What's New in Fetal DNA Testing

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It has long been the goal of obstetrics and genetics to be able to carry out non-invasive prenatal diagnosis and during the latter part of the last decade this has eventually started to become a reality [1]. Since the 1970s, testing for both chromosomal abnormalities and for single gene disorders has been an invasive procedure carrying a small but finite risk to the fetus. Amniocentesis, performed at around 14–16 weeks of pregnancy, and chorionic villous sampling, performed during the first trimester, both carry a risk of miscarriage of 1% and 1–2%, respectively.

Free Fetal DNA

In 1997 it was demonstrated that cell free fetal DNA (ffDNA) was present in the maternal plasma or serum [2] and this constitutes 3–6% of the total cell free DNA in maternal plasma. ffDNA levels rise during pregnancy and can reliably be detected from 6–9 weeks. A major advantage of ffDNA is that it is rapidly degraded following delivery and hence will not interfere with prenatal diagnosis in future pregnancies.

The basic premise of all work in this area is that the fetal DNA can be distinguished from the much higher background level of maternal DNA. There are two problems which need to be overcome.

Firstly, ffDNA is present at much lower levels than cell free maternal DNA. The fraction of ffDNA present can be increased somewhat by selective enrichment as ffDNA

is much shorter. Typically, maternal blood is separated into the plasma and cellular fractions and ffDNA is isolated from the former.

Secondly, the fetus inherits half of its genetic make-up from its mother, so half of the fetal DNA present will be identical to the maternal DNA. The simplest way to achieve high sensitivity and specificity is to use as a target a genetic variant which is absent from the mother. Initial methods have therefore examined paternally derived sequences.

Clinical Applications

Sex Determination

The first obvious use was to carry out sex determination for sex-linked disorders such as Duchenne muscular dystrophy or haemophilia A. This prevents an unnecessary invasive prenatal test if a woman is carrying a female fetus. Sex determination in women at risk of a fetus with congenital adrenal hyperplasia is also important as treatment during pregnancy can prevent masculinisation of female fetuses.

The approach used is to detect Y-derived sequences, such as the sex-determining region Y, SRY, or the multi-copy Y-specific marker, DYS14, from male fetuses using sensitive techniques such as real-time PCR. A female fetus is inferred from a negative result but as this could be due to a failure of the PCR reaction, or to a very low level of

ffDNA, the possibility of false negative results is inherent in the test. The recent introduction of fetal specific markers to ensure that fetal DNA is present can therefore reduce part of this risk. These markers depend on epigenetic differences between the maternal gene and the fetal gene, the best two studied examples being areas within the promoter of two tumour suppressor genes, *mapsin* and *RASS-F1A*. Overall the accuracy of sex determination has been shown to be very high with a sensitivity of over 99%.

Rhesus Testing

Rhesus D-negative pregnant women are routinely offered anti-D antibodies regardless of fetal Rhesus status to prevent Rhesus incompatibility. However, a significant number of these women receive the antibody unnecessarily if they carry a Rhesus-negative fetus. ffDNA analysis for determination of Rhesus D fetuses in women who have previously had a pregnancy affected with Rhesus disease has been introduced in many countries and enables appropriate and effective use of anti-D antibodies in this patient group. If ffDNA analysis can be introduced more widely for all D-negative women during pregnancy, the costs of anti-D treatment can be significantly reduced.

Diagnosis of Single Gene Disorders

ffDNA analysis of single gene disorders has the ability to significantly improve prenatal testing by removing the need for invasive tests. To date this has only proven to be possible by excluding the presence of paternally derived mutations or de novo mutations in the fetus. It has been applied to disorders such as Huntington's disease or myotonic dystrophy where the mutation has been confirmed as paternal in origin. It has also been applied in recessive disorders such as cystic fibrosis or β -thalassaemia where the mother and father carry different mutations and where it is therefore possible to exclude the paternally derived mutation.

Diagnosis of Common Aneuploidies

Much of the current excitement in the area of ffDNA is the ability to use this material as the target to identify

chromosomal aneuploidies such as trisomy 21 (Down's syndrome) and trisomy 18 (Edwards' syndrome) as the risk of these abnormalities is the most common indication for invasive prenatal diagnosis. Here the approach required is the accurate quantification of DNA from the relevant chromosome. One approach has been to look, not at DNA, but at mRNA expressed from the placenta using a gene on the relevant chromosome. mRNA from the *PLAC4* gene on chromosome 21 can be detected in the plasma of pregnant women and an informative polymorphic marker within that gene has been used to identify the presence of two copies of chromosome 21. In a normal fetus, the ratio of the two alleles on the two copies of chromosome 21 should be 1:1 whereas in a fetus with trisomy 21 the ratio will be 2:1. This technique has been limited by the need to find a highly informative polymorphism to differentiate the two chromosomes. Although the marker within the *PLAC4* gene is useful it is not on its own sufficient to provide reliable results on all cases and additional chromosome 21 markers within placentally transcribed genes will be required.

Two other promising alternative approaches have been reported. In very preliminary studies, on small numbers of samples, it has been shown that it is possible to measure chromosome dosage directly using highly sensitive techniques such as digital PCR or shot gun sequencing.

Overall the use of ffDNA for prenatal diagnosis should be with us as a routine procedure in the near future and will significantly reduce the number of invasive prenatal tests. Reliable detection of chromosomal aneuploidies could eventually replace biochemical tests as the front-line screening test during pregnancy.

References

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- 2 Lo YMD, Corbetta N, Chamberlain PF, Rai V, Sargent IL, Redman CW, Wainscoat JS: Presence of fetal DNA in maternal plasma and serum. *Lancet* 1997;350:485–487.