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COMMENTARY

Ready or not, genomic screening of fetuses is already here

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Over the past decade, genomic sequencing has transformed our ability to provide diagnoses for fetuses who have abnormal imaging findings. The diagnostic yield of exome sequencing is 31% in fetuses with sonographic abnormalities who previously had a non-diagnostic karyotype and chromosomal microarray; this yield is even higher in fetuses with specific anomalies.[1] Among apparently healthy fetuses and those with minor sonographic differences, one study found that 2.9%, or approximately 1 in 35, harbored pathogenic or likely pathogenic genetic variants.[2] As such, debate among those in the field of prenatal diagnosis about whether genomic sequencing should be offered to patients with non-anomalous fetuses has begun, with proponents suggesting that it offers access to a wider range of relevant results and improves autonomy during pregnancy.[3] At present, the International Society of Prenatal Diagnosis recommends that karyotype and microarray should be offered to all pregnant patients, whereas exome sequencing should be reserved only for cases in which fetal anomalies are present.[4] Yet, it seems only a matter of time until some patients begin inquiring about the use of this technology in apparently healthy pregnancies.

In practice, the line between the use of genomic sequencing for testing of fetuses with sonographic abnormalities versus screening of those who appear healthy has already been blurred. Cell-free fetal DNA screening for autosomal dominant disorders is commercially available and being marketed to pregnant patients who seek as much information as possible.[5] Patients who undergo exome sequencing for fetuses with ultrasound abnormalities may receive results that do not correspond specifically to their imaging findings and are associated with medical conditions that cannot be conclusively diagnosed until after birth. Additionally, because fetal exome sequencing offers an option to go beyond prenatal diagnosis and assess the genes on the current American College of Medical Genetics (ACMG) findings list, adult-onset disorders are already being queried in fetuses. Questions about the role of fetal exome sequencing have therefore become pressing. Now is the time to address 2 parallel issues: the development of

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standardized terminology used to describe fetal exome sequencing results and a reconsideration of the role of actionable genomic findings in pregnancy.

Similar to cytogenetic analysis of chromosomal copy-number variants, monogenic variant interpretation is nuanced. Importantly, the presence of a pathogenic monogenic variant alone is not always sufficient to make a diagnosis of a genetic disorder. Such a variant confers an increased risk of genetic disease but cannot be construed as a diagnosis unless it is corroborated by an orthogonal imaging finding, biomarker, or the clear assortment of the variant and disease within the fetus' family.[6] Historically, most pathogenic variants were identified in individuals with classic, recognizable features of genetic disease, leading to ascertainment bias and an overestimation of the penetrance and expressivity of these conditions.[7] New information from population and biobank studies has, in some cases, illustrated a surprisingly high prevalence and decreased penetrance of pathogenic alleles,[8] rendering the prognosis of fetuses with these variants increasingly murky.

In our own clinical practice, we counseled a patient who underwent fetal exome sequencing in the setting of a minor craniofacial abnormality seen on a second trimester ultrasound. The results revealed a pathogenic variant in *COL3A1*, associated with vascular Ehlers-Danlos syndrome (vEDS). Targeted pre-mRNA splicing analysis performed on amniocytes predicted that this variant would lead to exon-skipping.[9] However, a recent retrospective biobank study demonstrated an unexpectedly high number of individuals with pathogenic variants in *COL3A1* who were apparently healthy.[10] Despite the expectation that these variants would lead to haploinsufficiency or a dominant negative effect on the formation of procollagen, most participants who harbored them were alive in late adulthood without evidence of the aortic or visceral rupture that characterizes vEDS. What, then, were we to tell our patient about the likelihood that her fetus would develop clinical features of this disease?

In this case, correlating the *COL3A1* variant with the fetal phenotype was a challenge because there are no hallmark features of vEDS that can be observed on prenatal imaging. Many monogenic conditions ascertained by exome sequencing share this same obstacle to diagnosis because they do not give rise to congenital anomalies and may not manifest in anatomical differences until after birth, if at all. Fetal ultrasound and magnetic resonance imaging are unable to detect the subtle physical exam findings that medical geneticists and other rare disease specialists evaluate and the literature on prenatal phenotypes of monogenic disorders is scant.[11] Testing biomarkers of disease, such as in cases of possible fetal inherited metabolic disorders, is at times a challenge given that fetal blood cannot easily be sampled early in pregnancy and few labs will accept amniocytes as a test specimen, nor do they have norms for fetal results.

We must ensure, then, that all pregnant patients who opt for fetal exome sequencing have access to nuanced post-test counseling, performed with standardized terminology that is easy to understand and conveys an appropriate degree of uncertainty. For example, the popular phrase, "molecular diagnosis," used to describe the presence of pathogenic variants, is a misnomer. Patients may misconstrue that their fetus has been diagnosed with a genetic disease, but as noted above, a clinical diagnosis might not yet be established in the absence of supporting phenotypic features. Similarly, terms used describe steps in the process of cell-free fetal DNA screening, such as "diagnostic testing" (used a synonym for genetic testing performed on chorionic villus sampling or amniocentesis) or "true positive finding" (indicating a genetic finding observed in cell-free fetal DNA and corroborated by testing of the placenta or amniocytes) are also misleading. The positive predictive value of cell-free fetal DNA testing, defined as the rate with which the same genetic change is observed in placental or amniocytes, actually reveals little about the health of the fetus in cases in which disease penetrance is uncertain or ascertainment of the fetal phenotype is limited.[7]

Instead, pathogenic variants that do not clearly correspond to sonographic features of disease should be referred to as genetic risk factors, which increase the likelihood of, but do not ensure, the later onset of symptoms. Clinicians should also convey that a negative genomic sequencing result does not rule out the presence of genetic disease in the fetus. Because the dichotomous decision of whether to continue pregnancy must often be made by patients in a very short amount of time, the language we use must be as transparent as possible.

The ACMG list of genes for secondary findings[12] has long represented a form of opportunistic genomic screening for individuals undergoing clinical genetic testing. These genes have been selected because of their association with disorders that are actionable, prompting the initiation of treatment or surveillance. Yet, most of the conditions on the secondary findings list, such as heritable cancer predisposition and cardiac syndromes, cannot be corroborated by fetal imaging and do not cause symptoms until adulthood.

We suggest that an entirely new list of "prenatal actionable findings" should be offered alongside the ACMG secondary findings list when genomic sequencing of fetuses is performed. At a minimum, this list should include disorders with available fetal therapies, such as Pompe disease, other lysosomal storage disorders, and cobalaminopathies. Additionally, given that fetal exomes have the potential to improve neonatal management, disorders with severe, treatable, early-onset phenotypes that are not on the Recommended Uniform Screening Panel should be considered.[13] Although phenotypic correlates of these disorders may not be present in the fetus, supportive biomarkers could be measured in some cases, or the mere awareness of disease risk may lead to rapid evaluation of at-risk newborns. As more patients undergo fetal exome sequencing, a prenatal secondary findings panel could be proactively studied as a prototypic screening tool for fetuses.

Genomic sequencing has been a tremendous asset to prenatal diagnosis, and its role will continue to grow in

coming years, particularly as more cell-free DNA tools for monogenic disease risk assessment become available. Highly health-literate pregnant patients are likely to begin seeking out genomic sequencing, sometimes for minimal fetal anatomical differences, thereby worsening health care disparities among patients unaware of this technology. In time, improved characterization of prenatal phenotypes and genome-first studies will fill the gaps in fetal genetic diagnosis and prognostication. Until then, we must communicate to patients who are making reproductive decisions that pathogenic genetic variants are not always deterministic and negative genomic results do not rule out the presence of all genetic conditions. In addition, a list of actionable findings tailored to the prenatal setting should be designed and could be used to pave the way toward understanding the best uses of fetal genomic screening in the future.

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Conflict of Interest

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