Noninvasive Fetal Blood Grouping: Present and Future

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• Noninvasive prenatal diagnosis • Fee fetal DNA
• Blood groups • Rh • Genotyping

Hemolytic disease of the fetus and newborn (HDFN) is caused by immunoglobulin (Ig)G antibodies to red cell surface antigens crossing the placenta and facilitating the immune destruction of fetal red cells or erythroid progenitors. The most common culprit antibody is directed to the D (RH1) blood group antigen of the Rh system. The reason for testing for fetal D phenotype in pregnant women with anti-D is to assist in the management of pregnancy. If the fetus is D-positive, appropriate management of a pregnancy at risk from HDFN can be arranged. If the fetus is D-negative, then it is not at risk, and no unnecessary interventions are required. Obtaining fetal red cells for serologic testing is a difficult and risky procedure, but cloning of the Rh genes in early 1990s[1–3] and the subsequent elucidation of the molecular bases to the D polymorphism[4,5] made it possible to predict D phenotype from the genotype obtained from fetal DNA. This now can be done with a high degree of accuracy. Fetal typing from DNA has been provided as a service in England since 1994. Initially the source of this fetal DNA was amniocytes or chorionic villi, but the procedures for obtaining these materials are expensive, invasive, and present a risk to the fetus. Amniocentesis is associated with a 0.5% to 1% risk of spontaneous abortion.[6] In addition, amniocentesis is associated with a 17% risk of transplacental hemorrhage,[7] which, if the fetus were D-positive, could boost the maternal anti-D, enhancing the risk of severe HDFN.

MATERNAL PLASMA AS A SOURCE OF FETAL DNA

Detection of Fetal DNA in Maternal Plasma

When Lo and colleagues[8] found fetal Y-chromosome sequence in the plasma of pregnant women bearing male fetuses, the implications for prenatal diagnostics without the requirement for invasive procedures were obvious. The main complication is that a very low concentration of fetal DNA is present in the maternal plasma. Lo and

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colleagues[9] used quantitative real-time polymerase chain reaction (PCR) analysis on *SRY* and β-globin gene sequences in pregnant women with a male fetus to show that fetal DNA represents about 3% (mean 25 genome equivalents/mL, range 3 to 69) of cell-free DNA in maternal plasma during the first trimester of pregnancy, rising to about 6% (mean 292 genome equivalents/mL, range 77 to 769) in the third trimester. Higher levels, up to 10%, have been found in other studies.[10,11] Free fetal DNA has been detected as early as 4 weeks' gestation.[12] Cell-free DNA in plasma of pregnant women consists of longer fragments than in nonpregnant women, whereas fetal fragments are much shorter than maternal fragments, with most of the fetally derived DNA molecules less than 0.3 kilobase (kb) in length.[13,14] Although enrichment of fetal DNA can be achieved by exploiting this differences in fragment size between fetal and maternal DNA, complete separation has not proved possible. Consequently, the only diagnostic tests on free fetal DNA in maternal plasma that are used routinely are those where the target gene is not present in the mother. These are fetal sexing by detection of a Y-borne gene and fetal blood grouping in women whose red cells lack the corresponding antigen.

Fetal DNA is cleared rapidly from the maternal plasma following delivery, with a mean half-life of 16 minutes following Caesarean section,[15] but clearance may take longer following labor, possibly to as much as 2 weeks.[16]

The high turnover of fetal DNA in maternal plasma, demonstrated by its rapid disappearance following delivery, suggests that fetal DNA is liberated continuously into the maternal circulation in large quantities.[15] The principal source of the fetal DNA is the placenta: normal levels of free fetal DNA were detected in anembryonic pregnancies with a placenta but with no fetus.[17] Villous trophoblasts within the fetal compartment of the placenta are released into the maternal blood via the feto–maternal interface and destroyed rapidly by the maternal immune system.[18,19] In addition, apoptosis in the villous trophoblasts in situ might lead to release of DNA into the maternal circulation.[20]

Does Fetal DNA Persist in the Maternal Plasma After Delivery?

Microchimerism, the engraftment of fetal cells in maternal lymphoid organs or bone marrow, leads to the persistence of very small numbers of nucleated cells in the mother's circulation many years after the pregnancy.[21] In 2002, Invernizzi and colleagues[22] detected Y-chromosome-specific sequences in plasma from 22% of healthy women with sons, in some cases many years after their last male pregnancy. Lambert and colleagues[23] found that DNA from previous pregnancies could be detected in DNA prepared following gentle centrifugation (400 g) of maternal plasma, but it was removed by passing the plasma through a 0.45 μm filter. This indicates that the DNA detected from previous pregnancies originates from cellular material, rather than free DNA in the plasma. Chiu and colleagues[24] recommend that if filtration is not employed, plasma should be microcentrifuged for 10 minutes at full speed (approximately 16 000 g) to remove all cellular material after the initial plasma separation.

PREDICTION OF D PHENOTYPE FROM FETAL DNA

The D Polymorphism

The antigens of the Rh system are encoded by *RHD* and *RHCE*, a pair of paralogous genes on chromosome 1. Each gene has 10 exons, and they share 94% sequence identity. They produce homologous proteins of 417 amino acids that are palmitoylated but not glycosylated. The proteins traverse the red cell membrane 12 times, with both termini in the cytosol and six extracellular loops, the potential sites for antigen activity.[25]

In Caucasians, where the frequency of D-negative phenotype is around 15%,[25] almost all D-negative individuals are homozygous for a deletion of the whole of *RHD*