Non-invasive prenatal diagnosis — toward a new horizon

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Could a technique using fetal DNA from the maternal circulation mean the end of invasive prenatal testing?

Since the first application of ultrasound to it 50 years ago, the "black box" that is the pregnant uterus has gradually yielded to efforts to gain increasingly precise information about the condition of the fetus. Technological advances in ultrasound and, more recently, magnetic resonance imaging have seen the production of high-resolution displays of fetal anatomy and physiology. Specific information about the fetal genome, however, has until now only been available through the application of invasive techniques. Amniocentesis and chorionic villus sampling have been used to diagnose fetal aneuploidy (such as Down syndrome) and an ever-increasing range of genetic conditions. But these invasive procedures come at a cost — an associated 1% risk of pregnancy loss[1] limits their application and causes significant stress for women thinking of undertaking such testing. Screening tests such as ultrasound and maternal serum screening to assess aneuploidy risk have been developed to allow more judicious application of invasive procedures, but of themselves cannot accurately diagnose the fetal condition, and false negatives and positives both occur.

Now, the era of non-invasive prenatal diagnosis beckons — offering the ability to obtain specific and accurate genetic information from the fetus without the need for an invasive procedure and its associated risks. The article by Hyland and colleagues in this issue of the Journal (*page 21*) reports on the first potential clinical application of non-invasive prenatal diagnosis in an Australian population — the determination of fetal *RHD* status in Rhesus (Rh) D-negative women.[2]

About 17% of Australian women are RhD-negative.[3] These women are at risk of isoimmunisation, caused by the passage of RhD-positive fetal red blood cells across the placenta during pregnancy and childbirth. The resultant antibodies produced by the mother can cross the placenta and cause fetal anaemia due to haemolysis of fetal red blood cells. Women who are isoimmunised require intensive fetal surveillance, and are at risk for fetal death and neonatal brain injury if timely intervention is not performed. The incidence of isoimmunisation has been dramatically reduced since the introduction of preventive strategies, but these rely on administration of plasma-derived RhD immunoglobulin to all RhD-negative women.

The technique employed by Hyland et al involves detecting cell-free fetal DNA (ffDNA) in maternal plasma by real-time polymerase chain reaction (PCR).[2] ffDNA constitutes about 4%–6% of all cell-free DNA in the maternal circulation and can be reliably detected as early as 7 weeks after conception.[4] The use of ffDNA to detect the *RHD* gene in RhD-negative pregnant women was first described by Lo and colleagues in 1998.[5] Others have since applied the technique, and a recent meta-analysis demonstrated 96.1% sensitivity and 96.5% specificity for correctly determining fetal *RHD* status.[6] To minimise false-negative results, internal controls are used to confirm the presence of fetal DNA. The *SRY* gene was initially used for this purpose, but as this only detects male fetuses, Hyland and colleagues have also utilised the *RASSF1A* gene to confirm the presence of fetal DNA from female fetuses.[2] Where they were able to determine the fetal *RHD* status, their predictions showed 100% accuracy when compared with the infants' serotype determined from cord blood after delivery. In a small number of cases, determination of fetal status could not be achieved.

This technique requires further evaluation in larger studies to clarify the true performance characteristics of the test, and to allow algorithms to be developed for dealing with indeterminate results and the *RHD* gene variants seen in some ethnic groups. However, if these issues can be satisfactorily addressed, the potential applications are significant. In the small group of women with isoimmunised pregnancies, it will allow the determination of fetal *RHD* status without resorting to invasive tests. In addition to the risk of pregnancy loss, invasive procedures also have the potential to cause further sensitisation and worsen the natural history of the condition.

More broadly, this testing has the potential to reduce unnecessary exposure of pregnant women to RhD immunoglobulin. Currently, RhD immunoglobulin is administered for antenatal prophylaxis, potential sensitising events, and postnatally for RhD-positive infants. This regime has been very effective, reducing the incidence of sensitisation during pregnancy by more than 90%.[7] However, up to 40% of RhD-negative women receive treatment unnecessarily, as they will be carrying an RhD-negative fetus from a heterozygous partner.[8] Routine introduction of non-invasive fetal DNA testing would therefore be likely to have cost implications for delivery of this preventive treatment. Non-invasive detection of fetal *RHD* status is already being incorporated into clinical practice in Europe, and the feasibility of using this approach to make decisions about prophylaxis has been demonstrated.[9]

The use of ffDNA can also be applied more broadly than determination of fetal *RHD* status. Already, reports exist of the non-invasive prenatal diagnosis of Huntington disease,[10] cystic fibrosis[11] and other conditions. Detection of the *SRY* gene allows determination of fetal sex in women at risk of carrying a fetus with an X-linked condition.[12] The non-invasive diagnosis of Down syndrome and other aneuploidies remains challenging, but newer techniques such as methylation-dependent PCR and digital PCR hold promise.[13]

As with many technological advances involving reproductive choices, there are ethical concerns associated with this test. Sex determination for social rather than health reasons, for example, could result from unregulated use of such technologies. There is a pressing need for vigorous discussion of these issues.

Non-invasive diagnosis of specific fetal gene status using fetal DNA from the maternal circulation without any risk to the pregnancy may be the "holy grail" of prenatal diagnosis. The determination of fetal *RHD* status is nearing availability as a clinical tool, and is likely to be the first of many applications of non-invasive prenatal testing over the coming decade. Is a future without amniocentesis and chorionic villus sampling dawning? Not quite yet, but at least there is some light on the horizon.

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