From this analysis, we have shown that the novel c.612A→G mutation causes FHBL by disrupting splicing. We identified 3 family members who were heterozygous for this mutation but were largely asymptomatic because each still had 1 normal APOB allele.

This case highlights the difficulty of interpreting novel mutations identified in diagnostic laboratories and the need for a clear strategy to determine their significance. If sufficient family members are not available, linkage analysis may be uninformative and functional analysis is essential.

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Circulating Cell-Free Placental mRNA in the Maternal Plasma as a Predictive Marker for Twin-Twin Transfusion Syndrome

To the Editor:

Twin-twin transfusion syndrome (TTTS), which is a serious complication in monochorionic diamniotic twins (MCDA-T), involves unequal blood flow via the placental vascular anastomoses from the donor to the recipient twin. Although the placental anastomoses are present in all MCDA-T and both fetuses are genetically identical, TTTS occurs in only 15% of MCDA-T, and much of the pathophysiological basis of TTTS remains poorly understood. Clinically, a staging system based on the ultrasound features of TTTS is widely used for the management (1) but not for the prediction of TTTS. In addition, the known predictive findings observable by ultrasonographic examination are detectable only in a small portion of TTTS cases (2). New predictive markers are therefore desirable for the early detection and prevention of TTTS. Recently, placental mRNAs, such as human placental lactogen (PL) and some other hormones were detected in maternal plasma, and concentrations of each marker were measured with quantitative real-time reverse transcription (RT)-PCR assay (3, 4). Thus, circulating cell-free mRNA (cf-mRNA) in maternal plasma has become an attractive target for the noninvasive monitoring of pregnancy disorders (3, 5).

The purpose of the present study was to investigate the use of cf-mRNA concentration in maternal plasma as a predictive marker of later TTTS. The study participants included 17 pregnant women who visited the Obstetrics Clinic of Nagasaki University Hospital at 12–21 weeks of gestation for management of their pregnancy with MCDA-T. Included as a control group were 135 singleton pregnant women without medical complications at similar gestational age. All of the participants gave written informed consent, and the study was approved by the Research Ethics Committee of Nagasaki University. Although none of the 17 cases of MCDA-T were complicated by TTTS at the time of blood sampling, TTTS subsequently developed in 5 cases (TTTS group), but not in the remaining 12 cases (no-TTTS group). Gestational ages at diagnosis of TTTS were 15–25 weeks. The 3 groups had no significant differences in population characteristics, including the maternal age, the number of nulliparous women, and the gestational age at the time of sampling (data not shown).

The blood samples (8 mL) from each woman were collected into an EDTA tube, and the plasma sample was stored at −20°C until use. After cf-mRNA was extracted from maternal plasma, a quantitative 1-step real-time RT-PCR assay was performed using an ABI 7900T Sequence Detector (Perkin-Elmer) as described previously (4). Primer sets and TaqMan probes for each gene and single-strand, and synthetic DNA oligonucleotides from each amplicon used for a calibration curve were prepared as described previously (4). Then, plasma concentrations of cf-mRNA for human PL and for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were measured and converted into multiples of the median (MoM) of the controls adjusted for gestational age, as described previously (5). The differences between the TTTS and the no-TTTS groups were evaluated with the Mann–Whitney U-test. Significant difference was defined as a P value <0.05.

The median (minimum–maximum) cf-PL mRNA MoM values were 1.80 (0.89–3.81) in the TTTS group, 1.14 (0.77–1.35) in the no-TTTS group, and 1.00 (0.82–2.05) in the control group, respectively. At adjusted gestational age the cf-PL mRNA concentration was significantly higher in the TTTS group than in the no-TTTS group (Mann–Whitney U-test, P = 0.035), whereas there was no significant difference of cf-PL mRNA concentration between the no-TTTS group and the control group (P = 0.41; Fig. 1). In addition, the median cf-GAPDH mRNA MoM value in the maternal plasma was significantly higher in the TTTS group (1.80 (0.89–3.81) in the TTTS group, 1.14 (0.77–1.35) in the no-TTTS group, and 1.00 (0.82–2.05) in the control group, respectively. At adjusted gestational age the cf-PL mRNA concentration was significantly higher in the TTTS group than in the no-TTTS group (Mann–Whitney U-test, P = 0.035), whereas there was no significant difference of cf-PL mRNA concentration between the no-TTTS group and the control group (P = 0.41; Fig. 1). In addition, the median cf-GAPDH mRNA MoM value in the maternal plasma was significantly higher in the TTTS...
Fig. 1. Box and whiskers plots of cf-PL MoM distribution in the TTTS group, no-TTTS group, and control group.

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To the Editor:

Molecular tools are increasingly applied in epidemiological studies to unravel the relationship between environmental exposures and disease (1). DNA is required for analyses of genetic factors, such as polymorphisms, but currently used specimens, such as lymphocytes and buccal cells, have disadvantages related to collection, transport, storage, and processing of samples. A relatively infrequently used source of DNA that may overcome these problems is nail material. Human toenails have been collected in several epidemiological studies, predominantly for determination of trace elements as biomarkers for the intake of these compounds (2). Until now, none of these epidemiological studies have applied human toenails as a source of DNA.

We investigated whether toenail material collected 20 years ago in the Netherlands Cohort Study on Diet and Cancer (NLCS) (3) (n = 120,852) could be used as a source of DNA for analyses of multiple genetic polymorphisms. Approximately 90,000 participants provided toenail clippings (on average, 80 mg per participant) (2). We optimized a protocol for DNA isolation from nail material (4) and tested the suitability of this DNA in 2 PCR-based

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