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Quantitative Analysis of the Bidirectional Fetomaternal Transfer of Nucleated Cells and Plasma DNA

Y.M. Dennis Lo,[1*] Tze K. Lau,[2] Lisa Y.S. Chan,[1] Tse N. Leung,[2] and Allan M.Z. Chang[2]

Background: **Recently, much interest has been generated on the fetomaternal transfer of nucleated cells and plasma DNA. However, there has been no systematic quantitative comparison of these two directions and two modalities of trafficking within the same study population.**

Methods: **The fetus-to-mother transfer of nucleated cells and plasma DNA in pregnant women carrying male babies was studied using a real-time quantitative PCR assay for the *SRY* gene. For mother-to-fetus transfer, real-time quantitative PCR assays for the insertion/deletion polymorphisms involving the glutathione *S*-transferase M1 and angiotensin-converting enzyme genes were used.**

Results: **Of the 50 informative mother-baby pairs, maternal DNA was detected in the cellular fraction of umbilical cord blood in 24% of cases (12 of 50), at a median fractional concentration of 2.6×10^{-4} (interquartile range, 1.7×10^{-4} to 3.6×10^{-4}). In the plasma fraction of cord blood, maternal DNA was detected in 30% (15 of 50) of cases at a median fractional concentration of 3×10^{-3} (interquartile range, 1×10^{-3} to 1.6×10^{-2}). For the other direction of trafficking, fetus-to-mother transfer of nucleated cells was detected in 26% of cases (13 of 50) at a median fractional concentration of 3.2×10^{-4} (interquartile range, 0.6×10^{-4} to 7.6×10^{-4}). In the plasma fraction, fetal DNA was detected in 100% of maternal plasma (50 of 50) at a median fractional concentration of 3×10^{-2} (interquartile range, 1.4×10^{-2} to 5.3×10^{-2}).**

Conclusions: **This study indicated that significantly more fetal DNA is present in the plasma of pregnant women compared with DNA from the cellular fraction of maternal blood. In addition, maternal DNA was demonstrated in both the cellular and plasma fractions of cord blood after delivery. This study has therefore determined the fundamental quantitative values for the bidirectional fetomaternal cellular and plasma DNA traffic.**

The two-way transfer of nucleated cells between the mother and fetus is a phenomenon that is increasingly recognized to have important biologic and clinical implications *(1)*. For example, fetal nucleated cells have been detected in the maternal circulation from the first trimester of pregnancy onward *(2–5)* and represent a valuable source of fetal materials for noninvasive prenatal diagnosis *(6)*. Biologically, abnormal fetal nucleated cell traffic into the maternal circulation has been associated with certain pregnancy-related disorders such as preeclampsia *(7, 8)*. Recently, the persistence of fetal nucleated cells in the mother following delivery, sometimes for decades, has been demonstrated *(9, 10)*. This latter phenomenon has been postulated to be associated with the development of certain autoimmune diseases *(11–13)*.

The reverse transfer of nucleated maternal cells into the fetal circulation has also been demonstrated *(1, 14–16)*. This phenomenon has initially attracted attention because of the recent interest in the use of umbilical cord blood for bone marrow transplantation *(17)*. The potential existence of maternal immunocompetent cells or their precursors in cord blood has been postulated to constitute a theoretical risk of graft-vs-host disease *(18)*. Immunologically, the passage of maternal cells into the fetal circulation during pregnancy may also contribute to the observed partial immunological tolerance to the noninherited maternal HLA types *(19, 20)*.

Recently, in addition to the fetomaternal trafficking of nucleated cells, plasma DNA transfer from the fetus to the mother has also been demonstrated *(21)*. The concentration of fetal-derived plasma DNA has been shown to be several orders of magnitude higher than that of fetal

Departments of [1] Chemical Pathology and [2] Obstetrics and Gynecology, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, New Territories, Hong Kong SAR.

*Author for correspondence. Fax 852-2194-6171; e-mail loym@cuhk.edu.hk.

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nucleated cells per volume of maternal blood *(22)*. In addition, the clearance kinetics of fetal cells and plasma DNA appear to be very different, with plasma fetal DNA being cleared much more rapidly *(23)* than fetal nucleated cells *(24)*.

Several questions remain unanswered regarding the fetomaternal transfer of nucleated cells and plasma DNA. First, the possibility of plasma maternal DNA transfer into the fetal circulation in humans remains unexplored. Second, the relationship, if any, between the degree of fetomaternal transfer of nucleated cells and plasma DNA remains unknown. Third, most of the published data on the trafficking of maternal cells into the fetal circulation are qualitative in nature, with relatively little quantitative information. The recent development of real-time quantitative PCR *(22, 25)*, with its high accuracy and wide dynamic range, has allowed us to undertake the present study to address these important questions. To maximize the informativeness of our study, we explored this bidirectional and bimodal (i.e., nucleated cells and plasma DNA) fetomaternal transfer in the same patient cohort.

Materials and Methods

COLLECTION OF MATERNAL AND UMBILICAL CORD BLOOD SAMPLES

Pregnant women were recruited before normal vaginal delivery at the Department of Obstetrics and Gynecology, Prince of Wales Hospital, Hong Kong. None of the recruited subjects had preeclampsia or preterm labor in the current pregnancy. Ethics approval was obtained from the Clinical Research Ethical Committee of the Chinese University of Hong Kong. Informed consent was obtained in all cases. Three milliliters of maternal blood was collected from the antecubital fossa during the active phase of labor (typically 2 to 3 h before delivery) and stored in an EDTA-containing tube. Fetal sex was noted at delivery. Immediately after delivery, the umbilical cord was double clamped and transected from the umbilicus. The cord was carefully cleaned with saline, and umbilical venous blood was then withdrawn between the two clamps using a needle and syringe into an EDTA-containing tube.

PROCESSING OF BLOOD SAMPLES

Blood samples were centrifuged at 1600*g*, and plasma was carefully removed and transferred into plain polypropylene tubes. Great care was taken to ensure that the buffy coat was undisturbed when plasma samples were removed. The plasma was recentrifuged at 3000*g*, and the supernatants were collected into fresh polypropylene tubes. The plasma and buffy coat samples were stored at −20 °C until further processing.

DNA EXTRACTION FROM PLASMA AND BUFFY COAT SAMPLES

DNA from plasma samples was extracted with a QIAamp Blood Kit (Qiagen) using the "blood and body fluid protocol" (1997 version) as recommended by the manufacturer *(22)*. Eight hundred microliters of plasma sample was used for DNA extraction per column. An elution volume of 50 μL was used. Buffy coat DNA was extracted using a Nucleon DNA Extraction Kit from Scotlabs according to manufacturer's recommendations.

FETOMATERNAL GENOTYPING USING CONVENTIONAL PCR

For detection of the transfer of nucleated cells and plasma DNA from the fetus to the mother, the *SRY* gene that is present on the Y-chromosome of a male fetus was used as a fetal marker *(22)*. For the detection of the transfer of nucleated cells and plasma DNA from the mother to the fetus, two polymorphic markers were used, namely, the insertion/deletion polymorphism involving the glutathione *S*-transferase M1 (*GSTM1*) gene *(1, 26, 27)* and the insertion/deletion polymorphism involving intron 16 of the angiotensin-converting enzyme (*ACE*) gene *(1, 28)*. Fetomaternal pairs informative for these systems were those in whom the mother possessed an allele not present in the fetus.

For the *GSTM1* locus, ~50% of the population did not possess the gene *(27)*. Mother-baby pairs informative for this locus would have the mother possessing the *GSTM1* gene and the baby possessing the null allele. *GSTM1* genotyping of fetomaternal pairs was carried out using a low-sensitivity system based on conventional non-real-time PCR, using reagents from a GeneAmp DNA Amplification Kit (PE Biosystems). The primer sequences are listed in Table 1. *GSTM1* genotyping was carried out in 100-μL reaction volumes using 100 ng of maternal or cord buffy coat DNA in a mixture containing 1.5 mmol/L $MgCl_2$, 12.5 pmol of each of the primers GST-194F and GST-273R-2 (Table 1), 0.6 U of AmpliTaq Gold polymerase, and 200 μmol/L each of dATP, dGTP, dCTP, and dUTP. The reaction mixture was initially denatured at 95 °C for 12 min, followed by 35 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min, and ended with a final extension at 72 °C for 5 min using a PE Biosystems model 480 thermocycler. On agarose gel electrophoresis, DNA from individuals possessing a *GSTM1* gene would produce an 80-bp PCR product that was absent in *GSTM1*-null homozygotes.

For the insertion/deletion polymorphism involving a 287-bp fragment in intron 16 of the *ACE* gene, mother-baby pairs would be informative if the baby was homozygous for either the insertion (i.e., *II*) or deletion (i.e., *DD*) alleles, and the mother was heterozygous (*ID*). Fetomaternal genotyping was carried out using a low-sensitivity system involving primers ACE-P1 and ACE-P2 (Table 1). PCR was carried out in 100-μL reaction volumes using 100 ng of buffy coat DNA isolated from maternal and cord blood, as for the *GSTM1* system, except that $MgCl_2$ was kept at 3 mmol/L. Thermocycling was initiated with a 12-min incubation at 95 °C, followed by 35 cycles of 94 °C for 1 min, 58 °C for 1 min, and 72 °C for 1 min, and ended with a final extension at 72 °C for 5 min. On agarose gel

Table 1. Primer and probe sequences.

Primer name	Sequence	Reference/Sequence source
SRY-109F	5'-TGG CGA TTA AGT CAA ATT CGC-3'	*(22)*
SRY-245R	5'-CCC CCT AGT ACC CTG ACA ATG TAT T-3'	*(22)*
GST-194F	5'-GGA GAA GAT TCG TGT GGA CA-3'	GenBank X68676
GST-273R-2[a]	5'-CTG GAT TGT AGC AGA TCA TAC-3'	GenBank X68676
ACE-P1	5'-CTG GAG ACC ACT CCC ATC CTT TCT-3'	*(1)*
ACE-P2	5'-GAT GTG GCC ATC ACA TTC GTC AGA T-3'	*(1)*
ACE-1428F	5'-CCA TTT CTC TAG ACC TGC TGC C-3'	GenBank X62855
ACE-1721F	5'-GCT GGG ATT ACA GGC GTG ATA-3'	GenBank X62855
ACE-1826R	5'-GCC CTT AGC TCA CCT CTG CTT-3'	GenBank X62855
Beta-globin-354F	5'-GTG CAC CTG ACT CCT GAG GAG A-3'	*(22)*
Beta-globin-455R	5'-CCT TGA TAC CAA CCT GCC CAG-3'	*(22)*
Probe name	**Sequence[b]**	**Reference/Sequence source**
SRY-142T	5'-(FAM)-AGC AGT AGA GCA GTC AGG GAG GCA GA-(TAMRA)-3'	*(22)*
GST-215T	5'-(FAM)-TTT GGA GAA CCA GAC CAT GGA CAA C-(TAMRA)-3'	GenBank X68676
ACE-1745T	5'-(FAM)-TCA CTT TTA TGT GGT TTC GCC AAT TTT ATT CCA-(TAMRA)-3'	GenBank X62855
beta-globin-402T	5'-(JOE)AAG GTG AAC GTG GAT GAA GTT GGT GG(TAMRA)-3'	*(22)*

[a] The underlined nucleotide was an artificial mismatch to increase the specificity of the primer.

[b] FAM and JOE represent the fluorescent reporters and correspond to 6-carboxyfluorescein and 2,7-dimethyoxy-4,5-dichloro-6-carboxyfluorescein, respectively. TAMRA represents the quencher and corresponds to 6-carboxy-tetramethylrhodamine.

electrophoresis, the insertion and deletion alleles were indicated by 479- and 191-bp PCR products, respectively.

REAL-TIME QUANTITATIVE PCR

For the detection of the fetomaternal transfer of nucleated cells and plasma DNA, real-time quantitative PCR was used. Real-time quantitative PCR was based on the continuous optical monitoring of the progress of an amplification reaction that was coupled to the liberation of a fluorescent reporter *(22, 25)*. The fluorescent reporter was released when the 5′-to-3′ exonuclease activity of the *Taq* polymerase *(29)* cleaved it from the oligonucleotide probe hybridizing to the PCR product *(30)*. Fluorescent signals were captured in real-time using a PE Biosystems 7700 Sequence Detector (PE Biosystems).

Real-time quantitative PCR systems for the *SRY*, *GSTM1*, and *ACE* genes were used. The *SRY* system has been described in detail previously *(22)* and was used to detect the transfer of nucleated cells and plasma DNA from a male fetus into the maternal blood. Real-time PCR was set up with the primers SRY-109F and SRY-245R and the fluorogenic probe SRY-142T. The primer and probe sequences are listed in Table 1. Amplification reagents were obtained from a TaqMan PCR Core Reagent Kit (PE Biosystems). Detailed reaction setup conditions were as described previously *(22)*. dUTP and uracil-*N*-glycosylase were included in the reaction mixture as an anticontamination measure *(22, 31)*. The target consisted of either 500 ng of buffy coat DNA or 5 μL of plasma DNA (out of a total elution volume of 50 μL). Each assay was carried out in duplicate, and the mean results were used for further analysis. For quantitative measurement, a calibration curve was constructed using DNA from a healthy male individual. A conversion factor of 6.6 pg of DNA per cell was used for expressing the results as genome-equivalents *(32)*. One genome-equivalent was defined as the amount of *SRY* sequence present in one diploid male cell. Serial dilution experiments indicated that the *SRY* system was able to detect a minimum of 1 genome-equivalent of target.

The *GSTM1* and *ACE* systems were used to detect the transfer of maternal nucleated cells and plasma DNA into the baby's circulation. The real-time *GSTM1* system consisted of the primers GST-194F and GST-273R-2 and the fluorogenic probe GST-215T (Table 1). Reaction setup conditions were as those for the *SRY* system, with the target consisting of either 500 ng of buffy coat DNA or 5 μL of plasma DNA. The amplification conditions consisted of an initial incubation at 50 °C for 2 min (for the uracil-*N*-glycosylase to act), followed by incubation at 95 °C for 10 min, and 40 cycles of 95 °C for 15 s and 56 °C for 1 min. Each assay was carried out in duplicate, and the mean results were used for further analysis. For quantitative measurement, a calibration curve was constructed using DNA from an obligate *GSTM1* heterozygote (i.e., a *GSTM1*-positive mother of a *GSTM1*-null baby) possessing one copy of the target *GSTM1* gene. For the purpose of conversion to genome-equivalent units, one cell from this obligate *GSTM1* heterozygote was defined as possessing 0.5 genome-equivalent of the *GSTM1* sequence. Serial dilution experiments indicated that the *GSTM1* system was able to detect a minimum of 1 genome-equivalent of target.

Two real-time PCR systems were constructed for the *ACE* gene: one for the insertion allele and the other for the deletion allele. The assay for the insertion allele involved the primers ACE-1721F and ACE-1826R and the fluorogenic probe ACE-1745T (Table 1). For the deletion allele,

the primers ACE-1428F and ACE-1826R and the fluorogenic probe ACE-1745T (Table 1) were used. In addition, further allele specificity for the deletion allele was conferred by the digestion of the insertion allele using the restriction enzyme *Taq*I (10 U for 500 ng of buffy coat DNA or 5 μL of extracted plasma DNA for 2 h at 65 °C; Life Technologies) before DNA amplification *(1)*. One unit of *Taq*I was also included in each real-time PCR reaction to cleave any residual DNA containing the insertion allele and any PCR product amplified from the insertion allele. The thermal profile was same as that used for the *GSTM1* system, with 40 cycles being used for the *ACE* insertion system and 50 cycles being used for the deletion system. Each assay was carried out in duplicate, and the mean results were used for further analysis.

For quantitative measurement, calibration curves were constructed using DNA from individuals homozygous for either the insertion or deletion alleles (i.e., possessing two copies of the respective alleles). For the purpose of conversion to genome-equivalent units, a single cell from each of these individuals was defined as possessing 1 genome-equivalent of the respective allele. Serial dilution experiments indicated that the *ACE* insertion and deletion systems were able to detect a minimum of 1 and 3 genome-equivalents of target, respectively.

In addition to the *SRY*, *GSTM1*, and *ACE* systems, all buffy coat and plasma DNA samples were also amplified using a *β-globin* real-time quantitative PCR system using the primers beta-globin-354F and beta-globin-455R, and the fluorogenic probe beta-globin-402T (Table 1) *(22)*. This system was used to confirm the amplifiability of the extracted DNA, and the quantitative results were used to calculate the fractional concentration of the transferred cellular and plasma DNA involved in fetomaternal trafficking. A normal diploid cell was defined as possessing 1 genome-equivalent of *β-globin* sequence.

The data captured by the PE Biosystems 7700 Sequence Detector were analyzed using the Sequence Detection System software, Ver. 1.6.3 (PE Biosystems). The mean quantity of each duplicate was used for further concentration calculation. For buffy coat DNA, the software would calculate the amount of target sequences in the input DNA (500 ng). For plasma DNA, the concentration expressed in genome-equivalents/mL was calculated using the following equation:

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

where, c is the target concentration in plasma (genome-equivalents/mL); Q is the target quantity (genome-equivalents) determined by sequence detector in a PCR; V_{DNA} is the total volume of DNA obtained after extraction, typically 50 μL per Qiagen extraction; V_{PCR} is the volume of DNA solution used for PCR, typically 5 μL; and V_{ext} is the volume of plasma extracted, typically 0.8 mL.

STATISTICAL ANALYSIS

Statistical analysis was carried out using the Sigmastat 2.0 software.

Results

GENOTYPING OF MOTHER-BABY PAIRS

A total of 156 mother-baby pairs with male babies were recruited in this study. *GSTM1* genotyping revealed 21 mother-baby pairs in whom the babies were *GSTM1*-null and the mothers were *GSTM1*-positive (i.e., an obligate *GSTM1* heterozygote). *ACE* genotyping was carried out on the remaining cases and revealed 14 mother-baby pairs in whom the babies were homozygous for the insertion allele and the mothers were heterozygous. *ACE* genotyping also revealed 15 mother-baby pairs in whom the baby was homozygous for the deletion allele and the mother was heterozygous. In total, 50 mother-baby pairs were informative for studying the bidirectional transfer of nucleated cells and plasma DNA. As a negative control for the detection of fetal-derived DNA in maternal blood, an additional 20 mother-baby pairs with female babies were also recruited.

QUANTIFICATION OF MATERNAL NUCLEATED CELLS AND PLASMA DNA IN FETAL BLOOD

In the cellular fraction, maternal DNA sequences were detected in the cord blood buffy coat DNA in 12 of the 50 informative cases (24%; Table 2). Representative data illustrating the performance of the *GSTM1* system are shown in Fig. 1. For the positive cases, the median fractional concentration of maternal DNA (i.e., as a ratio to the *β-globin* real-time PCR results) in the buffy coat of cord blood was 2.6×10^{-4} (interquartile range, 1.7×10^{-4} to 3.6×10^{-4}; Table 2). In the plasma fraction, maternal DNA sequences were detected in cord plasma DNA in 15 of the 50 informative cases (30%; Table 2). For the positive cases, the median absolute concentration of maternal plasma DNA in cord plasma was 56 genome-equivalents/mL (interquartile range, 28–259 genome-equivalents/mL). When the results were expressed as a ratio of maternal to total cord plasma DNA (i.e., maternal plus fetal, using the *β-globin* real-time PCR results), the median fractional concentration was 3×10^{-3} (interquartile range, 1×10^{-3} to 1.6×10^{-2}; Table 2). As negative controls, none of the maternal buffy coat and plasma DNA samples from the 20 women carrying female babies were positive for the *SRY* PCR.

QUANTIFICATION OF FETAL NUCLEATED CELLS AND PLASMA DNA IN MATERNAL BLOOD

In the cellular fraction, fetal-derived *SRY* sequences were detected in the maternal buffy coat DNA in 13 of the 50 informative cases (26%; Table 2). For the positive cases, the median fractional concentration of fetal DNA in the total maternal buffy coat DNA (i.e., as a ratio to the *β-globin* real-time PCR results) was 3.2×10^{-4} (interquartile range, 0.6×10^{-4} to 7.6×10^{-4}; Table 2). In the plasma

Table 2. Quantification of two-way fetomaternal transfer of nucleated cells and plasma DNA.

Case no.	Gravidity	Parity	Gestational age, weeks	Fetus-to-mother plasma DNA transfer, fractional concentration × 10^2	Fetus-to-mother cellular transfer, fractional concentration × 10^4	Mother-to-fetus plasma DNA transfer, fractional concentration × 10^3	Mother-to-fetus cellular transfer, fractional concentration × 10^4
1	3	2	41	2.5	4.3	0	0.7
2	2	0	40	1.0	0	0	0
3	5	2	39	3.4	0	0	1.8
4	3	2	41	0.8	0	0	0
5	2	1	37	3.0	0	0	2.1
6	1	0	40	4.3	0	0	0
7	2	1	39	1.7	0	0	0
8	2	0	37	1.3	0	0.1	0
9	3	1	38	4.1	4.2	0	1.6
10	7	2	39	1.4	0	0.3	2.8
11	2	1	39	2.2	0	3.0	3.9
12	5	2	37	5.9	0	0	0
13	1	0	40	8.1	0.007	1.5	0
14	1	0	38	7.9	0	0	0
15	2	1	40	5.1	3.2	0	0
16	2	1	40	4.0	1.7	0	0
17	1	0	37	0.3	0	0	3.2
18	2	0	39	4.0	0	0	0
19	2	0	39	2.6	0	0.8	0.4
20	1	0	40	1.8	0	3.2	0
21	1	0	38	2.3	0	0	0
22	3	2	38	0.5	0	27.7	32
23	2	1	41	1.5	0	0	0
24	1	0	37	1.2	403.5	0	0
25	6	2	39	3.7	0.3	0	68
26	1	0	37	1.5	0	3.7	2.3
27	1	0	39	0.9	0	19.4	0
28	6	2	39	18.3	13.7	0	0
29	3	1	37	1.7	0	0	0
30	4	1	37	10.1	0	63.6	0
31	3	1	39	6.7	0	5.4	0
32	3	2	38	19.0	0	0	0
33	2	1	39	0.9	0	0	0
34	1	0	39	0.6	0	27.2	2.8
35	3	1	38	1.2	5.5	0	0
36	2	1	37	3.6	0	2.6	0
37	1	0	42	12.5	0	0	0
38	2	1	39	17.6	0.3	0	0
39	2	1	40	19.7	0	0	0
40	1	0	37	4.3	0	0	0
41	2	1	40	4.1	0	0	0
42	1	0	40	3.4	0	0	0
43	3	1	40	2.6	1.6	0	0
44	3	2	38	5.4	0	0.8	0
45	2	1	39	1.2	0	0	0
46	3	1	38	5.3	0	0	0
47	4	3	41	0.9	19.1	0	0
48	3	1	39	2.2	0	0	0
49	2	1	34	3.1	0	1.4	0
50	1	0	39	10.9	0.7	0	0

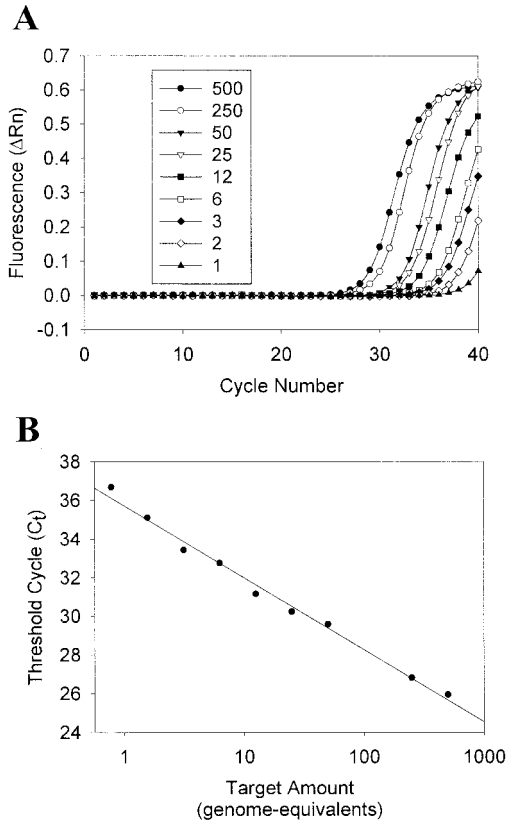

Fig. 1. Real-time quantitative PCR analysis of the *GSTM1* gene.

(*A*), amplification plots of the assay when it was applied to serial dilutions of genomic DNA from a *GSTM1*-positive individual. The numbers in the *key* represent the amounts of input DNA in genome-equivalents. Note that the amplification plots shift to the right with progressively smaller amounts of input target molecules. 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 *(25)*. (*B*), plot of the threshold cycle (C_t) against the input target quantity (common logarithmic scale). The correlation coefficient is 0.991.

fraction, fetal-derived *SRY* sequences were detected in maternal plasma DNA in 50 of the 50 cases (100%; Table 2). The median absolute concentration of fetal DNA in maternal plasma was 443 genome-equivalents/mL (interquartile range, 271–925 genome-equivalents/mL). The median fractional concentration of fetal DNA in maternal plasma was 3×10^{-2} (interquartile range, 1.4×10^{-2} to 5.3×10^{-2}; Table 2).

QUANTITATIVE-DIRECTIONAL CORRELATION OF FETOMATERNAL CELLULAR AND DNA TRAFFICKING

The number of cases with or without transfer of nucleated cells and plasma DNA from the mother to the baby are listed in Table 3A. The proportions of cases with or without cellular transfer were not affected by the plasma DNA transfer status (Fisher exact test, $P = 0.15$). Fifteen of the 50 informative cases (30%) had discordant results between the presence or absence of nucleated cell and plasma DNA traffic. For the cases with detectable cellular and/or plasma DNA transfer, there was no significant correlation in the amounts of these transfers (Spearman rank-order correlation, $r = -0.20$; $P = 0.37$). For the transfer of nucleated cells and plasma DNA from the fetus to the mother, the results are listed in Table 3B. The plasma DNA transfer status did not affect the proportions of cases with or without cellular transfer (Fisher exact test, $P = 1.0$). Among the 50 cases, 37 (74%) had detectable fetal-derived plasma DNA without detectable nucleated cell traffic. A comparison between the amounts of plasma fetal DNA in cases with and without detectable amounts of fetal nucleated cell traffic into the mother blood revealed no significant difference ($P = 0.20$, Mann–Whitney rank-sum test). For the cases with both detectable fetal cell and plasma DNA transfer into the maternal circulation, there was no significant correlation between the amounts of cellular and plasma DNA traffic (Spearman rank-order correlation, $r = -0.24$; $P = 0.41$).

Table 3. Number of cases tabulated with regard to the cellular and plasma DNA transfer status.

A. Mother-to-fetus transfer.

		Cellular DNA transfer[a]	
		+	−
Plasma DNA transfer[a]	+	6	9
	−	6	29

B. Fetus-to-mother transfer.

		Cellular DNA transfer[a]	
		+	−
Plasma DNA transfer[a]	+	13	37
	−	0	0

[a] + and − denote cases with detectable and undetectable trafficking, respectively.

Discussion

In this study we performed the first systematic quantitative analysis of the bidirectional transfer of nucleated cells and plasma DNA between mother and baby. Our data indicate that such transfers are common in human pregnancy. Thus, maternal nucleated cell and plasma DNA transfers into the baby's circulation could be detected in 24% and 30% of the studied cases, respectively. In the other direction of trafficking, fetal nucleated cell and plasma DNA transfer into the mother's circulation could be detected in 26% and 100% of the studied cases, respectively.

For nucleated maternal cell transfer into the fetal circulation, the proportion of cases with detectable maternal cell traffic into the fetal circulation was higher than those reported using less sensitive technologies, such as minisatellite analysis *(14, 33)*. Our data were qualitatively consistent with those reported previously using methods of comparable sensitivity *(1, 16, 34)*. However, our approach has the advantage that it is also quantitative in nature, thus allowing us to rapidly establish the amount of maternal cell transfer in each case. Our data indicated that maternal nucleated cells were present in umbilical

cord blood at a median fractional concentration of 2.6×10^{-4} (interquartile range, 1.7×10^{-4} to 3.6×10^{-4}). These results were consistent with those obtained by Hall et al. *(15)*, who used fluorescence in situ hybridization techniques and determined that the fractional concentration ranged from 4×10^{-4} to 2×10^{-2}. Our approach, however, was much more rapid and automated than that by Hall et al. *(15)* who had to manually score a minimum of 1000 nuclei for each case.

The current study was the first time maternal DNA was detected in human umbilical cord plasma. For the cases with detectable plasma DNA traffic, the median fractional concentration of maternal DNA in cord plasma was 3×10^{-3} (interquartile range, 1×10^{-3} to 1.6×10^{-2}). It is interesting to note that this fractional concentration was >10-fold higher than the comparable values determined for nucleated cell traffic discussed above. One explanation for the existence of 15 cases with discordant mother-to-fetus cellular and plasma DNA transfer is that the cell types responsible for cellular transfer and plasma DNA release may be different. Future studies are necessary to test this hypothesis. It is currently unknown whether this transfer of plasma DNA from the mother to the fetus may have any biological consequence. An interesting analogy can be drawn between the present data and animal experiments documenting the transplacental transfer of exogenous DNA administered either intravenously or orally to pregnant animals *(35, 36)*. In addition, recent data demonstrating the horizontal transfer of DNA *(37–39)* clearly suggest the interesting possibility that part of this plasma DNA may exert previously unknown biological functions. It is thus possible that some of the phenomena previously suggested to be mediated by cellular transfer, e.g., the induction of partial immunological tolerance to the noninherited maternal HLA antigens *(19, 20)*, might actually be mediated by plasma DNA.

Concerning the transfer of nucleated cells and plasma DNA from the fetus to the mother, our data represent the first quantitative comparison of the degree of fetus-to-mother trafficking of cells and plasma DNA in the same subject cohort. The most striking feature of this quantitative analysis was the lack of correlation in the amount of cellular and plasma DNA transfer into the maternal circulation. One possible explanation of this observation is that the cell populations predominantly involved in cellular traffic and plasma DNA liberation are different, similar to the hypothesis suggested above for mother-to-fetus transfer. For example, whereas fetal nucleated red cells have been regarded by many investigators to be a predominant cell type involved in fetus-to-mother transfer *(6, 40, 41)*, it is possible that other cell types, e.g., the trophoblasts, are more important for the liberation of plasma fetal DNA. Because of its ubiquitous presence in the studied samples, our data reemphasize our previous suggestion that plasma fetal DNA is a valuable and easily accessible source of fetal genetic material for prenatal diagnosis and monitoring *(22)*. Conversely, the detection rate of fetal nucleated cells in maternal blood was just 26% in the present cohort. This latter much lower detection rate of fetal nucleated cells in maternal blood was less than in our previous study *(1)*, possibly because of a reduction in the PCR reaction volume (from 100 μL to 50 μL), which thus concomitantly halved the amount of input DNA.

An impressive difference was observed between the detection rates of fetal DNA in maternal plasma (100%) and maternal DNA in cord plasma (30%). One possible explanation for this difference is the presence of a specialized "fetal-derived" structure, namely, the placenta, which separates the fetal and maternal circulation. If we postulate that the main constituent cell types of the placenta, i.e., the trophoblasts, are the predominant cell populations responsible for the liberation of fetal DNA into the maternal circulation, then the absence of an analogous maternal-derived cell type on the fetal side might contribute to the relatively low detection rate of maternal DNA in fetal plasma.

The present study establishes important baseline quantitative values for the two-way transfer of nucleated cells and plasma DNA between the mother and fetus in "normal" pregnancies. The biologic significance of the variations in the amount of trafficking in either direction is unclear at present. However, our methodology can be readily applied to answer the numerous unexplored areas in this field. First, relatively few data are available for the prenatal transfer of cells *(42, 43)* and plasma DNA from the mother to the fetus. It would therefore be very interesting to extend this type of quantitative bidirectional investigation to the prenatal scenario. Second, although it is established that the fetus-to-mother trafficking of nucleated cells and plasma DNA is perturbed in pathological conditions such as preeclampsia *(7, 8, 44)*, it is currently unclear whether such abnormalities could also be detected in the mother-to-fetus arm of trafficking. Third, relatively little is known regarding the clearance of plasma DNA from the circulation. Recent data indicate that fetal DNA is cleared very rapidly from maternal plasma *(23)*. It is currently unknown whether the same rapid clearance kinetics could also be observed for maternal DNA clearance from the fetal circulation. Because it is obviously difficult to carry out this type of analysis in humans, it would be highly informative to apply this bidirectional study approach to animal models of fetomaternal transfer. Fourth, van Wijk et al. *(45)* recently described the intriguing observation that apoptotic fetal and maternal nucleated cells are present in the plasma of pregnant women. The numbers of these "plasma-derived cells" (300–400 cells/mL of maternal blood, of which 1 in 500 to 1 in 2000 are of fetal origin, during gestational weeks 7–16) *(45)* appear to be much smaller than the total concentration of plasma DNA (mean of 3466 genome-equivalents/mL, of which an average of 3.4% is fetal-derived, during gestational weeks 11–17) *(22)*. These preliminary comparisons therefore indicate that most of

the DNA in the plasma fraction exists in a cell-free state, with the remaining minority being present in apoptotic cells. Further studies should attempt to confirm these deductions in the same subject cohort. In addition, it would be interesting to investigate whether apoptotic maternal and fetal cells are also present in fetal plasma. It is hoped that the answers to these questions would further improve our understanding of the fetomaternal relationship at a molecular level.

On a technological level, the real-time quantitative PCR systems that were developed as part of this study have numerous other applications. For example, these systems would be useful as sensitive and quantitative assays for the study of post-bone marrow transplantation chimerism. A second application would be for the study of autoimmune diseases in which recent data have suggested a possible role of fetal cell persistence in the mother's body *(11–13)*. The techniques described here might have application to study whether the persistence of maternal cells in the baby's body *(46)* may also contribute to the development of certain autoimmune diseases.

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