Prenatal and Postnatal Genetic Testing: Why, How, and When?

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ABSTRACT

There have been major advances in genetic testing especially over the last 10 years. We have advanced from looking at simple chromosomes under a microscope to more sophisticated analysis of the DNA makeup of chromosomes and from testing a single gene to sequencing almost all of our genetic material. Similarly, in the field of prenatal testing we have made great strides in screening and diagnostic testing in the hope of detecting significant abnormalities in the fetus while decreasing the risk to the pregnancy. In this article the major types of genetic screening and diagnostic testing, both prenatal and postnatal, will be reviewed. [Pediatr Ann. 2017;46(11):e423-e427.]

First of all, what is genetic testing? Genetic testing includes chromosomal analysis, genetic sequencing, and biochemical tests to diagnose hereditary diseases. Genetic testing does not imply investigating all of our genetic material. Genomic refers to the complete set of DNA contained within a cell of an organism and accordingly genomic testing refers to addressing all of the genes. This includes a karyotype, chromosomal microarray (CMA), whole exome sequencing (WES), and whole genome sequencing (WGS). These various techniques along with their detection rates, uses, and limitations will be discussed.

Many of these new techniques involve next generation sequencing (NGS). This involves fragmenting the DNA, amplification, parallel sequencing, and then mapping the pieces to reference genomes. The differences from the reference are then evaluated using bioinformatics. In NGS, the number of times that a particular nucleotide sequences is referred to as the number of reads. The number of reads reflects the depth of the sequencing, such that higher depth (or coverage) is desired, which occurs when there are a higher number of reads. A low number of reads may result in errors.

An important distinction in genetic testing is screening versus diagnostic testing. Screening implies analyzing people for increased risk, but it is not definitive. An ideal screening test has both high sensitivity and specificity with low false-positive and false-negative rates. Screening tests are designed to identify people who would benefit from further diagnostic testing. Diagnostic testing is specifically looking for a particular condition with higher sensitivity and specificity than screening tests, although there may be some limitations such as invasiveness or cost. The distinction between screening and diagnostic testing is very important in prenatal testing, especially in the context of counseling parents appropriately.

With the rapid advances of newer testing methodologies, it is very important to know the indications and the limitations of the various tests. In many cases, coverage by insurance lags behind these advances, and the ability to order the testing is hampered by insurance coverage.¹

WHY SHOULD WE DO GENETIC TESTING?

For prenatal testing, this may enable the family to be aware of the risk of having a child with various medical issues and to help guide decision-making regarding the course of the pregnancy. In these circumstances, however, it is essential to also stress the limitations of prenatal testing. For postnatal testing, having a diagnosis may give prognostic information and, in some cases, potentially identify targeted therapies. Making a diagnosis will eliminate additional testing that can present both emotional and financial burdens. A diagnosis will enable genetic counseling with accurate recurrence risk assessment and the potential for reproductive options in future
pregnancies such as preimplantation genetic diagnosis and prenatal testing. In addition, testing results oftentimes enable the families to network with people who may be affected similarly.

PRENATAL TESTING

Prenatal testing can involve prenatal screening and diagnostic testing (Table 1). There can be screening for various inherited typically autosomal recessive conditions or testing for chromosome abnormalities. Carrier testing is conducted on the parents (or the donors of the genetic material of the offspring) requiring blood or saliva sampling. Carrier testing for various autosomal recessive conditions has traditionally been ethnicity-based, such as cystic fibrosis screening for Caucasians, Tay-Sachs testing for people of Ashkenazi Jewish descent, and hemoglobinopathy testing for people of Mediterranean, African, and Asian descent. However, with the advancement of NGS, coupled with mixed ancestry of many people, panethnic carrier testing is being used more widely.²

Screening for chromosome aneuploidies during pregnancy has evolved over time, from isolated second trimester alpha fetoprotein (AFP) levels to triple screen to quad screen and to first trimester screening. The latest advance in prenatal first trimester screening is cell free DNA (cfDNA) testing. Traditional first trimester screening includes measurement of free beta-human chorionic gonadotropin (β-hCG), pregnancy-associated plasma protein A (PAPP-A), and measurement of nuchal translucency by ultrasound. The quadruple screen done during the second trimester consists of beta-hCG, AFP, inhibin, and unconjugated estriol (UE3). Integrated screening includes a combination of first and second trimester blood and ultrasound screening and the results are obtained at the end of the second trimester. Sequential screening consists of first trimester screening followed by second trimester screening with interim results given to the patient.³ These tests have variable sensitivities and specificities for detection of the various aneuploidies. With any of the screening tests, it is important to know the sensitivity and specificity for the detection of the condition.

cfDNA is circulating fetal DNA (not contained within a cell) in the maternal blood that is derived from placental trophoblasts. The fetal DNA is distinguished from the maternal DNA using various techniques. Then the amount of DNA from particular chromosomes is matched to a standard. It has been used for detection of the common aneuploidies with high sensitivities. Meta-analysis of various studies on the sensitivity and specificity of cfDNA has shown a detection rate for trisomy 21 of 99.7% with a false-positive rate of 0.04%, a detection rate of 98.2% for trisomy 18 with a false-positive rate of 0.05%, a detection rate of trisomy 13 of 99% with a false-positive rate of 0.04%, and a detection rate of 95.8% for monosomy X with a false-positive rate of 0.38%.⁴ Overall, these detection rates appear higher than the traditional screening; however, there are some caveats. There are conditions in which the consequence is no results, such as low fetal fraction, maternal obesity, and small placental mass.⁵,⁶ In fact, pregnancies with aneuploidies have a higher test failure rate.⁵ Because cfDNA reflects placental DNA, confined placental mosaicism is a concern in which there is an abnormal cell line only in the placenta and does not reflect the fetus. There can be maternal mosaicism in which an abnormal maternal cell line will skew the results, and occult maternal malignancies have also been detected.⁷,⁸

Some companies are offering enhanced cfDNA testing that includes some micro-deletions. There is a lower sensitivity for these micro-deletions with detection of deletions or duplications >7 Mb (megabase).⁹ cfDNA is a screening test and not a diagnostic test. It has been recommended that it be offered to women who are high risk, such as those with advanced maternal age,¹⁰ but in reality it is being used more widely. When an increased risk is found on cfDNA, diagnostic testing is recommended. With the advances in screening, the number of invasive diagnostic procedures has decreased substantially.¹¹

Diagnostic testing for chromosome abnormalities during the pregnancy includes amniocentesis and chorionic villus sampling (CVS). The testing done by amniocentesis and CVS is typically a karyotype, plus a CMA when there are fetal anomalies and demise.¹² The greatest limitation for these tests is their invasive nature, leading to increased risk of miscarriage, especially for CVS, which is performed earlier in pregnancy. As for more advanced testing, there are some limited studies on prenatal exome sequencing for fetuses with multiple congenital anomalies with a detection rate similar to that found postnatally.¹³

POSTNATAL GENETIC TESTING

The type of testing ordered postnatally depends upon the indications, which may include confirmation of potential diagnoses by prenatal screening (including cfDNA), the presence of multiple congenital anomalies, intrauterine growth retardation, microcephaly, macrocephaly, dysmorphic features, hypotonia, hearing loss, abnormal newborn screen, developmen-
tal delay/intellectual disability, or abnormal newborn screen.

A karyotype is indicated in the evaluation of aneuploidies, mosaicism, translocation, and a family history of multiple miscarriages and/or multiple congenital anomalies. Detection of deletion or duplications is limited to those ≥5 Mb. Assessment of deletions and duplications is better by CMA. A CMA (Figure 1) can be an oligoarray or a single nucleotide polymorphism (SNP) array. An oligoarray uses a small segment of DNA as the probe whereas the SNP uses a single nucleotide. It is useful in the detection of micro-duplications and deletions, as well as detecting copy number variants (CNVs). It is the first-line testing for developmental delay, intellectual disability, autism spectrum disorder, and multiple congenital anomalies. About 6% of people with intellectual disability and 10% of those with dysmorphic features have an abnormal CMA. With the SNP array, areas of homozygosity can be detected. This can unmask uniparental disomy (in which both copies or portions of both chromosomes come from one parent), autosomal recessive conditions, consanguinity, and incest. Results include pathogenic or likely pathogenic variants, variants of unknown significance (VUS), and benign or likely benign variants. Deletions can vary in size and small CNVs can be pathogenic harboring single gene or exon deletions. Duplications are more problematic as the effects of gene dosage are not known for all genes. A CMA cannot detect structural rearrange-
ments or single-gene defects, and is limited in the detection of mosaicism.

**Fluorescent In Situ Hybridization**

Fluorescent *in situ* hybridization (FISH) is a targeted hybridization of segments of DNA. Multiplex ligation-dependent probe amplification (MLPA) is a polymerase chain reaction based amplification of a particular segment of DNA. Both techniques are used for micro-deletions and micro-duplications. FISH can detect the location of the segment on the chromosome, detect chromosomal rearrangements, identify supernumerary extra marker chromosomes, and evaluate hematologic malignancies, which MLPA cannot. One needs to know specifically the area of interest as testing is limited to the probe used. For example, velocardiofacial syndrome (22q11.2 deletion) is commonly diagnosed using FISH or MLPA.

Single-gene testing is appropriate when a particular condition is suspected. For example, diagnostic testing for achondroplasia is by testing of the *FGFR3* gene. There are situations in which there are multiple genes that can cause a specific disorder or there are groups of disorders caused by different genes. In this situation, a gene panel would be indicated. For example, five genes are known to cause Cornelia de Lange syndrome so a panel would be the most appropriate and cost effective. There are broad groups of disorders in which there are multiple responsible genes in which a gene panel would be the most appropriate, such as epilepsy or inherited neuropathy panels. Only a specific set of genes (with good coverage) is included and incidental findings are not found. There are fewer VUS identified using gene panel testing, and it may be less expensive than other genomic methodologies.  

**Whole Exome Sequencing/Whole Genome Sequencing**

WES analyzes about 1% of the genome, specifically the genes that code for proteins. The process involves NGS, capture of the exonic genes, and then analysis for variants compared to a reference. Indications include negative prior testing, unusual presentation of a condition, and consanguinity with an unknown disorder. The detection rate for significant abnormalities is about 25% to 30%. There are ongoing studies looking at the utility for rapid diagnosis with WES in the neonatal population. Limitations include unequal gene coverage and it cannot detect triplet repeat mutations, some deletions, duplications, mosaicism, and methylation abnormalities (such as for Prader Willi and Angelman syndromes). Incidental or secondary findings can be found for which a consent process is indicated. There is an American College of Medical Genetics and Genomics policy statement on recommendations for reporting of secondary findings. The list of these genes includes various cancer predisposition genes, connective tissue disorders, cardiomyopathy genes, some metabolic conditions, and susceptibility to malignant hyperthermia.

WGS covers approximately 98% of the genome, has more uniform genome coverage and includes areas outside of the exome. In contrast to WES and panel testing, capture of exonic genes is not done and therefore regulatory regions, structural changes, deletions and duplications, and copy number variants may be found. It is more expensive than WES and more variants will be found. There are studies currently looking at WGS in critically ill newborns.

**SUMMARY**

There have been multiple advances in genetic technology; therefore, it is important to know the indications and the limitations of this technology. The type of testing done depends upon the indication and specifically what type of condition is being evaluated.

**REFERENCES**