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International Society for Prenatal Diagnosis (ISPD) Updated Position Statement from the on the use of genome-wide sequencing for prenatal diagnosis

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Abstract

The research and clinical use of genome-wide sequencing for prenatal diagnosis of fetuses at risk for genetic disorders have rapidly increased in recent years. Current data indicate that the diagnostic rate is comparable and for certain indications higher than that of standard testing by karyotype and chromosomal microarray. Responsible clinical implementation and diagnostic use of prenatal sequencing depends on standardized laboratory practices and detailed pre-test and post-test counseling. This updated position statement on behalf of the International Society for Prenatal Diagnosis (ISPD) recommends best practices for the clinical use of prenatal exome and genome sequencing from an international perspective. We include several new points for consideration by researchers and clinical service and laboratory providers.

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INTRODUCTION

In 2018, the International Society for Prenatal Diagnosis (ISPD), joined by the Society for Maternal Fetal Medicine (SMFM) and the Perinatal Quality Foundation (PQF) published the first position statement specifically on the use of genome-wide sequencing for the prenatal diagnostic work-up of pregnancies complicated by fetal structural anomalies[1]. At that time this was an emerging genomic diagnostic method being evaluated in a limited number of centers[2]. Since then, both its research and clinical use have rapidly increased[3]. This has led to the development of local guidance or "points to consider" statements by national professional societies in some countries, but for many parts of the world, such guidance is still lacking. To our knowledge there is currently no updated statement by an international society, for broadly applicable use. To bridge this gap, the listed authors have been tasked by the ISPD to update its position statement on prenatal genome-wide sequencing. This now includes several new points for consideration by researchers and clinical service and laboratory providers. It replaces the 2018 joint statement and has been reviewed and approved by the ISPD Board of Directors.

Genome-wide DNA sequencing, which includes both exome (ES) and genome (GS) sequencing, focuses on finding disease-causing variants in the genome. Presently, ES, which evaluates the coding sequence of human genes, comprising 1.5–2% of the genome, is now a well-established tool in the diagnosis of pediatric and adult genetic disease. This has led to some professional societies supporting its use as a first-line test in children and adults with developmental and intellectual disabilities. ES can be used along with a method to assess for chromosomal imbalance (such as a chromosomal microarray). New data indicate that in these populations, GS, which analyzes most of the 3 billion base pairs on the genome, has equal to superior diagnostic rate compared to exome sequencing[4,5], but has a higher incidence of variants of uncertain significance (VUS) and possibly incidental and secondary findings (IF and SF)[6–8].

Presently, prenatal exome sequencing (pES), is the predominant approach used for genome-wide sequencing in prenatal clinical practice and research[3]. Multiple studies, small series and case reports have illustrated the value of pES[2,3], but it is important to recognize the differences between these studies and the study-specific sequencing and interpretive approaches (Table 1). Some include sequencing and interpretation of variants in the exons of nearly all genes, while others focus on the "clinical exome", a collection of 4000–5000 genes causatively associated with known single gene disorders catalogued in OMIM, or alternatively, on the ~1600 genes clearly associated with genetic conditions known to present with malformations detectable in the fetus or neonate[3]. Furthermore, small series of prenatal genome sequencing (pGS) are now emerging[9] and there are ongoing trials evaluating pGS. The use of pES and pGS is likely to increase as interpretive tools and appropriate data sources continue to be improved, and costs continue to fall. Although wider integration of genome-wide sequencing into prenatal care is now considered appropriate for specific indications, it remains a complex test, particularly when used clinically for prenatal diagnosis of fetuses with suspected genetic disorders.

EXPERIENCE TO DATE

A recent systematic literature review[3] showed that pES offered for unselected pregnancies complicated by sonographically detected fetal anomalies with a normal karyotype and chromosomal microarray analysis (CMA) has an overall diagnostic yield of 31%. The diagnostic yield in unselected cohorts of fetuses with anomalies is 15% but increases to 42% in cases series selected because they have a higher suspicion of a single gene disorder based on the phenotypic presentation and/or family history. Although for some categories more evidence is needed to refine numbers, there are now sufficient data to begin differentiating diagnostic yields by specific organ system or number of organ systems affected (Table 2). Reported diagnostic yields vary but must be tempered by a number of factors including the type of sequencing used and the laboratories' practices on defining pathologic variants. To date, the available data is insufficient to recommend which categories of abnormalities warrant sequencing. As use of sequencing continues to increase, so does 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, disclosure of secondary findings, impact on family members and responsibility for future re-analysis.

The current literature, including systematic reviews, cohort analyses, policy-guideline reviews and expert opinions, was examined to support the development of this updated statement on the use of diagnostic genome-wide sequencing for prenatal diagnosis. This updated position statement addresses important points for consideration by those who perform pES or pGS and report results, including those who counsel and obtain informed consent from parents.

ISSUES FOR CONSIDERATION

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.
    a. The trio approach currently benefits timeliness of result interpretation and aids assignment of pathogenicity for detected sequence variants.
    b. If only proband 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 still limited genotype-phenotype correlation for the genetic disorders identified in the fetal period since ultrasound and/or MRI imaging is frequently limited, the fetal phenotypes of many conditions have not been well described and new fetal phenotypes for conditions recognised postnatally are now being described[10,11]. Approaches to sequence analysis may vary from examination of genes known to be associated with fetal or neonatal phenotypes to a broader genome-wide strategy (Table 1). 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, or if a new approach restricting reporting to severe childhood conditions should be considered[12]. This may vary according to local national practice and guidelines already in place for testing generally.

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. This is typically within the domain of a genetic health provider, or a relevant other specialist with extensive gentic training. 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 pertinent clinical records, sequencing results and fetal imaging studies.

4. Expert pre-test patient 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 when possible.

    b. Counseling requires communicating detailed and often complex genetic information in a manner that balances the reality of variable genetic literacy and time constraints. Patient counseling, both consistency and knowledge, is aided by educational tools.

    c. As diagnostic sequencing can reveal genetic information about the fetus that can impact one or both parents and the family unit, ideally if possible, both biological parents should provide consent for fetal sequencing.

        i. If trio sequencing is undertaken, each parent should provide separate informed consent for the sequencing of his or her own sample.

        ii. 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.

        iii. The pregnant woman can provide consent for the fetal genetic assessment if the biologic father is unavailable and cannot be contacted.

d. Pre-test counseling and informed consent must address the following for each genome analyzed (*i.e.* the fetus and each biological parent) and should reflect the sequencing analysis and reporting policy of their local testing laboratory.

- **i.** The types of results to be conveyed (variants that are pathogenic, likely pathogenic, of uncertain significance, likely benign, and benign). The approach to reporting variants of uncertain significance should be disclosed during pretest counseling and included in consenting.
- **ii.** Realistic expectations about the chance that a clinically significant result will be obtained. The understanding that even with non-diagnostic result an underlying genetic disorder may still be possible.
- **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 in a timely fashion to influence pregnancy or neonatal management.

e. There is no universal consensus on the management of IF and SF and each center should convey their policy detailing whether they are or are not reported, and if reported what is included for parents and fetus.

- **i.** Secondary findings (SF): where appropriate, the option for inclusion or exclusion of SF in the fetal and parental sequence should be addressed. SF are pathogenic and likely pathogenic variants in a defined set of genes that are medically actionable[13,14], i.e. cause disorders for which a healthcare intervention can improve outcome in asymptomatic individuals (i.e. they are medically actionable and include for example cancer susceptibility genes) and if disclosed, the implications for other family members.
  1. Parental SF: Each parent should consent separately to inclusion or exclusion of SF for their own sequencing results.
  2. Fetal SF: Fetal SF with a moderate to severe childhood condition should be discussed for inclusion / exclusion consent.
  3. If using a panel approach to analysis (Table 1) parents should be advised that SFs are not looked for.
- **ii.** Incidental findings (IF): where appropriate, the option for inclusion or exclusion of IF should be addressed. IF are pathogenic or likely pathogenic variants in genes not related

to the testing indication, and not in the defined SF list of medically actionable genes. These are variants that cause late-onset conditions including neurological, neuromuscular, cardiovascular, or inherited cancer syndromes, and if disclosed, have implications for other family members[15]. They can also include genetic carrier states (autosomal recessive, dominant and X-linked) which should be considered separately

1. Parental IF: Each parent should consent to inclusion or exclusion separately
2. Fetal IF: Fetal IF should be discussed for inclusion / exclusion consent. IF findings in genes associated with neurodevelopmental disorders, intellectual disability or metabolic conditions, are highly penetrant and are known to cause moderate to severe childhood disorders. These conditions may present without ultrasound findings.
3. If using a panel approach to analysis (Table 1) parents should be advised that the risk of detecting IFs is small and will be restricted to genes on the panel used.

f. The possibility of uncovering non-paternity or close parentage (e.g. consanguinity or an incestuous relationship between the biological parents of the fetus) should be discussed. Pretest counselling should include how a specimen from a nonbiological parent will be analyzed and reported.

g. The importance of data sharing in de-identified databases is crucial for genetic healthcare[16].

- **i.** 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.

h. It is recommended that all individuals undergoing sequencing always receive post-test counseling, including those for whom sequencing has not yielded clinically useful information. Such counseling should be provided by individuals with relevant genetic expertise.

- **i.** 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.

ii. Result disclosure and post-test counseling will be based on knowledge that is current at the time of result interpretation and disclosure.

iii. Potential changes over time are likely to occur in our knowledge of disease genes, pathogenicity of sequence variants and fetal phenotypes.

1. This may result in reclassification either upward or downward of identified variants.

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

3. Reanalysis should be considered as an option if indicated clinically, for example if additional phenotype information is available from the proband after birth or during development, or if a future pregnancy is planned. Parents should be made aware of this possibility at post-test counselling and know how to contact their genetic health provider in these eventualities.

iv. Results disclosure should include a discussion regarding the future implications for the parents' reproductive and testing options.

v. Parents should be given written information about the results, the genetic counseling, implications for family members and their reproductive options in a language appropriate for non-experts, in a format that is easily accessible for future reproductive decisions.

CLINICAL INDICATIONS

Although much remains to be learned about its science and clinical application, fetal diagnostic sequencing has increased over the last five years, providing sufficient experience to permit the development of suggestions for clinical use.

1. The current existing data support that prenatal sequencing is beneficial for the following indications:

   a. A current pregnancy with a fetus having a major single anomaly or multiple organ system anomalies:

      i. For which no genetic diagnosis was found after CMA and a clinical genetic expert review considers the phenotype suggestive of a possible genetic etiology.

ii. For which the multiple anomaly "pattern" strongly suggests a single gene disorder with no prior genetic testing. As pES is not currently validated to detected all CNVs, CMA should be run before or in parallel with pES in this scenario.

b. A personal (maternal or paternal) history of a prior undiagnosed fetus (or child) affected with a major single or multiple anomalies:

i. With a recurrence of similar anomalies in the current pregnancy without a genetic diagnosis after karyotype or CMA for the current or prior undiagnosed pregnancy. Point **a.i.** above also applies in these circumstances.

ii. 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[17,18]. However, where possible, obtaining tissue from a previous abnormal fetus or child for pES is preferable.

2. There is currently no evidence that supports routine testing (including upon parental request) on fetal tissue obtained from an invasive prenatal procedure (amniocentesis, CVS, cordocentesis, other) for indications other than fetal anomalies

a. There may be special settings when prenatal sequencing in the absence of a fetal phenotype visible on prenatal imaging can be considered, such as with a strong family history of a recurrent childhood-onset severe genetic condition with no prenatal phenotype in previous children for whom no genetic evaluation was done and is possible. Such scenarios should be reviewed by an expert multidisciplinary team preferentially in the context of a research protocol. If sequencing is done for this indication, it must be done as trio sequencing, using an appropriate analytical approach (Table 1).

LABORATORY RECOMMENDATIONS

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. Technical workflows and bioinformatic pipelines should be fully

validated with known sensitivity settings clearly communicated in the laboratory report.

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. This should include details of family and parental medical histories and details of all prenatal imaging (ultrasound, MRI etc) performed. The imaging reports should include details of fetal biometry. Whilst use of human phenotype ontology terms is preferred, development of these terms as pertains to the fetus is still in development. In some circumstances, review of images may be helpful. 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 filtering is best performed by the diagnostic laboratory. A clear variant filtering strategy should be employed and made known to the referring clinician and stated on the laboratory report. When a trio has been sequenced, inheritance filtering can be used. However, if no candidate variant is found by this approach it is recommended to analyze variants without taking into account assumed inheritance patterns, such that for example de novo variants can be uncovered. This can be done by comparison of identified variants to those listed in databases of known genes associated with genetic disease, as well as those that contain variants identified in healthy people (e.g. ClinVar, HGMD, gnomAD).

4. Variant classification is also best performed initially by the diagnostic laboratory according to local best practice guidelines such as ACMG[19]. Interpretation of pathogenicity and attributed clinical significance should be informed by the fetal phenotype and other relevant clinical information. We therefore recommend that variants of interest are discussed using a multidisciplinary team approach that includes clinical scientists, specialists in imaging, 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.

5. 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.

6. 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.

7. 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 which 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.

CONCLUSION

This Position Statement reflects the data and technology available for consensus review at the time of its preparation in March 2022. 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 changing rapidly. We also recognise that there may be geographical variations in practice and this statement outlines the broad principles that should be applied when offering pES. Widespread health professional education is required to enable appropriate implementation and delivery of clinically effective and beneficial fetal sequencing. Further 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.

As use of sequencing in clinical practice continues to increase, the need for enhanced understanding and a framework to address patient and health professional education is growing. This testing presents significant challenges, including incidental findings in the parents and/or fetus, impact on family members and responsibility for future re-analysis. The routine use of pES or pGS as a diagnostic test on all pregnancies cannot currently be supported due to insufficient validation data and knowledge about its benefits and pitfalls, in particular when there are no known congenital anomalies in the fetus. To evaluate the potential of this application of prenatal sequencing, prospective studies with adequate population numbers for validation are needed and when completed may result in confirmation or revision of this position.

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Bulleted topics:

What is already known about this topic?

In 2018, the ISPD published the first position statement on the use genome-wide sequencing, which was then an emerging technology, in the diagnostic work-up of pregnancies complicated by fetal congenital anomalies.

What does this study add?

Since then, there has been a significant growth in the experience with prenatal genome-wide sequencing. This new position statement replaces the 2018 statement with updated information on the technologies, experience, and recommended practices.

Table 1:

Summary of sequencing approaches.

Method	What is sequenced?	What is analyzed?	Depth	Advantage	Disadvantage	comment
(Whole) Genome	~95% of 3 billion bp (introns, exons, non-coding)	~95% of 3 billion bp (introns, exons, non-coding)	30–40 X	Comprehensive; NC genome data; Potential for SV, CNV, aneuploidy detection	More VUS/GUS; possible more SF/IF; Limited data on SV and NC-V interpretation / disease association; Expensive; lower coverage	Research only
Capture (Whole) Exome	Exons and flanking intron sequence of ~20,000 genes (1.5% of genome)	Exons and flanking intron sequence of ~20,000 genes (1.5% of genome)	~100 X	All sequenceable exons analyzed Higher coverage	Only coding sequences, not all genes equally captured; more risk for GUS/ VUS. SF, IF than clinical, prenatal phenotype and phenotype-driven panels.	Acceptable clinical option with MDT expert involvement
Digital (Whole) Exome	~95% of 3 billion bp (introns, exons, non-coding)	Exons and flanking sequence of ~20,000 genes (1.5% of genome)	30–40 X	All sequenceable exons analyzed; Easily adaptable analysis pipeline if new gene discovery	Same as above; Lower coverage than capture (whole) exome	Research only
(Digital) prenatal phenotype panel	Exons and flanking intron sequence of ~4,000 genes	Exons and flanking intron sequence of ~1,300 genes	~100 X	Comprehensive for known genes with prenatal phenotypes; Lower risk for GUS/VUS than whole/clinical exome; High coverage: may include method for CNV detection; reanalysis possible. AFs not detected. Infrequent IFs	Only coding sequences of known genes for prenatal phenotypes not all genes equally captured; regular panel update required.	Acceptable clinical option with MDT expert involvement
(Digital) phenotype-driven gene panel	Exons and flanking intron sequence of ~4,000 genes	Exons and flanking intron sequence of few to 100's of genes	~100 X	Known genes for specific syndrome / organ phenotype: Lower risk for GUS/VUS than whole/clinical/prenatal exome; higher coverage; may include method for CNV detection; reanalysis possible. AFs not detected. Infrequent IFs	Only coding sequences of known genes for prenatal phenotypes not all genes equally captured; regular panel update required.	Acceptable clinical option with MDT expert involvement
Clinical/ Medical Exome Capture	Exons and flanking intron sequence of ~4,000 genes	Exons and flanking intron sequence of ~4,000 genes	~100 X	Known disease genes; includes childhood disorders w/o prenatal phenotype; Lower risk for GUS than whole exome; Higher coverage	Only coding sequences of known disease genes, not all genes equally captured; more risk for GUS/VUS. SF/IF than prenatal phenotype and phenotype-driven panels; Can become outdated with new disease gene discovery	Acceptable clinical option with MDT expert involvement
Gene Panel Capture	Exons and flanking intron sequence of few to 100's of genes	Exons and flanking intron sequence of few to 100's of genes	~100 X	Known genes for specific syndrome / organ phenotype: Lower risk for GUS/VUS than whole/clinical/prenatal exome; higher coverage; may include method for CNV detection. AFs not detected. Infrequent IFs	Only coding sequences of known genes for specific phenotypes; Can become more quickly outdated with new disease gene discovery; panels usually designed based on postnatal presentations	Not preferred, but acceptable option if exome not available

CNV: copy number variants, IF: incidental findings, GUS: genes of uncertain significance, MDT: multidisciplinary team with genetics expertise, NC: non-coding, SF: secondary findings, SV: structural variants, VUS: variants of uncertain significance,

Table 2.

Diagnostic yield of fetal sequencing in fetuses with a normal karyotype/microarray.

(Data are largely taken from the systematic review by Mellis 2022 which covered publications from 1st January 2010 until 31st October 2021, as other reviews do not break down the categories by system.) Additional data is provided from publications identified more recently.

Category	No.	Added diagnostic yield	Reference
Multisystem, selection not defined	698 694	31% 33%	Mellis 2022[3] Pauta 2022[20]
Selected for likely monogenic aetiology	140 1293	40% 42%	Pauta 2021[21] Mellis 2022[3]
Any abnormality(ies), no selection	2771	15%	Mellis 2022[3]
Isolated Skeletal	424	53%	Mellis 2022[3]
Neuromuscular/Fetal akinesia deformation sequence (FADS)	33	37%	Mellis 2022[3]
Isolated Hydrops/oedema	137	22%	Mellis 2022[3]
Isolated cardiac abnormalities	773	11%	Mellis 2022[3]
Isolated increased NT (at presentation and throughout pregnancy)	290	2%	Mellis 2022[3]
Increased NT plus other anomaly at presentation or later	91	26%	Mellis 2022b[22]
Isolated CNS (single and complex)	417	17%	Mellis 2022[3]
Isolated congenital anomalies of kidneys and urinary tract (CAKUT)	278	9%	Mellis 2022[3]
Isolated echogenic kidneys	11	72%	Deng 2022[23]
Isolated agenesis of the corpus callosum	45	29%	Lei 2022[24]; Baptiste 2022[25]