UPDATE Prenatal exome sequencing

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Prenatal genetic testing is boldly going to the next frontier: exome sequencing. Here, experts consider studies that explore the technology's potential utility and offer practical society guidance on use.

IN THIS ARTICLE

Prenatal diagnosis of genetic anomalies is important for diagnosing lethal genetic conditions before birth. It can provide information for parents regarding pregnancy options and allow for recurrence risk counseling and the potential use of preimplantation genetic testing in the next pregnancy. For decades, a karyotype was used to analyze amniocentesis and chorionic villus sampling specimens; in recent years, chromosomal microarray analysis provides more information about significant chromosomal abnormalities, including microdeletions and microduplications. However, microarrays also have limitations, as they do not identify base pair changes associated with single-gene disorders.

The advent of next-generation sequencing has substantially reduced the cost of DNA sequencing. Whole genome sequencing (WGS) can sequence the entire genome—both the coding (exonic) and noncoding (intronic) regions—while exome sequencing analyzes only the protein-coding exons, which make up 1% to 2% of the genome and about 85% of the protein-coding genes associated with known human disease. Exome sequencing increasingly is used in cases of suspected genetic disorders when other tests have been unrevealing.

In this Update, we review recent reports of prenatal exome sequencing, including studies exploring the yield in fetuses with structural anomalies; the importance of prenatal phenotyping; the perspectives of parents and health care professionals who were involved in prenatal exome sequencing studies; and a summary of a joint position statement from 3 societies regarding prenatal sequencing.

Prenatal whole exome sequencing has potential utility, with some limitations

Petrovski S, Aggarwal V, Giordano JL, et al. Whole-exome sequencing in the evaluation of fetal structural anomalies: a prospective cohort study. Lancet. 2019;393:758-767.

Lord J, McMullan DJ, Eberhardt RY, et al; for the Prenatal Assessment of Genomes and Exomes Consortium. Prenatal exome sequencing analysis in fetal structural anomalies detected by ultrasonography (PAGE): a cohort study. Lancet. 2019;393:747-757.

Exome sequencing has been shown to identify an underlying genetic cause in 25% to 30% of children with an undiagnosed suspected genetic disorder. Two studies recently published in the *Lancet* sought to determine the incremental diagnostic yield of prenatal whole exome sequencing (WES) in the setting of fetal structural anomalies when karyotype and microarray results were normal.

Details of the studies

In a prospective cohort study by Petrovski and colleagues, DNA samples from 234 fetuses with a structural anomaly (identified on ultrasonography) and both parents (parent-fetus "trios") were used for analysis. WES identified diagnostic genetic variants in 24 trios (10%). An additional 46 (20%) had variants that indicated pathogenicity but without sufficient evidence to be considered diagnostic.

The anomalies with the highest frequency of a genetic diagnosis were lymphatic, 24%; skeletal, 24%; central nervous system, 22%; and renal, 16%; while cardiac anomalies had the lowest yield at 5%.

In another prospective cohort study, known as the Prenatal Assessment of Genomes and Exomes (PAGE), Lord and colleagues sequenced DNA samples from 610 parent-fetus trios, but they restricted sequencing to a predefined list of 1,628 genes. Diagnostic genetic variants were identified in 52 fetuses (8.5%), while 24 (3.9%) had a variant of uncertain significance that was thought to be of potential clinical usefulness.

Fetuses with multiple anomalies had the highest genetic yield (15.4%), followed by skeletal (15.4%) and cardiac anomalies (11.1%), with the lowest yield in fetuses with isolated increased nuchal translucency (3.2%).

Diagnostic yield is high, but prenatal utility is limited

Both studies showed a clinically significant diagnostic yield of 8% to 10% for prenatal exome sequencing in cases of fetal structural anomalies with normal karyotype and microarray testing. While this yield demonstrates the utility of prenatal exome sequencing, it is significantly lower than what has been reported in postnatal studies. One of the reasons for this is the inherent limitation of prenatal phenotyping (discussed below).

WHAT THIS EVIDENCE MEANS FOR PRACTICE

The cohort studies by both Petrovski and Lord and their colleagues show the feasibility and potential diagnostic utility of exome sequencing in cases of fetal structural anomalies where karyotype and microarray are not diagnostic. However, the lower yield found in these studies compared with those in postnatal studies highlights in part the limitations of prenatal phenotyping.

The importance of prenatal phenotyping

Aarabi M, Sniezek O, Jiang H, et al. Importance of complete phenotyping in prenatal whole exome sequencing. Hum Genet. 2018;137:175-181.

In postnatal exome sequencing, the physical exam, imaging findings, and laboratory results are components of the phenotype that are used to interpret the sequencing data. Prenatal phenotyping, however, is limited to the use of fetal ultrasonography and, occasionally, the addition of magnetic resonance imaging. Prenatal phenotyping is without the benefit of an exam to detect more subtle anomalies or functional status, such as developmental delay, seizures, or failure to thrive.

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When a structural anomaly is identified on prenatal ultrasonography, it is especially important that detailed imaging be undertaken to detect other anomalies, including more subtle facial features and dysmorphology.

Value of reanalyzing exome sequencing data

Aarabi and colleagues conducted a retrospective study of 20 fetuses with structural anomalies and normal karyotype and microarray. They performed trio exome sequencing first using information available only prenatally and then conducted a reanalysis using information available after delivery.

With prenatal phenotyping only, the investigators identified no pathogenic, or likely pathogenic, variants. On reanalysis of combined prenatal and postnatal findings, however, they identified pathogenic variants in 20% of cases.

Significance of the findings

This study highlights both the importance of a careful, detailed fetal ultrasonography study and the possible additional benefit of a postnatal examination (such as an autopsy) in order to yield improved results. In addition, the authors noted that the development of a prenatal phenotype-genotype database would significantly help exome sequencing interpretation in the prenatal setting.

WHAT THIS EVIDENCE MEANS FOR PRACTICE

Careful prenatal ultrasonography is crucial to help in the interpretation of prenatal exome sequencing. Patients who have undergone prenatal clinical exome sequencing may benefit from reanalysis of the genetic data based on detailed postnatal findings.

FAST TRACK

Most parents reported that they would like to be told about uncertain results of prenatal WES, but that desire decreased as the certainty of results decreased

Social impact of WES: Parent and provider perspectives

Wou K, Weitz T, McCormack C, et al. Parental perceptions of prenatal whole exome sequencing (PPPWES) study. Prenat Diagn. 2018;38:801-811.

Horn R, Parker M. Health professionals' and researchers' perspectives on prenatal whole genome and exome sequencing: 'We can't shut the door now, the genie's out, we need to refine it.' PLoS One. 2018;13:e0204158.

As health care providers enter a new era of prenatal genetic testing with exome sequencing, it is crucial to the path forward that we obtain perspectives from the parents and providers who participated in these studies. Notably, in both of the previously discussed *Lancet* reports, the authors interviewed the participants to discuss the challenges involved and identify strategies for improving future testing.

What parents want

To ascertain the perceptions of couples who underwent prenatal WES, Wou and colleagues conducted semi-structured interviews with participants from the Fetal Sequencing Study regarding their experience. They interviewed 29 parents from 17 pregnancies, including a mix of those who had pathogenic prenatal results, terminated prior to receiving the results, and had normal results.

Expressed feelings and desires. Parents recalled feelings of anxiety and stress around the time of diagnosis and the need for help with coping while awaiting results. The majority of parents reported that they would like to be told about uncertain results, but that desire decreased as the certainty of results decreased.

Parents were overall satisfied with the

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prenatal genetic testing experience, but they added that they would have liked to receive written materials beforehand and a written report of the test results (including negative cases). They also would like to have connected with other families with similar experiences, to have received results sooner, and to have an in-person meeting after telephone disclosure of the results.

Health professionals articulate complexity of prenatal genomics

In a qualitative interview study to explore critical issues involved in the clinical practice use of prenatal genomics, Horn and Parker conducted interviews with 20 health care professionals who were involved in the previously described PAGE trial. Patient recruiters, midwives, genetic counselors, research assistants, and laboratory staff were included.

Interviewees cited numerous challenges involved in their day-to-day work with prenatal whole genome and exome sequencing, including:

- the complexity of achieving valid parental consent at a time of vulnerability
- management of parent expectations
- transmitting and comprehending complex information
- the usefulness of information
- the difficulty of a long turnaround time for study results.

All the interviewees agreed that prenatal exome sequencing studies contribute to knowledge generation and the advancement of technology.

The authors concluded that an appropriate next step would be the development of appropriate guidelines for good ethical practice that address the concerns encountered in genomics clinical practice.

WHAT THIS EVIDENCE MEANS FOR PRACTICE

The prenatal experience can be overwhelming for parents. Pretest and posttest counseling on genetic testing and results are of the utmost importance, as is finding ways to help support parents through this anxious time.

FAST TRACK

Professional societies recommend extensive parental pretest education, counseling, and informed consent, as well as posttest counseling

Societies offer guidance on using genome and exome sequencing

International Society for Prenatal Diagnosis, Society for Maternal and Fetal Medicine, Perinatal Quality Foundation. Joint Position Statement from the International Society for Prenatal Diagnosis (ISPD), the Society for Maternal Fetal Medicine (SMFM), and the Perinatal Quality Foundation (PQF) on the use of genome-wide sequencing for fetal diagnosis. Prenat Diagn. 2018;38:6-9.

In response to the rapid integration of exome sequencing for genetic diagnosis, several professional societies—the International Society for Prenatal Diagnosis, Society for Maternal Fetal Medicine, and Perinatal Quality Foundation—issued a joint statement addressing the clinical use of prenatal diagnostic genome wide sequencing, including exome sequencing.

Guidance at a glance

The societies' recommendations are summarized as follows:

- Exome sequencing is best done as a trio analysis, with fetal and both parental samples sequenced and analyzed together.
- Extensive pretest education, counseling, and informed consent, as well as posttest counseling, are essential. This should include:

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—the types of results to be conveyed (variants that are pathogenic, likely pathogenic, of uncertain significance, likely benign, and benign)
—the possibility that results will not be obtained or may not be available before the birth of the fetus
—realistic expectations regarding the likelihood that a significant result will be obtained
—the timeframe to results
—the option to include or exclude in the results incidental or secondary findings (such as an unexpected childhood disorder, cancer susceptibility genes, adult-onset disorders)
—the possibility of uncovering nonpaternity or consanguinity
—the potential reanalysis of results over time
—how data are stored, who has access, and for what purpose.

- Fetal sequencing may be beneficial in the following scenarios:
  —multiple fetal anomalies or a single major anomaly suggestive of a genetic disorder, when the microarray is negative
  —no microarray result is available, but the fetus exhibits a pattern of anomalies strongly suggestive of a single-gene disorder
  —a prior undiagnosed fetus (or child) with anomalies suggestive of a genetic etiology, and with similar anomalies in the current pregnancy, with normal karyotype or microarray. Providers also can consider sequencing samples from both parents prior to preimplantation genetic testing to check for shared carrier status for autosomal recessive mutations, although obtaining exome sequencing from the prior affected fetus (or child) is ideal.
  —history of recurrent stillbirths of unknown etiology, with a recurrent pattern of anomalies in the current pregnancy, with normal karyotype or microarray.
- Interpretation of results should be done using a multidisciplinary team-based approach, including clinical scientists, geneticists, genetic counselors, and experts in prenatal diagnosis.
- Where possible and after informed consent, reanalysis of results should be undertaken if a future pregnancy is planned or ongoing, and a significant amount of time has elapsed since the time the result was last reported.
- Parents should be given a written report of test results.

FAST TRACK

A multidisciplinary team-based approach should be used to interpret prenatal exome sequencing results

WHAT THIS EVIDENCE MEANS FOR PRACTICE

Three professional societies have convened to issue consensus opinion that includes current indications for prenatal exome sequencing and important factors to include in the consent process. We follow these guidelines in our own practice.

Summary

Exome sequencing is increasingly becoming mainstream in postnatal genetic testing, and it is emerging as the newest diagnostic frontier in prenatal genetic testing. However, there are limitations to prenatal exome sequencing, including issues with consent at a vulnerable time for parents, limited information available regarding the phenotype, and results that may not be available before the birth of a fetus. Providers should be familiar with the indications for testing, the possible results, the limitations of prenatal phenotyping, and the implications for future pregnancies. ●