
Macedonia


Sullenberger


Wu


Larkin

Cell-Free DNA:

10 Things Every Health Care Provider Should Know

Christian Macedonia, MD | Rebecca Sullenberger, MS, MPH, LCGC | Serena Wu, MD | Robert Larkin, MD
LGHP – Maternal-Fetal Medicine

BACKGROUND

Whether you are a practitioner of women's health care or just someone who regularly reads medical journals, you have probably heard about cell-free fetal DNA testing or cfDNA. You may have heard it called by other names such as Non-Invasive Prenatal Testing or Non-Invasive Prenatal Screening (NIPT or NIPS), but regardless of the name, it is revolutionizing prenatal diagnostics.

Although the presence of fetal material in the maternal circulation has been known since the late 1800s, it wasn't until recently when Bianchi and others developed the practical techniques for isolating fetal cells from the maternal circulation of healthy subjects, that fetal DNA testing became an actuality.[1,2] But even these impressive early research experiments revealed some practical limitations. Fetal cells in the maternal circulation are limited in quantity, which makes them difficult to find. In addition, fetal cells can "immortalize" and be found in the maternal circulation outside of pregnancy as well as in subsequent pregnancies.[3]

Toward the end of the 1990s a new approach emerged that simply looked in the maternal circulation for free floating, or "cell-free," fragments of circulating DNA from the fetus, rather than searching for intact fetal cells.[4] Early work showed that these fetal DNA fragments were not only abundant, but could be differentiated from circulating maternal DNA because fetal fragment length tends to be significantly shorter than that of maternal DNA. Also, unlike DNA from intact fetal cells, cfDNA tends to clear quickly from the maternal circulation once the fetus and placenta are delivered.[5]

Two key products of the NIH Human Genome Project made cfDNA a practical reality by the beginning of the current decade.[6] The first was the development of automated DNA sequencing technologies; the second was the rapid development of multiple genomic libraries and bioinformatics programs to help with the massively complex task of aligning genomic data.[7] Sequencing the millions of tiny maternal and fetal fragments is important, but the sequence reads would simply be gibberish without the ability to line them up and to make sense out of them.

The first human genome was sequenced from the Human Genome Project using Sanger Sequencing, a time consuming and manpower intensive method.[8] At the end of the project, large investments into massively parallel pyrosequencing (first pioneered in Sweden in the late 1990s) advanced the field of genomics into an entirely different realm.[9] Instead of costing billions of dollars and taking many years to sequence a single human genome, it now costs hundreds of dollars and takes only hours. In all of human history, no other technology has advanced so fast nor dropped so far in price.

While the advances in human genome sequencing were not developed with the specific purpose of analyzing fetal DNA in the maternal circulation, the advances arrived at a time that was ripe for a change in prenatal diagnostics. Amniocentesis has been around since the 1950s, and though it had been often used to obtain genetic material from the fetus, it is an invasive procedure that has always been refused by a substantial proportion of pregnant mothers. Their objections include the fear of losing the pregnancy; concern about the discomfort and cost of the procedure; and religious or philosophical opposition to a procedure that has often been connected to elective abortion.[10] For these reasons, a non-invasive screening method

emerged that became known as the "First Trimester Screen," and consisted of ultrasound combined with serum analyses.[11] While this screening is an improvement over the previous prenatal genetic screening (quadruple and triple screening), it does not have the diagnostic accuracy of an amniocentesis; it is and will remain only a "screening" test.

IMPLICATIONS OF PRENATAL TESTING

Because of a growing consensus both within and without the obstetrical community, the American College of Obstetrics and Gynecology issued a statement in 2007 that decoupled genetic testing from the issue of pregnancy termination.[12] Indeed, though some people choose to terminate their pregnancies based on the results of genetics tests, far more women continue their pregnancies after testing. Genetic testing is revolutionary in regard to the information it reveals, but in the end it is a medical test. Like all medical tests, it is meant only to inform the decision-making process, which may involve preparations for special needs at delivery, the decision to deliver at a tertiary care center, or the decision to take a medication to mitigate the genetic disorder and optimize birth outcome.

The fetus can indeed be considered a "patient" when the fetus reaches a point at which medical or surgical therapy can be administered, generally after 20 weeks. A broad variety of medical and surgical procedures have emerged to treat fetuses for various conditions with the goal of either curing or mitigating the effects of disease or deformity. A clear example is Rh sensitization of Rh-negative mothers, who may carry fetuses that have critical hemolytic anemia. Without the ability to test the fetus for anemia we would not know which fetuses need life-saving transfusions in-utero. Until recently, only invasive prenatal testing could determine the Rh genotype of the fetus in cases where the father is heterozygous for the Rh D gene. Knowing the fetal Rh genotype is important so that maternal fetal medicine specialists can determine if intensive fetal monitoring is warranted.[13] This is just one example of how non-invasive determination of the fetal genotype can benefit the health of the fetus, while easing the burden of frequent perinatology visits and decreasing parental anxiety.

By 2011, enough laboratory and clinical evidence was presented to the FDA to allow for the first approval of cell-free fetal DNA testing to screen specifically for Trisomy 21.[14] Other data soon followed to support testing for non-Trisomy 21 aneuploidy (abnormal numbers of chromosomes) and testing of twin gestations.[15]

LIMITATIONS OF CELL-FREE FETAL DNA TESTING

The advent of reliable cell-free fetal DNA sampling has had a major impact on the decision by patients whether or not to seek information about the genetics of their fetus. Even though more women than ever before are seeking genetic testing, the proportion choosing invasive procedures is declining.[16] This change alarms some leaders in the maternal fetal medicine community because they point to the key limitations of cell-free fetal DNA testing at this time.[17]

First, cfDNA testing is primarily a sampling of the DNA released from cells of the placenta and not from the fetus.[18] This means that if there is confined placental mosaicism (when placental tissue is genetically discordant from the fetus) then the test may be falsely reassuring or falsely alarming. Overall, confined placental mosaicism (CPM) is estimated to occur in 1-2% of all pregnancies, though the actual incidence of CPM varies according to the specific chromosomes involved. Second, cell-free fetal DNA testing was originally created to target the most common chromosome abnormalities of live-born neonates, so its sensitivity and specificity are highest for these most common conditions: Trisomy 21, 18, 13, and the sex chromosomal abnormalities.

To a lesser degree the companies that perform these tests have expanded the capability of cfDNA testing to include Rh genotyping, paternity testing, and screening for select microdeletions, including DiGeorge Syndrome, Cri-du-chat, Prader-Willi/Angelman, Jacobsen, Langer-Giedion, Wolf-Hirschhorn, and 1p36 deletion syndromes. While there is even a cell-free fetal DNA test that samples the whole genome (for gains and losses of chromosome material greater than or equal to 7 Mb), the sensitivities and specificities are not uniformly distributed across the genome. In other words, a positive result for Trisomy 21 is more likely to be correct than a positive result for Trisomy 13. The last limitation that should raise a note of caution is in the "no call" or "low fetal fraction result" that is found in about 0.8-8% of samples depending on the laboratory, although the number is declining due to advances in the tests.[19] One major reason for a "no call" result is high maternal BMI; another reason for a low fetal fraction may be low fetal cell turnover, which can occur particularly in fetuses with aneuploidy. Other reasons for no call rate include the use of heparin products and maternal lupus. The last concern is simply that the tests are so new that there is less experience with them than with older testing and screening techniques.

10 THINGS EVERY HEALTH CARE PROVIDER SHOULD KNOW

1. Cell-free fetal DNA is a sampling of placental DNA fragments found in the maternal circulation. Roughly 5-10 percent of the DNA fragments in the maternal circulation are from the pregnancy. These fragments tend to be of lower molecular weight and length than maternal fragments, and they clear rapidly following delivery of the fetus and placenta.

2. The cell-free DNA fragments from the pregnancy found in the maternal circulation are primarily of placental origin. A placenta with "confined placental mosaicism" may result in a significant test error.

3. Not all "fetal fragments" are of "fetal" in origin. If there is a maternal tumor, the tumor can produce DNA fragments resembling DNA of fetal origin.[20]

4. The fraction of "fetal" DNA in the maternal circulation is contingent on a number of factors including maternal BMI, gestational age, vanishing twins, and the presence or absence of aneuploidy. It can sometimes be influenced by the medications and health conditions of the mother (including heparin products and maternal lupus). An indeterminate or "no-call" result should not be construed as a laboratory error and may be a cause for concern; low fetal fraction can indicate that a serious problem is decreasing the rate of cell division – e.g. in the case of an autosomal aneuploidy.

5. Genetic testing, be it in the form of invasive testing such as amniocentesis with karyotype or in the form of cell-free fetal DNA, should not be construed as a test to determine the need or lack of need for termination of pregnancy. These tests provide important information regarding the state of a pregnancy and the possible future outcomes of that pregnancy.

6. As sequencing technology has improved, the cost of sequencing has declined; the cost of sequencing is now decreasing at a rate far faster than the decline in prices of microprocessors or computer storage.[21] There is no single technology in human history that has fallen faster in price.

7. The amount of information that is allowed to be revealed from the cell-free fetal DNA tests is a tiny fraction of the information derived from the actual laboratory tests. This is particularly true in cfDNA testing involving Next Generation Sequencing (NGS) technologies. As these technologies evolve, so too will the diversity of the testing available, as well as the accuracy of tests that are already in use.

8. Cell-free fetal DNA testing is a "screening" test rather than a "diagnostic" test and is considered so by the American College of Obstetricians and Gynecologists (ACOG) and the Society for Maternal Fetal Medicine (SMFM).[22] They jointly advocate that all positive screens be followed by "diagnostic testing," usually in the form of an amniocentesis.

9. The distinction between "screening" and "diagnostic" designation depends on the positive predictive value of the test. In many clinical settings, cell-free fetal DNA testing can near the standard accepted definition of a diagnostic test if the "statistical priors" (other factors such as age-related risks and sonographic findings) change the predictive value to where confident clinical decisions can be made.[23]

10. Cell-free fetal DNA testing has opened the door to cell-free genomic testing for all. Cell-free epigenetic DNA and cell-free RNA testing will soon follow, and will provide a rich opportunity to understand the state of wellness and disease in all individuals, not just mothers or fetuses. Cell-free fetal DNA testing is not simply a boon to obstetrics, but can be credited with pioneering a pathway for precision molecular diagnostics for all humanity.

CONCLUSIONS

Cell-free fetal DNA testing is not a fad. It is a revolutionary new way of non-invasively gathering huge amounts of information about the makeup of a developing human. The coupling of advanced sequencing techniques with big data analytics means that what we know today about the genome of the developing fetus will change in coming years. Furthermore, as new facets of these technologies emerge, such as the sampling of fetal RNAs, we will undoubtedly address other problems that were either unknown, or were believed to be intractable.[24] What is certain is that the momentum behind non-invasive genomic testing is changing the practice of obstetrics, is changing our view of the fetus as a patient, and is advancing the development of better genomic technologies for the benefit of all medical disciplines.

Christian R. Macedonia, M.D.
Rebecca Sullenberger, M.S., M.P.H., L.C.G.C.
Serena Wu, M.D.
Robert W. Larkin, M.D.
LGHP — Maternal-Fetal Medicine
690 Good Drive
Lancaster, PA 17604-3750
Phone: 717-544-3514

Table 1.

Sequencing METHOD		SNP METHOD	Digital Analysis of Selected Regions (DANSR) METHOD
Illumina (Verifi) SAFeR algorithm MPS	**Sequenom** (MaterniT 21 and MaterniT GENOME) Quantitative MPS	**Natera** (Panorama)	**Ariosa** (Harmony)
Perkin Elmer (Verifi)	**Quest** (QNATAL—Illumina HiSeq v4)	**GenPath** (Panorama)	
Progenity (Verifi)	**Recombine** (Chromomap—send out to Illumina)		
Counsyl (Informed Pregnancy Screen—send out to Illumina for Verify	**Integrated/LabCorp** (InformaSeq) Quantitative MPS		

Key: BOLD—The Company Performing Service; (In Parenthesis)—The Brand Name of the Analytic Technique; SAFeR—"Selective Algorithm for Fetal Results." Proprietary technology uses Normalized Chromosome Value analysis, or NCV; MPS—Massively Parallel Sequencing; SNP—Single Nucleotide Polymorphism; DANSR—Digital Analysis of Selected Regions. A proprietary targeted variation of SNP testing.

REFERENCES

1. Bianchi DW, Parker RL, Wentworth J, Madankumar R, Saffer C, Das AF, Craig JA, Chudova DI, Devers PL, Jones KW, Oliver K, Rava RP, Sehnert AJ; CARE Study Group. DNA sequencing versus standard prenatal aneuploidy screening. N Engl J Med. 2014 Feb 27;370(9):799-808.
2. Schmoerl, G. Pathologisch-anatomische untersuchungen ueber Publereklapmsie. Vogel, Leipzig. 1893.
3. Steele CD, Wapner RJ, Smith JB, et al. Prenatal diagnosis using fetal cells isolated from maternal peripheral blood: a review. Clin Obstet Gynecol. 1996 Dec; 39(4):801-13. Review.
4. Lo YM, Corbetta N, Chamberlain PF, et al. Presence of fetal DNA in maternal plasma and serum. Lancet. 1997 Aug 16;350(9076):485-7.
5. Dennis YM, Zhang J, Leung TN, et al. Rapid clearance of fetal DNA from maternal plasma. Am J Hum Genet. 1999:64:218-224
6. Fan HC, Blumenfeld YJ, Chitkara U, et al. Noninvasive diagnosis of fetal aneuploidy by shotgun sequencing DNA from maternal blood. Proc Natl Acad Sci U S A. 2008 Oct 21;105(42):16266-71.
7. Li H, Homer N. A survey of sequence alignment algorithms for next-generation sequencing. Brief Bioinform. 2010 Sep;11(5):473-83.
8. Bentley DR. The Human Genome Project-an overview. Med Res Rev. 2000. (May)20(3):189-96.
9. Ahmadian A, Ehn M, Hober S. Pyrosequencing: history, biochemistry and future. Clin Chim Acta. 2006 Jan;363(1-2):83-94.
10. Al-Jader LN, Parry-Langdon N, Smith RJ. Survey of attitudes of pregnant women towards Down syndrome screening. Prenat Diagn. 2000 Jan;20(1):23-9.
11. Noble PL, Abraha HD, Snijders RJ, et al. Screening for fetal trisomy 21 in the first trimester of pregnancy: maternal serum free beta-hCG and fetal nuchal translucency thickness. Ultrasound Obstet Gynecol. 1995 Dec;6(6):390-5.
12. ACOG Committee on Practice Bulletins. ACOG Practice Bulletin No. 77: screening for fetal chromosomal abnormalities. Obstet Gynecol. 2007 Jan;109(1):217-27.
13. Teitelbaum L, Metcalfe A, Clarke G, et al. Costs and benefits of non-invasive fetal RhD determination. Ultrasound Obstet Gynecol. 2015 Jan;45(1):84-8.
14. Food and Drug Adminstration (FDA). Ultra High Throughput Sequencing for Clinical Diagnostic Applications - Approaches to Assess Analytical Validity, June 23, 2011.
15. Canick JA, Kloza EM, Lambert-Messerlian GM et al. DNA sequencing of maternal plasma to identify Down syndrome and other trisomies in multiple gestations. Prenat Diagn 2012; 32(8):730-45.
16. Shah FT, French KS, Osann KE, et al. Impact of Cell-Free Fetal DNA Screening on Patients' Choice of Invasive Procedures after a Positive California Prenatal Screen Result. J Clin Med. 2014 Jul 24;3(3):849-64.
17. Boon EM, Faas BH. Benefits and limitations of whole genome versus targeted approaches for noninvasive prenatal testing for fetal aneuploidies. Prenat Diagn. 2013 Jun;33(6):563-8.
18. Hall AL, Drendel HM, Verbrugge JL, et al. Positive cell-free fetal DNA testing for trisomy 13 reveals confined placental mosaicism. Genet Med. 2013 Sep;15(9):729-32.
19. Yaron Y. The implications of non-invasive prenatal testing failures: a review of an under-discussed phenomenon. Prenat Diagn. 2016 Mar 4.
20. Bianchi DW, Chudova D, Sehnert AJ, et al. Noninvasive Prenatal Testing and Incidental Detection of Occult Maternal Malignancies. JAMA. 2015 Jul 14;314(2):162-9.
21. Muir P, Li S, Lou S, Wang D, Spakowicz DJ, Salichos L, Zhang J, Weinstock GM, Isaacs F, Rozowsky J, Gerstein M. The real cost of sequencing: scaling computation to keep pace with data generation. Genome Biol. 2016 Mar 23;17(1):53.
22. Committee Opinion No. 640: Cell-Free DNA Screening For Fetal Aneuploidy. Obstet Gynecol. 2015 Sep;126(3):e31-7.
23. Wax JR, Cartin A, Chard R, Lucas FL, Pinette MG. Noninvasive prenatal testing: impact on genetic counseling, invasive prenatal diagnosis, and trisomy 21 detection. J Clin Ultrasound. 2015 Jan;43(1):1-6.
24. Hui WW, Chiu RW. Noninvasive prenatal testing beyond genomic analysis: what the future holds. Curr Opin Obstet Gynecol. 2016 Apr;28(2):105-10.