used to design therapeutic strategies. Almost 75% of the patients we assessed were eligible for pharmacological treatment. Thus, the need for an accurate estimation of LDLc to determine therapeutic intervention is evident. Nevertheless, unlike in the present study, this point has, to our knowledge, previously been assessed based on lipid concentrations alone. Both forms of the Friedewald equations underestimated cardiovascular risk and the need for drug intervention, which would be omitted inappropriately in ~10% vs in no cases according to LDL-apoB.

In conclusion, equations used to calculate LDLc concentrations in type 2 diabetes are far from ideal. The inclusion of apoB in the estimation decreases its bias and allows identification of additional patients at risk. Until direct LDLc methods have been thoroughly assessed, we may recommend that the proposed formula be used for LDLc estimation in type 2 diabetic patients.

References

5. Bachorik PS, Ross JW. National Cholesterol Education Program recommenda-
20. Sugluchi H, Uji Y, Okabe H, Irie T, Uekama K, Kayahara M, Miyauchi K. Direct measurement of high-density lipoprotein cholesterol in serum with polyethyl-

Presence of Fetal RNA in Maternal Plasma, Leo L.M. Poons,1 Tse N. Leung,2 Tze K. Lau,2 and Y.M. Dennis Lo1* (Departments of 1Chemical Pathology and 2Obstetrics and Gynecology, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, New Territories, Hong Kong SAR; * address correspondence to this author at: Department of Chemical Pathology, The Chinese University of Hong Kong, Prince of Wales Hospital, Room 38023, 1/F Clinical Sciences Building, 30-32 Ngan Shing Street, Shatin, New Territories, Hong Kong SAR; fax 852-2194-6171, e-mail loym@cuhk.edu.hk)

The discovery of fetal DNA in maternal plasma (1) has opened up a new horizon on prenatal molecular diagnosis. Many groups have since shown that fetal genetic traits, such as RhD status and inherited genetic diseases, can be determined from fetal DNA in maternal plasma (2–5). However, it is not known whether fetal RNA is also present in maternal plasma. Here, using a two-step reverse transcription (RT)-PCR assay, we demonstrate the presence of fetal-derived, male-specific mRNA in plasma of pregnant women carrying male fetuses.

Pregnant women attending the Prenatal Diagnosis Unit at the Department of Obstetrics and Gynecology, Prince of Wales Hospital, Hong Kong were recruited with informed
consent. The study was approved by the Clinical Research Ethics Committee. Women early and late in their pregnancies (n = 21 and 37, respectively) were recruited in this study. The mean gestational ages of the subjects in early and late pregnancies were 16 weeks (range, 11–19 weeks) and 33 weeks (range, 26–40 weeks), respectively. All early-pregnancy samples were obtained before any invasive procedure. On the other hand, late-pregnancy samples were collected either from women who had invasive procedures in early pregnancy (n = 21) or from women who did not have any prenatal invasive procedure (n = 16). All plasma samples were harvested within 30 min from EDTA-blood samples as described previously (1). Total RNA from plasma samples was isolated with the Trizol LS Reagent (Life Technologies) as instructed by the manufacturer. In general, RNA isolated from 1 mL of plasma was dissolved in 50 μL of RNase-free water.

In this study, we chose to detect fetal-derived, Y-chromosome-specific zinc finger protein (ZFY) mRNA (6, 7) in maternal plasma. As shown in Fig. 1, RT-PCR products corresponding to ZFY mRNA were observed only when male placental total RNA was used in the RT-PCR assay (Fig. 1, lane 1). By contrast, no positive signal was detected when either reverse transcriptase was omitted (Fig. 1, lane 2) or female placental total RNA was used (Fig. 1, lane 3) in the RT-PCR assays. Among 20 women carrying male fetuses in late pregnancy, ZFY-positive signals were detected (Fig. 1, middle panel, lanes 6–10) in 13 plasma samples. Positive signals were observed in two of nine women carrying male fetuses in early pregnancy. The identities of ZFY mRNA-specific RT-PCR products in the positive cases were confirmed by DNA sequencing (data not shown). By contrast, of 20 women carrying female fetuses either in early (n = 12) or in late (n = 8) pregnancy, all but 1 case were negative in the assay (Fig. 1, middle panel, lanes 11–14). The only false-positive case was presumably attributable to contamination during RNA processing. As a control for the quality of the extracted RNA, we also subjected all samples to a RT-PCR assay for HLA-G mRNA (8). The HLA-G gene is expressed by both fetal [e.g., trophoblasts (8)] and maternal [e.g., lymphocytes (9)] tissues. As shown in the bottom panel of Fig. 1, RT-PCR products specific for HLA-G mRNA were detected in all tested plasma samples, demonstrating the presence of amplifiable RNA in these samples.

Recently, it has been demonstrated that a proportion of maternal plasma fetal DNA circulates in the form of intact fetal cells (10). Thus, theoretically it is possible that the fetal RNA that we detected in the current study could have originated from these “plasma-derived” cells. To conclusively test whether fetal RNA can be detected in the “cell-free” form in maternal circulation, maternal plasma samples were filtered by a 0.2 μm membrane (Nalgene), and the RNA extracted from these filtered plasma samples was tested by the ZFY RT-PCR assay. Of nine filtered plasma samples collected from women carrying male fetuses in late pregnancies, positive ZFY mRNA signals were detected in two of nine women carrying male fetuses in early pregnancy. The identities of ZFY mRNA-specific RT-PCR products in the positive cases were confirmed by DNA sequencing (data not shown). By contrast, of 20 women carrying female fetuses either in early (n = 12) or in late (n = 8) pregnancy, all but 1 case were negative in the assay (Fig. 1, middle panel, lanes 11–14). The only false-positive case was presumably attributable to contamination during RNA processing. As a control for the quality of the extracted RNA, we also subjected all samples to a RT-PCR assay for HLA-G mRNA (8). The HLA-G gene is expressed by both fetal [e.g., trophoblasts (8)] and maternal [e.g., lymphocytes (9)] tissues. As shown in the bottom panel of Fig. 1, RT-PCR products specific for HLA-G mRNA were detected in all tested plasma samples, demonstrating the presence of amplifiable RNA in these samples.

![Fig. 1. Detection of fetal ZFY mRNA from maternal plasma.](image-url)
were detected in six samples (data not shown). These results indicate that at least a portion of fetal RNA in maternal plasma exists in the cell-free form. This observation is consistent with the recent finding that tumor-derived RNA can be detected in the circulation of cancer patients (11, 12).

Our data demonstrate that fetal RNA can be detected in maternal plasma. The detection rates of plasma fetal RNA in early and late pregnancies were 22% and 63%, respectively. The detection rate of fetal RNA in early pregnancy cases was lower than that in late pregnancy cases, suggesting that the concentration of plasma fetal RNA is lower in early pregnancy. This observation is similar to our previous finding that the concentration of fetal DNA in maternal plasma increases with gestation (13). We also realized that the detection rate of plasma fetal RNA in this study is lower than that of plasma fetal DNA (1). It is possible that fetal RNA is more susceptible to degradation in maternal blood. As a result, the amount of fetal RNA in plasma is much lower than plasma fetal DNA. This is supported by the fact that Y-specific DNA was detected in all plasma samples from women carrying male fetuses in this study (data not shown). To improve the sensitivity of maternal plasma fetal RNA detection, we are now developing a highly sensitive real-time quantitative RT-PCR assay for this purpose.

In conclusion, we have shown for the first time that fetal RNA can be detected in maternal plasma, and our data provide a novel means of noninvasive prenatal diagnosis. Plasma fetal DNA analysis can provide data on the presence and concentration of fetal genetic material in the maternal circulation. Plasma fetal RNA analysis, in addition, can provide valuable information regarding the gene expression patterns of fetal tissues. For example, abnormal pregnancies, such as those with preeclampsia, often are associated with abnormal gene expression patterns in fetal tissues (14). Thus, with the development of further RNA markers, maternal plasma RNA analysis may allow the noninvasive monitoring of fetal gene expression in a multitude of physiological and pathological conditions.

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References


CYP3A4-V Polymorphism Detection by PCR-Restriction Fragment Length Polymorphism Analysis and Its Allelic Frequency among 199 Dutch Caucasians, Ron H.N. van Schaik,1,2 Saskia N. de Wildt,2 Nienieke M. van Iperen,2 Andre G. Lüttlerinden,3,4 John N. van den Anker,2 and Jan Linde mans2

Interindividual variation in drug metabolism is a complicating factor in pharmacotherapy. Enzymes of the cytochrome P450 system are involved in the metabolism of several endogenous substrates and a broad range of foreign compounds, such as drugs, environmental pollutants, and carcinogens (1). The cytochrome P450 enzyme family consists of several subfamilies, with CYP3A4 being the most abundant P450 enzyme in human liver (2). CYP3A4 is involved in the metabolism of >50% of all drugs used in humans (3, 4). Interindividual differences in CYP3A4 expression may account for the observed interindividual differences in pharmacokinetics of drugs metabolized by this enzyme (4–6). Variations in CYP3A4 expression may be caused by factors inhibiting or stimulating transcription and/or translation (e.g., concomitant drug administration) and by genetic polymorphisms.

In a recent study, an A→G (290)G substitution was described in the 5′ regulatory region of the CYP3A4 gene (7, 8). This allele was termed CYP3A4*V but was recently also referred to as CYP3A4*1B (9). The allelic frequency, determined by conformation-sensitive gel electrophoresis,