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Quantitative Analysis of Fetal DNA in Maternal Plasma and Serum: Implications for Noninvasive Prenatal Diagnosis

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Summary

We have developed a real-time quantitative PCR assay to measure the concentration of fetal DNA in maternal plasma and serum. Our results show that fetal DNA is present in high concentrations in maternal plasma, reaching a mean of 25.4 genome equivalents/ml (range 3.3–69.4) in early pregnancy and 292.2 genome equivalents/ml (range 76.9–769) in late pregnancy. These concentrations correspond to 3.4% (range 0.39%–11.9%) and 6.2% (range 2.33%–11.4%) of the total plasma DNA in early and late pregnancy, respectively. Sequential follow-up study of women who conceived by in vitro fertilization shows that fetal DNA can be detected in maternal serum as early as the 7th wk of gestation and that it then increases in concentration as pregnancy progresses. These data suggest that fetal DNA can be readily detected in maternal plasma and serum and may be a valuable source of material for noninvasive prenatal diagnosis.

Introduction

Noninvasive prenatal diagnosis is a long-sought goal in human genetics. The passage of fetal nucleated cells into maternal blood is a well-recognized phenomenon (Walknowska et al. 1969; Lo et al. 1989; Lo et al. 1996), and it makes possible the use of these cells for noninvasive prenatal diagnosis (Simpson and Elias 1993). Significant advances have been made in the enrichment and isolation of fetal cells for analysis (Bianchi et al. 1990; Cheung et al. 1996). However, most techniques are time consuming, labor intensive, or difficult to implement on a large scale. These limitations prompted us to investigate alternative sources of fetal genetic material for molecular analysis.

There has been much recent interest in the use of DNA derived from plasma or serum, for molecular diagnosis (Boland 1996). In particular, plasma and serum DNA from cancer patients has been shown to contain large quantities of tumor DNA (Chen et al. 1996; Nawroz et al. 1996; Anker et al. 1997). Prompted by such reports, we recently demonstrated that fetal DNA is present in maternal plasma and serum (Lo et al. 1997). Detection of fetal DNA sequences was possible, in 80% and 70% of cases, with just 10 μl of boiled plasma and serum, respectively (Lo et al. 1997).

These observations suggest that maternal plasma/serum DNA may be a useful source of material for the noninvasive prenatal diagnosis of certain genetic disorders. However, a number of important questions need to be answered before clinical application of this new approach can be contemplated. First, fetal DNA in maternal plasma and serum would need to be shown to be present in sufficient quantities for reliable molecular diagnosis to be carried out. Second, data on the variation of fetal DNA concentrations in maternal plasma and serum, in relation to gestational age, would be required to determine the applicability of this technology to early prenatal diagnosis.

We have addressed both of these issues by developing a real-time quantitative TaqMan PCR assay (Heid et al. 1996) for measuring the copy numbers of fetal DNA molecules in maternal plasma and serum. This technique permits continuous optical monitoring of the progress of an amplification reaction, giving accurate target quantitation over a wide concentration range. Our data show that fetal DNA is present in maternal plasma and serum at concentrations similar to those achieved by many fetal cell–enrichment protocols. We have also investigated the changes of fetal DNA concentration in maternal serum at different gestational ages. Using this plasma- or se-

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rum-based approach, we show that reliable detection of fetal DNA is achievable and may potentially be useful for the noninvasive prenatal diagnosis of selected genetic disorders.

Subjects and Methods

Subjects

Pregnant women attending the Department of Obstetrics and Gynecology at the Prince of Wales Hospital, Shatin, Hong Kong were recruited, and informed consent was obtained. The Research Ethics Committee of the Chinese University of Hong Kong approved the study protocol. For women studied at a single time point, early-pregnancy samples were obtained prior to amniocentesis or chorionic villus sampling (CVS), whereas late-pregnancy samples were collected just prior to delivery. We collected 5–10 ml of maternal peripheral blood into each of two tubes, one of which contained EDTA. Subjects studied at multiple time points were recruited, prior to conception, from the in vitro fertilization (IVF) program. We collected 5–10 ml of maternal blood from these subjects into a plain tube at each studied time point. For women undergoing prenatal diagnosis, the sex of the fetus was ascertained from cytogenetic results from amniocentesis or CVS. For women recruited just prior to delivery or from the IVF program, the baby's sex was noted at the time of delivery.

Preparation of Samples

Blood samples were centrifuged at 3,000*g*, and plasma and serum were carefully removed from the EDTA-containing and plain tubes, respectively, and were transferred into plain polypropylene tubes. Great care was taken to ensure that the buffy coat or the blood clot was undisturbed when plasma or serum samples, respectively, were removed. The plasma and serum samples were recentrifuged at 3,000*g*, and the supernatants were collected into fresh polypropylene tubes. The samples were stored at −20°C until further processing.

DNA Extraction from Plasma and Serum Samples

DNA was extracted from plasma and serum samples by use of a QIAamp Blood Kit (Qiagen) according to the "blood and body fluid protocol" recommended by the manufacturer (Chen et al. 1996). We used 400–800 μl of the plasma/serum sample, per column, for DNA extraction. The exact amounts used were documented, to enable calculation of target DNA concentration.

Real-Time Quantitative PCR

Theoretical and practical aspects of real-time quantitative PCR were described elsewhere by Heid et al. (1996). Real-time quantitative PCR analysis was performed by use of a PE Applied Biosystems 7700 Sequence Detector, which is essentially a combined thermal cycler/fluorescence detector with the ability to monitor, optically, the progress of individual PCR reactions. The amplification and product-reporting system used is based on the 5′ nuclease assay (Holland et al. 1991) (the TaqMan assay, Perkin-Elmer). In this system, in addition to the two amplification primers, as in conventional PCR, a dual-labeled fluorogenic hybridization probe is also included (Lee et al. 1993; Livak et al. 1995*a*). One fluorescent dye (6 carboxyfluorescein [FAM]) serves as a reporter, and its emission spectrum is quenched by a second fluorescent dye (6 carboxy-tetramethylrhodamine [TAMRA]). During the extension phase of PCR, the 5′ to 3′ exonuclease activity of the *Taq* DNA polymerase cleaves the reporter from the probe, thus releasing it from the quencher, resulting in an increase in fluorescent emission at 518 nm. The PE Applied Biosystems 7700 Sequence Detector is able to measure the fluorescent spectra of the 96 amplification wells continuously during DNA amplification, and the data are captured onto a Macintosh computer.

The SRY TaqMan system consisted of the amplification primers SRY-109F, 5′-TGG CGA TTA AGT CAA ATT CGC-3′; SRY-245R, 5′-CCC CCT AGT ACC CTG ACA ATG TAT T-3′; and a dual-labeled fluorescent TaqMan probe SRY-142T, 5′-(FAM)AGC AGT AGA GCA GTC AGG GAG GCA GA(TAMRA)-3′. The β-globin TaqMan system consisted of the amplification primers β-globin-354F, 5′-GTG CAC CTG ACT CCT GAG GAG A-3′; β-globin-455R, 5′-CCT TGA TAC CAA CCT GCC CAG-3′; and a dual-labeled fluorescent TaqMan probe β-globin-402T, 5′-(FAM)AAG GTG AAC GTG GAT GAA GTT GGT GG(TAMRA)-3′. The TaqMan probes contained a 3′-blocking phosphate group, to prevent probe extension during PCR. Primer/probe combinations were designed by use of the Primer Express software (Perkin-Elmer). Sequence data were obtained from the GenBank Sequence Database (accession numbers L08063 [SRY gene] and U01317 [β-globin gene]).

TaqMan amplification reactions were set up in a reaction volume of 50 μl by use of components (except TaqMan probes and amplification primers) supplied in a TaqMan PCR Core Reagent Kit (Perkin-Elmer). TaqMan probes were custom-synthesized by PE Applied Biosystems. PCR primers were synthesized by Life Technologies. Each reaction contained 5 μl of 10 × buffer A; 300 nM of each amplification primer; 100 nM of the corresponding TaqMan probe; 4 mM $MgCl_2$; 200 μM each dATP, dCTP, and dGTP; 400 μM dUTP; 1.25 U AmpliTaq Gold; and 0.5 U AmpErase uracil N-glycosylase. We used 5–10 μl of the extracted plasma/serum DNA for amplification. The exact amount used was re-

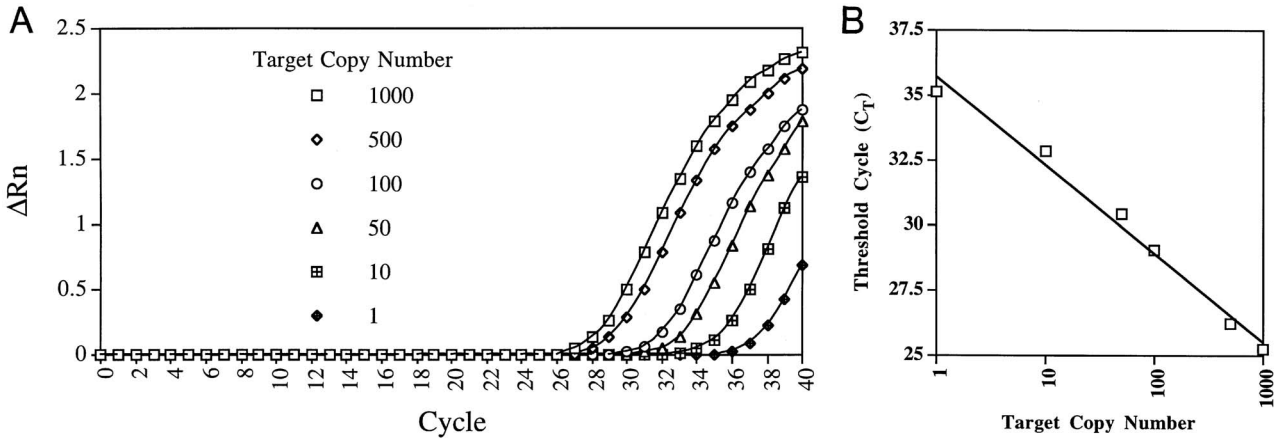

Figure 1 Real-time quantitative PCR. *A,* Amplification plots obtained using real-time quantitative PCR for the SRY gene. Each plot corresponds to a particular input target quantity, marked by a corresponding symbol. The *X*-axis denotes the cycle number of a quantitative PCR reaction. The *Y*-axis denotes the ΔRn, which is the fluorescence intensity over the background (Heid et al. 1996). *B,* Plot of the threshold cycle (C_T) against the input target quantity (common log scale). The correlation coefficient is 0.986.

corded, for subsequent concentration calculations. DNA amplifications were carried out in 96-well reaction plates that were frosted, by the manufacturer, to prevent light reflection and were closed by use of caps designed to prevent light scattering (Perkin-Elmer). Each sample was analyzed in duplicate. A calibration curve was run in parallel and in duplicate with each analysis. The conversion factor of 6.6 pg of DNA per cell was used, for expression of results as copy numbers.

Identical thermal profiles were used for both the SRY and the β-globin TaqMan systems. Thermal cycling was initiated with a 2-min incubation at 50°C, to allow the uracil N-glycosylase to act, followed by a first denaturation step of 10 min at 95°C and then 40 cycles of 95°C for 15 s and 60°C for 1 minute.

Amplification data collected by the 7700 Sequence Detector and stored in the Macintosh computer were then analyzed by use of the Sequence Detection System software developed by PE Applied Biosystems. The mean quantity of each duplicate was used for further calculation. The concentration, expressed in copies per milliliter, was calculated by use of the following equation:

$$C = Q \times \frac{V_{DNA}}{V_{PCR}} \times \frac{1}{V_{ext}},$$

where C = target concentration in plasma or serum (copies per milliliter); Q = target quantity (copies) determined by sequence detector in PCR; V_{DNA} = total volume of DNA obtained after extraction, typically 50 μl per Qiagen extraction; V_{PCR} = volume of DNA solution used for PCR, typically 5–10 μl; and V_{ext} = volume of plasma or serum extracted, typically 400–800 μl.

Anticontamination Measures

Strict precautions against PCR contamination were taken (Kwok and Higuchi 1989). Aerosol-resistant pipette tips were used for all handling of liquids. Separate areas were used for the preparation of amplification reactions, the addition of DNA template, and the carrying out of amplification reactions. The 7700 Sequence Detector offered an extra level of protection in that its optical detection system obviated the need to reopen the reaction tubes after completion of the amplification reactions, thus minimizing the possibility of carryover contamination. The TaqMan assay also included a further anticontamination measure in the form of preamplification treatment by use of uracil N-glycosylase, which destroyed uracil-containing PCR products (Longo et al. 1990). Multiple negative water blanks were included in every analysis.

Results

Development of Real-Time Quantitative PCR

To determine the dynamic range of real-time quantitative PCR, we made serial dilutions of male DNA, in water, consisting of the DNA equivalent from 1,000 cells to 1 cell, and we subjected the dilutions to analysis by the SRY TaqMan system. Figure 1*A* demonstrates that the amplification curve shifted to the right as the input target quantity was reduced. This was expected, since reactions with fewer target molecules required more amplification cycles to produce a certain quantity of reporter molecules than did reactions with more target

Table 1

Quantitative Analysis of Maternal Plasma and Serum by the β-globin TaqMan Assay

	Mean (copies/ml)	Median (copies/ml)	Range (copies/ml)
Plasma (early + late pregnancy)	3,466	1,594	356–31,875
Serum (early + late pregnancy)	50,651	34,688	5,813–243,750
Plasma (early pregnancy)	986	975	356–1,856
Plasma (late pregnancy)	5,945	4,313	1,125–31,875

molecules. The system was sensitive enough to detect the DNA equivalent from a single target cell.

Figure 1*B* shows a plot of the threshold cycle (C_T) against the input target quantity, with the latter plotted on a common log scale. The threshold was set at 10 SD above the mean baseline fluorescence, calculated from cycles 1–15, and the C_T was proportional to the starting target copy number used for amplification (Heid et al. 1996). The linearity of the graph demonstrates the large dynamic range and accuracy of real-time quantitative PCR. Similar results were obtained by use of the β-globin TaqMan system (data not shown).

The real-time quantitative SRY system was insensitive to the existence of background female DNA from 0 to 12,800 female genome equivalents. This greatly simplified the application of this system since, within this range, separate calibration curves did not have to be constructed for different cases because of the presence of different concentrations of fetal and maternal DNA.

The reproducibility of DNA extraction from plasma and serum with the Qiagen protocol was tested by replicate extractions (10 for each case) from plasma and serum samples from normal individuals. These replicate extractions were then subjected to real-time quantitative PCR by use of the β-globin system. The coefficient of variation of C_T values of these replicate extractions was 1.1%.

Table 2

Quantitation of Fetal DNA in Maternal Plasma and Serum: Relationship with Gestational Age

	SRY Concentration (copies/ml)			
	Early Pregnancy		Late Pregnancy	
	Plasma	Serum	Plasma	Serum
Range	3.3–69.4	4.0–58.1	76.9–769	33.8–900
Mean	25.4	28.7	292.2	342.1
Median	20.6	19.5	244.0	286.0

Quantitative Analysis using the Real-Time β-globin TaqMan System

The concentration of β-globin sequences in maternal plasma and serum samples was used as a measure of the total amount of extracted DNA, both maternal *and* fetal. DNA extracted from plasma and serum samples from 50 pregnant women was analyzed by the β-globin TaqMan system. Twenty-five pregnant women were recruited during the first and second trimesters (11–17 wk gestation), and samples were termed "early-pregnancy samples." The other 25 women were recruited just prior to delivery (37–43 wk gestation), and samples were termed "late-pregnancy samples." The concentrations of β-globin sequences in maternal plasma and serum are listed in table 1. These results show that serum contains more DNA than plasma (Wilcoxon signed rank test, $P < .0005$), with a mean concentration of serum DNA 14.6 times that of plasma DNA in our studied population. The concentration of β-globin sequences in maternal plasma from early- and late-pregnancy samples are compared in table 1. These data show that the total amount of plasma DNA increases as pregnancy progresses (Mann-Whitney rank sum test, $P < .0005$).

Quantitative Analysis of Fetal SRY Gene from Maternal Plasma and Serum

Real-time quantitative analysis using the SRY TaqMan system was carried out on DNA extracted from maternal plasma and serum, to determine the amount of fetal DNA. Of the 25 early-pregnancy samples (11–17 wk gestation), 13 were from women bearing male fetuses, and 12 were from women bearing female fetuses. Of the 25 late-pregnancy samples (37–43 wk gestation), 14 were from women bearing male fetuses, and 11 were from women bearing female fetuses. A positive signal was obtained for each of the 27 women bearing male fetuses, and no signal was detected for each of the 23 women bearing female fetuses. Fourteen women had a history of previous delivery of a male baby, and 5 of these were carrying a female baby in the current pregnancy.

Quantitative SRY data from the 27 women bearing male fetuses are summarized in table 2. These data show that the concentrations of fetal DNA in plasma and serum are higher in late gestation than in early gestation (Mann-Whitney rank sum test, $P < .0005$). The mean concentrations of fetal DNA in maternal plasma and serum are 11.5 times and 11.9 times higher, respectively, in late gestation than in early gestation. The absolute concentrations of fetal DNA in maternal plasma and serum were similar in individual cases. The fractional concentration of fetal DNA in early pregnancy is 0.39%–11.9% (mean 3.4%) in plasma and

0.014%–0.54% (mean 0.13%) in serum. In late pregnancy, the fraction of fetal DNA is 2.33%–11.4% (mean 6.2%) in plasma and 0.032%–3.97% (mean 1.0%) in serum.

Sequential Follow-Up of Women Who Conceived by IVF

Twenty women who conceived by IVF were followed up at preconception and at multiple time points during pregnancy. All 20 subjects had singleton pregnancies, as determined by ultrasound scanning. Twelve women delivered male babies, and the remaining 8 delivered female babies. None of the women carrying male fetuses had a history of pregnancy-associated complications. Subject S-51 (fig. 2) underwent CVS at 12 wk. Subjects S-1 and S-56 (fig. 2) had amniocentesis at 16 and 17 wk, respectively. A total of 163 serum samples from these 20 women were analyzed by use of the real-time quantitative SRY TaqMan system. None of the 65 serum samples from the 8 women bearing female babies gave a positive SRY signal. The concentrations of fetal DNA in the 98 serum samples from women carrying male babies are plotted in figure 2.

Discussion

In this study, we have developed an accurate real-time quantitative PCR system for determining the concentration of fetal DNA in maternal plasma and serum. This system has a number of advantages: (1) a large dynamic range of over five orders of magnitude (Heid et al. 1996); (2) a high throughput and fast turnaround time—96 samples could be simultaneously amplified and quantified in ~2 h; and (3) the use of a homogeneous amplification/detection system that requires no post-PCR processing and therefore minimizes the risk of carryover contamination.

The most important observation in this study is the very high concentration of fetal DNA in maternal plasma and serum. Bianchi et al. (1997) reported that the average number of fetal cells in maternal blood in normal pregnancies was 19 cells/16 ml of maternal blood, or 1.2 cells/ml, during the second trimester. Therefore, the mean concentration of fetal DNA in maternal plasma and serum is 21.2 (25.4/1.2) and 23.9 (28.7/1.2) times, respectively, higher than that in the cellular fraction of maternal blood at the same gestational stage. The relative concentration of fetal to total plasma DNA is even higher. Thus, in early pregnancy, fetal DNA in maternal plasma constitutes a mean of 3.4% of the total plasma DNA. The respective figure in late pregnancy is 6.2%. Hamada et al. (1993) reported that the frequency of fetal cells in the second trimester was 0.0035%, whereas that in the third trimester was 0.008%. The fetomaternal ratio is therefore 970-fold and 775-fold higher in maternal plasma than in the cellular fraction, at the respective gestational age. Indeed, the fetomaternal ratio in plasma DNA is comparable to that following many fetal cell–enrichment protocols. For example, Bianchi et al. (1994) reported that after fetal nucleated–red cell enrichment performed with fluorescence-activated cell sorting, the resulting fetal cells constituted 0.001%–5% of the sorted cell populations, as determined by quantitative PCR analysis. In a similar study using cell sorting and fetal cell detection by use of fluorescence in situ hybridization, Sohda et al. (1997) found that, on average, 4.6% of the sorted cells were of fetal origin. Maternal plasma, therefore, offers an easily accessible source of fetal DNA for prenatal genetic analysis.

We have demonstrated that the absolute concentration of fetal DNA in maternal plasma is similar to that in maternal serum. The main difference lies in the presence of a larger quantity of background maternal DNA in serum compared to plasma, possibly as a result of the liberation of DNA during the clotting process. Although this exerts no noticeable effect on the efficiency of fetal DNA detection by means of the real-time TaqMan system, it is possible that, with the use of less sensitive methods, such as conventional PCR followed by ethidium-stained agarose gel electrophoresis, maternal plasma may be preferable to maternal serum, for robust fetal DNA detection.

The high concentration of fetal DNA in maternal plasma and serum has allowed us to detect reliably the presence of fetal genetic material. Of the 263 serum or plasma samples analyzed in this study, we were able to detect fetal SRY genes in maternal plasma or serum from every subject who was carrying a male baby at the time of venesection. This robust detection rate was obtained with DNA extracted from just 40–80 μl of maternal plasma and serum. This volume represents a 4–8-fold increase over the 10 μl of boiled maternal plasma or serum reported in our previous study (Lo et al. 1997), and it results in significant improvement in sensitivity. The specificity was preserved, since we did not observe amplification signals from samples obtained prior to conception or from subjects carrying a female fetus. From the data obtained thus far, plasma/serum analysis does not appear to be affected significantly by the persistence of fetal cells from previous pregnancies (Bianchi et al. 1996). Thus, we did not obtain any false-positive results from women who had previously carried a male fetus but were carrying a female fetus at the time of blood sampling for this study.

The sequential study of patients undergoing IVF yielded a number of important results. First, all of the 12 patients carrying male babies were shown to be negative for SRY sequences in their sera prior to conception. This provided convincing evidence that the SRY se-

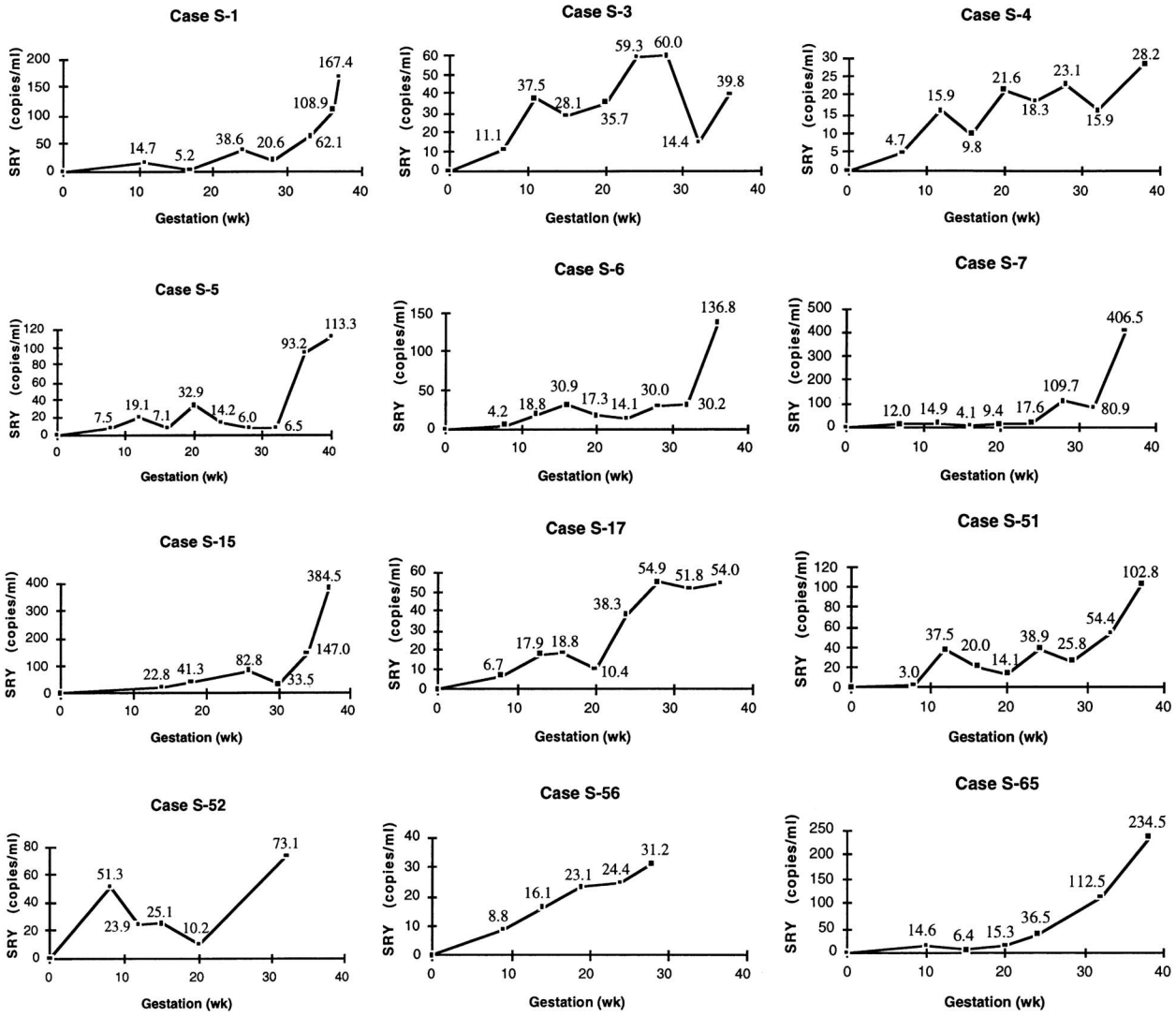

Figure 2 Sequential study of 12 women, bearing male fetuses, who conceived by IVF. Each case is denoted by a unique recruitment case number. The *X*-axis denotes the gestational ages at which serum samples were obtained. A gestational age of zero denotes the preconception sample. The *Y*-axis denotes the concentration of fetal SRY in maternal serum, expressed in copies per milliliter. The scale has been optimized for the concentration range for each case.

quence detected by the TaqMan assay did indeed originate from the male fetus in the *current* pregnancy. Second, we were able to detect fetal SRY sequences as early as the 7th wk of gestation, thus indicating that fetal genetic analysis in maternal plasma/serum could be used in the first trimester. Third, we showed that fetal DNA concentration increased as pregnancy progressed (fig. 2). This last point was also confirmed by data obtained from women studied at a single time point. Women recruited late in pregnancy had higher fetal DNA concentrations in their plasma and serum (table 2).

In addition to the increase in fetal DNA concentration as pregnancy progresses, our data also indicate that maternal plasma DNA also increases with gestation (table 1). The biologic basis for this phenomenon is unclear at present. Possible explanations include the increase in size of the fetomaternal interface, as gestation progresses, and a possible reduction in DNA clearance associated with other physiologic changes during pregnancy.

For selected disorders, fetal genetic information could be acquired more economically and rapidly from maternal plasma or serum than by use of fetal cells isolated from maternal blood. We envisage that fetal DNA analysis in maternal plasma and serum would be most useful in situations where the determination of fetal-derived paternally-inherited polymorphisms/mutations or genes would be helpful in clinical prenatal diagnosis (Lo et al. 1994). Examples include fetal sex determination, for

prenatal diagnosis of sex-linked disorders; fetal rhesus D status determination in sensitized rhesus-negative pregnant women (Lo et al. 1993); autosomal dominant disorders in which the father carries the mutation; and autosomal recessive genetic disorders in which the father and mother carry different mutations (Lo et al. 1994)—for example, certain hemoglobinopathies (Camaschella et al. 1990) and cystic fibrosis. Because of the much-reduced maternal background and high fetal DNA concentration in maternal plasma and serum, we predict that this type of analysis would be much more robust when based on plasma or serum DNA than when unsorted fetal cells in maternal blood are used. The ability for allelic discrimination (Lee et al. 1993; Livak et al. 1995*b*) allows the homogeneous TaqMan assay to be used for this purpose. The high throughput and anticontamination capability of this system makes it an attractive candidate for large-scale clinical application.

Bianchi et al. (1997) recently reported that fetal cells in maternal blood were increased in aneuploid pregnancies, and it would be very interesting to investigate whether the fetal DNA concentration in maternal plasma and serum would also be elevated in these pregnancies. This would potentially provide a new screening test for fetal chromosomal disorders. For this potential application, fetal DNA quantitation systems must be developed for polymorphic markers outside the Y chromosome so that quantitation can be applied to female fetuses. Autosomal polymorphic systems that might be used for this purpose have already been described (Lo et al. 1996). However, fetal cell isolation techniques would still be necessary for a definitive cytogenetic diagnosis. Similarly, fetal cell isolation would also be required for direct mutational analysis of autosomal recessive disorders caused by a single mutation. It is likely that fetal cell isolation and analysis of fetal DNA in maternal plasma/serum would be used as complementary techniques for noninvasive prenatal diagnosis.

The biological basis by which fetal DNA is liberated into maternal plasma remains to be elucidated. It is possible that fetal DNA is released from cell lysis resulting from physical and immunologic damage or through developmentally associated apoptosis of fetal tissues. It is also likely that increased amounts of fetal DNA may be found in conditions associated with placental damage, such as pre-eclampsia. The real-time quantitative PCR system described here offers a powerful tool to study these unexplored pathophysiologic aspects of fetal DNA in maternal plasma, and it may improve our understanding of the fetomaternal relationship.

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References

Anker P, Lefort F, Vasioukhin V, Lyautey J, Lederrey C, Chen XQ, Stroun M, et al (1997) K-ras mutations are found in DNA extracted from the plasma of patients with colorectal cancer. Gastroenterology 112:1114–1120

Bianchi DW, Flint AF, Pizzimenti MF, Knoll JH, Latt SA (1990) Isolation of fetal DNA from nucleated erythrocytes in maternal blood. Proc Natl Acad Sci USA 87:3279–3283

Bianchi DW, Shuber AP, DeMaria MA, Fougner AC, Klinger KW (1994) Fetal cells in maternal blood: determination of purity and yield by quantitative PCR. Am J Obstet Gynecol 171:922–926

Bianchi DW, Williams JM, Sullivan LM, Hanson FW, Klinger KW, Shuber AP (1997) PCR quantitation of fetal cells in maternal blood in normal and aneuploid pregnancies. Am J Hum Genet 61:822–829

Bianchi DW, Zickwolf GK, Weil GJ, Sylvester S, DeMaria MA (1996) Male fetal progenitor cells persist in maternal blood for as long as 27 years postpartum. Proc Natl Acad Sci USA 93:705–708

Boland CR (1996) Setting microsatellites free. Nat Med 2: 972–974

Camaschella C, Alfarano A, Gottardi E, Travi M, Primignani P, Caligaris CF, Saglio G (1990) Prenatal diagnosis of fetal hemoglobin Lepore-Boston disease on maternal peripheral blood. Blood 75:2102–2106

Chen XQ, Stroun M, Magnenat J-L, Nicod LP, Kurt A-M, Lyautey J, Lederrey C, et al (1996) Microsatellite alterations in plasma DNA of small cell lung cancer patients. Nat Med 2:1033–1035

Cheung MC, Goldberg JD, Kan YW (1996) Prenatal diagnosis of sickle cell anemia and thalassemia by analysis of fetal cells in maternal blood. Nat Genet 14:264–268

Hamada H, Arinami T, Kubo T, Hamaguchi H, Iwasaki H (1993) Fetal nucleated cells in maternal peripheral blood: frequency and relationship to gestational age. Hum Genet 91:427–432

Heid CA, Stevens J, Livak KJ, Williams PM (1996) Real time quantitative PCR. Genome Res 6:986–994

Holland PM, Abramson RD, Watson R, Gelfand DH (1991) Detection of specific polymerase chain reaction product by utilizing the 5′–3′ exonuclease activity of the *Thermus aquaticus* DNA polymerase. Proc Natl Acad Sci USA 88: 7276–7280

Kwok S, Higuchi R (1989) Avoiding false positives with PCR. Nature 339:237–238

Lee LG, Connell CR, Bloch W (1993) Allelic discrimination by nick-translation PCR with fluorogenic probes. Nucleic Acids Res 21:3761–3766

Livak KJ, Flood SJ, Marmaro J, Giusti W, Deetz K (1995*a*) Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization. PCR Methods Appl 4: 357–362

Livak KJ, Marmaro J, Todd JA (1995*b*) Towards fully auto-

mated genome-wide polymorphism screening. Nat Genet 9: 341–342

Lo YMD, Bowell PJ, Selinger M, MacKenzie IZ, Chamberlain P, Gillmer MDG, Littlewood TJ, et al (1993) Prenatal determination of fetal RhD status by analysis of peripheral blood of rhesus negative mothers. Lancet 341:1147–1148

Lo YMD, Corbetta N, Chamberlain PF, Rai V, Sargent IL, Redman CWG, Wainscoat JS (1997) Presence of fetal DNA in maternal plasma and serum. Lancet 350:485–487

Lo YMD, Fleming KA, Wainscoat JS (1994) Strategies for the detection of autosomal fetal DNA sequence from maternal peripheral blood. Ann N Y Acad Sci 731:204–213

Lo YMD, Lo ESF, Watson N, Noakes L, Sargent IL, Thilaganathan B, Wainscoat JS (1996) Two-way cell traffic between mother and fetus: biologic and clinical implications. Blood 88:4390–4395

Lo YMD, Patel P, Wainscoat JS, Sampietro M, Gillmer MDG, Fleming KA (1989) Prenatal sex determination by DNA amplification from maternal peripheral blood. Lancet 2: 1363–1365

Longo MC, Berninger MS, Hartley JL (1990) Use of uracil DNA glycosylase to control carry-over contamination in polymerase chain reactions. Gene 93:125–128

Nawroz H, Koch W, Anker P, Stroun M, Sidransky D (1996) Microsatellite alterations in serum DNA of head and neck cancer patients. Nat Med 2:1035–1037

Simpson JL, Elias S (1993) Isolating fetal cells from maternal blood: advances in prenatal diagnosis through molecular technology. JAMA 270:2357–2361

Sohda S, Arinami T, Hamada H, Nakauchi H, Hamaguchi H, Kubo T (1997) The proportion of fetal nucleated red blood cells in maternal blood: estimation by FACS analysis. Prenat Diagn 17:743–752

Walknowska J, Conte FA, Grumbach MM (1969) Practical and theoretical implications of fetal/maternal lymphocyte transfer. Lancet 1:1119–1122