Fetal Exome Sequencing on the Horizon

K. Wou

Karen Wou, MD;[1,*] Isabelle DeBie, MD;[2] June Carroll, MD;[3] Jo-Ann Brock, MD, PhD;[4] R. Douglas Wilson, MD, MSc[5]

[1]Department of Gynecology and Obstetrics, McGill University Health Centre, Montréal, QC

[2]Department of Medical Genetics, McGill University, Montréal, QC

[3]Department of Family & Community Medicine, University of Toronto, Toronto, ON

[4]Department of Obstetrics & Gynecology, Dalhousie University, Halifax, NS

[5]Department of Obstetrics & Gynaecology, University of Calgary, Calgary, AB

Abstract

Prenatal whole exome sequencing has recently been introduced. It is evolving and although not currently ready for everyday clinical practice, it will likely become part of the diagnostic arsenal available to clinicians caring for couples carrying a pregnancy for which fetal anomalies have been identified. This commentary discusses what it is, its indications, its benefits, and its limitations.

Résumé

Le séquençage prénatal de l'exome complet a récemment commencé à être employé. Il est encore en évolution et, bien qu'il ne soit pas prêt pour la pratique clinique quotidienne, il fera probablement partie du futur arsenal diagnostique des cliniciens qui prennent soin de couples dont le fœtus présente des anomalies. Ce commentaire explique de quoi il s'agit, et présente ses indications, ses avantages et ses limites.

INTRODUCTION

Congenital anomalies occur in 2% to 5% of pregnancies and explain up to 20% of perinatal deaths[1–4]. In spite of chromosomal microarray and next-generation sequencing, the diagnostic yield for structural fetal anomalies remains low at 30% to 40%, partly because many genetic syndromes are incompletely characterized for the fetal period[5,6].

Corresponding author: Dr. Karen Wou, Department of Gynecology and Obstetrics, McGill University Health Centre, Montréal, QC.
karen.wou@muhc.mcgill.ca

SOGC Genetics Committee members: Francois Audibert, MD; Richard Brown, MD; Carla Campagnolo, CCGC; Shannon Dwinnell, MD; Nanette Okun, MD; Melanie Pastuck, RN; Karine Vallee-Pouliot, MW; Rhonda Zwingerman, MD

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Prenatal whole exome sequencing has recently been introduced, on the basis of pediatric experience, demonstrating an incremental diagnostic yield of 20% to 30% when all other technologies have failed to secure a diagnosis[7,8]. It is postulated that prenatal WES, used to investigate the etiology of structural fetal anomalies, can improve diagnostic yield, facilitate genetic counselling and prenatal management, and eventually permit the offering of tailored in utero therapy or neonatal care[9]. Unfortunately, prenatal WES was introduced clinically before validation studies demonstrated its clinical utility in the prenatal setting. There are no clinical guidelines on the indications, benefits, and limitations of prenatal WES and whole genome sequencing[10].

The American College of Medical Genetics and Genomics recommends considering WES to investigate further the cause of fetal anomalies suggestive of a genetic etiology when available investigations have not yielded a diagnosis, including targeted gene panels to specific phenotype[11], with a more cautious approach regarding fetal WGS expressed in a recent joint position statement from the International Society of Prenatal Diagnosis, the Society of Maternal-Fetal Medicine, and the Perinatal Quality Foundation[12].

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WHAT IS WES?

WES is a molecular technique that evaluates the protein-coding portion of the genome, known as the exome. The exome represents approximately 1.5% of the entire human genome. Noncoding regions of the genome, such as regulatory or structure-maintaining elements, are not covered by WES. WES focuses solely on the known coding regions of the genome, which include about 20 000 genes, covering 180 000 exons or 30 million base pairs.

WES methodologies are based on targeting the desired sequences; "capturing them," usually by binding them with complementary DNA sequences; and enriching them for sequencing using any high-throughput DNA sequencing technology. WES is highly accurate in sequencing only the coding regions. By comparing sequences obtained from an affected individual with known "normal" reference sequences, WES can identify genetic variants or alterations in protein sequences in genes that may lead to Mendelian disorders and other polygenic diseases. WES has demonstrated an incremental diagnostic yield of 30% in patients with previously undiagnosed disorders.

WHAT IS WGS?

WGS is the process whereby the *entire* human genome of 3 billion base pairs is sequenced to include exons as well as noncoding regions such as introns, promoters, regulatory elements, noncoding RNA, and mitochondrial DNA. WGS aims to detect variants present in disease-causing and disease-susceptibility genes or genomic regions and to identify genetic variations associated with genetic predispositions. Current WES methodologies encounter issues related to insufficient coverage of the entire exome, so WGS may be more likely to provide complete coverage of the genome's entire coding regions. Genome-wide coverage may allow reliable detection of copy number variations currently not systematically detected by WES. Cost is a major impediment to the wide-scale clinical use of WGS, and validation studies are lacking to develop interpretive tools.

ABBREVIATIONS

ACMG	American College of Medical Genetics and Genomics
CMA	chromosomal microarray
ISPD	International Society of Prenatal Diagnosis
PQF	Perinatal Quality Foundation
SMFM	Society of Maternal-Fetal Medicine
WES	whole exome sequencing
WGS	whole genome sequencing

INDICATIONS

Although WES has relatively simple technical premises, the interpretation of data is complex and must take into consideration of biological, interpretative, and technical variables. A multidisciplinary team approach comprising clinical scientists, bioinformaticians, clinical geneticists, genetic counsellors, and maternal-fetal medicine specialists is advisable when providing interpretation of WES results.

A recent review of prenatal WES included only 16 studies with five or more fetuses and demonstrated diagnostic rates of 6.2% to 80%[13]. This wide range of diagnostic yield was explained by various inclusion criteria from fetuses with any type of anomaly, including isolated increased nuchal translucency, to multiple anomalies. The two largest series reported detection rates of 7.7% and 6.2%, with a higher diagnostic rate in fetuses with multiple anomalies, likely reflecting higher a priori risk[14,15]. These heterogeneous findings illustrate the need for more peer-reviewed data and validation studies to determine appropriate clinical indications for prenatal WES before this technology becomes widely used.

The ISPD/SMFM/PQF joint position statement on the use of WES, WGS, and targeted gene panels for fetal diagnosis argues that these technologies should be used only

Table. Joint Position Statement from the ISPD, the SMFM, and the PQF on the use of genome-wide sequencing for fetal diagnosis

1. 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.
2. A personal (maternal or paternal) history of an undiagnosed fetus (or child) affected with a major single or multiple anomalies suggestive of a genetic etiology and a recurrence of similar anomalies in the current pregnancy without a genetic diagnosis after karyotype or CMA.
3. 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.
4. There is currently no evidence that supports routine testing on fetal tissue obtained from an invasive prenatal procedure (amniocentesis, chorionic villus sampling, cordocentesis, other) for indications other than fetal anomalies.

after chromosomal studies do not identify an etiology. They should not be routinely offered prenatally as a diagnostic method but instead studied under a research protocol for specific indications or used clinically on a case-by-case basis when a genetic disorder is suspected and requires sequencing for quick and accurate diagnosis[12]. This testing is best done as a *trio analysis* to include fetal and parental samples. Because of the limited genotype-phenotype knowledge in the prenatal setting, interpretation of variants may be complex, and a more restrictive approach may be considered compared with approaches in pediatric and adult medicine. Pretest and posttest counselling requires genetic expertise and should include discussion of the benefits and risks of WES and WGS, possible results, secondary findings, time frame, and informed consent from both parents.

Fetal indications cited in the ISPD/SMFM/PQF statement for offering diagnostic sequencing are provided in the Table[12].

BENEFITS

Prenatal WES and WGS have the potential to improve diagnostic yield in cases of fetal anomalies, and this can lead to informed reproductive decision making and pregnancy management and more accurate genetic counselling about prognosis and recurrence risk. Rapid WES for critically ill neonates has shown that a single comprehensive test can avoid the diagnostic odyssey that involves time and costs from multiple serial investigations. Reaching a diagnosis rapidly can lead to improved management and cost savings. Prenatal diagnosis can reduce morbidity and mortality by guiding delivery options, neonatal care, and, when available, in utero therapy to improve clinical outcomes. In the case of stillbirth, a specific diagnosis will permit the family to reach closure and allow for reproductive decision making, taking into account recurrence risks and the possibility of preimplantation genetic diagnosis. Prenatal diagnosis of rare and/or lethal genetic disorders allows for expansion of the clinical phenotype of known Mendelian disorders, previously unrecognized in the prenatal setting.

LIMITATIONS

Although prenatal WES is feasible and has potential clinical value, important limitations must be overcome before its clinical implementation can be recommended. Despite advances in fetal imaging, fetal phenotyping (dysmorphology) remains difficult, making prenatal WES challenging[16]. Fetal phenotypes of many monogenic disorders have been poorly studied, and their clinical presentations may have variable expressivity, incomplete penetrance, and late manifestations during gestation. Early multiple fetal anomaly associations with differential diagnosis lists are a start to enhance prenatal phenotype-genotype opportunities. Although neurostructural variants can be identified, neurodevelopmental symptoms cannot be detected prenatally. All these factors make the identification of candidate genes difficult.

The interpretation of variants found with prenatal WES is complex. On average, every human carries about a dozen novel variants that have not been inherited from either parent. Detailed and precise phenotyping for interpretation of variants requires the expertise of a multidisciplinary team including clinical and molecular geneticists, biostatisticians, genetic counsellors, and maternal-fetal medicine specialists. The reporting of variants of uncertain significance in prenatal WES may create more anxiety and uncertainties for families and their health care providers. Currently, there is no fetal variant database equivalent to ClinVar (aggregation of information about genomic variation and its relationship with human health) or the Human Gene Mutation Database and no organized algorithm to determine pathogenicity of genomic variants for prenatal WES. Laboratories do not have any formal curation procedure for prenatal genomic variant interpretation.

A dilemma in prenatal genomic studies is the identification of secondary findings, findings unrelated to the reason WES or WGS was undertaken, and the subsequent impact on pregnancy management. Although this issue is not unique to this technology, the larger number of variants potentially identified from sequencing, with a range of clinical consequences, makes it a greater ethical concern. The ACMG recommends the reporting of known or expected pathogenic variants in 59 "targeted" genes that are medically actionable, even when they are unrelated to the indication for testing[17]. Although the reporting of secondary findings may allow disease prevention, it also has significant potential medical, legal, social, and economic implications for the proband and parents. There are no guidelines on the reporting of secondary findings for prenatal WES and WGS, and most laboratories are following the postnatal ACMG recommendations[17].

Turn-around time is another challenge for prenatal WES because results may be used to make decisions for an ongoing pregnancy. This requires that testing be completed quickly, on the basis of rapid DNA sequencing and data interpretation. Pediatric studies have shown that results can be obtained within hours or days or 2 to 3 weeks.

Pretest counselling by genetic professionals, both for the individual and the family, is crucial to cover issues such as

possible identification of nonpaternity, consanguinity, and parents' own secondary findings that are medically actionable. Posttest counselling is essential to review the implications, clinical decisions, and need for referrals following negative or positive test results.

CONCLUSION

Prenatal WES is evolving, and although it is not currently ready for everyday clinical practice, it will likely become part of the diagnostic arsenal available to clinicians caring for couples carrying a pregnancy in which fetal anomalies have been identified. Prenatal genomic sequencing faces many challenges before its wider use can be considered, such as ethical concerns, test performance, turnaround time, interpretation of variants of unknown significance, secondary findings, and pretest and posttest counselling. Practical challenges include the availability of genetics expertise and resources, education of health care providers, insurance coverage, provider counselling time, and costs.

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