Prenatal Diagnosis of Chromosome Abnormalities: Past, Present, and Future

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For decades, conventional chromosome analysis using G-banded karyotyping has been the gold standard for detecting cytogenetic abnormalities in fetuses for prenatal diagnosis and pregnancy loss. Although chromosome studies easily identify such chromosome abnormalities as aneuploidy (gain or loss of an entire chromosome), balanced rearrangements, and large unbalanced structural rearrangements, they have limited resolution. Pathogenic copy number changes smaller than approximately 5–10 Mb are not detectable; however, recent advances in chromosomal microarray (CMA) analysis now allow the detection of chromosomal deletions and duplications at much higher resolutions (down to only a few kilobases, depending on the microarray), producing a higher diagnostic yield. In fact, a recently published study that compared standard karyotyping with postnatal CMA testing for patients with unexplained developmental delay, autism spectrum disorders, or multiple congenital anomalies demonstrated an increased diagnostic yield of about 12%–15% with CMA analysis, vs. 4% with karyotyping. These findings led to the recommendation by the American College of Medical Genetics that CMA testing replace chromosome analysis as the first-tier test for such patients (1, 2).

Given these findings in the postnatal setting, the incremental diagnostic yields of CMA analysis and chromosome analysis are now being evaluated for prenatal genetic testing. Recent publications in the New England Journal of Medicine have described approaches to answering this question, not only with prenatal samples (3) but also with stillbirths (defined as pregnancy loss at or after 20 weeks’ gestation) (4). The multicenter study by Wapner et al. successfully analyzed 4182 samples of amniotic fluid and chorionic villi with both chromosome and CMA analysis (3). The women were tested for indications that included advanced maternal age, a positive result in maternal-serum screening, and fetal anomalies detected on ultrasonography. CMA was equally efficacious in identifying all aneuploidies and unbalanced rearrangements that had been identified by karyotyping. This methodology, however, was unable to detect balanced translocations and triploidy. These features are known limitations of this type of testing, although truly balanced rearrangements are rarely clinically relevant for the fetus. Nevertheless, microarrays that use probes to interrogate single-nucleotide polymorphisms in addition to copy number changes can, in fact, detect triploidy. In samples with a normal karyotype, CMA analysis detected clinically relevant copy number changes in 6% of pregnancies with structural anomalies identified by ultrasound and in 1.7% of pregnancies with an older maternal age and positive results in serum screening tests. These results demonstrate—as in the postnatal setting—that CMA analysis has greater clinical utility than chromosome studies.

Karyotype analysis reveals an abnormality in approximately 6% to 13% of stillbirths; however, 25% to 60% of stillbirths remain unexplained, even after postmortem examination and karyotype analysis. Therefore, the Stillbirth Collaborative Research Network conducted a population-based study to determine whether CMA analysis would yield additional genetic diagnoses (4). In this study, CMA yielded a result (whether normal or abnormal) in 87.4% of the 532 samples analyzed, whereas chromosome studies yielded a result only 70.5% of the time. Chromosome analyses were able to identify clinically relevant abnormalities in 5.8% of stillbirths, whereas CMA detected aneuploidy or a pathogenic copy number variant (CNV) in 8.3% of samples. Including CNVs of uncertain clinical relevance increases the abnormality-detection rate for CMA to 13%, a 122.6% increase compared with karyotype analyses. Although CMA was unable to detect 2 low-level mosaic cases (≤10%), the authors point out that the clinical implications of these 2 low-level mosaic cases are unclear; hence, CMAs have increased clinical utility in cases of miscarriage as well as for prenatal diagnosis.

The increased diagnostic yield is the primary benefit of CMA, but CMA has additional benefits compared with karyotyping. First, CMA is performed on genomic DNA, often without the need to culture the
sample first. That is beneficial because avoiding culture decreases the turnaround time, allowing patients to receive results and make decisions regarding the pregnancy days to weeks sooner. Second, potential cell culture artifacts can thus be ruled out. The absence of a sample-culture requirement is particularly useful for miscarriage and stillbirth samples, because culturing tissues derived from these sources can be challenging, as Reddy et al. have demonstrated (4).

Finally, the use of CMA in the prenatal setting has the potential to decrease maternal anxiety in certain cases and in other cases helps prepare the family for the delivery of a neonate with a particular clinical diagnosis. Despite these advantages, CMA testing has some drawbacks. One is that this platform cannot detect balanced rearrangements (translocations and inversions). Although these types of rearrangements are unlikely to produce an abnormal fetus or miscarriage/stillbirth, they are important to detect because they are most often inherited. Such information is valuable because it identifies the possibility of future chromosomally unbalanced offspring. Another drawback is that CMA testing may also yield "incidental" findings, such as small deletions or duplications that cause adult-onset conditions, that may be irrelevant at the time of testing and may even have been inherited from a parent who does not yet exhibit symptoms. Such findings can lead to ethical issues of reporting and thus highlight the need for appropriate pretest genetic counseling.

Another major drawback regarding CMA analysis is the detection and reporting of CNVs of uncertain clinical significance (VUSs). Detection of a VUS presents a major challenge for counseling and can cause anxiety for the family in a prenatal setting. When such findings are detected, efforts are made to test parental samples to determine whether a given VUS has been inherited. In the event of inheritance from a normal parent, the VUS is much less likely to produce an abnormal phenotype in the offspring. Such genetic properties as incomplete penetrance (a phenotype due to a mutation not expressed in all carriers) and variable expression (variation in a phenotype in different individuals) can be complicating factors, however. Despite efforts to clarify findings by the laboratory to adjust reporting criteria to minimize the reporting of such variants, circumstances in which a variant remains of uncertain clinical relevance will undoubtedly arise, making clinical genetic-counseling decisions challenging.

As experience in the field grows, the number of VUSs will gradually decline, particularly because of such efforts as those of the International Collaboration for Clinical Genomics (ICCG) consortium, which is currently curating genes and genomic regions via an evidence-based process for assessing gene dosage sensitivity, with the ultimate goal of creating a genomewide dosage-sensitivity map (http://www.ncbi.nlm.nih.gov/projects/dbvar/ISCA/). The ICCG is also collecting CMA and phenotype data from clinical laboratories and housing this information in a large database so that more precise genotype/phenotype correlations can be made over time (http://www.ncbi.nlm.nih.gov/gap). These efforts will help further our understanding of such CNVs and therefore decrease the number of VUSs. This process is already apparent in the report of Wapner et al. (3), who used the most-current literature at the end of the study to reinterpret the VUS data. These efforts led to the reclassification of several CNVs.

Practice guidelines that recommend CMA as a first-tier test for prenatal and stillbirth samples may soon be issued. Even as we move in that direction, however, new methods of conducting prenatal diagnosis are already appearing on the horizon. The recent study by Talkowski et al. used whole-genome sequencing to demonstrate a prenatal diagnosis of CHARGE syndrome (5). Karyotype analysis of an amniotic fluid sample from a fetus with ultrasound abnormalities at 18.8 weeks’ gestation identified a de novo balanced translocation between the long arms of chromosomes 6 and 8. CMA analysis did not detect any clinically relevant copy number changes. The observation of a de novo balanced translocation confers an approximately 6.1% risk of congenital anomalies; however, until recently no subsequent clinical testing options were available to determine whether the translocation had any clinical importance. The genes potentially disrupted by this translocation were identified by paired-end sequencing of large inserts in DNA isolated from amniocytes. This work demonstrated that this translocation disrupts the CHD7 (chromodomain helicase DNA binding protein 7) gene on chromosome 8, findings consistent with a diagnosis of CHARGE syndrome. A clinical postnatal diagnosis of CHARGE syndrome was subsequently made on the basis of clinical features that supported the whole-genome sequencing results.

In summary, although these technological advances clearly lead to increases in clinical utility, including increased diagnostic yield, shorter turnaround times, and more-accurate genetic diagnoses, both cost and ethics constraints may limit the speed at which these technologies are implemented into routine clinical practice. The costs of novel technologies are decreasing, but increased costs must be weighed against the incremental clinical utility when choosing a test. This concept is exemplified in noninvasive prenatal screening, in which next-generation sequencing of free fetal DNA from maternal serum is used to detect common trisomies and sex chromosome aneuploidies. Despite its high cost, such screening is increasingly replacing older methodologies, including first-trimester screening to determine aneuploidy risk to the fetus.

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Currently, noninvasive prenatal screening is used as a screening test specific for certain aneuploidies, with any positive results requiring confirmation with an invasive methodology. Given the high clinical sensitivity and specificity of this testing, however, along with the potential to expand this type of testing to include high-resolution copy number detection, it has the potential to ultimately replace much of current chromosome and CMA prenatal testing.

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References


