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The Use of Chromosomal Microarray Analysis in Prenatal Diagnosis

ABSTRACT: Chromosomal microarray analysis is a technique that identifies chromosomal abnormalities, including submicroscopic abnormalities that are too small to be detected by conventional karyotyping. Like conventional fetal karyotyping, prenatal chromosomal microarray analysis requires direct testing of fetal tissue and thus can be offered only with chorionic villus sampling or amniocentesis. Based on the results of a *Eunice Kennedy Shriver* National Institute of Child Health and Human Development multicenter trial and of prior studies, prenatal chromosomal microarray analysis is most beneficial when ultrasonographic examination identifies fetal structural anomalies. The potential for complex results and detection of clinically uncertain findings identified by prenatal chromosomal microarray testing can result in substantial patient anxiety. This underscores the critical need for comprehensive patient pretest and posttest genetic counseling from qualified personnel about the benefits, limitations, and results of testing so that patients can make informed decisions. The American College of Obstetricians and Gynecologists and the Society for Maternal-Fetal Medicine offer background information as well as recommendations regarding the application of chromosomal microarray technology in the prenatal setting.

Chromosomal microarray analysis is a method of measuring gains and losses of DNA throughout the human genome. It is a high-resolution whole-genome screening that can identify major chromosomal aneuploidy as well as the location and type of specific genetic changes that are too small to be detected by conventional karyotyping. It is considered to be a first-tier test in the genetic evaluation of infants and children with unexplained intellectual disability, congenital anomalies, or autism spectrum disorder. Within this population, chromosomal microarray analysis has been useful in detecting causative genomic imbalances or genetic mutations in as many as 15% of children with a normal conventional karyotype (1, 2).

The utility of microarray in the diagnosis of genetic abnormalities in infants and children stimulated interest in its application in the prenatal setting. Several early descriptive studies demonstrated the potential benefit of chromosomal microarray analysis for fetal abnormalities beyond conventional fetal karyotyping (3–7). Until recently, however, the broad application of this technology was limited by a lack of large population-based studies. In December 2012, researchers published the results of a large cohort study supported by the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD) that compared the efficacy of chromosomal microarray analysis with conventional karyotyping in prenatal diagnosis (8). In this joint document, the American College of Obstetricians and Gynecologists (the College) and the Society for Maternal-Fetal Medicine offer recommendations regarding the application of chromosomal microarray technology in the prenatal setting. For recommendations on prenatal testing for aneuploidy, please refer to College Practice Bulletin Number 88, *Invasive Prenatal Testing for Aneuploidy* (9).

Microarray Technology

Chromosomal microarray analysis is a technique that can identify major chromosomal aneuploidy as well as submicroscopic abnormalities that are too small to be detected by conventional karyotyping. In contrast to the

conventional karyotype, which detects primarily genetic abnormalities resulting from large changes in the number or structure of chromosomes, microarray analysis also can provide information at the submicroscopic level throughout the human genome. Duplicated or deleted sections of DNA are known as copy number variants. These submicroscopic rearrangements may account for a sizable portion of the human genetic disease burden, with some estimates as high as 15% (10). The probability of finding significant copy number variants is highly correlated with the presence of structural fetal abnormalities, although significant copy number variants also can be identified in structurally normal fetuses. Another type of DNA alteration is a single-nucleotide polymorphism (SNP). An SNP is a DNA variation in which a single nucleotide in the genome sequence is altered. This can occur between two different individuals or between paired chromosomes of the same individual and may or may not cause disease. In contrast with Down syndrome and other common trisomies, copy number variants or SNPs identified using chromosomal microarray analysis are not associated with increasing maternal age.

There are two types of microarrays used in clinical prenatal testing: comparative genomic hybridization (CGH) and SNP arrays. Although both of these techniques detect copy number variants, they identify different types of genetic variation. With each of these technologies, DNA from a fetal sample is hybridized to a DNA chip or array containing DNA fragments of known identity (known sequences). The fetal DNA to be studied is typically derived from amniocytes or chorionic villi samples. With CGH, the fetal DNA is labeled with one color of fluorescent dye, while the control DNA (of known genetic sequences) is labeled with another color. The relative intensity of the different colors (the relative amount of fetal DNA versus control DNA) is compared. Duplications or deletions are detected as regions with a higher or lower hybridization signal than the control sample. Comparative genomic hybridization detects copy number variation for relatively large deletions or duplications, including whole-chromosome duplications (trisomy), but CGH cannot detect triploidy. With SNP arrays, only fetal DNA is hybridized to the array platform, and the presence or absence of specific known DNA sequence variants is evaluated by signal intensity to provide a genome-wide copy number analysis. Single-nucleotide polymorphism arrays detect homozygosity or heterozygosity (identical or different stretches of DNA) and, therefore, can demonstrate the extent of consanguinity (shown as regions of homozygosity), as well as triploidy and uniparental disomy.

Arrays also can be "targeted" and focus on copy number variants of known pathogenicity instead of testing the entire genome. Targeted arrays are designed to primarily detect copy number variants known to cause clinical findings, while minimizing the detection of variants of uncertain clinical significance. Variants of unknown significance describe identified DNA changes that either have not yet been reliably characterized as benign or pathogenic or that are associated with a variable phenotype (variable penetrance). In contrast, whole-genome arrays are designed to provide greater coverage across the genome and, therefore, optimize detection, but may be more likely to identify differences that have uncertain clinical consequences. Because such a large number of potential findings are possible with any type of microarray technology, databases are used to determine if specific copy number variants have been previously reported and whether they are considered pathogenic, benign, or of unknown significance.

Chromosomal Microarray Versus Karyotype

The primary advantage of chromosomal microarray analysis over the conventional karyotype is the higher resolution, which yields more genetic information. In addition, because DNA usually can be obtained from uncultured specimens, results are usually available more quickly than with karyotyping, which requires cultured cells. Because chromosomal microarray analysis does not require dividing cells, it may be useful in the evaluation of fetal demise or stillbirth, in which the culturing of macerated tissue is frequently unsuccessful (11). In addition, chromosomal microarray analysis is a standardized procedure that involves the use of computerized analysis, whereas karyotyping involves microscopic examination of stained chromosomes and may be more subjective and prone to human error.

In the 2012 NICHD multicenter trial that compared prenatal chromosomal microarray analysis with traditional fetal karyotyping, analysis performed using array CGH identified all clinically significant aneuploidies and unbalanced translocations diagnosed with traditional fetal karyotyping (8). Consistent with previous studies (12), array CGH identified additional clinically significant abnormalities in approximately 6% of fetuses with ultrasonographic abnormalities and a normal conventional karyotype. Further, array CGH detected an abnormality in 1.7% of fetuses with a normal ultrasonographic examination result and a normal karyotype (8). Thus, based on the results of the NICHD multicenter trial and prior studies, prenatal chromosomal microarray analysis is most beneficial when ultrasonographic examination identifies fetal structural anomalies. Unlike conventional karyotyping, chromosomal microarray analysis cannot detect balanced inversions, balanced translocations, or all cases of tissue mosaicism. In addition, not all microarrays can detect triploidy, although most triploid fetuses can be identified by ultrasonography. In the NICHD trial, as anticipated, neither triploidies nor balanced translocations were identified by array CGH, and samples demonstrating chromosomal mosaicism were excluded from the analysis.

A limitation of chromosomal microarray analysis is the potential to identify copy number variants of unknown clinical significance. This occurred in 3.4% of cases in the NICHD trial (8). Such results were classified as "likely benign" in 1.8% of cases and "likely pathogenic" in 1.6%. In some cases, the significance was uncertain because the findings were rare or novel, whereas some results were known to have variable penetrance. That is, such results indicate a susceptibility to a particular outcome, such as autism, but not a certainty that this will occur. In some cases, evaluation of parental samples can help clarify whether or not this is an inherited finding or a new finding in the offspring; however, the clinical outcome may remain unclear. Of note, the interpretation of many such results changed over the course of the study as additional information became available regarding the significance of some copy number variants. Thus, interpretation of results is expected to improve as knowledge of the human genome grows and the use of databases to link clinical findings with copy number variants becomes more robust.

Need for Patient Counseling

In addition to the data regarding genetic testing results, the NICHD study raised several important considerations for the clinical application of chromosomal microarray analysis in the prenatal setting. The potential for detection of clinically uncertain and complicated findings with prenatal chromosomal microarray analysis can result in substantial patient anxiety. This underscores the critical need for comprehensive patient pretest and posttest genetic counseling from qualified personnel such as a geneticist or genetic counselor about the benefits, limitations, and results of testing so that patients can make informed decisions. Information that should be shared with patients who are considering prenatal chromosomal microarray analysis is provided for use before referral for genetic counseling (see Box 1).

In the NICHD study, an independent multidisciplinary advisory group composed of clinical geneticists, cytogeneticists, and a genetic counselor was convened to evaluate all copy number variants not known to be benign to determine how patients with these findings should be counseled. Following the NICHD trial, a subset of women in the study who received abnormal results was interviewed regarding their experience (13). In general, the women reported a need for extensive support and counseling regarding the analysis. Although the NICHD trial included an informed consent process, many of these women reported a lack of good understanding of the potential for uncertain results and noted feeling great distress on receiving such information and then needing to decide how to proceed with the pregnancy (13).

In addition to copy number variants of uncertain clinical significance, chromosomal microarray analysis can detect genetic abnormalities associated with adult-onset disorders (eg, *BRCA* mutations or Charcot-Marie-Tooth disease), which may be inherited from an asymptomatic parent. In addition, some types of arrays can identify evidence of consanguinity and nonpaternity. The type and amount of information reported varies depending on the type of array used as well as the policy of the laboratory that performs the analysis (14). Therefore, genetic counseling and informed consent is essential before patients undergo testing with this technology.

Box 1. Information to Share With Patients Before Prenatal Chromosomal Microarray Analysis

- Chromosomal microarray analysis will identify almost all of the abnormalities that are identified by fetal karyotyping and may identify additional specific genetic diseases. It will not identify all genetic disorders.
- Diseases may be identified for which the clinical presentation may vary greatly and range from mild to severe. It may not be possible to predict what the outcome will be in a given patient.
- The test may identify consanguinity (a close blood relationship or incest) or nonpaternity.
- Genetic changes may be identified that may or may not cause disease. Samples from both parents may be required to help understand the significance of these results.
- Test results may identify adult-onset diseases that will not affect health during the newborn period or childhood but may have unknown severity later in life. Identification of such findings may also indicate that one of the parents has the same adult-onset disease but has not yet developed symptoms.

Recommendations

The College and the Society for Maternal-Fetal Medicine offer the following recommendations for the use of chromosomal microarray analysis in prenatal diagnosis:

- In patients with a fetus with one or more major structural abnormalities identified on ultrasonographic examination and who are undergoing invasive prenatal diagnosis, chromosomal microarray analysis is recommended. This test replaces the need for fetal karyotype.
- In patients with a structurally normal fetus undergoing invasive prenatal diagnostic testing, either fetal karyotyping or a chromosomal microarray analysis can be performed.
- Most genetic mutations identified by chromosomal microarray analysis are not associated with increasing maternal age; therefore, the use of this test for prenatal diagnosis should not be restricted to women aged 35 years and older.

- In cases of intrauterine fetal demise or stillbirth when further cytogenetic analysis is desired, chromosomal microarray analysis on fetal tissue (ie, amniotic fluid, placenta, or products of conception) is recommended because of its increased likelihood of obtaining results and improved detection of causative abnormalities.
- Limited data are available on the clinical utility of chromosomal microarray analysis to evaluate first-trimester and second-trimester pregnancy losses; therefore, this is not recommended at this time.
- Comprehensive patient pretest and posttest genetic counseling from qualified personnel such as a genetic counselor or geneticist regarding the benefits, limitations, and results of chromosomal microarray analysis is essential. Chromosomal microarray analysis should not be ordered without informed consent, which should be documented in the medical record and include discussion of the potential to identify findings of uncertain significance, nonpaternity, consanguinity, and adult-onset disease.

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