (https://decipher.sanger.ac.uk/) represent such an effort. In the meantime, many novel CNVs are being discovered in clinical samples with the help of high-resolution whole-genome arrays. Databases derived from clinical samples are also a rich source for evaluating genotype–phenotype correlations. Eventually, this knowledge will make it possible to minimize the detection of bCNVs and maximize the detection of clinically important regions.

CLINICAL-DIAGNOSTIC UTILITY

The first-generation arrays designed for clinical use were targeted BAC CGH arrays developed in house at the Baylor College of Medicine *(34)* and Signature Genomic Laboratories *(71)*. Both arrays were designed to cover regions of known genomic disorders, as well as all subtelomeric regions. Array-CGH and FISH results showed 100% concordance. In addition, array CGH detected changes missed by prior karyotyping or FISH. A similar design was also available commercially, the Constitutional Chip by Spectral Genomics *(72)*. The Constitutional Chip also included backbone clones to cover the rest of the genome with an interval of about 1 Mb. Since that time, more BAC clones (from 589 to >4600) have been selected to cover additional clinically relevant regions (approximately 40–150 recognized genomic disorders/syndromes), improving detection rates from 5.6% in the first-generation BAC arrays to 10.8% in later arrays *(68*, *73)*.

The clinical utility of oligonucleotide arrays has not yet been evaluated in large clinical studies; however, the current data indicate that the detection rate is higher than for BAC arrays (Table 4). High-resolution whole-genome oligonucleotide arrays have identified small novel microdeletions and microduplications in 16p11.2 that are associated with mental retardation/autism *(50*, *53)*, have helped to circumscribe critical regions such as 17q21.31 *(74)*, and have identified intragenic deletions/duplications in large genes, such as *PARK2* [Parkinson disease (autosomal recessive, juvenile) 2, parkin] and *DMD* (dystrophin) (Y. Shen et al., unpublished data).

Common clinical indications for MGDP-based diagnostics testing include developmental delay, multiple congenital anomalies, dysmorphic features, unexplained mental retardation, seizure disorders, ASDs, and other neurologic/psychiatric disorders such as schizophrenia, as well as prenatal diagnosis, including spontaneous abortion or fetal demise.

Conclusion and Future Development

Microarray-based clinical molecular diagnostics technologies such as MGDP have gone beyond the stage of "coordinated and concurrent FISH analysis" *(75)* and extended into loss-of-heterozygosity testing and possibly methylation profiling. Current MGDP technologies are capable of detecting genomic imbalances of any size but do not reveal positional or orientational information. FISH analysis has largely been responsible for providing positional information. FISH is also instrumental in testing for translocations in parental samples for de novo CNVs that have been identified in probands. MGDP has much better resolution than FISH, however. The field would benefit immensely from a tool with the localization capability of FISH and the resolution of whole-genome oligonucleotide arrays. Advanced next-generation sequencing platforms may eventually provide digital profiling for detecting copy numbers and positional alterations simultaneously. Therefore, DNA-diagnostics profiling may soon extend beyond microarray-based technology, from analog analysis to digital analysis.

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