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Prenatal diagnosis by chromosomal microarray analysis

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Chromosomal microarray analysis (CMA) is performed either by array comparative genomic hybridization or by using a single nucleotide polymorphism array. In the prenatal setting, CMA is on par with traditional karyotyping for detection of major chromosomal imbalances such as aneuploidy and unbalanced rearrangements. CMA offers additional diagnostic benefits by revealing sub-microscopic imbalances or copy number variations that are too small to be seen on a standard G-banded chromosome preparation. These submicroscopic imbalances are also referred to as microdeletions and microduplications, particularly when they include specific genomic regions that are associated with clinical sequelae. Not all microdeletions/duplications are associated with adverse clinical phenotypes and in many cases, their presence is benign. In other cases, they are associated with a spectrum of clinical phenotypes that may range from benign to severe, while in some situations, the clinical significance may simply be unknown. These scenarios present a challenge for prenatal diagnosis, and genetic counseling prior to prenatal CMA greatly facilitates delivery of complex results. In prenatal diagnostic samples with a normal karyotype, chromosomal microarray will diagnose a clinically significant subchromosomal deletion or duplication in approximately 1% of structurally normal pregnancies and 6% with a structural anomaly. Pre-test counseling is also necessary to distinguish the primary differences between the benefits, limitations and diagnostic scope of CMA versus the powerful but limited screening nature of non-invasive prenatal diagnosis using cell-free fetal DNA. (Fertil Steril® 2018;109:201–12. ©2017 by American Society for Reproductive Medicine.)

Key Words: Chromosomal microarray, prenatal diagnosis, microdeletion, microduplication, VOUS

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Prenatal diagnosis of chromosome abnormalities has been offered since the mid-1960s (1). For the bulk of the past 50 years, cytogenetic testing of the fetus has been accomplished by standard G-banded karyotyping. The diagnostic yield using conventional cytogenetic analysis by karyotype is dependent on the indication. For the most common indications such as advanced maternal age and positive biochemical screening, the diagnostic yield at the time of chorionic villus sampling (CVS) and amniocentesis is approximately 6% and 3%, respectively (data from additional analysis of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development [NICHD] microarray data set) (2). For fetuses with structural anomalies, the diagnostic yield is approximately 49% in the first trimester and 17% in the second (data from additional analysis of the NICHD microarray data set) (2).

The advent of newer molecular cytogenomic technologies such as chromosomal microarray analysis (CMA) brought about the prospect of greater diagnostic resolution. CMA, which detects imbalances in the kilobase range, readily demonstrates its superiority over standard karyotyping which is limited to imbalances greater than 7–10 million bases. In postnatal studies of children with congenital abnormalities, developmental delay or intellectual disability, CMA will have an additional diagnostic yield of clinically relevant sub-chromosomal abnormalities of about 12% to 15% (3, 4). In 2013, Wapner and colleagues (2) published a large multicenter NICHD sponsored study that demonstrated the clinical utility of CMA in prenatal diagnosis. The prospective cohort study demonstrated that in pregnancies with fetal structural anomalies and a normal karyotype there was an incremental diagnostic yield of about 6% above what a karyotype would detect. For all other indications this was about 1.7% (2).

CMA works by detecting imbalances in DNA copy number. These imbalances are referred to as copy number variants (CNVs), which in and of itself, does not imply an abnormal or pathogenic phenotype. In fact, a significant number of CNVs are clinically insignificant and are found in apparently normal individuals (5–9). The majority of these "benign" CNVs are very small in size (<50 Kb) and do not have clinically significant coding

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regions (5–9). CNVs are often referred to as microdeletions (sub-microscopic losses) or microduplications (sub-microscopic gains) and are undetectable by conventional karyotype. The medical relevance of CNVs relates to the functional impact of the micro-deletion/duplication which is more likely to have a phenotypic effect when the region of imbalance occurs in critical gene/s or an important regulatory region.

CMA TECHNIQUES

There are two CMA techniques used in identifying submicroscopic imbalances: comparative genomic hybridization (CGH) and single nucleotide polymorphisms (SNP).

CGH based arrays (aCGH) compare a patient's DNA to a normal control DNA sample to identify areas that are either over- or under-represented in the patient sample (10). In the aCGH approach, the patient and control DNA samples are cut into fragments then labeled with different fluorescent colors (usually green and red). They are mixed together in equal proportions and placed onto an array (glass slide) containing multiple probes from representative sequences from across the human genome. The DNA mixture binds (hybridizes) in a competitive manner to complimentary sequences located within the probe DNA on the array. The fluorescence intensity of every probe is measured using digital imaging software. After a normalization process, a ratio of the fluorescence intensities between the patient and the control sample is calculated. A ratio of one indicates equal contributions from the patient and control sample which in turn represents a normal copy number at that locus. A ratio that is significantly greater than one indicates that more of the patient's DNA hybridized at a particular location compared to the control DNA. This represents a gain of patient chromosomal material (a duplication or trisomy). Conversely, a loss of genetic material (a deletion or monosomy) in the patient would yield a ratio that is significantly less than 1 due to more hybridization of the control DNA sequences compared to patient's DNA. The location and size of the gain/loss can be determined by the number of consecutive probes that show a ratio above or below one. A typical clinical CGH array contains a few hundred thousand probes while the number of probes on research CGH arrays may reach into the millions. The resolution and diagnostic capability of aCGH depends on the number and types of probes used and their distribution across the entire genome (11). Most clinical laboratories performing aCGH will report clinically significant imbalances in the range of 50–100 Kb in postnatal studies. The reporting size range is usually larger in prenatal studies and may vary according to the indication for testing.

SNP microarray analysis (SOMA) uses high-density oligonucleotide-based arrays in which target probes are chosen from DNA locations known to vary between individuals by a single base pair (i.e. SNPs) (12). In the SOMA approach, only the patient's DNA (fetal) is labelled and hybridized to the SNP array. Copy number changes are determined by measuring the absolute fluorescence probe intensities of the patient sample compared with the intensities of multiple normal controls that were independently hybridized (in silico comparison) (Fig. 1). Most SNP arrays used in a clinical setting are in fact hybrid arrays that contain both SNP probes and copy number probes. The density of probes on some of these hybrid arrays may be as high as 2.7 million probes. Clinical laboratories performing SOMA usually report CNVs of known clinical significance in the range of 50–100 Kb and higher. In addition to detecting CNVs, other clinically useful information may be extracted from the genotype plots generated from the SNPs. This includes uniparental disomy (UPD), mosaicism (Fig. 2), zygosity, maternal cell contamination, parent of origin and consanguinity. Lastly, triploidy which cannot be detected by aCGH, can easily be identified by SOMA by assessing the SNP allele patterns on the array (Fig. 3) (13, 14).

THE DIAGNOSTIC YIELD OF CMA AND RATIONAL FOR ITS UTILIZATION OVER STANDARD KARYOTYPE

CMA in Fetuses without Ultrasound Anomalies

The biggest advantage to using CMA over classic cytogenetic and fluorescence in situ hybridization (FISH) techniques for prenatal genetic diagnosis of chromosomal abnormalities lies with CMA's ability to detect much smaller imbalances. Typical karyotype analysis by G-banding may be able to delineate deletions and duplications that are 5–10 Mb in size (15). However, given the variation in banding resolution from one prenatal preparation to the next, 10–20 Mb and greater is a more realistic threshold of detection for conventional karyotype analysis. Standard FISH probes for microdeletion/duplication syndromes are usually around 100–200 Kb in size and require clinical features to guide probe selection, a challenging task for prenatal samples. It is possible to multiplex FISH probes but the limited number of spectrally unique commercial fluorophores that can simultaneously be used to interrogate multiple diseases is limited to a handful. To increase the diagnostic yield, one could perform sequential testing of many FISH probes but this is inefficient, time-consuming and very expensive. CMA offers the benefit of detecting submicroscopic imbalances (<5 Mb) anywhere in the genome in a single test and its resolution is only limited by the probes present on the chip. CMA is 100% accurate in identifying the common aneuploidies in prenatal specimens compared to karyotype (2, 16, 17) and in the NICHD study, it demonstrated an increased diagnostic yield over standard karyotyping of 1.7% in patients referred for advanced maternal, parental anxiety and positive serum screening (2). A recent meta-analysis assessing CMA on 10,614 fetuses from 10 large studies found a pathogenic, clinically significant CNV in 0.84% (1:119) of cases referred for AMA and parental anxiety (18).

Another recent meta-analysis evaluated the onset/penetrance of genomic disorders diagnosed by CMA. 10,314 fetuses from 8 large studies showed that CNVs associated with early onset syndromic disorders occurred in 1:270 (0.37%) pregnancies (18). Approximately 1:909 (0.11%) cases involved late onset diseases and a susceptibility CNV was observed in 1:333 (0.3%) cases (18). By adding the individual risk for pathogenic CNVs to the individual risk for cytogenetically visible chromosome aberrations, Srebniak

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