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EDITORIAL

Fetal screening and whole genome sequencing: where are the limits?

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1. Introduction

Genetic conditions contribute significantly to human morbidity and mortality. The importance of genetics is clear in the setting of pregnancy, not only to prenatal healthcare providers, but also to pregnant patients, who acknowledge that the genetic contributions of the parents have significant impact on the offspring. For patients, this often is displayed in a hopeful wondering about their future child, presenting itself in the near universal dialogue of what characteristics may be expected: Will they have my eyes? Will they have the family's musical talent? Almost always, patients ask a more serious question: Will my baby be healthy? Therefore, it is not surprising that prenatal testing, particularly genetic screening, is well incorporated into prenatal care, with the offer of prenatal aneuploidy screening and carrier screening universally recommended to pregnant patients by influential medical organizations such as the American College of Obstetricians and Gynecologists (ACOG), the UK National Health Service, and the International Society for Prenatal Diagnosis [1–4].

The broad purpose of prenatal genetic screening in a general population is to identify those patients whose offspring are at high-risk for a genetic condition, affording them an opportunity to consider diagnostic testing and other appropriate follow-up and to facilitate informed decision-making in line with their personal goals and values. The incorporation of whole-genome sequencing (WGS) into prenatal screening via cell-free DNA analysis has been the single biggest innovation in fetal genetic screening since aneuploidy screening was first introduced. Prenatal genetic screening is sure to undergo significant changes in the years and decades to come, with rapid improvements in the technological possibilities and reduced costs of genome sequencing.

2. History of fetal genetic screening

The universal question 'Will my baby be healthy?' most often reflects a desire to understand general risks to the fetus rather than a precise desire to assess the risk for a particular aneuploidy or other specific disease. Assessment of fetal growth and development via ultrasound, aneuploidy screening, and carrier screening are all modalities utilized by providers to help answer this patient query and guide pregnancy management. Definitive answers are often available via diagnostic testing like chorionic villus sampling and amniocentesis, but by and large, patients are more likely to pursue the noninvasive option of screening as a first-line test in hopes of avoiding an unnecessary invasive procedure and the subsequent small risk of a loss of a healthy pregnancy.

Prenatal aneuploidy screening, specifically for Down syndrome, and carrier screening for inherited genetic conditions like cystic fibrosis and hemoglobinopathies, have existed for decades. At first, high risk for aneuploidy was identified using maternal age alone. However, incremental improvements in performance were achieved over time with the addition of maternal serum and ultrasound markers. In general, these traditional modes of aneuploidy screening led to improved sensitivity for Down syndrome, but specificity remained relatively low, with a 5% false-positive rate (FPR) held as the acceptable threshold, resulting in positive predictive values (PPV) of <5% in even the most comprehensive forms of screening. Carrier screening has also improved incrementally, with initial approaches based on reported patient ethnicity alone, such as sickle cell screening by hemoglobin analysis for individuals of African descent. As the deleterious genetic variants for a variety of inherited conditions were characterized, carrier screening recommendations expanded to include more conditions for more ethnicities, but disparities persisted because of low detection rates in some patient populations.

3. Impact of WGS on fetal screening

Fetal genetic screening has been revolutionized by the incorporation of WGS. Noninvasive aneuploidy screening methods were limited to evaluating associations of serum and ultrasound markers with these conditions. Investigation of the fetal genome was available only through invasive chorionic villus sampling or amniocentesis. In 1997, the discovery of fetal DNA in maternal blood was first published [5]. Subsequently, in 2010, noninvasive prenatal screening (NIPS) via analysis of cell-free DNA derived from the placenta and circulating in maternal blood became available, providing a direct, noninvasive method to investigate the copy number of chromosomes present in the sample. The most widely used approach to NIPS employs low-pass WGS to detect abnormalities in copy number. This low-pass WGS allows for detection of sizable changes (typically several MBs in size or larger) across the

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entire fetal genome, but does not detect point mutations or very small CNVs across the genome. NIPS rapidly changed the face of routine fetal screening, as sensitivities for Down syndrome were shown to be >99%, but perhaps more importantly, specificity was dramatically improved over traditional methods and PPVs shown to be >20x higher in the general obstetric population, with false-positive results occurring infrequently [6,7]

While not whole genome based, carrier screening has benefitted from full gene sequencing in a similar fashion and timeframe. Whereas genetic carrier screening was at first limited to targeted mutation analysis, full sequencing of genes has improved both carrier and at-risk couple detection. These advancements have been instrumental in leveling the playing field for equitable use of carrier screening, as it is now possible for patients from minority ethnic groups to access screening with higher detection rates than were ever previously attainable. For example, a panel that detects the 23 most common *CFTR* pathogenic variants fails to identify nearly three-quarters of affected pregnancies in Hispanic persons compared to full sequencing of the gene with deletion and duplication analysis [8]. Similarly, reduced costs of sequencing have allowed for much broader lists of genes that can be screened, no longer limiting the identification of at-risk couples to only those of certain ethnicities for small sets of conditions historically thought – often incorrectly – to be most prevalent in those ethnicities [9,10]. At present, ACOG recognizes both ethnicity-based screening and expanded carrier screening (offered without regard ethnicity) as acceptable options for carrier screening [11]. While a minimum targeted mutation panel is still considered acceptable for carrier screening, use of full gene sequencing better facilitates equitable screening since it is not limited to variants that have predominantly been well characterized in Caucasian populations.

4. Now and the near future: possibilities of fetal screening with WGS

NIPS offers the best example of the impact of WGS on fetal screening, pushing the limits of what has been considered possible to ascertain noninvasively. While various technological approaches to NIPS exist, the WGS-based platform offers the most promise for expanding the number and types of conditions screened with the technology. WGS-based NIPS is already widely used to assess risk for the common aneuploidies (T18 and T13, in addition to T21 Down syndrome), but only this method of NIPS can currently provide insight into risk for abnormalities across all 24 chromosomes, including copy number variants (CNV) (deletions/duplications) and the rare autosomal aneuploidies (RAA) that involve autosomes other than 21, 18, and 13 and that are mostly associated with placental dysfunction. This expanded NIPS, which provides additional important information to answer the question, 'Will my baby be healthy?', has been implemented into clinical care to varying degrees across the globe, but is not yet considered standard to offer to patients, and hurdles to access exist for many. The innovation of fetal fraction enrichment [12], which increases the fetal fraction of a sample and thereby increases resolution of CNVs identifiable with NIPS, promises to decrease the size of CNVs discernible and allow for insight typically only gathered via prenatal microarray. Fetal fraction enrichment is also a key improvement in equitable access for current and future NIPS innovations, as it dramatically reduces the test failures common in patients with high Body Mass Index (BMI), which disproportionately impacts certain minority ethnic groups [13].

As cell-free DNA originating from the pregnancy is specifically derived from the trophoblast layer of the placenta, this offers unprecedented insight into the health of the placenta, an organ of great importance during pregnancy and one for which a noninvasive method of assessing its chromosomal health has never before existed. It is well established that chromosome aneuploidy, even when isolated to the placenta and not present in the fetus itself, can have devastating impacts on pregnancy health, resulting in serious placental insufficiency and perinatal morbidity and mortality as a result of severe fetal growth restriction and preterm delivery [14]. While serum markers via traditional aneuploidy screening have commonly been used to help identify pregnancies at risk for such placental complications, the PPV and FPR have been unimpressive. Traditional screening has therefore not been uniformly adopted as a way to identify compromised pregnancies that may benefit from additional surveillance, with the goal of providing intervention to improve outcomes. NIPS also promises to assist in the assessment of risk for single-gene conditions. Currently, very few clinical screens assess risk for conditions such as cystic fibrosis and Noonan syndrome via cell-free DNA analysis. While cell-free DNA analysis is being used in some regions of the world as a noninvasive diagnostic for single-gene conditions, concerns still exist given that datasets are small and the source of DNA from the pregnancy is placental rather than fetal. NIPS to screen for single-gene conditions is sure to expand as technology advances to allow for highly sensitive and specific identification of fetuses at risk for both inherited and sporadic conditions.

5. The long vision: future of fetal screening with WGS

Typically, innovations in fetal genetic screening have initially been reserved for a high-risk subset of patients before access is expanded to the general population. This is the likely path for the aforementioned newer applications, with the limits based on data available first in the high risk and then subsequently in the general populations. We are entering a world in which fetal screening can include conditions previously considered only within the realm of WGS-related diagnostic testing, like establishing the sequence of the fetal genome via cell-free DNA analysis [15].

In line with answering that essential parent question, 'Will my baby be healthy?', the future will move toward a single blood draw for a WGS-based evaluation that screens directly for hundreds of conditions: aneuploidy, CNVs, and multitudes of monogenic conditions, offered on a general population basis. The number and type of conditions to screen will not

be limited so much by what is technically possible, but by careful consideration of the other essential elements in panel design: an understanding of what information patients desire, how the screen can be offered, and how it can benefit various patients equitably, regardless of individual characteristics such as ethnicity or BMI. These elements are closely tied to the clinical utility of the screen, and incorporate the limitations of curating variants in healthy populations across ethnicities, establishing prenatal phenotypes for even well-known conditions, and providing guidance on the clinical follow-up needed in the setting of such variants. This type of comprehensive screen will also require revisiting the parameters of what is and is not reportable in the prenatal period.

WGS-based noninvasive analysis of the pregnancy will eventually move from cell-free DNA, the mixture of placental and maternal components, to whole-cell analysis. While circulating trophoblasts are a promising source of DNA, they still suffer the limitation of being placentally rather than fetally derived. However, isolation of fetally derived nucleated red blood cells circulating in maternal blood removes this final technical barrier to providing noninvasive diagnostic WGS of the fetus [16]. At a timepoint in the near future, prenatal screening will be antiquated, replaced by noninvasive prenatal diagnosis as standard of care.

6. Conclusion

Ultimately, the limits of WGS in prenatal screening will be based upon what is deemed acceptable in terms of performance and clinical utility, as the technological limitations will continue to fall away with continuous improvement over time. The concept of screening may be overshadowed by the emergence of widely available noninvasive prenatal diagnosis. Equity in care and the voice of the patient should be considered of utmost importance when determining the acceptable and desired use of WGS of the fetus.

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References

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. American College of Obstetricians and Gynecologists Committee on Genetics. Committee opinion No. 691: carrier screening for genetic conditions. Obstet Gynecol. 2017;129(3):e41–e55. .
2. International Society for Prenatal Diagnosis. Position statement from the chromosome abnormality screening committee on behalf of the board of the international society for prenatal diagnosis. 2015 [cited 2021 Jan 10]. Available from: https://www.ispdhome.org/docs/ISPD/Society%20Statements/PositionStatement_Current_8Apr2015.pdf
3. National Health Service. NHS Fetal Anomaly Screening Programme (FASP): programme overview. 2013 [cited 2021 Jan 10]. Available from: https://www.gov.uk/guidance/fetal-anomaly-screening-programme-overview
4. Rose NC, Kaimal AJ, Dugoff L, et al. American college of obstetricians and gynecologists' committee on practice bulletins-obstetrics and committee on genetics and the society for maternal-fetal medicine. Screening for fetal chromosomal abnormalities: ACOG practice bulletin, number 226. Obstet Gynecol. 2020;136(4):e48–e69.
5. Lo YM, Corbetta N, Chamberlain PF, et al. Presence of fetal DNA in maternal plasma and serum. Lancet. 1997 Aug 16;350(9076):485–487.
6. Norton ME, Jacobsson B, Swamy GK, et al., Cell-free DNA analysis for noninvasive examination of trisomy. N Engl J Med. 372(17): 1589–1597. 2015. .

•• **Seminal study showing that cell-free DNA screening for trisomy 21 had higher sensitivity, lower false-positive rate, and higher positive predictive value than traditional screening.**

7. Bianchi DW, Parker RL, Wentworth J, et al. DNA sequencing versus standard prenatal aneuploidy screening. N Engl J Med. 2014 Feb 27;370(9):799–808.
8. Beauchamp KA, Johansen Taber KA, Grauman PV, et al. Sequencing as a first-line methodology for cystic fibrosis carrier screening. Genet Med. 2019;21(11):2569–2576. .
9. Haque IS, Lazarin GA, Kang HP, et al. Modeled fetal risk of genetic diseases identified by expanded carrier screening. JAMA. 2016;316 (7):734–742.
10. Kaseniit KE, Haque IS, Goldberg JD, et al. Genetic ancestry analysis on >93,000 individuals undergoing expanded carrier screening reveals limitations of ethnicity-based medical guidelines. Genet Med. 2020;22(10):1694–1702.
11. American College of Obstetricians and Gynecologists Committee on Genetics. Committee opinion No. 690: carrier screening in the age of genomic medicine. Obstet Gynecol. 2017 Mar;129(3):e35–e40. .
12. Welker NC, Lee AK, Kjolby RAS, et al. High-throughput fetal fraction amplification increases analytical performance of noninvasive prenatal screening. Genet Med. 2020;23:443–450. Epub 2020/ 11/16. .

•• **Reports a new technology that increases fetal fraction, allowing for improved detection of copy number variants by noninvasive prenatal screening.**

13. Muzzey D, Goldberg JD, Haverty C. Noninvasive prenatal screening for patients with high body mass index: evaluating the impact of a customized whole genome sequencing workflow on sensitivity and residual risk. Prenat Diagn. 2020;40(3):333–341.
14. Toutain J, Goutte-Gattat D, Horovitz J, et al. Confined placental mosaicism revisited: impact on pregnancy characteristics and outcome. PLoS One. 2018;13(4):e0195905.
15. Kitzman JO, Snyder MW, Ventura M, et al., Noninvasive whole-genome sequencing of a human fetus. Sci Transl Med. 4(137): 137ra76. 2012. .

•• **Proof-of-concept that the fetal genome can be sequenced using cell-free DNA.**

16. Huang CE, Ma GC, Jou HJ, et al. Noninvasive prenatal diagnosis of fetal aneuploidy by circulating fetal nucleated red blood cells and extravillous trophoblasts using silicon-based nanostructured microfluidics. Mol Cytogenet. 2017;10:44.