Detective of fetal-derived DNA and RNA molecules in maternal plasma is a promising approach for noninvasive prenatal diagnosis (1). Analysis of circulating fetal RNA, unlike analysis of fetal Y-chromosomal DNA, can be used in pregnancies with fetuses of either gender (2). Placenta-derived mRNA species are readily detectable in maternal plasma (2).

The fetal hematopoietic compartment may be another source of nucleic acids in maternal plasma (3). Watagana et al. (4) reported detection of γ-globin mRNA in maternal plasma. However, expression of γ-globin is not a fetus-specific phenomenon and is shared by maternal erythroid cells (5), and γ-globin mRNA is readily detectable in nonpregnant individuals (4). In this study, we assessed whether rhesus D (RHD gene) mRNA derived from fetal erythroid cells is detectable in the plasma of rhesus D-negative pregnant women.

Fifteen rhesus D-negative women at 11–40 weeks of gestation (Table 1), attending the University Women’s Hospital, Basel, were recruited with informed consent and institutional ethics approval. Trisomy 21 was subsequently confirmed in 1 pregnancy, which was excluded from the analysis. We collected 15 mL of maternal peripheral blood into EDTA tubes. Cord blood was collected from 3 of the term pregnancies after delivery. The blood samples were stored at 4 °C until processing within 3 h by centrifugation (2). Trizol LS reagent (4 mL) was mixed with 3.2 mL of plasma and stored frozen at −80 °C. The plasma samples were sent to Hong Kong in dry ice.

The maternal plasma and cord blood samples were analyzed for RHD, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and human placental lactogen (hPL) mRNA by 1-step real-time quantitative reverse transcription-PCR assays. The latter 2 assays had been described previously (2). The RHD mRNA assay was designed by use of Primer Express software, Ver. 2.0 (Applied Biosystems), with the fluorescent probe crossing the junction between exons 7 and 8 (GenBank accession no. BN000065). Specificity of the assay for RHD was conferred by the forward primer. The primer and probe sequences were as follows: forward primer, 5′-TGC TTT ATG CCG TCG GAG C-3′; reverse primer, 5′-TGA GTT CCC CAA TGC TGA GGG-3′; fluorescent probe, 5′-(FAM) AAT GGC ATG ATT GCG TTC CAG GTC C (TAMRA)-3′, where FAM is 6-carboxyfluorescein and TAMRA is 6-carboxytetramethylrhodamine. The reactions were set up according to the manufacturer’s instructions (EZ rTth RNA PCR reagent set; Applied Biosystems) in a reaction volume of 25 μL with 200 nM of each primer and 50 nM of the fluorescent probe. We used 5 μL of extracted plasma RNA for each reaction, which was performed in a thermal cycler with a fluorometric detector (ABI Prism 7900; Applied Biosystems) at 50 °C for 2 min for the activation of uracil N-glycosylase, 60 °C for 30 min for reverse transcription, and 95 °C for 5 min, followed by 45 cycles of 94 °C for 20 s and 1 min at 60 °C. The calibration curve was constructed with serial dilutions of an HPLC-purified single-stranded synthetic DNA oligonucleotide (5′-CTG GTT CTT GAT ACC GTC GGA GCC GCC AAT GGC ATG ATT GGCTTC CAG GTC CTC CTC AGC ATT GGG GAA CTC AGC TT-3′) at 2.5 × 10^{-2} to 2.5 copies. Specificity of the assay was confirmed by the lack of amplification for a buffy coat RNA of rhesus D-negative individuals. Sensitivity of the assay was assessed by use of buffy coat and plasma RNA from 10 rhesus D-positive nonpregnant individuals. Positive amplification was noted from all samples, and the assay could detect 5 copies/mL of plasma.

Sample analysis was performed without knowledge of the fetal rhesus D status. GAPDH mRNA was detectable in all samples, whereas hPL mRNA, a placenta-specific transcript (2), was detectable in all maternal plasma samples (data not shown). RHD mRNA was detected in the cord plasma sample collected from a rhesus D-positive neonate, but in no maternal plasma samples (Table 1).

Although RHD mRNA has been detected in fetal erythroid cells isolated from maternal circulation (6,7), we found no RHD mRNA in maternal plasma. We suggest that fetal erythroid cells are unlikely to contribute a large portion of the fetal mRNA in maternal plasma. We believe that detection of plasma RHD mRNA in rhesus D-negative preg-
due to fetal erythroid cells contribute to a predominant fraction of fetal mRNA in maternal plasma.

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Differences in Glomerular Filtration Rate Estimates by 2 Cystatin C–Based Equations

To the Editor:

Glomerular filtration rate (GFR) represents the best overall index of kidney function (1), and the National Kidney Foundation has recommended that clinical laboratories routinely report an estimate of GFR (1, 2). Several cystatin C–based equations for calculation of GFR have been reported (3, 4). The most recent equations have been published by Larsson et al. (GFR\textsubscript{Larsson} = 99.43 × cystatin C\textsuperscript{-1.5837}) (4) and Grubb et al. (GFR\textsubscript{Grubb} = 84.69 × cystatin C\textsuperscript{-1.680} (× 0.948 if female)) (5).

Both groups report on GFR estimates obtained from particle-enhanced turbidimetric immunoassay measurements of cystatin C (DakoCytomation). After correcting the Larsson estimate for body surface according to the Du Bois and Du Bois formula (6), we compared both equations, using data from 29 adult renal transplant patients who also had undergone 125I-iothalamate clearance determination as a reference measurement of GFR (7, 8). For cystatin C measurements, we used a particle-enhanced turbidimetric immunoassay (Dako) run on a Cobas Mira instrument (Roche Diagnostics), as described earlier (7, 8).

The correlation of the Larsson and Grubb cystatin C-based GFR estimates was highly significant (r = 0.98; P < 0.001); however, evaluation of these methods by linear regression showed a slope substantially different from 1, indicating 23% higher GFR values obtained with the Larsson estimate (Fig. 1). The Larsson estimates were substantially higher than the Grubb estimates even when body surface area was not performed (data not shown).

In comparison with the 125I-iothalamate clearance, the linear regression line for the Grubb estimate was (numbers in parentheses are the SD):