A Noninvasive Test to Determine Paternity in Pregnancy

TO THE EDITOR: Five percent of women who are raped become pregnant, which results in an estimated 32,000 pregnancies annually in the United States. In many circumstances, it is unclear whether the pregnancy resulted from the rape or from consensual intercourse. The only options available for prenatal paternity determination are invasive tests, such as the sampling of chorionic
villi and amniocentesis, that carry a risk of miscarriage and are not performed before 10 to 15 weeks of gestation. Because 78.9% of terminations of unintended pregnancies are carried out before 10 weeks,² it seems likely that many rape victims terminate pregnancies before testing for paternity. A noninvasive prenatal paternity test based on cell-free fetal DNA present in maternal blood, performed at 8 weeks of gestation or later, could provide a safe option for determining paternity.

Previous studies of noninvasive prenatal paternity testing have shown that amplification of fetal alleles from maternal blood is suppressed by the presence of cell-free maternal DNA.³ Furthermore, fetal DNA in maternal plasma is highly degraded. These limitations can be overcome by first adding a fixative to maternal blood samples.

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**Figure 1. The Use of Informative Single-Nucleotide Polymorphisms (SNPs) to Determine Paternity.**

An informative SNP is one in which the mother and one of the two potential fathers are homozygous for the same allele, whereas the other potential father is homozygous for the alternative allele. For example, the maternal (M) DNA and paternal 2 (P2) DNA in Panel A are homozygous (AA genotype) and the paternal 1 (P1) DNA is homozygous (GG genotype) at this informative SNP. In the maternal plasma (PL), the fetal DNA has the maternal allele (A allele) and paternal allele (G allele) at the informative SNP site. Paternal 2 does not have a G allele and therefore does not match the fetal DNA signal in maternal plasma. Paternal 2 can thus be excluded as a potential father. In Panels B and C, sequencing gel images of two informative SNPs show that the man with paternal 2 DNA cannot be the father and that paternal 1 is the biologic father of the fetus.
to stabilize cell membranes and prevent the release of maternal DNA into the plasma. By using single-nucleotide polymorphisms to distinguish fetal DNA from maternal DNA (Fig. 1), one can use short amplicons (shorter than 75 bp) to minimize allele dropout (absence of a fetal DNA signal when one should be present).

We collected blood samples from 30 women with pregnancies of 8 to 14 weeks of gestation. Each maternal blood sample was paired with blood from the biologic father and then randomly grouped with 1 of 29 samples from unrelated men. The 3 samples in each group were processed in a blinded manner. We determined paternity correctly for all 30 samples, by comparing the genetic profile of fetal DNA in maternal blood with those of the 2 “paternal” samples (1 genuine, 1 not) (Table 1 in the Supplementary Appendix, available with the full text of this letter at NEJM.org). The odds of identifying the correct father for all 30 samples are less than 1 out of 1 billion ($P = 1.86 \times 10^{-9}$). Our approach shows that noninvasive prenatal paternity testing can be performed within the first trimester with the use of a maternal blood sample.

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