

Array-based technology and recommendations for utilization in medical genetics practice for detection of chromosomal abnormalities

Melanie Manning, MD, MS FACMG¹ and Louanne Hudgins, MD, FACMG¹
For the Professional Practice and Guidelines Committee

Disclaimer: This guideline is designed primarily as an educational resource for healthcare providers to help them provide quality medical genetic services. Adherence to this guideline does not necessarily assure a successful medical outcome. This guideline should not be considered inclusive of all proper procedures and tests or exclusive of other procedures and tests that are reasonably directed to obtaining the same results. In determining the propriety of any specific procedure or test, the geneticist should apply his or her own professional judgment to the specific clinical circumstances presented by the individual patient or specimen. It may be prudent, however, to document in the patient's record the rationale for any significant deviation from this guideline.

INTRODUCTION

Laboratory evaluation of patients with developmental delay/intellectual disability (DD/ID), congenital anomalies and dysmorphic features has changed significantly in the last several years with the introduction of microarray technologies (array-based comparative genomic hybridization and SNP array analysis) into the menu of tests available to the practicing clinician. With these techniques, a patient's genome is examined for detection of gains or losses of genetic material that typically are too small to be detectable by standard G-banded chromosome studies. Guidelines for the application of array-based technology in the practice of medical genetics were originally published in this journal in 2007.¹ However, because of the rapidly expanding use of genomic copy number microarrays in the clinical setting, we herein update the recommendations.

MICROARRAY FOR EVALUATION OF COPY NUMBER VARIATION

Diagnostic cytogenetic testing has undergone a marked evolution since the introduction of chromosome banding techniques in the late 1960's.² DNA-based techniques such as genomic copy number microarrays (cytogenetic microarrays, or CMA) are the latest tools available for clinical use.¹ The initial technology, comparative genomic hybridization (CGH) was developed for genome-wide screening for unbalanced rearrangements in a single

experiment.^{1,3-5} However, the resolution of this "conventional" CGH was only 3-10 Mb⁴ comparable to high resolution karyotyping,¹ thus CGH was further adapted for use on microarrays.⁶ With array CGH, cloned [e.g. bacterial artificial chromosomes (BACs)] or synthesized [e.g. oligonucleotides (oligos)] DNA fragments representing precise chromosomal loci across the genome are immobilized on a glass surface.^{1,7-8} Copy number variations (CNV) are determined by the differences in hybridization pattern intensities between patient DNA and control DNA.⁷ Hybrid single nucleotide polymorphism (SNP)/oligo arrays have been developed for the analysis of CNVs and detection of copy number neutral regions of homozygosity. For these analyses, the patient DNA is labeled and hybridized to the microarray and the patient results are compared to a well-studied reference DNA.⁹ It should be noted that not all CNVs are pathologic, in that it has been demonstrated that the mean number of benign CNVs per person could be as high as 800 or more.¹⁰

The resolution and yield of an array is limited by the genomic coverage (the length of and spacing between probes) on the microarray,^{3,11-12} and by the specific statistical algorithms used to set the criteria for gains and losses. The increased resolution of microarray technology over conventional cytogenetic analysis allows for identification of chromosomal imbalances with greater precision, accuracy and technical sensitivity.

CLINICAL UTILITY

Rauch et al.¹³ investigated the diagnostic yield of various genetic tests utilized in the evaluation of patients with unexplained cognitive impairment. In their study, molecular karyotyping would only miss ~0.6% of cases with disease-causing balanced de novo aberrations. They noted that mole-

From the ¹Department of Pathology/Pediatrics, Stanford University School of Medicine, Stanford, CA.

Melanie Manning, MD, MS, FACMG, Department of Pathology/Pediatrics, Stanford University School of Medicine, 300 Pasteur Drive, H315, Stanford, CA 94305. E-mail: mmanning@stanford.edu.

cular karyotyping in every patient with cognitive impairment would have the highest diagnostic yield of any single test (28.9%) and was thus suggested by the authors to be considered a first tier test. Recently Miller et al.¹⁴ reviewed the evidence for utilization of CMA as a first tier test for the investigation of developmental delay/intellectual disability (DD/ID), multiple congenital anomalies, and/or autism spectrum disorders (ASD). These authors' recommendation for use of CMA as a first-tier test was based on studies of 21,698 patients referred for the above-listed indications, in whom the diagnostic yield was 12.2% higher than that of a G-banded karyotype. After a review of 36,325 patients with DD/ID, Hochstenbach et al.¹⁵ also recommended that CMA be a first tier test in this group of patients. In their group, a pathogenic anomaly was found in 19%. Shen et al.¹⁶ specifically investigated the utility of CMA in the investigation of children with ASD. They also provided evidence that analysis should be the first tier test for children with autism. They found that whereas abnormalities were found on karyotyping and fragile X testing 2.23% and 0.46% of the time respectively; a microarray identified deletions or duplications in 18.2% of patients (N = 848), with 7% of those clearly abnormal.

In addition to the linking of submicroscopic *de novo* deletions and duplications identified on CMA to the patients' disorders,¹⁷⁻¹⁸ *de novo* copy number variants (CNVs) have also been implicated in increasing the risk for ASD and point to regions of the genome that may house candidate genes for autism.¹⁹⁻²⁰

Additional uses for CMA include the investigation of individuals with physical or cognitive impairment but in whom conventional cytogenetic studies demonstrated an apparently balanced translocation. Subsequent CMA investigation demonstrated that the karyotype was actually unbalanced; several studies have shown that approximately 20% of individuals with an apparently balanced translocation (*de novo* or familial) have loss or gain of genetic material as identified by CMA.²¹⁻²² More recently, CMA has been shown to be valuable in uncovering chromosomal regions of medical importance apart from the original indication of the study. Adam et al.²³ reported three patients evaluated for developmental delay or dysmorphic features/multiple anomalies who were found by CMA to have microdeletions encompassing known tumor susceptibility genes. In a larger study, Adams et al.²⁴ found that 0.18% of patients with an identified gain or loss of genetic material had the inclusion of a gene associated with a cancer-predisposing condition. Both sets of authors emphasized that information gained from CMA, such as the unexpected finding of tumor susceptibility, can have a direct bearing on the future medical management of patients with developmental delay/multiple congenital anomalies in addition to providing an explanation of the general phenotype.

In addition to identifying copy number variants that affect the number of copies of a particular gene or genes, microarray analysis could theoretically identify genes that are disrupted by breakpoints in the genome. Disruption could be on the basis of interruption of a coding sequence or of a sequence that affects transcription and translation, e.g. sequence changes in a promoter region. Examples include duplications and deletions involving the NRXN1 and CNTN4

genes.²⁵⁻²⁶ Finally, as pointed out by Moeschler et al.²⁷⁻²⁸ and Saam et al.,²⁹ an accurate diagnosis for patients will provide the clinician the opportunity to discuss treatment options, prognosis, and recurrence risks as well as to avoid unnecessary future testing.

PLATFORMS

While microarray analysis is very proficient in characterizing chromosomal imbalances (which ultimately improves patient care),²⁹ clinicians ordering the test need to be aware of the different clinical platforms (e.g. BAC vs oligo, targeted vs whole genome, SNP), the variation in resolution among arrays and the information each provides. For example, many clinicians are unaware that a whole genome oligoarray can detect clinically significant copy number changes missed on a targeted BAC array³⁰ or that a SNP array can detect long contiguous stretches of homozygosity (LCSH) that can be associated with uniparental disomy or consanguinity, both of which increase the risk for autosomal recessive conditions.

Array resolution is dependent on the number and types of probes used and how they are distributed across the genome.³¹ BAC probes are larger than oligonucleotide probes used for oligo and SNP arrays (BACs are ~75,000-150,000 base pairs in length, whereas oligos are usually about 50-60 base pairs long). This translates into reduced breakpoint specificity of copy number abnormalities for the BAC arrays. Higher probe density on oligo arrays allows for copy number evaluation to be based on multiple adjacent probes, enhancing the accuracy of the interpretation. Oligonucleotide array construction tends to have better reproducibility and less batch-to-batch variation than does BAC construction.³¹

Single nucleotide polymorphism (SNP) microarrays are applications of microarray technology that also provide genome-wide copy number analysis. In addition to copy number changes, SNP arrays are able to detect so-called "copy number neutral" abnormalities such as segmental uniparental disomy and areas of LCSH that can give rise to disease, congenital anomalies, or cognitive impairment.³²⁻³³ SNP arrays are increasingly being used in the assessment of cognitive impairment or developmental delay, with or without associated anomalies and are likely to be employed in the diagnosis of these conditions.^{9,11}

When ordering a CMA, the clinician should be aware of the various platforms currently in use and their limitations. Questioning the laboratory performing the test about coverage of the array in specific regions of interest (e.g. telomeres, X chromosome, common microdeletions) is justified. The clinician also should understand what type of follow up tests will be performed, and on whom, in the event of abnormal results. Further, for deletions and duplications, parental studies (by FISH or metaphase preparations, if possible) should be conducted to rule out the presence of a chromosomal rearrangement such as an insertion or inherited duplication. Although rare, for a family in which such a rearrangement is found, recurrence risk can be as high as 50%. With increased utilization of a diagnostic test comes a better appreciation of the range of possible and sometimes unexpected results. This is certainly the case with aCGH and identification of what we now understand to be benign CNVs. An international con-

sortium of over 75 laboratories has been formed to address questions surrounding array-based testing. The International Standard Cytogenomic Array consortium (ISCA consortium, <https://isca.genetics.emory.edu/iscaBrowser/>) is investigating the feasibility of establishing a standardized, universal system of reporting and cataloging CGH results, both pathologic and benign, in order to provide the clinician with the most accurate and up-to-date information.¹⁴ Databases currently available for referencing gene location and function, CNV listings and up-to-date clinical information for specific abnormalities include the UC Santa Cruz Database (<http://www.genome.uscs.edu>), the Toronto Database of Genomic Variants (<http://projects.tcag.ca/variation/>), DECIPHER (<http://www.sanger.ac.uk/PostGenomics/decip>) and ECARUCA.³⁴

Even though CMA technology has greatly improved since it was initially described,⁴ clinicians ordering these tests must be aware of the limitations that remain. Array CGH cannot identify balanced chromosomal rearrangements such as translocations or inversions or differentiate free trisomies from unbalanced Robertsonian translocations.^{13,35-36} Some aneuploidies can be missed, such as XYY if the wrong gender control is used.³¹ Marker chromosomes may also be missed, depending on the size, marker composition and array coverage of the specific chromosomal region present on the marker.³⁵ Detection of mosaicism has been reported, but the accuracy of detecting low levels described by some groups³⁷ has been questioned by others.³⁵⁻³⁶ Recently, Scott et al.³⁸ suggested that mosaicism for an extra chromosome could be detected at the 10% level, whereas mosaicism for deletion or duplication of part of a chromosome could be detectable at the 20-30% level. These findings remain to be replicated by others. Interpretation of the significance of a rare copy number change can be incomplete if parental samples are unavailable for comparison and published data on the CAN are lacking. Finally, triploidy will not be detected by some forms of microarray.

A microarray should not be ordered when a rapid turnaround time is needed (a STAT newborn analysis, for example), especially if a chromosomal trisomy is suspected. Currently, a STAT G-banded chromosome analysis can be performed within 48 hours. With some aCGH platforms, hybridization alone can take 48 hours. While technically some arrays may be run in 3-5 days in some laboratories, analysis and confirmation of results with FISH (development of a unique probe can take weeks) and analysis of parental samples and interpretation may take much longer.

Although microarray is a powerful diagnostic tool for the evaluation of chromosomal copy number changes, its use as a first tier test may not always be appropriate. For example, conventional karyotyping may be more appropriate when a common aneuploidy (e.g., trisomy 21, trisomy 18, or a sex chromosome aneuploidy) is suspected. FISH with a single probe to confirm a suspected diagnosis of a well-described syndrome, such as Williams syndrome, would be a more cost-effective testing methodology. CMA also should not be used in cases of family history of chromosome rearrangement in a phenotypically normal individual or in cases of multiple

miscarriages.¹⁴ Finally, CMA cannot detect low-level mosaicism or, in some arrays, polyploidy.

Recommendations:

1. Cytogenetic microarray (CMA) testing for copy number variation (CNV) is recommended as a first-line test in the initial postnatal evaluation of individuals with the following:
 - A. Multiple anomalies not specific to a well-delineated genetic syndrome
 - B. Apparently non-syndromic developmental delay/intellectual disability
 - C. Autism spectrum disorders
2. Further determination of the use of CMA testing for the evaluation of the child with growth retardation, speech delay, and other less-well studied indications is recommended, particularly via prospective studies and after-market analysis.
3. Appropriate follow up is recommended in cases of chromosome imbalance identified by CMA, to include cytogenetic/FISH studies of the patient, parental evaluation, and clinical genetic evaluation and counseling.

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