EDITORIAL

Maternal plasma DNA: a major step forward in prenatal testing

J Med Screen 2012;**19**:57–59
DOI: 10.1258/jms.2012.012059

Almost twenty-five years have passed since the introduction of prenatal screening for Down's syndrome using multiple markers. During that time there has been steady improvement in performance and clinical acceptance worldwide. Such screening has been the way to best determine who, among the population of pregnant women, are at the highest risk of having an affected pregnancy and, therefore, are most appropriately offered invasive procedures (amniocentesis and chorionic villus sampling) for karyotype analysis. Using the most informative screening tests now available, 90% of all Down's syndrome cases can be identified among a high-risk group consisting of 2% of all screened pregnancies; the group risk is about 1 in 20. While this is relatively good performance, it means that 10% of Down's syndrome pregnancies are missed while 19 of every 20 pregnancies classified as screen-positive are false-positive and will be offered invasive diagnosis with the small, but real, possibility of a procedure-related miscarriage.

With the publication of two 'proof of concept' papers in 2008,[1,2] and with a number of larger studies published this past year,[3–9] a major improvement in prenatal screening for Down's syndrome has emerged and appears ready for clinical implementation. It relies on a genotypic rather than a phenotypic approach and utilizes 'next-generation' sequencing, the simultaneous sequencing of many thousands or millions of DNA fragments. These fragments can be found in maternal plasma, where a small proportion is of fetal/placental rather than maternal origin.[10] The proportion of DNA fragments mapping to chromosome 21 can then be assessed to identify a Down's syndrome fetus. Similarly, chromosomes 18, 13, X and others can be assessed for aneuploidy.

The 'fetal fraction' of cell-free DNA in the maternal circulation is usually in the range of 5% to 25% of the total circulating DNA. This hallmark finding provided the biological basis on which to develop a testing strategy. Contrast that with the intensively studied search for fetal cells in the maternal circulation, which represent at best a few cells per million maternal cells.[11] It is clear that measurement of fetal DNA provides much higher initial concentration. What was needed was a breakthrough in DNA sequencing technology. Next-generation sequencing has provided laboratories with the ability to sequence (or partially sequence) millions of DNA fragments at a time. This allows for computer matching of each sequenced fragment (or as many fragments as reasonable) to a particular chromosome. Thus, the ability to discern a fetal trisomy on a background of cell-free DNA from a euploid mother is an exercise in analytical precision. It is simple in concept, but massive in scale, relying on the latest methods in molecular genetics.[1–5] A fetus affected by a trisomy will have a 50% increase in that chromosome's contribution to the total genome sequenced. That increase is diluted according to the proportion of fetal DNA present in the maternal plasma sample. For example, if the fetal fraction is 10%, then the 50% increase in the expected chromosome percentage is diluted 10-fold, so that the increase measured in the maternal plasma will be, on average, only 5% (one-tenth of the maximum 50% increase). Consequently, the expected increase in the percentage of the trisomic chromosome will be, on average, one-half the fetal fraction of the sample. Sequencing must be precise enough to reliably measure these small differences.

There are various ways to implement and interpret such testing using next-generation sequencing. One method is to sequence fragments representing the entire genome.[4,5] Another is to target sequencing to fragments that map only to chromosomes of interest.[9] A third relies on the comparison of highly heterogeneous neighboring single nucleotide polymorphisms (tandem SNPs) measured in maternal plasma with those same SNPs from the mother (by examining maternal white blood cell DNA in the same sample).[12] The latter two methods require fewer fragments to be sequenced, but each method has its advantages and disadvantages. When all chromosomes are examined, the determination of many more full and partial numerical disorders is possible, but potentially at lower throughput and higher cost. When only specific chromosomes are examined, a more limited interpretation is possible, but with higher throughput and potentially lower cost. At this time, all published methods appear to be effective; it is too early to say if any one will be clearly superior.

How well does maternal plasma DNA sequencing identify fetal Down's syndrome? A cumulative estimate of test performance, based on the studies that have been published since the 2008 proof-of-concept papers, indicates that detection rates (sensitivity) of the tests for the common aneuploidies can be as high as 99%, with overall false-positive rates (1-specificity) of less than 0.5%. Such high performance, however, is still not quite as good as that of invasive testing and karyotype analysis, in which rare mistakes are thought to be the result of sampling errors or contamination, rather than test error. With a karyotype, the numerical disorder is seen or not seen, while with DNA sequencing a statistical result is reported. For this reason, and because the false-positive rate is not zero, it is important that, at least in the near future, a positive DNA sequencing result be followed by the offer of an invasive diagnostic test. In addition to positive results, the DNA test may be uninformative, or may fail. The most common reason for a failure is inadequate fetal fraction in the maternal plasma sample (usually less than 4% or 5%) or a technical problem in

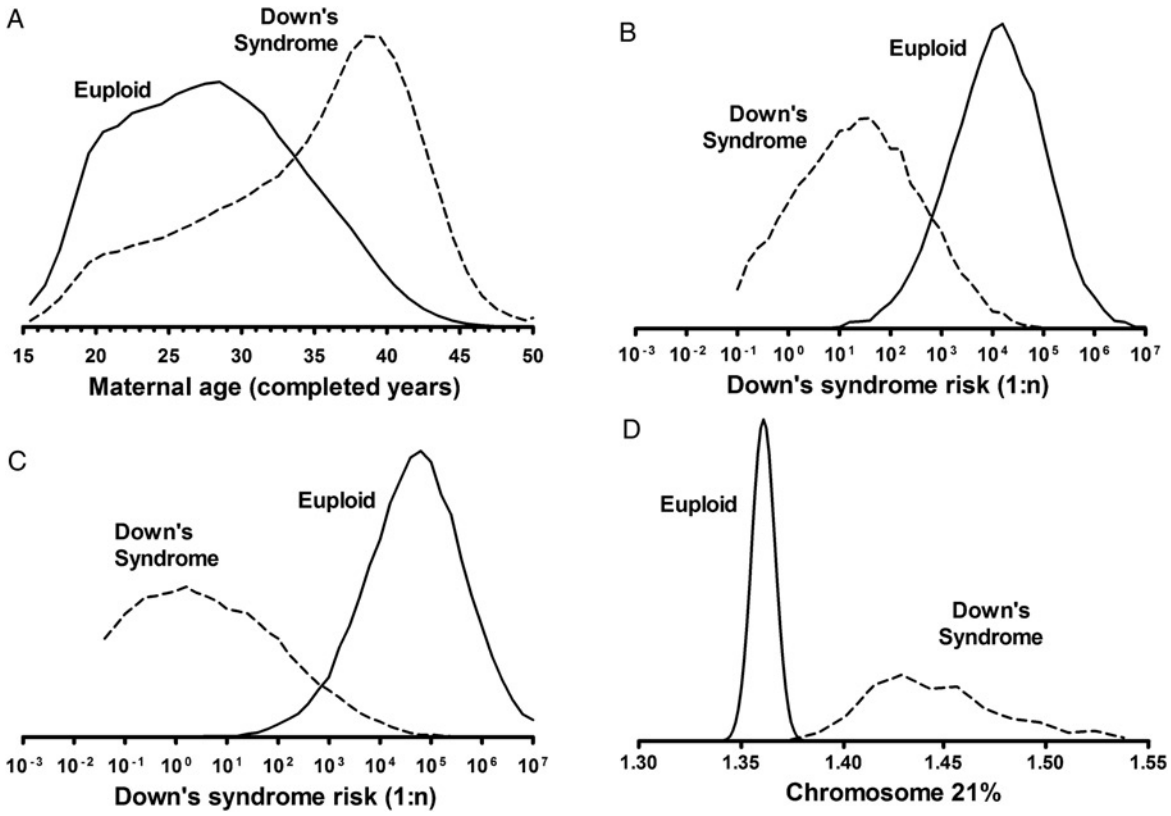

Figure 1 Sets of overlapping distributions for four Down's syndrome tests. Panel A shows the modelled performance of maternal age to identify Down's syndrome, using the 2009 maternal age distribution among the 4,248,000 deliveries in the United States. Panel B shows modelled Down's syndrome risks for the quadruple test (combining maternal age and four second trimester serum markers), in 20,000 Down's syndrome and 20,000 euploid pregnancies based on SURUSS parameters.[13] In like manner, panel C shows modeled Down's syndrome risks for the integrated test (an ultrasound measurement of nuchal translucency and one first trimester serum marker, combined with the quadruple second trimester markers), based on SURUSS parameters.[13] Panel D shows the overlapping distribution of adjusted chromosome 21 percentages from a clinical validation study involving 212 Down's syndrome and 1,484 euploid pregnancies.[6] In all panels, the dashed lines indicate the distribution for Down's syndrome pregnancies, with the solid line showing the distribution for euploid pregnancies.

preparing or running the DNA library. In our recent collaborative study,[6] the uninformative rate was less than 1%. These women could be offered invasive diagnostic testing or, potentially, a repeat sampling might be successful.

An important question at this early point in clinical implementation is whether the results of maternal plasma DNA testing can be interpreted dichotomously or must remain quantitative. In other words, is there a high test result above which the likelihood of a false-positive is so small, and a low value below which the likelihood of a false-negative is also so small that a definitive interpretation can be provided? The answer lies in the shape and overlap of the distributions of test results among known trisomic and euploid pregnancies. A visual comparison of the distributions of results for selected Down's syndrome tests helps provide the answer (see Figure 1). Historically, maternal age (panel A) was the first screening test, and women aged 35 (or aged 37) and older at delivery were offered diagnostic testing. A major gain in test performance was including results of multiple serum tests, culminating in the second trimester 'quadruple' test (panel B) and the first trimester 'combined' test using ultrasound measurement of nuchal translucency and two serum markers, with similar improvement in separation between the test results (risks) in euploid and Down's syndrome pregnancies.[13] The best current test is the integrated test and its sequential variant (a combination of first trimester serum and ultrasound markers with second trimester serum markers)[13,14] (panel C). Again, the separation is improved. The last panel (D) shows the performance of massively parallel sequencing, with the distribution of chromosome 21 percentages (after correcting for plate-to-plate variability) in the two populations.[6] There is very little overlap. It is possible that some day only those results in this 'grey zone' will require diagnosis by CVS or amniocentesis. And, we can expect that the 'grey zone' will get smaller with improvements in methodology.

Currently, all published DNA studies have been performed in cohorts of 'high-risk' pregnancies; those identified through prenatal screening, abnormal ultrasound findings, advanced maternal age, or previous history of aneuploidy. This fact, along with practical issues with the test's clinical application, such as limited availability, turnaround time of seven days or more, high costs and lack of universal coverage, have led to the view that only women with 'high-risk' pregnancies are eligible for maternal plasma DNA testing. DNA testing can identify nearly all of the common trisomies in this high risk group, while avoiding invasive testing for the vast majority of women with euploid fetuses. While these practical issues currently limit test availability, there is no clear scientific reason for limiting the test to high-risk pregnancies. A

'low-risk' pregnant woman may differ from one at high-risk because she is younger, or because she has unremarkable serum marker values, or a negative ultrasound scan, not because the underlying cause of a certain trisomy is different. The DNA test determines a difference in genotype, not in phenotype. Therefore, there is no reason to expect the DNA test not to perform in all pregnant women. Some indirect evidence of this comes from our large collaborative study on high-risk pregnancies. Regardless of the reason for high risk, the performance of the DNA test did not change.[6] Independent of the reason for referral, women with a Down's syndrome pregnancy have similarly high test results and women with a euploid pregnancy have similarly low test results. It will be necessary to plan and examine the introduction of such testing into the general pregnancy population. Appropriate validated education materials need to be developed, and research into the ethical and social aspects of a new paradigm of screening will no doubt occur.

Where might DNA testing of maternal plasma move in the future? One can expect that test performance will improve, and that current implementation issues will be solved (e.g. turn-around time and sample collection), as methods, platforms and supplies continually improve. Sequencing more base pairs on each fragment, and sequencing more fragments might also allow for the identification of a large number of rare, but clinically important deletion/duplication syndromes. This methodology has already been used to identify the fetal carrier status for β-thalassemia,[15] suggesting that mutation-based testing for monogenic diseases is also possible.

In addition, intellectual property lawsuits have been filed and their outcome, as well as the technical aspects of the different methodologies, will influence how testing will proceed in the future. Whatever DNA testing methodologies emerge, we can look forward to a new, safer chapter in prenatal screening and diagnosis.

Declaration of interests: From November 2008 through to February 2012, Drs Canick and Palomaki were co-principal investigators of the InFANet Study through a research agreement between WIH and Sequenom Inc.

Jacob A Canick and Glenn E Palomaki

Division of Medical Screening and Special Testing, Department of Pathology and Laboratory Medicine, Women & Infants Hospital and Alpert Medical School of Brown University, Providence, RI, USA

Correspondence to: Jacob A Canick, PhD, Medical Screening and Special Testing, 70 Elm Street, Providence, RI 02903 USA
jcanick@wihri.org

REFERENCES

1 Fan HC, Blumenfeld YJ, Chitkara U, Hudgins L, Quake SR. Noninvasive diagnosis of fetal aneuploidy by shotgun sequencing DNA from maternal blood. *Proc Natl Acad Sci USA* 2008;**105**: 16266–71
2 Chiu RW, Chan KC, Gao Y, *et al.* Noninvasive prenatal diagnosis of fetal chromosomal aneuploidy by massively parallel genomic sequencing of DNA in maternal plasma. *Proc Natl Acad Sci USA* 2008;**105**: 20458–63
3 Chiu RW, Akolekar R, Zheng YW, *et al.* Non-invasive prenatal assessment of trisomy 21 by multiplexed maternal plasma DNA sequencing: large scale validity study. *BMJ* 2011;**342**:c7401
4 Ehrich M, Deciu C, Zwiefelhofer T, *et al.* Noninvasive detection of fetal trisomy 21 by sequencing of DNA in maternal blood: a study in a clinical setting. *Am J Obstet Gynecol* 2011;**204**:205 e1–11
5 Sehnert AJ, Rhees B, Comstock D, *et al.* Optimal detection of fetal chromosomal abnormalities by massively parallel DNA sequencing of cell-free fetal DNA from maternal blood. *Clin Chem* 2011;**57**:1042–9
6 Palomaki GE, Kloza EM, Lambert-Messerlian GM, *et al.* DNA sequencing of maternal plasma to detect Down's syndrome: an international clinical validation study. *Genet Med* 2011;**13**:913–20
7 Palomaki GE, Deciu C, Kloza EM, *et al.* DNA sequencing of maternal plasma reliably identifies trisomy 18 and trisomy 13 as well as Down's syndrome: an international collaborative study. *Genet Med* 2012;**14**:296–305
8 Bianchi DW, Platt LD, Goldberg JD, *et al.* Genome-wide fetal aneuploidy detection by maternal plasma DNA sequencing. *Obstet Gynecol* 2012;**119**:890–901
9 Sparks AB, Wang ET, Struble CA, *et al.* Selective analysis of cell-free DNA in maternal blood for evaluation of fetal trisomy. *Prenat Diagn* 2012;**32**:3–9
10 Lo YM, Corbetta N, Chamberlain PF, *et al.* Presence of fetal DNA in maternal plasma and serum. *Lancet* 1997;**350**:485–7
11 Bianchi DW, Simpson JL, Jackson LG, *et al.* Fetal gender and aneuploidy detection using fetal cells in maternal blood: analysis of NIFTY I data. National Institute of Child Health and Development Fetal Cell Isolation Study. *Prenat Diagn* 2002;**22**:609–15
12 Rabinowitz M, Gemelos G, Banjevic M, *et al.* A novel and highly accurate method for noninvasive prenatal diagnosis using parental gentoypes. Abstract. Presented at the Society for Maternal-Fetal Medicine Meeting, Dallas, TX, February 6–11, 2012
13 Wald NJ, Rodeck C, Hackshaw AK, *et al.* First and second trimester antenatal screening for Down's's syndrome: the results of the Serum, Urine and Ultrasound Screening Study (SURUSS). *J Med Screen* 2003;**10**:56–104
14 Wald NJ, Watt HC, Hackshaw AK. Integrated screening for Down's syndrome on the basis of tests performed during the first and second trimesters. *N Engl J Med* 1999;**341**:461–7
15 Lo YM, Chan KC, Sun H, *et al.* Maternal plasma DNA sequencing reveals the genome-wide genetic and mutational profile of the fetus. *Sci Transl Med* 2010;**2**:61ra91