

Prenatal Diagnosis of Chromosomal Abnormalities—Shifting Paradigm

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It is very likely that in 10 years time, invasive prenatal diagnostic tests like amniocentesis and chorionic villus sampling (CVS) will join the club of forgotten obstetric procedures like vaginal breech delivery and rotational forceps delivery. In 1968, Henry Nadler¹ and his team were the first to report prenatal diagnosis of trisomy 21 from full karyotyping of cultured amniocytes obtained by amniocentesis. Over the next 40 years, both amniocentesis and CVS became commonplace diagnostic tools to obtain fetal cells for antenatal diagnosis of chromosomal abnormalities in high-risk pregnant mothers. The methods of screening and identifying the high-risk mother have changed over time. Initially, invasive testing methods like amniocentesis and CVS were offered to women of advanced maternal age (typically women above 35 years). It was soon realised that age was a poor screening method for fetal chromosomal abnormalities, especially Down syndrome, that most deliveries occurred in mothers aged less than 35, and while their individual risk is lower, this group makes a significant contribution to missed diagnoses. Various screening tests were therefore introduced. The most sensitive and most recommended is the first trimester combined screen (FTS), which includes ultrasound-measured fetal nuchal translucency and serum biochemistry at 11 to 14 weeks gestation. The FTS detects 85% to 90% of all fetuses with Down syndrome but has a significant false positive rate (3% to 5%). These 3% to 5% of pregnant mothers require invasive diagnostic tests to confirm the diagnosis, which will be truly positive in only 3% to 4% of these screen-positive women.

Fetal Genetic Material in Mother's Blood

However, the whole paradigm of prenatal screening and diagnosis of chromosomal abnormalities started to shift with the identification of cell-free fetal DNA (cffDNA) in maternal circulation.² The discovery of this promising source of fetal genetic material propelled forward commercial

exploration of non-invasive prenatal testing (NIPT) at a tremendous pace. The origin of these cffDNA is believed to be from trophoblastic fragments entering maternal circulation. The dissolved DNA in maternal plasma is a mixture of fragmented maternal and fetal genomic DNA (10% to 20%) and becomes detectable 5 weeks after conception. The concentration of the fetal fraction (cffDNA) increases with gestational age and falls with increasing maternal weight. The sea of background maternal DNA, however, poses a great challenge for assessing fetal chromosomal status from the small amount of cffDNA. The total absolute amount of fetal DNA is typically less than 1 µg in 20 ml of maternal blood and unfortunately, there is no reliable way to separate the ostensibly similar fetal cell-free DNA (cfDNA) from the maternal cfDNA at the present time. However, many different technical approaches have been used to circumvent this and a number of non-invasive prenatal tests have become commercially available since 2011. The rapid availability of the tests have been made possible by the development of very rapid DNA sequencing and computing capability of matching hundreds of millions of DNA fragments and sequences in hours.

Looking for the Needle in the Haystack

The most commonly used aneuploidy testing technique randomly amplifies all available cfDNA in the maternal plasma sample with polymerase chain reaction (PCR) and then uses a DNA sequencing method called massive parallel sequencing (MPS) or next-generation sequencing (NGS). The chromosomal origin of each DNA fragment or “read” is then determined by comparing its sequence with known chromosome-specific human genome sequences. The relative amount of each chromosome's DNA is then compared in this MPS approach. Trisomies are detected based on excess DNA from a specific chromosome, relative to all other chromosome pairs. Mathematical modelling methods are used to determine thresholds (deviations from

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the expected mean amount of particular chromosomes DNA), which is strongly associated with the diagnosis of aneuploidy. The absolute increase in the trisomic chromosomal DNA is, however, dependent on 2 important facts—firstly, the proportion of fetal fraction in the maternal plasma cfDNA. Hence, these tests may not yield results before 9 weeks gestation and may be insufficient for prenatal diagnosis in obese women. Secondly, the differential amplification efficiency of DNA fragments of different chromosomes. The variability of amplification is dependent on the guanine-cytosine (GC) content, and GC content is lower in chromosomes 13 and X than in chromosomes 21 and 18. Hence, the detection of trisomy 13 and monosomy X is less efficient. However, this problem of amplification has been answered by mathematical methods correcting for each chromosome's GC content. Majority of commercial tests available (MaterniT21 test from Sequenom, veriFi from Verinata/Illumina, iGeneScreen from Inex and the NIFTY test from BGI) are using the MPS method.

A second cfDNA testing strategy uses a more targeted approach rather than the “shot-gun” approach of the MPS method. In this technique, selectively amplified genomic regions of target chromosomes are sequenced, rather than all available cfDNA. These specific sequences from target chromosomes are then quantified to determine if there is trisomy-related overexpression. This approach reduces the number of sequences to read, which accelerates reporting time and allows use of benchtop sequencers, thereby reducing cost. The Harmony test from Ariosa utilises this approach. This test has a high sensitivity for trisomy 21 and 18, but considerably less sensitivity for trisomy 13.

A third approach uses targeted amplification of chromosome-specific polymorphic loci (SNPs). It then integrates maternal and paternal (if available) allelic data to model a set of hypotheses based on monosomy, disomy or trisomy for each chromosome and incorporates all possible inheritance patterns and crossover locations. Bayesian statistical analysis is then used to assign a probability to each hypothesis, and select the most likely hypothesis. This approach claims to have a low false positive result. Since this technique does not require a reference chromosome, it is equally sensitive for all autosomal trisomies, sex chromosomal aneuploidy and triploidy detection. The Panorama test from Natera utilises this approach.

Many other approaches are being developed and many more companies are developing newer tests and waiting in the wings. A number of cell-free fetal nucleic acids (cffNA) tests are already reporting on common deletions and will soon be detecting common insertions and single-gene mutations.

Application in Clinical Practice

While the technology used by different tests vary, they all have very high sensitivity (approximately 99%) for the detection of trisomy 21. These tests also have a very high specificity for detection of trisomy 21 (above 99%). This was initially tested in a number of studies with high-risk samples. These were mostly validation studies by the testing companies and utilised case-control formats with known abnormal samples or prospective cohort studies in high-risk populations. A meta-analysis of these studies confirmed the efficacy of these cffDNA tests in high-risk singleton pregnancies.³ Pooled detection rates and false-positive rates for trisomy 21 were 99.0% (95% CI, 98.2 to 99.6) and 0.08% (95% CI, 0.03 to 0.14), respectively. Detection rates for trisomy 18 and 13 were also well above 90% with very low false positive rates (below 0.2%). However, detection rates for sex chromosome aneuploidies, like Turner syndrome, were slightly lower than 90%.

Given the performance of NIPT in high-risk pregnancies, many professional societies such as the International Society for Prenatal Diagnosis (ISPD), the National Society of Genetic Counselors (NSGC), and the American College of Obstetrics and Gynecology (ACOG) approve, though do not proactively recommend cffDNA testing only for high-risk women. These professional bodies advise the deployment of NIPT as a “secondary” screening tool for women who are deemed to be at increased risk due to age, history or positive primary screening tests. NIPT using cffDNA is still a screening test and an abnormal result must always be confirmed with invasive prenatal diagnosis.

While the results from the studies in high-risk cohorts were very encouraging, until recently, it was quite unclear about the performance of NIPT in a general average-risk population. Recently, Bianchi et al⁴ published the results of a multicentric cohort comparison of standard aneuploidy screening and cffDNA test in a low-risk population with a mean maternal age of 29.6 years. The authors found that both the tests achieved 100% detection rate for trisomy 21 and 18, but the cffDNA test was 10 times better in predicting cases of Down syndrome compared to the standard blood test and ultrasound screening, and 5 times better in predicting trisomy 18. The number of false-positive results was also greatly reduced, thereby reducing the number of unnecessary invasive tests. Dr Bianchi commented that “9 out of 10 women who are currently being referred for further testing would not need invasive tests”. Given the result of this study and another large study from China,⁵ it is likely that cffDNA testing will increasingly be offered to all pregnant women within the next few years as a primary screening tool. Already, the most recent guidelines from the American College of Medical Genetics (ACMG) do not distinguish between ‘high-risk’ and ‘low-risk’ populations, although

they continue to recommend confirmatory invasive testing for definitive diagnosis. Indeed, the appeal of these non-invasive tests to the consumer are such that the industry has managed to popularise these tests even before large prospective trials, and before regulatory and academic bodies are able to issue any guidelines regarding the use of these tests. Test companies have pursued various strategies to build consumer demand including reaching out to expectant mothers through the effective use of social media like Facebook and YouTube.

However, patients need to be informed that tests using cfDNA do not provide information about some disorders that are identified through standard combined ultrasound and serum biochemistry screening. False positives of cfDNA tests do occur. Possible underlying causes for such discordance could be confined placental mosaicism, maternal chromosomal abnormalities and very rarely, an occult maternal malignancy.

Pandora's Box?

A concern is the push from industry to introduce less validated tests in the screening package, in order to surge ahead in the race. Companies are hurrying to add various chromosomal abnormalities, such as microdeletions associated with DiGeorge syndrome, to the list of disorders for which they can test. On one hand, routine screening for microdeletions could enable more families to prepare for children with special needs. However, the physical and mental effects of such microdeletion syndromes are not always entirely predictable. A positive test result might lead to unnecessary invasive tests and, in some instances, prompt difficult decisions for families that receive such diagnoses. There are more difficult ethical issues on the horizon. In 2010, Lo et al⁶ reported that the entire fetal genome was represented in short cfDNA fragments in the maternal plasma and suggested that the reconstruction of substantially complete inherited complement was technically attainable. Genome-wide inherited and de novo variations can be determined prenatally without risk to the mother or fetus.⁷ The problem no longer lies with the technique, but with the meaningful translation of the findings and ethics. A serendipitous finding and discovery of genetic variants of uncertain significance will certainly lead to challenging clinical situations.

Conclusion

Without doubt, introduction of NIPT based on cfDNA testing is the big-bang development that has taken place in obstetrics in the 21st century. It is rapidly moving from a secondary screening tool for high-risk pregnant patients to a primary screen for all pregnant women. Unfortunately,

cfDNA testing is still expensive and cost remains prohibitive for many women, although the cost is heading downwards. The outcome of several ongoing patent lawsuits in the United States between all the major test companies may alleviate or exacerbate this cost issue. In Singapore, these tests have been available since 2013, but at a cost almost similar to an invasive diagnostic test. However, there are no local laboratories performing the test here and hence, the maternal plasma samples need to be sent to laboratories overseas. Establishment of a local laboratory would certainly help to reduce cost and minimise turnaround time. Unlike invasive diagnostic tests such as amniocentesis and CVS, there are no government subsidies or Medisave cover for NIPT at present. As a direct consequence, NIPT is becoming a popular screening test for the privately cared, while low-income families infrequently take up the test. This raises concerns that unless subsidies are made quickly available, it may lead to significant disparity in access to optimum prenatal care.

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