AMPATHCHAT no. 99

CHROMOSOMAL MICROARRAY ANALYSIS IN PRENATAL DIAGNOSTICS

Now offered by Ampath Genetics

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BACKGROUND

Traditional karyotyping has been the cornerstone of prenatal cytogenetic analysis for decades. However, karyotyping is limited by its resolution, detecting only large chromosomal abnormalities (>5–10 Mb).[1]

Chromosomal microarray (CMA), introduced in the early 2000s, revolutionized cytogenetics by enabling high-resolution, genome-wide analysis of copy number variations (CNVs). In addition to the numerical abnormalities detected by karyotyping, CMA can detect sub-microscopic deletions and duplications that are not visible by standard karyotyping, offering significantly improved diagnostic yields.[1]

In the last decade, guidelines from the American College of Obstetricians and Gynecologists (ACOG), the Society for Maternal-Fetal Medicine (SMFM) and the Royal College of Pathologists have all endorsed CMA as a first-tier test in prenatal diagnosis for certain clinical indications.[2,3]

INDICATIONS FOR PRENATAL CMA

Prenatal CMA is recommended, and can replace standard karyotyping, in the following scenarios:

- One or more foetal structural anomalies detected on ultrasound (e.g. cardiac, central nervous system (CNS), skeletal)
- Increased nuchal translucency or other soft markers suggestive of aneuploidy
- Unexplained intrauterine growth restriction (IUGR)
- Normal karyotype with persistent suspicion of a genetic condition
- Family history of chromosomal rearrangements
- Intrauterine foetal death / still born (where a common aneuploidy or acquired cause is excluded or not suspected)[2]

ARRAY COMPARATIVE GENOMIC HYBRIDIZATION (Array CGH)

Array CGH is one of two platforms for CMA, the other being a single-nucleotide polymorphism (SNP) array, which is not currently offered in South Africa.[4]

LABORATORY METHOD

Array CGH utilises DNA extracted from a foetal sample (usually amniotic fluid (AF) or a chorionic villus sample (CVS)), which is combined with DNA from a normal reference, and hybridised to a slide containing DNA probes. The relative intensities of the foetal and reference DNA, which are labelled with two different fluorescent dyes, are then compared to localise deletions/duplications of foetal DNA across the genome (Figure 1).[5] The slide design utilised at Ampath (Agilent GenetiSure 60K CGH) contains approximately 60 000 high quality oligonucleotide probes spread across the genome, with a median spacing of about 50 kilobases (kb), and increased density in clinically relevant areas.[6]

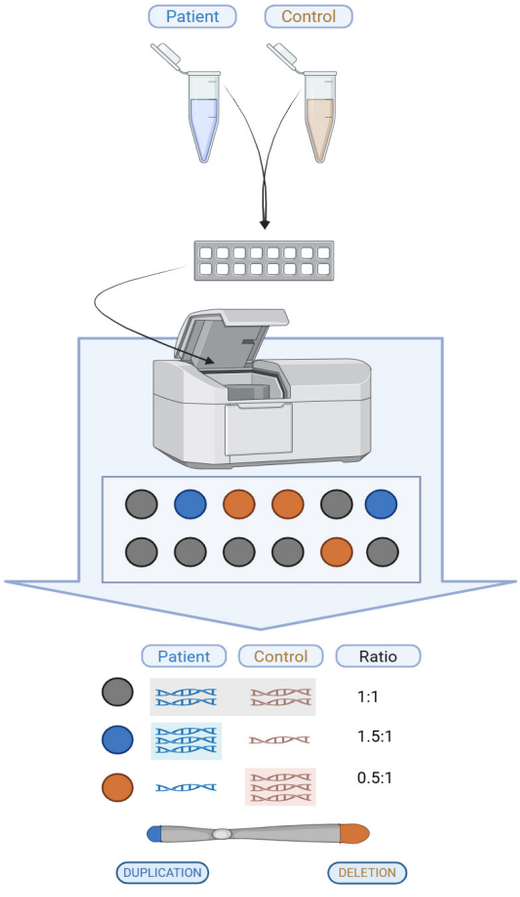

FIGURE 1: ARRAY CGH LABORATORY METHOD*

ANALYSIS AND REPORTING

Copy number variations (CNVs) are identified using Agilent's Cytogenomics software. The CNVs identified by the CMA are compared to databases of known genetic variation and classified according to American College of Medical Genetics and Genomics (ACMG) and ClinGen standards (Figure 2).[7]

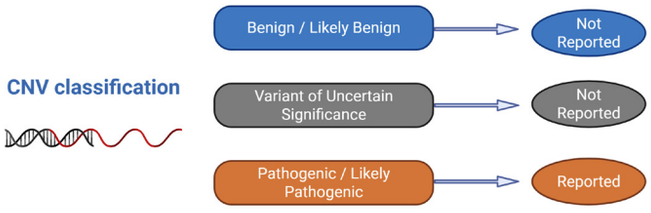

FIGURE 2: CNV CLASSIFICATION AND REPORTING*

All pathogenic/likely pathogenic variants that correlate with the phenotype are reported, whilst benign/likely benign variants are not reported. Incidental or unsolicited pathogenic variants without a direct relationship to the indication for testing are not reported, unless they could potentially inform the management of the pregnancy or family. However, CNVs that are not linked to potential phenotypes for the pregnancy, have low penetrance, or have no actionable consequence, would not be reported.[3]

Classification, interpretation, and reporting in the context of the clinical presentation, is performed by a team of experienced medical scientists, a medical geneticist and genetic counsellors, and often involves direct discussion with the referring clinician. Ampath Genetics has been performing array CGH testing in the postnatal setting for more than eight years and draws on this experience in the prenatal context.

DIAGNOSTIC YIELD

Numerous large-scale studies have evaluated the incremental yield of CMA in foetuses with ultrasound anomalies. A meta-analysis published in 2013 identified an increased diagnostic yield of 7–10% over karyotype in pregnancies with structural foetal abnormalities.[8] Ultrasound anomalies observed in multiple organ systems (excluding nuchal abnormalities), increase the frequency of relevant CNVs by 13.6%.[1] Overall, the literature shows that CMA will provide additional information over karyotype in about 6–7% of pregnancies when an anomaly is identified on foetal ultrasound[1], with ACOG recommending CMA as the first-tier test in the diagnostic evaluation of foetal structural anomalies.[2]

CMA VERSUS KARYOTYPING

While karyotyping can detect numerical and large structural abnormalities, CMA's resolution allows detection of sub-microscopic deletions and duplications (Table 1), leading to the increased diagnostic yields. CMA does not require dividing cells (unlike karyotype, which can only be performed after cell culture). However, a back-up culture may be required for CMA in cases where the sample is contaminated by maternal cells (MCC) or is of small volume.

TABLE 1: A COMPARISON OF CHROMOSOME ABNORMALITIES DETECTED BY METHOD

	Array CGH	Karyotype
Aneuploidy	+	+
Sub-microscopic CNVs	+	-
Unbalanced translocations	+	+/-
Balanced rearrangements	-	+
Triploidy	-	+

LIMITATIONS OF CMA TESTING

CMA is unable to detect chromosomal rearrangements where there is no nett loss/gain of genetic material (e.g. balanced translocations or inversions). The vast majority of balanced rearrangements result in a normal outcome. CMA cannot provide direct evidence of genetic mechanism (for example, CMA cannot distinguish between trisomy 13 due to a non-disjunction versus a translocation). CMA may also not detect low-level mosaicism and array CGH cannot detect triploidy or loss of heterozygosity (such as uniparental disomy).[8] CMA cannot detect imbalances below the resolution of the platform used or point mutations in single gene disorders.

COUNSELLING AND CONSENT

Providing patients with CMA related information (and genetic counselling, if possible) is essential prior to testing. Patients should be informed about:

- The test scope and limitations
- The possibility of reportable incidental findings (such as high penetrance neuro-susceptibility loci)
- The potential need for parental testing to aid interpretation of results

Informed written consent is required prior to testing. A dedicated request form will be provided with relevant information and consent sections to be completed by the referring clinician and patient respectively.

CONCLUSION

CMA is a high-resolution technique for detection of chromosomal abnormalities and is considered the first-tier genetic investigation for certain foetal indications. Rapid PCR testing/karyotype remains indicated when a common aneuploidy is suspected. Close collaboration between the referring specialist and laboratory team is essential. Genetic counselling (pre-and/or post testing) is available on referral from the clinician.

For further information or referrals, please contact Ampath Genetics on 012 678 0645 or geneticsclinic@ampath.co.za.

KEY INFORMATION

Test mnemonic	**ACGHPREN**
Sample Requirements	1. CVS or 15-20ml amniotic fluid or 3ml cord blood 2. Maternal EDTA blood to exclude maternal cell contamination
Documentation	1. Completed prenatal genetic request form 2. Signed patient consent
Turnaround time	Two weeks, provided sample is adequate for direct analysis (i.e. no culture is required)

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*Figures 1 and 2 were created using www.biorender.com.

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