Noninvasive Prenatal Testing Moves Beyond Chromosomal Abnormalities to Single-Gene Disorders

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SAN FRANCISCO (GenomeWeb) – Early this year, Baylor Genetics launched a noninvasive prenatal 30-gene test that focuses on identifying *de novo* mutations that indicate autosomal dominant or X-linked disorders.

It is the first time individual genes have been analyzed in a noninvasive prenatal test in the US and it pushes the field closer to analyzing entire fetal genomes for disease risk.

Christine Eng, chief medical officer and chief quality officer at Baylor Genetics, said that the test filled a gap in Baylor's menu of genetic test offerings that include NIPT for chromosomal abnormalities and mirodeletions, as well as universal carrier testing and diagnostic exome sequencing for rare diseases.

"There was no option to screen in a non-invasive fashion for a category of disorders that account for a significant proportion of diagnoses made by whole-exome sequencing — *de novo* dominant or X-linked disorders," Eng said.

However, similar to the NIPTs that look at chromosomal alterations, such a test raises questions about what should be screened for prenatally and how to counsel couples who receive positive results, particularly as more firms and clinical laboratories begin offering such tests. Natera has been working with Baylor and plans to launch its own version. China’s Berry Genomics has indicated it plans to offer single-gene NIPT and Dennis Lo’s group at the Chinese University of Hong Kong has been developing techniques to screen for single-gene disorders in the fetus genome-wide, including a method that requires both maternal and paternal samples to be sequenced, and another that incorporates 10x Genomics’ linked-read technology to haplotype the fetal genome.
"It's a brave new world in prenatal diagnostics," said Michael Greene, chief of obstetrics at Massachusetts General Hospital.

Baylor first launched PreSeek, which targets 30 genes known to be responsible for a number of disorders often caused by de novo mutations, in January. In May, Natera said that it was working with Baylor and planned to launch its version of the test, Vistara, first in a limited fashion and then more broadly.

Eng noted that although PreSeek and Vistara analyze the same genes, PreSeek was developed and validated at Baylor, and that Baylor collaborated with Natera for the clinical introduction and to help establish the lower limit of fetal fraction.

Eng said that PreSeek has a sensitivity and specificity of greater than 99 percent, as well as an analytical positive predictive value of more than 99 percent. Unlike other NIPTs, which require just a maternal blood sample, for PreSeek, both maternal and paternal blood samples are required, which helps distinguish true de novo mutations and increases the test's accuracy.

The disorders screened for include syndromic disorders like Rett syndrome, CHARGE, and intellectual disability; as well as craniosynostosis syndromes, Noonan spectrum disorders, and skeletal disorders such as hypochondroplasia and osteogenesis imperfecta.

The test is available for any patient who is interested in it, Eng said, but is particularly suitable for patients with ultrasound abnormalities that might be related to one of the genes on the PreSeek panel, as well as couples where the father is of advanced age, a risk factor for de novo fetal mutations. Only pathogenic or likely pathogenic variants are reported, Eng said, and diagnostic testing is recommended to confirm a positive result.

For at least some of these disorders, interventions can be taken during pregnancy to reduce the impact. For instance, Brian Krishon, a maternal-fetal medicine specialist at Houston Perinatal Associates, previously said in a statement that screening and confirmatory diagnosis for osteogenesis imperfecta can "result in reduced bone fractures through adjusted delivery and post-natal management."

Eng declined to disclose the number of PreSeek tests that have been run or the number of positive results the lab has reported, but said that orders have increased each month since launch and that the lab has identified "positive results that correlate with ultrasound abnormalities as well as cases where the father is affected with one of the PreSeek disorders." Interest in the test has been primarily from maternal-fetal medicine specialists, geneticists, and genetic counselors, she said.

CUHK's Lo said that although he couldn't comment specifically on the PreSeek test since he had not seen data, in general, taking a targeted approach to looking for de novo fetal mutations in cell-free DNA would enable deeper sequence coverage than a whole-genome approach and increase the accuracy of being able to detect de novo variants. The whole-genome approach, which his team published in the Proceedings of the National Academy of Sciences, was "challenging because of sequencing errors," he said. And, increasing coverage increases the cost, "so targeting a select group of well-documented genes seems like a reasonable approach."

Lo added that it would be interesting to see what patient population is targeted and how such single-gene testing would be incorporated with NIPT for chromosomal aneuploidies. For instance, older women are at a higher risk for fetal chromosomal aneuploidies, but for many of the single-gene disorders targeted by PreSeek, it's the father's age that matters, he said, so the tests could potentially target different populations. And for some couples, both tests would be relevant, while for others, only one would be.

In addition, Lo said, if an ultrasound finding shows an abnormal result, it could be argued that the patient should just go straight to diagnostic testing rather than another screening test — although those are all...
studies that would have to be done.

Since the launch of the first NIPT in 2011, bioethicists have raised concerns about the proliferation of such tests without appropriate education, understanding, or counseling. In particular, they have taken issue with a critical metric of the tests, the positive predictive value (PPV), and are concerned that companies often do not disclose PPV, choosing instead to focus on sensitivity and specificity. But PPV is important to understand since, for rare disorders especially, it can be very low even when sensitivity and specificity are both high.

Although Baylor’s PreSeek test analyzes disorders that are individually very rare, the test manages to have a high PPV of more than 99 percent, partly because it requires both maternal and paternal samples to run the test, Eng said. By contrast, tests that look for fetal chromosomal aneuploidies only analyze maternal samples.

"One concern I always have about cell-free DNA screening is PPV, especially as conditions are increasingly rare," said Marsha Michie, an assistant professor at the University of California, San Francisco, whose current research focuses on the social and ethical issues of NIPT. "But, Baylor uses both maternal and paternal samples, and that makes a difference in test accuracy," she said.

Nonetheless, Michie added that it would be important to see published clinical data on such single-gene NIPTs. "A consistent problem with commercial cell-free DNA tests is that they become available without peer-reviewed data," she said, and providers often do not know how to properly use them. "Provider education, including clinical data and literature that goes beyond marketing material facilitates informed consent."

Natera CEO Matt Rabinowitz has said that the firm does plan to publish a peer-reviewed study of its version of the test, Vistara. "That’s why we’re rolling it out in a limited domain, to flesh out how well sensitivity and specificity work in commercial practice," he said.

As NIPT continues to expand to single-gene disorders and beyond, it will be important to continue to discuss which disorders should and should not be included on screening tests, said Catherine Joynson, assistant director at the Nuffield Council on Bioethics.

"The crux of ethical issues for prenatal screening is: what’s a serious enough condition to warrant an intervention in pregnancy?" she said. "That’s an extremely difficult question to answer. Clearly there are some conditions that are very serious, but people have different views about what’s going to impact their family."

In addition, conditions have variable phenotypes. Even Down syndrome, which has been included in prenatal testing for years, has a variable phenotype, Michie said. "We still can’t say by looking at a fetus with trisomy 21 how severely it will be affected in terms of functionality and physical condition, and that is also certainly true with the microdeletion syndromes, and will be the case with each condition that is added."

Eng said that although some disorders on the PreSeek test have variable phenotypes, she said that Baylor’s genetic counselors address that variability. "Families are counseled regarding the range of phenotypes and severity that may be seen" for a given condition, she said. In addition, she noted, the issue of phenotype variability is not unique to NIPT, but is common in all of genetic testing.

Ultimately, the concerns that are sure to arise in response to single-gene NIPTs will be very similar to the concerns that arose when NIPT for chromosomal aneuploidies first hit the market and even when maternal serum screening was first introduced, MGH’s Greene said.
“There’s nothing new about this," Greene said. "Serum screening was introduced well before doctors understood the meaning of the tests and didn't appreciate the false positive rate of abnormal test results. That happened again with the roll out of cell-free DNA tests," he said, and would likely continue as more conditions are included.

Greene added that MGH offers NIPT to women with both average-risk and high-risk pregnancies, but because MGH is a major academic medical center it has the resources to have genetic counselors on staff to provide appropriate counseling, something the average Ob-Gyn doesn’t tend to have, he said.

Going forward, Greene said that stakeholders should keep pushing for more transparency around NIPT in general, especially as increasing numbers of rare disorders are screened for. Increased transparency would help ensure that the tests are used properly and that patients are adequately informed, he said.