Frequently Asked Questions: Chromosome Microarray Analysis (CMA)

Chromosome microarray is a high-resolution cytogenetic test that, for most indications, has replaced karyotype ("chromosome analysis") for the assessment of losses (deletions) and gains (duplications) of DNA.

The Division of Genome Diagnostics (DGD) uses a microarray platform that assesses single nucleotide polymorphisms using SNP-probes. This has important consequences for the types of changes that may be detected (see below)[1].

What can CMA detect?

- **Large copy number gains or losses, termed copy number variants** (**CNVs**). These are gains or losses of a portion of the chromosome that exceed a minimum threshold of size and probe coverage. CNVs are classified according to supporting information from clinical databases and scientific literature. CNVs may be classified as pathogenic, likely pathogenic or unclear clinical significance. Variants that are considered benign or likely benign are not reported. CNV interpretation relies on accurate clinical information provided on the requisition to help clarify the significance of the results and minimize the likelihood of receiving variants of uncertain clinical significance. *It is important to know that variant classification may change over time as scientific literature develops.*
- **Absence of heterozygosity (AOH).** CMA analyses that include SNP-probes are capable of detecting long stretches of homozygosity across the genome, which may be indicative of uniparental disomy (UPD) or identity by descent. Large AOH restricted to a single chromosome may be suggestive of UPD. Results in keeping with UPD are reported for chromosomes 6, 7, 11, 14, 15, as UPD of these chromosomes may be clinically significant. Suspected UPD requires confirmation by molecular genetic testing (see www.genebc.ca). AOH suggestive of identity by descent or parental consanguinity is not generally reported.
- **Incidental findings**. These can include copy number changes associated with previously unknown adult onset conditions or cancer predisposition. Thus, thorough pre-test counselling is recommended.
- **Chromosomal aneuploidy*.** Gain or loss of entire chromosomes.
  ***CMA is not the appropriate test to assess for common aneuploidy such as Down syndrome; see below.**

Are there any limitations to what CMA can detect?

Yes; CMA cannot detect:

- **Balanced structural rearrangements.** These include balanced reciprocal translocations or inversions. Balanced rearrangements can be detected by karyotype analysis.
- **Exon-level CNVs (deletions or duplications).** CMA is designed to detect large CNVs and is, thus, limited by probe distribution over genomic regions. It is not designed to confidently detect small CNVs, including small exon level deletions or duplications, which are detected using other methods (see www.genebc.ca for details).
- **Single nucleotide changes**. CMA is designed to assess for copy number variation, it is not designed to assess for other types of DNA variation. Single nucleotide changes or repeat disorders (eg. Fragile X), are detected using other methods. See www.genebc.ca for details.
- **Low levels of mosaicism.** Chromosomal abnormalities that are present in a small percentage of cells may not be detected by CMA.
- **All instances of UPD.** CMA is not a diagnostic assay for UPD.

What are the clinical indications for postnatal CMA?

- Unexplained intellectual disability, global developmental delay or autism spectrum disorders (ASD)[1-4].
  OR
- Multiple congenital anomalies[2,3]. A detailed description of the anomalies must be included on page 2 of the requisition, as this will aid in interpretation of the results.
  OR
- Individuals with global developmental delay/intellectual disability/multiple congenital anomalies where karyotype has previously been performed and was apparently balanced[1]. CMA can detect sub-microscopic deletions or duplications that are below the resolution of karyotype analysis.

What conditions are not clinically indicated for CMA?

- Suspected common chromosomal aneuploidy: Trisomy 13 (Patau syndrome), trisomy 18 (Edwards syndrome) and trisomy 21 (Down syndrome)[3]. Karyotype should be requested for confirmation of diagnosis.
- Suspected sex chromosome aneuploidy: Turner syndrome, Klinefelter syndrome, etc. Karyotype should be requested for confirmation of diagnosis.
- Suspected UPD disorder. Molecular testing should be performed to confirm UPD. See www.genebc.ca for more information.
- Single gene or intragenic deletion or duplication disorder. CMA may not have sufficient probe coverage to detect deletion or duplication of the gene of interest. Therefore, alternative approaches such as multiplex ligand probe amplification (MLPA) should be used.
- Delays in a single developmental domain (eg. ADHD) or isolated forms of learning disability. The utility of using CMA for these indications has not been well established[5].

Will additional testing be required if an abnormality is detected by CMA?

- Parental testing may be required to determine if a CNV is inherited. This will require a blood draw for the parents. Depending on the size of the abnormality, follow-up testing may be performed by CMA or by fluorescence in situ hybridization (FISH). Follow-up by CMA will only require a blood sample from the parents; however, when FISH testing is required, a second blood draw will also be required for the proband. Instructions will be provided on the clinical report.
- If follow-up is requested on the patient's CMA clinical report, please provide the requested blood samples, select "CMA Follow-up" on the Genome Diagnostics requisition and provide all relevant information (i.e. Proband Sample ID #, relationship to proband).
- If an abnormality detected on CMA is suggestive of a structural chromosomal rearrangement, this may require additional analysis by karyotype for the proband and/or parents. An additional blood draw for the proband will be necessary. This information will be provided on the clinical report.
- Alternative molecular methods may be required to confirm suspected abnormalities detected by CMA. This includes, but is not limited to, molecular genetic testing for UPD and methylation analysis if abnormalities encompass known imprinted regions (eg. Prader-Willi/Angelman syndrome region).
- Carrier status for autosomal recessive conditions is not typically reported unless the patient's phenotype is consistent with the disorder and / or the carrier frequency in the population is sufficiently high. In these cases, additional molecular testing may be warranted.

Sources:

(1) CCMG Guidelines for Genomic Microarray Testing, CCMG Laboratory Practice Committee (2016)
(2) Miller *et al* 2010. Consensus statement: chromosomal microarray is a first-tier clinical diagnostic test for individuals with developmental disabilities or congenital anomalies. PMID: 20466091
(3) Manning *et al* 2010. Array-based technology and recommendations for utilization in medical genetics practice for detection of chromosomal abnormalities. PMID: 20962661
(4) Bélanger and Caron 2018. Evaluation of the child with global developmental delay and intellectual disability. PMID: 30919832
(5) Beaudet 2013. The utility of chromosomal microarray analysis in developmental and behavioral pediatrics. PMID: 23311723