panorama™ prenatal screen

PANORAMA™ NON-INVASIVE PRENATAL SCREENING FOR MICRODELETION SYNDROMES

MEGAN P. HALL, PH.D.

INTRODUCTION

Panorama™ is a non-invasive prenatal screening test for fetal chromosomal anomalies. The screening test analyzes fetal cell-free DNA (cfDNA) isolated from maternal plasma and can be performed as early as 9 weeks of gestation with high accuracy. Panorama was originally designed to screen for Trisomy 21 (Down syndrome), Trisomy 18 (Edwards syndrome), Trisomy 13 (Patau syndrome), Monosomy X (Turner syndrome), sex chromosome trisomies, triploidy and, if requested, fetal sex (1-5). Recently, the clinical scope of Panorama was expanded to include screening for five microdeletion syndromes. This Panorama Extended Panel now screens for the 22q11.2 deletion (DiGeorge), 1p36 deletion, Cri-du-chat, Prader-Willi, and Angelman deletions. Validation studies have demonstrated sensitivities of greater than 93% and specificities of greater than 99% for each microdeletion condition.

MICRODELETION PREVALENCE AND TRADITIONAL MICRODELETION DETECTION

Clinically relevant microdeletions and microduplications are more common than previously thought, occurring in up to 1 in 60 pregnancies, and can occur in pregnancies lacking ultrasound anomalies (6). The combined at-birth incidence of the 5 microdeletion syndromes covered by this screening test is approximately 1 in 1,000 (7-11), approaching the overall rate observed for Down syndrome (12). The most common microdeletion, 22q11.2 deletion, is more common than Edwards syndrome and Patau syndrome combined, and is more common than cystic fibrosis (Figure 1)(10,12,13). Further, the risk for microdeletions is independent of maternal age, unlike whole chromosome aneuploidies like Trisomy 21 (Down syndrome) that are more prevalent in women of advanced maternal age. For pregnant women under the age of 29, this means they are more likely to have a fetal microdeletion than fetal Down syndrome (Figure 2). Most concerning is that these microdeletion syndromes are severe and can result in serious physical and/or intellectual impairment.

Until now, prenatal diagnosis of microdeletion syndromes required an invasive procedure, like amniocentesis or chorionic villus sampling (CVS), followed by fluorescence *in situ* hybridization (FISH) or chromosomal microarray analysis. Recently, the American College of Obstetricians and Gynecologists (ACOG) and the Society for Maternal-Fetal Medicine (SMFM) released joint guidelines

supporting the use of microarray testing for pregnant women who are undergoing invasive testing, regardless of maternal age (14). However, due to the risk of pregnancy loss associated with invasive procedures (15, 16), most women do not undergo invasive testing without another high-risk indication.

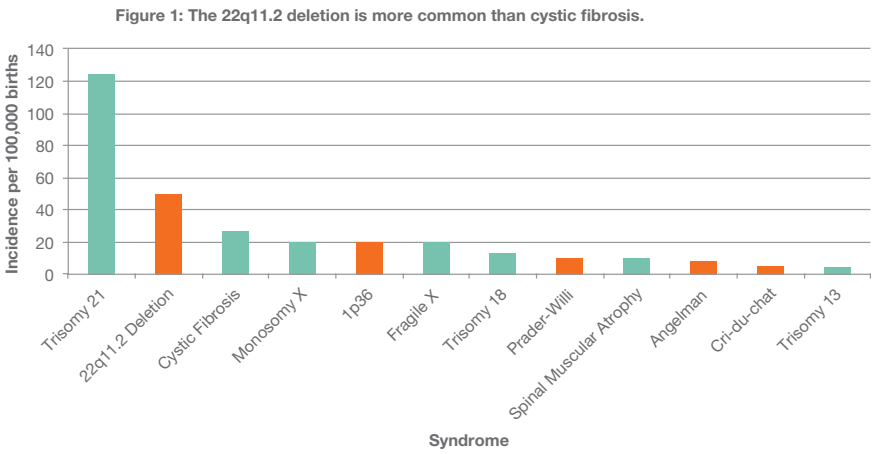

FIGURE 1:
The 22q11.2 deletion is more common than cystic fibrosis.

Traditional non-invasive tests such as serum screening and ultrasound have very low detection rates for these microdeletions. This means that many babies with microdeletions go undiagnosed until after birth, oftentimes well into adulthood. Thus, there is a clear need for a prenatal screening approach that is both accurate and non-invasive.

NON-INVASIVE DETECTION OF MICRODELETIONS USING CELL-FREE DNA

Prenatal screening for fetal aneuploidies has recently been revolutionized by non-invasive prenatal tests (NIPT) that analyze fetal cfDNA present in maternal plasma. This cfDNA is a mixture of maternal and fetal cfDNA, with the fetal percentage referred to as the “fetal fraction” (17). Among cfDNA-based screening tests, Panorama is the only one that incorporates genotypic information from the mother’s white blood cells and thus can differentiate between maternal and fetal genotypes in the plasma. Panorama targets 19,488 SNPs covering chromosomes 21, 18, 13, X, and Y, and it uses a patented algorithm called Next- Generation Aneuploidy Test Using SNPs (NATUS) to screen for whole chromosomal fetal aneuploidy and fetal sex. In clinical trials, this approach delivered a more robust fetal signal and greater quality control capabilities than first-generation quantitative “counting” methods that do not incorporate genotypic information (2, 5, 18-21).

Panorama uses similar methodology for detecting microdeletions. By specifically targeting SNPs within the microdeletion regions-of-interest, Panorama determines whether the full region is present or absent, and at the same time avoids inadvertent identification of “variants of unknown significance” (VUS), or abnormalities without clear clinical manifestations. Also, because Panorama analyzes maternal white blood cells separately from the plasma, it may also identify a previously undetected microdeletion in the mother, which would be reported.

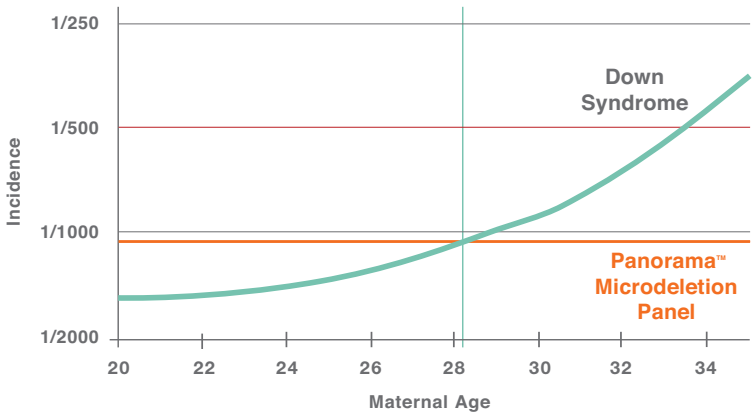

FIGURE 2:
Microdeletions are more common than Down syndrome in younger women

VALIDATION OF PANORAMA FOR MICRODELETIONS

The validation study for Panorama Extended Panel reported sensitivities of greater than 93% and specificities of greater than 99% for each microdeletion condition (22). These sensitivities reflect the presence or absence of the entire targeted region. The study examined 358 pregnant women, including 6 women carrying fetuses affected with the 22q11.2, Cri-du-Chat, and 1p36 microdeletions.

In addition to the 358 pregnancy plasmas, Natera's research team also developed a novel scientific method called "plasmART" to validate assay performance in detecting rare disorders. PlasmART samples are mixtures of fragmented genomic DNA collected post-natally from a mother and her affected child. They are designed to replicate the cfDNA profile in maternal plasma at numerous fetal fractions. This approach is important for two reasons. First, some microdeletions are exceedingly rare, such that collecting a high number of affected pregnancy samples would take many years. Second, the pregnancy samples available for research are generally collected later in pregnancy, after diagnosis by an invasive procedure. Because fetal fraction increases with gestational age (23), these research samples have higher fetal fractions than clinical samples collected from women undergoing NIPT in the first trimester.

To address this problem, Natera's plasmART method can mimic fetal fraction profiles expected during the first trimester of pregnancy (24), which allows for rapid validation that more closely represents clinical performance. In the future, this method may be used to validate new assays for screening other disorders that generate circulating cfDNA, such as cancer.

Furthermore, the Panorama Extended Panel achieves high sensitivity with a limited increase in sequencing depth. By contrast, "counting"-based screening tests require much greater sequencing power (and expense) to detect a microdeletion, especially for cases with low fetal fraction. The counting methods do not differentiate between maternal and fetal genotypes; instead they compare the number of sequence reads from a region-of-interest, such as chromosome 22q11.2, to the number of sequence reads from a reference chromosome that is presumed to be euploid (normal copy number). A low ratio indicates a deletion. The challenge, however, is that microdeletion regions are much smaller than whole chromosomes, so the only way to get enough data is to sequence more – a lot more. Indeed, published studies have shown that the counting method required 20 to 50 times greater sequencing power to detect microdeletions when compared to the number

of reads required for detecting Trisomy 21 (25, 26). The Panorama Extended Panel is the only available method that targets SNPs and does not use a counting method. This difference means that Panorama is a more efficient approach to microdeletion screening, which should allow for broader clinical adoption.

CLINICAL UTILIZATION

Since microdeletions occur independently of maternal age and other traditional risk factors, conventional notions of "high-risk" and "low-risk" pregnancies may be revised. As a result, the Panorama Extended Panel is already being discussed by many physicians as a test for all pregnant women, not just those considered high-risk. Panorama can be ordered as early as nine weeks gestation and consists of a simple blood draw with an optional buccal swab from the father. The father's sample maximizes the chances that a sample will return a result, but is not required. For sample collection, special blood tubes are used which protect the cfDNA. Samples are sent at room temperature to Natera's CLIA-certified laboratory in San Carlos, California. For pregnancies identified as high-risk by the Panorama Extended Panel, follow-up invasive testing with SNP microarray analysis is recommended. Genetic counseling is recommended for all patients.

CONCLUSIONS

Panorama, already established as a highly-accurate non-invasive screen for fetal whole-chromosome aneuploidies, has now been scientifically validated as a screening tool for the most common and severe microdeletions. As the incidence of microdeletion syndromes is not related to maternal age, it may be important to screen all pregnancies, not only those women of advanced maternal age. The initial panel of microdeletions includes 22q11.2 deletion (DiGeorge), 1p36, Cri-du-chat, Prader-Willi, and Angelman syndromes. This panel may expand in the future.

While NIPT cannot currently screen for all of the conditions identified by amniocentesis with microarray analysis, the clinical scope of the Panorama Extended Panel offers expectant parents accurate results regarding determining the health of their unborn child through a safe, non-invasive screening test which can be performed during routine first-trimester screening.

REFERENCES

1. Pergament E, Cuckle H, Zimmermann B, Banjevic M, Sigurjonsson S, Ryan A, et al. Single-nucleotide polymorphism-based non-invasive prenatal testing in a high- and low-risk cohort. Obstetrics and Gynecology.
2. Samango-Sprouse C, Banjevic M, Ryan A, Sigurjonsson S, Zimmermann B, Hill M, et al. SNP-based non-invasive prenatal testing detects sex chromosome aneuploidies with high accuracy. Prenat Diagn. 2013;33(7):643-9.
3. Zimmermann B, Hill M, Gemelos G, Demko Z, Banjevic M, Baner J, et al. Noninvasive prenatal aneuploidy testing of chromosomes 13, 18, 21, X, and Y, using targeted sequencing of polymorphic loci. Prenat Diagn. 2012;32(13):1233-41.
4. Nicolaides K, Syngelaki A, Gil M, Quezada M, Zinevich Y. Prenatal Detection of Fetal Triploidy from Cell-Free DNA Testing in Maternal Blood. Fetal Diagnosis and Therapy. 2013.
5. Nicolaides KH, Syngelaki A, Gil M, Atanasova V, Markova D. Validation of targeted sequencing of single-nucleotide polymorphisms for non-invasive prenatal detection of aneuploidy of chromosomes 13, 18, 21, X, and Y. Prenat Diagn. 2013;33(6):575-9.
6. Wapner RJ, Martin CL, Levy B, Ballif BC, Eng CM, Zachary JM, et al. Chromosomal microarray versus karyotyping for prenatal diagnosis. The New England journal of medicine. 2012;367(23):2175-84.
7. Battaglia A. 1p36 deletion syndrome. Seattle, WA: University of Washington; 2008 [cited 2014 May 4]. Available from: http://www.ncbi.nlm.nih.gov/books/NBK1191/.
8. Dagli AI, Williams CA. Angelman syndrome Seattle, WA: University of Washington; 1998 [updated Jun 16 2011; cited 2014 May 4]. Available from: http://www.ncbi.nlm.nih.gov/books/NBK1144/.
9. Driscoll DJ, Miller JL, Schwartz S, Cassidy SB. Prader-Willi syndrome Seattle, WA: University of Washington; 1998 [updated Jan 23 2014; cited 2014 May 4]. Available from: http://www.ncbi.nlm.nih.gov/books/NBK1330/.
10. McDonald-McGinn DM, Emanuel BS, Zackai EH. 22q11.2 Deletion syndrome Seattle, WA: University of Washington; 1999 [updated Feb 28 2013; cited 2014 May 5]. Available from: http://www.ncbi.nlm.nih.gov/books/NBK1523/.
11. Genetics Home Reference Bethesda, MD: National Library of Medicine; 2014 [cited 2014 May 4]. Available from: http://ghr.nlm.nih.gov/condition/cri-du-chat-syndrome.
12. Nussbaum RL, McInnes RR, Willard HF. Thompson & Thompson Genetics in Medicine2007.
13. Grosse SD, Boyle CA, Botkin JR, Comeau AM, Kharrazi M, Rosenfeld M, et al. Newborn Screening for Cystic Fibrosis: Evaluation of Benefits and Risks and Recommendations for State Newborn Screening Programs. 2004 October 15, 2004. Report No.: RR13
14. Committee opinion no. 581: the use of chromosomal microarray analysis in prenatal diagnosis. Obstetrics and gynecology. 2013;122(6):1374-7.
15. Tabor A, Vestergaard CH, Lidegaard O. Fetal loss rate after chorionic villus sampling and amniocentesis: an 11-year national registry study. Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology. 2009;34(1):19-24.
16. ACOG Practice Bulletin No. 88, December 2007. Invasive prenatal testing for aneuploidy. Obstet Gynecol. 2007;110(6):1459-67.
17. Norton ME, Brar H, Weiss J, Karimi A, Laurent LC, Caughey AB, et al. Non-Invasive Chromosomal Evaluation (NICE) Study: results of a multi center prospective cohort study for detection of fetal trisomy 21 and trisomy 18. Am J Obstet Gynecol. 2012;207(2):137.e1-8.
18. Palomaki GE, Deciu C, Kloza EM, Lambert-Messerlian GM, Haddow JE, Neveux LM, et al. DNA sequencing of maternal plasma reliably identi fies trisomy 18 and trisomy 13 as well as Down syndrome: an international collaborative study. Genet Med. 2012;14(3):296-305.
19. Palomaki GE, Kloza EM, Lambert-Messerlian GM, Haddow JE, Neveux LM, Ehrich M, et al. DNA sequencing of maternal plasma to detectDown syndrome: an international clinical validation study. Genet Med. 2011;13(11):913-20.
20. Bianchi DW, Platt LD, Goldberg JD, Abuhamad AZ, Sehnert AJ, Rava RP. Genome-wide fetal aneuploidy detection by maternal plasma DNA sequencing. Obstet Gynecol. 2012;119(5):890-901.
21. Ashoor G, Syngelaki A, Wang E, Struble C, Oliphant A, Song K, et al. Trisomy 13 detection in the first trimester of pregnancy using a chromosome-selective cell-free DNA analysis method. Ultrasound Obstet Gynecol. 2013;41(1):21-5.
22. Natera Internal Data (www.panoramatest.com).
23. Wang E, Batey A, Struble C, Musci T, Song K, Oliphant A. Gestational age and maternal weight effects on fetal cell-free DNA in maternal plasm Prenat Diagn. 2013;33(7):662-6.
24. Fan HC, Blumenfeld YJ, Chitkara U, Hudgins L, Quake SR. Analysis of the size distributions of fetal and maternal cell-free DNA by paired-end sequencing. Clin Chem. 2010;56(8):1279-86.
25. Srinivasan A, Bianchi DW, Huang H, Sehnert AJ, Rava RP. Noninvasive detection of fetal subchromosome abnormalities via deep sequencing of maternal plasma. American journal of human genetics. 2013;92(2):167-76.
26. Jensen TJ, Dzakula Z, Deciu C, van den Boom D, Ehrich M. Detection of microdeletion 22q11.2 in a fetus by next-generation sequencing of maternal plasma. Clin Chem. 2012;58(7):1148-51.