CCMG Position Statement:
Use of array genomic hybridization technology in constitutional genetic diagnosis in Canada

Approved by the CCMG Board of Directors September 22, 2009.

Purpose: The aim of this statement is to provide recommendations for Canadian genetic counsellors, medical geneticists, pediatricians and other physicians regarding the use of array genomic hybridization technology in constitutional genetic diagnosis.

Methods of Statement Development: Members of the Canadian College of Medical Geneticists (CCMG) Clinical Practice and Cytogenetics committees reviewed the relevant literature on the use of microarray genomic hybridization in constitutional genetic diagnosis. Input was also sought from the CCMG Prenatal Diagnosis committee. Guidelines were developed for health care providers in Canada. The guidelines were circulated for comment to the CCMG members-at-large and, following appropriate modification, approved by the CCMG board of directors.

Introduction and Literature Review: The search for the etiology of unexplained developmental delay, congenital anomalies, dysmorphism and other features suggestive of a genetic cause traditionally has involved light and fluorescence microscopy chromosomal investigations. The ‘gold’ standard 550 G-band karyotype is limited to a resolution of 5-10Mb (1) and detects abnormalities in 5-10% of cases, depending on the ascertainment criteria. Locus-specific FISH (fluorescence in situ hybridization) detects subtelomeric and interstitial submicroscopic chromosomal rearrangements (usually 3-5 Mb in size) associated with particular phenotypes in an additional 3-6% of patients (2). Array genomic hybridization reveals chromosomal imbalances across the entire genome. New developments in array genomic hybridization have demonstrated its greater sensitivity over traditional microscopic chromosome and locus-specific FISH tests for the identification of unbalanced chromosome anomalies of 1 Mb or smaller (3). Array resolution is dependent on the distance between probes across the genome and is limited by the size of the probes used i.e. bacterial artificial chromosomes (BACs) versus synthetic oligonucleotides. As a result, the effective resolution between various platforms can vary significantly. Array genomic hybridization analysis of phenotypically normal individuals demonstrated that copy number variants (CNVs) occur at many loci throughout the human genome (4-7). The Database of Genomic Variants maintains a summary of copy number variation in the human genome that has now been identified in dozens of research studies (see http://projects.tcag.ca/variation/ and references therein). In patients, CNVs may thus represent pathogenic genomic imbalances or benign copy number alterations. In addition, some CNVs have been shown to contribute to complex traits. It is imperative to distinguish between pathogenic and benign copy number variants by incorporating family studies, gene content, and data from CNV databases and current literature.

In 2005, the American College of Medical Genetics (ACMG) published its guidelines for the cytogenetic evaluation of the individual with developmental delay (DD) and mental retardation.
(MR) (8). The guidelines recommend routine chromosome analysis (minimum 550 band resolution) for investigation of MR/DD. They do not recommend higher resolution (> 850 G-band) chromosome analysis in a diagnostic laboratory setting. FISH or other molecular techniques are recommended when a specific syndrome associated with microdeletion or microduplication is suspected. Subtelomeric FISH studies may be considered if routine chromosome analysis yields normal results. The 2007 ACMG practice guidelines for the use of array-based technologies (9) recommend that array genomic hybridization be used as an adjunct to standard chromosome testing including targeted FISH. The use of array genomic hybridization as a first-line test in prenatal diagnosis is specifically not recommended.

Multiple reviews on the topic of array genomic hybridization have been published (9-24). Advantages over-and-above increased detection of unbalanced anomalies with greater resolution by array genomic hybridization are: the equivalent of hundreds or thousands of FISH tests can be performed simultaneously and, therefore, there is less reliance on the clinician’s suspicion of a specific diagnosis; array genomic hybridization does not require dividing cells and therefore studies can be performed on post-mortem samples (3). The disadvantages of array genomic hybridization include: balanced chromosome rearrangements such as translocations or inversions cannot be identified; triploidy can only be detected using a SNP-based platform; and the location or orientation of a chromosome segment duplication cannot be established. Conventional cytogenetics or FISH are often required as follow-up studies to abnormal array genomic hybridization results. Furthermore, the interpretation and follow-up of CNVs is labour-intensive and requires well-developed data management strategies and resources.

There are two main formats or platforms for array genomic hybridization: 1) targeted and 2) whole genome array (3). Targeted arrays use DNA probes corresponding to regions of predefined clinical significance, but gaps exist for genomic regions that do not include previously identified clinically relevant loci. The whole genome array uses probes equally spaced over the entire genome. However, these arrays may not have adequate coverage for clinically relevant regions such as telomeres, centromeres, and syndromes associated with microdeletion or microduplication. Although the resolution with the latter technique is much greater across the genome, it reveals more copy number variations (CNVs) of unknown clinical significance.

Early CGH arrays were made up of clone inserts of bacterial artificial chromosomes or BACs (~150 Kb). BAC arrays have a limited resolution, albeit higher than standard G-band analysis. Copy number changes that extend beyond the genomic boundary of the BAC cannot accurately be determined. BACs may be inaccurately mapped in existing libraries and updates to a BAC array require much validation. An alternative to the use of BAC clones was the development of synthetic oligonucleotides for genome wide copy number assessments. Oligonucleotide arrays are based on the reference human genome sequence, and thus allow for any sequence of interest to be a potential target. Oligonucleotide arrays provide a greater density of coverage, thereby increasing the resolution of detection. However, they also reveal significant numbers of uncharacterized CNVs that challenge clinical correlation and interpretation.

A third array design is a modified whole genome array that combines increased targeted enhancement for clinically relevant regions, with genome-wide chromosomal backbone coverage. These arrays can be BAC or oligonucleotide based. It has been proposed that an
additional 3-5% of clinically significant imbalances will be identified by an oligonucleotide array with this design, as compared to the targeted BAC array platform which has a 5 to 10% abnormality rate in patients with a normal karyotype by standard G-band analysis (2, 26, 27).

In addition, a fourth microarray format has been developed in which an oligonucleotide array incorporates dense, single nucleotide polymorphisms (SNPs) in conjunction with DNA copy number changes. This type of array provides genotype information in addition to copy number, thus enabling testing for loss of heterozygosity (LOH), uniparental disomy (UPD), paternity, parent-of-origin, homozygosity mapping and consanguinity.

**General Recommendations:**

1) The field of array CGH technology is evolving rapidly and new platforms are likely to be developed in the future. At present, there are insufficient data to recommend a specific platform for array genomic hybridization. Higher resolution arrays detect more abnormalities but also identify more CNVs of uncertain significance. It is recommended that the appropriate CCMG committees provide guidance to establish a minimum standard for the coverage of specific clinically relevant regions, and a minimum resolution for whole genome coverage in specific patient populations i.e. postnatal versus prenatal.

2) Except in cases where the finding is a recognized syndrome caused by microdeletion or microduplication that is concordant with clinical presentation, all positive array genomic hybridization results should be confirmed by appropriate methods. Standard cytogenetic studies may be required to rule out a chromosome rearrangement such as a translocation. FISH analysis may be helpful in delineating the structure of a chromosomal imbalance detected by array CGH.

3) Parental studies may be required to determine if a deletion or duplication is de novo and/or to rule out a parental balanced rearrangement as a cause of a deletion/duplication in the proband. Furthermore, family studies may be required to provide supportive evidence for the association between an abnormal array genomic hybridization finding and the clinical presentation. Exceptions to the practice of performing parental studies include (but are not limited to) well-described syndromes resulting from microdeletion or microduplication that are not known to be inherited such as Williams syndrome and Prader-Willi syndrome.

4) The establishment and validation of array genomic hybridization techniques and the definition of reporting standards, including questions of resolution, sensitivity, specificity, turn around time and independent confirmation of test results are the responsibility of the laboratories performing the assays. This should be guided by laboratory norms developed by the appropriate CCMG committees and approved by the College. These committees will also address issues relating to the ethics of disclosing information concerning predictive information for adult-onset conditions.

5) The CCMG recognizes that there will be a need for most centres to phase-in array genomic hybridization as the replacement for traditional chromosome testing as the first-line laboratory investigation for patients that meet specific criteria (see the sections that follow).
This transition will require the allocation of resources for training and purchase of new equipment for the establishment of this new technology.

6) The CCMG strongly urges the collaboration of clinicians and laboratory directors in establishing and supporting readily accessible anonymized databases for clinical correlates with laboratory findings. These will be of importance for understanding the genotype/phenotype correlation of array genomic hybridization results. In this regard, it will be important to standardize the collection of clinical and pedigree information submitted when requesting array genomic hybridization (to be developed by the CCMG Clinical Practice Committee).

7) Array genomic hybridization testing involves complex counseling issues including difficult interpretations due to CNVs, carries the potential of discovering non-paternity with parental testing, may require further parental studies when a deletion/duplication is detected and may not provide information in cases with single gene disorders or other syndromes. There is also potential to identify deletions or duplications associated with adult onset disorders. All clinicians ordering array-based tests should fully understand the limitations and potential pitfalls of this type of testing and ensure that their test subject or parent/guardian thereof is also made aware of these to allow them to give appropriate informed consent.

**Recommendations for testing postnatal samples:**

Analysis should be performed on array designs that cover known clinically relevant loci as well as some form of whole genome coverage. A minimum resolution of whole genome coverage for postnatal samples should be recommended by the appropriate CCMG committees. This will prevent multiple testing and better utilize limited resources.

1) Array genomic hybridization should be the first line laboratory investigation for the patient whose DD/MR, autism, multiple congenital anomalies or dysmorphic features is unexplained after a thorough history and physical examination. Array genomic hybridization could also be used for the study of post-mortem samples, including stillbirths with abnormalities as described above, if future literature supports its usefulness in this regard.

2) Chromosome studies and FISH tests are not routinely required for the investigation of the above mentioned patients who have normal array genomic hybridization studies using a platform that includes whole genome coverage. It follows that array genomic hybridization replaces the previous testing modalities of chromosome analysis by G-banding and FISH for targeted microdeletions or subtelomeric rearrangements.

3) For patients with developmental delay and/or dysmorphisms of suspected chromosomal etiology, array genomic hybridization should be the first line investigation, with the possible exception of those suspected to have a common standard aneuploidy or triploidy. Array genomic hybridization is not recommended for the investigation of the child or adult suspected of having either Down syndrome, trisomy 13, trisomy 18, Turner syndrome, Klinefelter syndrome, XXX or XYY, since, in many cases, confirmatory chromosome
studies would be required if the diagnosis were made with array genomic hybridization. This is especially true for Down syndrome and trisomy 13, which can be associated with Robertsonian translocations not detectable by array genomic hybridization. Therefore, if the clinician has a strong suspicion of one of these conditions, it may be more economical to request chromosome studies first.

4) Array genomic hybridization is not an appropriate investigation for couples experiencing repeated pregnancy losses, infertility, oligospermia or azoospermia, since balanced translocations or inversions are frequent causes of these conditions (28),

5) Array genomic hybridization should not be used for the investigation of suspected triploidy unless a SNP-based array is used.

6) When there is a strong clinical suspicion of a disorder known to be caused by copy number variations in single genes, such as Duchenne muscular dystrophy, where less expensive targeted molecular genetics testing is available, array genomic hybridization should not be used as a first line of investigation.

Recommendations for testing prenatal samples:

1) Array genomic hybridization is not recommended in pregnancies at low risk for a structural chromosomal abnormality e.g. advanced maternal age, positive maternal serum screen, previous trisomy, or the presence of “soft markers” on fetal ultrasound. Since the vast majority of fetuses in these situations are clinically unaffected, the positive predictive value of a detected CNV would likely be low.

2) Array genomic hybridization may be an appropriate investigative measure in cases with fetal structural abnormalities detected on ultrasound or fetal MRI. Array genomic hybridization could be done in lieu of a karyotype if interphase FISH or qPCR fail to diagnose a chromosomal aneuploidy or triploidy (which is not detectable with some array technology), provided the turn around time for results is comparable or less than karyotyping.

3) Any prenatal patient that qualifies for microarray genomic hybridization testing should be seen in consultation by a medical geneticist prior to testing so that the benefits, limitations and possible outcomes of the analysis can be discussed in detail with the patient. The difficulties of interpretation of some CNVs should also be discussed. This will allow patients to make an informed decision as to whether or not they wish to pursue such prenatal testing.
References:


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