allele-specific PCR, or TaqMan-type assays), our method is more sensitive but requires two steps [two rounds of amplification (PCR + LDR) are needed]. The costs for setup are larger (PCR primers and three labeled oligonucleotides are needed per SNP) with respect to PCR/RFLP and allele-specific PCR but are similar for the TaqMan assay. The main advantage consists in the inherent multiplexing capability that can increase throughput, which decreases cost and time per scored SNP. The capability of performing multiple assays on the same slide further enhances this feature.

To improve the molecular characterization of AD, several other polymorphisms potentially involved in the disease could be included within this analytical system

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References


Placenta Increta: Postpartum Monitoring of Plasma Cell-free Fetal DNA, Masatoshi Jimbo, Akihiko Sekizawa,* Yumi Sugito, Ryu Matsuoka, Kiyotake Ichizuka, Hiroshi Saito, and Takashi Okai (Department of Obstetrics and Gynecology, Showa University School of Medicine, Tokyo 142-8666, Japan; * address correspondence to this author at: Department of Obstetrics and Gynecology, Showa University School of Medicine, 1-5-8, Hatanoda, Shinagawa-ku, Tokyo 142-8666, Japan; fax 81-33784-8355, e-mail sekizawa@med.showa-u.ac.jp)

Antenatal prediction of abnormal adherence of the placenta to the uterine wall is very important in clinical practice because it is associated with high maternal morbidity and a high risk of mortality. We previously reported that the concentration of fetal DNA in maternal plasma is increased in cases of placenta previa, especially in patients with placenta increta and placenta accreta (1). It has been suggested that invasion of trophoblasts into the uterine muscle of these patients produces increased plasma concentrations of cell-free fetal DNA because of the destruction of trophoblasts by the maternal immune system on invasion of the uterine muscle. We earlier proposed that antenatal prediction of abnormal conditions, such as placenta increta, might be achieved by an analysis of fetal DNA in the plasma of high-risk pregnant women, including those with placenta previa and/or a previous history of uterine surgery (1). In the present report, we describe a case of placenta increta in which a small part of the placenta remained adherent despite manual removal of the placenta at the time of delivery. The patient was followed by monitoring the concentrations of plasma human chorionic gonadotropin human chorionic gonadotropin β (hCGβ) and fetal DNA (DYS14) after delivery.

A 37-year-old Japanese woman, gravida 0, para 0, was admitted to Showa University Hospital because of the onset of labor pain at 37 weeks and 3 days gestation. During pregnancy, she did not have vaginal bleeding or symptoms of preeclampsia or preterm labor. The course of her pregnancy was normal until delivery. Her previous medical history was unremarkable, with no history of pelvic inflammatory disease, uterine surgery, or use of intrauterine devices. After the onset of labor, her delivery progressed, and an infant boy (2842 g) was delivered vaginally with an Apgar score of 10 (1 min). After delivery, because the placenta was not delivered spontaneously within 30 min, manual removal of the placenta was performed. The placenta, however, was tightly adherent to the lower posterior part of the uterus, and a small part of the placenta (7 cm in diameter) could not be removed. Through magnetic resonance imaging findings, we diagnosed the patient as having placenta increta and followed her clinical symptoms.

To assess withdrawal of placental villi from the uterus, plasma concentrations of DYS14 and hCGβ were measured (Fig. 1). Maternal blood samples (7 mL) were collected into tubes containing EDTA, and within 3 h the plasma was separated by centrifugation at 3000g for 10 min. DNA was extracted from 1.5 mL plasma with QIAamp Blood mini Kit (Qiagen) in accordance with the manufacturer’s “blood and body fluid protocol”, with only minor modifications. Total DNA was eluted from the columns with 50 μL of water. Subsequently, quantitative PCR of a Y-chromosome-specific DYS14 sequence was performed with maternal plasma and a LightCycler (Roche Diagnostics), as described previously (1,2). The concentration of hCGβ in the maternal plasma was measured with a Ball ELSA free βhCG reagent set (CIS bio international) (2). This study received institutional approval, and written informed consent was obtained.

As shown in Fig. 1, the concentrations of DYS14 gene
and hCGβ in the plasma at 14 h postpartum were 1104 genome-equivalents/mL and 10.5 µg/L, respectively. DYS14 DNA was detected until 10 weeks postdelivery, whereas plasma hCGβ was undetectable by 11 days postpartum. The patient had intermittent vaginal bleeding until DYS14 DNA could no longer be detected in her plasma. The remaining portion of the placenta was spontaneously delivered with a slight increase in uterine bleeding at 10 weeks postpartum. After removal of the placenta, uterine bleeding was not observed.

Previous reports have documented the ability to predict abnormal adherence of the placenta with the use of gray-scale ultrasonography, color Doppler imaging (3), and magnetic resonance imaging. Increased concentrations of creatine kinase (4) or α-fetoprotein (5) in maternal serum have also been reported as biochemical markers of the disease. Predicting the abnormal adherence of the placenta to the uterine wall antepartum, however, is still difficult.

We recently reported that concentrations of cell-free fetal DNA may be increased in the plasma of pregnant women with placenta accreta and placenta increta. Thus, we earlier proposed that fetal DNA in the plasma of pregnant women with placenta previa and/or a previous history of uterine surgery might serve as a molecular marker for detection of abnormal adherence of the placenta (1). In the present report, we describe a case of placenta increta. In this case, we monitored the patient’s plasma concentrations of DYS14 gene and hCGβ after delivery. Although fetal DNA cannot usually be detected in maternal plasma more than 2 h after delivery (6), we observed more than 1000 genome-equivalents of DYS14 gene per milliliter in the plasma of our patient with placenta increta 14 h after delivery. Because the placenta is the only remaining fetal tissue after delivery, the high concentration of DYS14 likely originated from the trophoblasts. The definite origin of fetal DNA within the maternal circulation during pregnancy, however, has not yet been established. That being said, if the observed increase in fetal DNA within the plasma of our patient with placenta increta was from retained placental trophoblasts, it seems reasonable to suggest that trophoblasts at the interface between the fetus and mother might be responsible for the basal concentration of fetal DNA noted in maternal plasma during normal pregnancy.

In this patient, fetal DNA was detected until 10 weeks after delivery, whereas plasma hCGβ could not be detected by 11 days postpartum. The patient had intermittent vaginal bleeding until fetal DNA could no longer be detected in her plasma. Fetal DNA in the plasma of patients with retained placental tissue might reflect trophoblast destruction, whereas plasma hCGβ might reflect trophoblast viability. Because of the high sensitivity of the DNA detection system, however, fetal DNA might possibly be detected at lower concentrations and, thus, over a longer period of decrease than plasma hCGβ.

In conclusion, the decrease in fetal DNA in the patient’s plasma correlated well with her clinical improvement after delivery. We propose that the concentration of fetal DNA in the plasma might be a useful marker by which to follow patients with retained placental tissue after delivery.

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References