

Joint Position Statement from the International Society of Prenatal Diagnosis (ISPD), the Society of Maternal Fetal Medicine (SMFM) and the Perinatal Quality Foundation (PQF) on the use of genome-wide sequencing for fetal diagnosis¹

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Consensus Position Statement:

Introduction

Driven by technological advances, DNA sequencing has rapidly become an important tool in the diagnosis of genetic disease. Presently, exome sequencing, either using targeted clinical panels or whole exome sequencing (WES) is the predominant approach used in clinical practice. However, whole genome sequencing (WGS) is likely to increase in use as interpretive tools and appropriate data sources are developed, and costs fall. In response to the integration of sequencing into care and in recognition of its complexity, particularly when used for prenatal diagnosis of fetuses with suspected genetic disorders, the Board of Directors of the International Society for Prenatal Diagnosis (ISPD), the Society for Maternal-Fetal Medicine (SMFM), and the Perinatal Quality Foundation (PQF) have developed these “points requiring consideration” for practitioners and laboratories offering this service.

This is an ISPD Position Statement that has not undergone peer review by this journal. Since *Prenatal Diagnosis* is the official society journal of the ISPD, it is being published as a courtesy to the ISPD. Please cite this article as doi: 10.1002/pd.5195

Exome sequencing is an emerging technology for the evaluation of the fetus with structural anomalies that are detected on ultrasound. A recent systematic literature review (2014 to 2017) revealed a broad range (6.2 – 80%) of diagnostic yield in fetuses with structural anomalies¹. With refinement of testing criteria including trio analysis, a focus on multiple anomalies and use of structured variant curation, the likelihood of a positive finding increases.¹ Along with the implementation of this new technology, there is the need for enhanced understanding and a framework to address patient and health professional education. This testing presents significant challenges, including incidental findings in the parents and/or fetus, impact on family members and responsibility for future re-analysis.² The current literature, including systematic reviews, cohort analyses, policy-guideline reviews and expert opinions, was examined to support the development of this statement on the use of diagnostic genome-wide sequencing for prenatal diagnosis. We also included elements and comments from a panel discussion at the 21st Annual Conference on Prenatal Diagnosis and Fetal Therapy of the ISPD.

While there are 3 billion nucleotides (base pairs) within the 23 chromosomes, only about 1.5% of the genetic information codes for the 20,000 genes in the human genome. A smaller number of disease associated genes (4000-5000 identified genes) are termed 'clinical exomes' and these selected genes may be evaluated in certain laboratory settings.

The underlying principle of this document is that the introduction of new genetic technologies into reproductive care must take into account lessons learned from existing prenatal genetic testing, consideration of the impact of innovations, attention to patient and provider education, and consideration of the wider ethical and societal concerns¹.

Using this approach, the authors came to the following consensus opinion on the clinical use of prenatal diagnostic genome wide sequencing including whole exome sequencing, targeted analysis using clinical panels and whole genome sequencing hereafter referred to as sequencing:

- The use of diagnostic sequencing is currently being introduced for evaluation of fetuses for whom standard diagnostic genetic testing, such as chromosomal microarray analysis (CMA), has already been performed and is uninformative or is offered concurrently according to accepted practice guidelines, or for whom expert genetic opinion determines that standard genetic testing is less optimal than sequencing for the presenting fetal phenotype.
- The routine use of prenatal sequencing as a diagnostic test cannot currently be supported due to insufficient validation data and knowledge about its benefits and pitfalls.¹⁻³ Prospective studies with adequate population numbers for validation are needed and when completed may result in confirmation or revision of this position. Currently, it is ideally done in the setting of a research protocol. Alternatively, sequencing may be performed outside a research setting on a case-by-case basis when a genetic disorder is suspected for which a confirmatory genetic diagnosis can be obtained more quickly and accurately by sequencing. Such cases should be managed after consultation with and under the expert guidance of genetic professionals working in multidisciplinary teams with expertise in the clinical diagnostic application of sequencing, including interpretation of genomic sequencing results and how they translate to the prenatal setting, as well as expertise in prenatal imaging and counseling.

Points to consider

It is recommended that for all diagnostic applications of genome-wide sequencing, whether in a research setting or offered clinically, the following important points are considered:

1. Diagnostic sequencing for fetal indications is best done as a trio analysis, where fetal and both parental samples are sequenced and analyzed together. The trio approach currently benefits timeliness of result interpretation and aids assignment of pathogenicity for detected sequence variants. If proband-only sequencing is performed, validation of diagnostic or potentially diagnostic findings best includes a determination of inheritance through targeted testing of samples from biological parents.
2. There is currently limited genotype-phenotype correlation for the genetic disorders identified in the fetal period since ultrasound imaging is frequently limited and the fetal phenotypes of many conditions have not been well described. It is therefore uncertain if sequencing should focus on a limited panel of genes suspected to be associated with the fetal phenotype or whether a genome-wide approach should be used. It is also uncertain whether interpretation of variants found by genome-wide sequencing should follow the general guidelines for interpretation and reporting of results for children and adults, or whether a more restrictive approach, limited to those variants that explain the phenotype, is preferable in the prenatal setting.
3. The provider or providers who offer sequencing for fetal indications and who conduct the pre-test education and counseling, obtain informed consent, and conduct post-test counseling and result disclosure must have an in-depth understanding of the benefits

and risks to the fetus and parents of trio-based sequencing. Interpretation of results and post-test counseling are highly complex and are best conducted in consultation with a multidisciplinary team with expertise and experience in both the clinical and laboratory aspects of prenatal diagnosis and fetal sequencing. Ideally, members of the team will have access to all clinical records, sequencing results and fetal imaging studies.

4. Extensive pre-test education, counseling and informed consent, as well as post-test counseling are essential. It is recommended that the following minimal elements be considered:
 - a. Pre-test education and counseling should be individualized and offered to both parents if at all possible. Such counseling requires communicating detailed and often complex genetic information in a manner that balances explaining possible knowledge gaps with the reality of variable genetic literacy and time constraints.
 - b. The effectiveness of alternative patient education tools to replace or supplement individualized in-person genetic counseling should be assessed prior to their introduction into clinical care.
 - c. As diagnostic sequencing can reveal genetic information about the fetus that can impact one or both parents and the family unit, ideally, both biological parents (if at all possible) should provide consent for fetal sequencing. However, as for all prenatal procedures, the pregnant woman alone can provide consent for the invasive procedure that is performed on her to obtain the fetal genetic material.
 - d. If trio sequencing is undertaken, each parent should provide separate informed consent for the sequencing of his or her own sample.

e. Pre-test counseling and informed consent must address the following for each genome analyzed (*i.e.* the fetus and each biological parent):

- i. The types of results to be conveyed (variants that are pathogenic, likely pathogenic, of uncertain significance, likely benign, and benign).
 - ii. Realistic expectations about the chance that a clinically significant result will be obtained.
 - iii. The timeframe (range) when a result can be expected.
 - iv. The possibility that no result is obtained (e.g. related to sample quality), or that a result may not be available before the birth of the fetus in ongoing pregnancies.
 - v. Inclusion or exclusion of incidental findings (e.g. an unexpected childhood disorder) in the results disclosure.
 - vi. Inclusion or exclusion of secondary findings (e.g. cancer-susceptibility genes) in the results disclosure.
 - vii. The handling of discoveries related to adult-onset conditions on fetal samples.
 - viii. The possibility of uncovering non-paternity or close parentage (e.g. consanguinity or an incestuous relationship between the biological parents of the fetus).
 - ix. Result disclosure and post-test counselling will be based on knowledge that is current at the time of result interpretation and disclosure.
- Potential changes over time are likely to occur in our knowledge of disease genes, pathogenicity of sequence variants and fetal phenotypes.

This should include information on available strategies for sample and/or data storage, and re-analysis.

- x. The importance of data sharing in de-identified databases (ISPD, SMFM, and PQF endorse the position of the ACMG that laboratory and clinical genomic data sharing is crucial for genetic healthcare).⁴ Where this is available, consent should be obtained for storing this data and parents should be advised of who will have access and for what purpose.

- f. Post-test counseling and return of results should take into account the documented patient and provider pre-test discussions of options and choices including which results will be returned. It is recommended that all individuals undergoing sequencing always receive post-test counseling, including those for whom sequencing has not yielded clinically useful information.

The science and clinical application of fetal diagnostic sequencing is still an evolving field wherein large studies are lacking and evidence is limited, although rapidly increasing. Early experience supports the following recommendations for laboratories and clinicians offering diagnostic sequencing for fetal indications:

1. Although experience is still limited, the current existing data suggest that the following indications are scenarios where fetal sequencing may be beneficial:
 - a. A current pregnancy with a fetus with a single major anomaly or with multiple organ system anomalies that are suggestive of a possible genetic etiology, but no genetic diagnosis was found after CMA; or in select situations with no CMA result, following a multidisciplinary review and consensus, in which there is a

fetus with a multiple anomaly 'pattern' that strongly suggests a single gene disorder.

- b. A personal (maternal or paternal) history of a prior undiagnosed fetus (or child) affected with a major single or multiple anomalies suggestive of a genetic aetiology, and a recurrence of similar anomalies in the current pregnancy without a genetic diagnosis after karyotype or CMA. In addition, when such parents present for preconception counseling and no sample is available from the affected proband, or if a fetal sample cannot be obtained in an ongoing pregnancy, it is considered appropriate to offer sequencing for both biological parents to look for shared carrier status for autosomal recessive mutations that might explain the fetal phenotype.⁵ However, where possible, obtaining tissue from a previous abnormal fetus or child for exome sequencing is preferable.
- c. Fetal diagnostic sequencing in families with a history of recurrent stillbirths of unknown etiology after karyotype and/or CMA, where the fetus in the current pregnancy has a recurrent pattern of anomalies.
- d. There is currently no evidence that supports routine testing on fetal tissue obtained from an invasive prenatal procedure (amniocentesis, CVS, cordocentesis, other) for indications other than fetal anomalies.

Although evidence is still limited, early experience also supports the following recommendations for diagnostic or research laboratories pertaining to quality standards, variant interpretation and the return of results:

1. Laboratory quality standards, analysis and variant annotation principles outlined for other uses of clinical diagnostic sequencing should be followed. As with all diagnostic

testing, this should only be performed in accredited diagnostic laboratories with relevant experience in prenatal genomic diagnostic testing and interpretation.

2. Clinical information about the phenotype is an integral component of interpretation of sequencing data. Before testing is initiated, clinical information must be submitted by the referring clinician in a standardized format, preferentially using human phenotype ontology terms. In addition, clinicians should provide imaging data (at a minimum reports and ideally supplemented by relevant images) to support the fetal phenotypic findings. Laboratories are encouraged to set up systems to facilitate submission of standardized phenotype information as part of the test requisition process.
3. Initial variant annotation and classification is best performed by the diagnostic laboratory. Interpretation of pathogenicity and attributed clinical significance should be informed by the fetal phenotype and other relevant clinical information. This is best done using a multidisciplinary team-based approach that includes clinical scientists, clinical geneticists or genetic counselors with prenatal expertise, as well as experts in prenatal diagnosis in order to take into account all relevant clinical information.
4. Considering the complexity of sequencing data, dialogue between laboratories and referring clinicians, with support of relevant clinical experts for final interpretation or possible revision of interpretation is highly recommended.
5. Result reporting from sequencing data on fetal samples is best focused on pathogenic and likely pathogenic variants in genes that are relevant to the fetal phenotype.
6. It is recognized that some laboratories may report variants of uncertain significance in strong candidate disease genes for the fetal phenotype, for example, in an autosomal recessive gene that is relevant for the fetal phenotype, when a pathogenic (or likely pathogenic) variant is inherited from one parent along with a variant of uncertain

significance from the other parent. This should be addressed in pre-test counselling and in these situations expert genetic post-test counselling is highly recommended.

7. It is recognized that some laboratories may report pathogenic and likely pathogenic variants in genes that cause moderate to severe childhood-onset and adult-onset disorders. Such practice may vary and should always be addressed in detail during pre-test counselling. In these circumstances post-test counselling should be delivered by those with expertise in genetic counselling and with paediatric expertise relevant to the condition.
8. When trio-sequencing is performed, the laboratory may report other clinically significant findings, for example parental carrier status for severe childhood disorders that could have implications for the currently tested or for future pregnancies. Strategies for disclosure of results on parental samples should be addressed during pre-test counseling and in the informed consent process.
9. It is recommended that inconclusive or uninformative sequencing results on a prior fetus, infant, or other relevant family member that may place a future pregnancy at risk be reviewed. Where possible, re-analysis should be undertaken (after informed consent) if a future pregnancy is planned or ongoing and a significant amount of time has elapsed since the result was last reported.
10. It is recommended that results disclosure includes a discussion regarding the future implications for the parents' reproductive and testing options.
11. It is recommended that parents be given written information about the results, the genetic counseling, and their reproductive options in a language appropriate for non-experts, in a format that is easily accessible for future reproductive decisions.

Conclusion

This Joint ISPD, SMFM, and PQF Position Statement reflects the data and technology available for consensus review at the time of its preparation in November 2017. The authors recognize that genomic technologies are developing rapidly, and that scientific and clinical knowledge about their use for prenatal diagnostic evaluation for fetal disease and malformations is still incomplete and constantly changing. Widespread health professional education is required to enable appropriate implementation and delivery of clinically effective and beneficial fetal sequencing. Clinical and translational research in this area is needed and its funding should be prioritized. The results of such studies are likely to inform further refinement of this statement, which will require regular review and modification to take into account the evolving scientific, clinical, ethical and societal context.

Acknowledgements for the creation and peer-review of this Joint ISPD and SMFM Position

Statement include for the ISPD Position Statement Committee: R D Wilson MD (Chair), I Van den Veyver MD, L Wilkins-Haug MD PhD, LS Chitty PhD MBBS, J Friedman MD PhD (Member at Large), R Wapner MD (SMFM representative), M Norton MD (PQF representative). The statement was reviewed and approved by the ISPD Board of Directors and representatives of SMFM and PQF.

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