

# Maternal Plasma Cell-Free Fetal and Maternal DNA at 11–13 Weeks' Gestation: Relation to Fetal and Maternal Characteristics and Pregnancy Outcomes

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## Key Words

Cell-free fetal DNA · Cell-free maternal DNA · First-trimester screening · Preeclampsia · Small for gestational age · Preterm birth

## Abstract

**Objective:** To examine the possible relationship between maternal and fetal characteristics and pregnancy outcomes on fetal and maternal cell-free (cf) DNA in maternal plasma at 11–13 weeks' gestation. **Methods:** cfDNA was extracted from maternal plasma of 1,949 singleton pregnancies and chromosome-selective sequencing was used to determine the proportion of cfDNA and total cfDNA counts which was of fetal and maternal origin. Multivariate regression analysis was used to determine whether specific maternal and fetal characteristics and pregnancy complications, such as pre-eclampsia (PE), early spontaneous preterm birth (SPB) and delivery of small for gestational age (SGA) neonates, were significant predictors of fetal and maternal cfDNA in maternal plasma. **Results:** The fetal and maternal cfDNA plasma concentration increased with serum pregnancy-associated plasma protein-A and free  $\beta$ -human chorionic gonadotropin level, was higher in women of Afro-Caribbean and East-Asian

racial origin than in Caucasians, and lower in smokers, but it was not significantly altered in pregnancies complicated by PE, SPB or SGA. The fetal cfDNA level was inversely related to maternal weight and uterine artery pulsatility index, and maternal cfDNA increased with maternal weight. **Conclusions:** The fetal and maternal cfDNA level in maternal plasma is affected by maternal and fetal characteristics, but it is not altered in pregnancy complications.

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## Introduction

Analysis of cell-free DNA (cfDNA) in maternal blood can detect more than 99% of pregnancies with trisomy 21 at a false-positive rate of less than 1% [1–9]. Consequently, this method is far superior to all currently available approaches to screening for trisomy 21 [10]. A potential issue with analysis of cfDNA as a universal screening test in all pregnant women is the failure rate in providing a result, which primarily depends on the relative proportion of fetal cfDNA to maternal cfDNA in maternal plasma. In trisomic pregnancies the number of molecules derived from the extra fetal chromosome, as a proportion of

all sequenced molecules, is higher than in disomic pregnancies. The ability to detect this small increase in the amount of a given chromosome in maternal plasma in a trisomic pregnancy is directly related to the fetal fraction. If the fetal fraction is below 4%, noninvasive prenatal testing is currently unable to provide a result [2, 3, 7].

In a recent study of 1,949 singleton pregnancies undergoing first-trimester screening for aneuploidies as part of their routine antenatal care at 11–13 weeks' gestation, the median fraction of fetal cfDNA in maternal plasma was 10% [11]. Fetal fraction cfDNA in maternal plasma was determined by chromosome-selective sequencing of nonpolymorphic and polymorphic loci, where fetal alleles differ from maternal alleles [8]. The fetal fraction decreased with maternal weight, increased with fetal crown rump length (CRL), maternal serum level of free  $\beta$ -human chorionic gonadotropin ( $\beta$ -hCG) and pregnancy-associated plasma protein-A (PAPP-A), was higher in smokers and in the presence of fetal trisomy 21, and lower in women of Afro-Caribbean racial origin than in Caucasians. However, this study did not clarify whether the changes in fetal fraction were the consequence of altered levels in fetal or maternal cfDNA. For example, the finding that the fetal fraction was decreased in women of Afro-Caribbean racial origin could have been the consequence of decrease in fetal cfDNA, increase in maternal cfDNA, decrease in both fetal and maternal cfDNA (but more so for fetal cfDNA), or increase in both fetal and maternal cfDNA (but more so for maternal cfDNA).

The increase in fetal fraction with serum level of free  $\beta$ -hCG and PAPP-A has been attributed to the size of the placental mass, which is the common source of both cfDNA and metabolites [11]. However, several studies in pregnancies with male fetuses in which maternal plasma cfDNA level was estimated by amplification loci on the Y-chromosome reported that there is a 2- to 5-fold increase in cfDNA in preeclampsia (PE), which is associated with impaired placentation reflected in low first-trimester serum PAPP-A [12–16].

The objectives of this study comprising roughly 2,000 women undergoing routine screening for aneuploidies and adverse pregnancy outcomes at 11–13 weeks' gestation were (1) to examine the possible relationship between fetal and maternal characteristics on the maternal plasma concentrations of fetal and maternal cfDNA, and (2) secondly, to evaluate if there is a significant association between fetal and maternal cfDNA with PE, delivery of small for gestational age (SGA) neonates and early spontaneous preterm birth (SPB), which are thought to be associated with impaired placentation.

## Methods

The data for this study were derived from analysis of stored maternal plasma obtained during prospective first-trimester combined screening for aneuploidies and adverse pregnancy outcomes in women with singleton pregnancies attending for their routine first hospital visit in pregnancy [17]. Patients agreeing to participate in the study, which was approved by the NHS National Research Ethics Service, provided written informed consent.

At 11<sup>+0</sup>–13<sup>+6</sup> weeks' gestation, we recorded maternal characteristics and medical history, including maternal racial origin (Caucasian, Afro-Caribbean, South Asian, East Asian and mixed), method of conception (spontaneous or assisted conception requiring the use of ovulation drugs or in vitro fertilization), cigarette smoking during pregnancy (yes or no), and measured maternal weight and height. We then performed a transabdominal ultrasound scan to (1) determine gestational age from the measurement of the fetal CRL, (2) diagnose any major fetal abnormalities, (3) measure fetal nuchal translucency (NT) thickness and (4) use color Doppler ultrasound to visualize the left and right uterine artery; we then measured the pulsatility index (PI) in each vessel and calculated the mean PI [18–21]. In addition, the maternal serum PAPP-A and free  $\beta$ -hCG were determined within 10 min of blood collection using automated machines that provide reproducible results (DELFIAXpress system; PerkinElmer Life and Analytical Sciences, Waltham, Mass., USA). The measured NT was expressed as a difference from the expected normal mean for gestation (delta value) [20]. Similarly, the measured free  $\beta$ -hCG and PAPP-A were converted into the multiple of median (MoM) for gestational age adjusted for maternal weight, racial origin, smoking status, method of conception, parity and machine for the assays [22]. Biochemical and biochemical markers were combined to estimate the patient-specific risk for aneuploidies; women who considered their risk to be high were offered chorionic villus sampling for fetal karyotyping.

The population for this study included 1,949 pregnancies examined between October 2010 and January 2011 and in which cfDNA analysis was carried out in 2 ml of stored maternal plasma. This is the same cohort as published in a previous report where sensitivity and specificity for trisomy detection was determined [9].

### Outcome Measures

Demographic characteristics, ultrasonographic measurements and biochemical results were recorded in computer databases. Karyotype results, obtained from genetic laboratories, and details on pregnancy outcomes, obtained from the maternity computerized records or the general medical practitioners of the women, were added into the database as soon as they became available.

The definition of PE was that of the International Society for the Study of Hypertension in Pregnancy [23]. SGA was defined as birth weight below the 5th percentile for gestational age of a normal range derived from our population [24]. Spontaneous preterm delivery was defined as delivery before 34 weeks' gestation as a result of presentation with preterm premature rupture of membranes and/or contractions. The obstetric records of patients with adverse pregnancy outcomes were examined to confirm the diagnosis.

### Laboratory Analysis

Venous blood was collected in ethylene diamine tetraacetic acid (EDTA) BD Vacutainer™ tubes (Becton Dickinson UK Ltd., Oxford, UK) and within 15 min of collection it was centrifuged at 2,000 g for 10 min and subsequently at 16,000 g for 10 min. Plasma samples were then stored at  $-80^{\circ}\text{C}$  until subsequent analysis. The samples were sent overnight on dry ice from London, UK to the USA for analysis using a chromosome-selective assay (Harmoney™ Prenatal Test; Ariosa Diagnostics Inc., San Jose, Calif., USA). The laboratory personnel, who were unaware of the fetal karyotype, provided risk scores for trisomies 21 and 18 and the plasma concentrations of fetal and maternal cfDNA as determined by sequencing counts [8, 25].

In this study we report the maternal plasma concentrations in genomic equivalent (GE)/ml of fetal and maternal cfDNA. The estimated risks for trisomies 21 and 18 were published previously [9].

### Statistical Analyses

Descriptive data were presented as the median and interquartile range for continuous variables and in numbers and percentages for categorical variables. Comparisons between outcome groups was by  $\chi^2$  or Fisher's exact test for categorical variables and Mann-Whitney U test for continuous variables, with post hoc Bonferroni correction with an adjusted p value of  $<0.0125$ .

The measured maternal plasma concentrations of fetal and maternal cfDNA were  $\log_{10}$ -transformed to make the distribution Gaussian. Normality of distribution was assessed using probability plot. Regression analysis was used to determine which of the factors amongst maternal weight, height, racial origin, smoking status, method of conception,  $\log_{10}$  PAPP-A,  $\log_{10}$  free  $\beta$ -hCG, fetal CRL, delta NT, fetal gender and karyotype,  $\log_{10}$  uterine artery PI, and adverse pregnancy outcomes were significant predictors of  $\log_{10}$  values of fetal and maternal cfDNA.

The statistical software package SPSS 20.0 (SPSS Inc., Chicago, Ill., USA) was used for data analyses.

## Results

Maternal characteristics, results of the combined test and maternal plasma concentrations of fetal and maternal cfDNA of the study population of 1,949 cases are presented in table 1. The population included 8 cases of trisomy 21, 2 of trisomy 18 and 1,939 euploid pregnancies [9]. In the euploid group there were 46 cases that developed PE, 68 cases that delivered SGA neonates in the absence of PE and 20 cases of SPB before 34 weeks' gestation.

In PE patients, the median maternal weight and uterine artery PI were significantly higher, and there was a higher prevalence of women of Afro-Caribbean racial origin. In SGA without PE, the median maternal weight and height were significantly lower, the uterine artery PI was higher, and there was a higher prevalence of cigarette smokers. The median maternal plasma concentrations of

fetal and maternal cfDNA in the total population were 13.30 GE/ml (10.05–17.86) and 114.93 GE/ml (80.53–164.01), respectively. There was no significant difference in plasma concentration of fetal cfDNA between the outcome groups, but in PE the concentration of maternal cfDNA was increased.

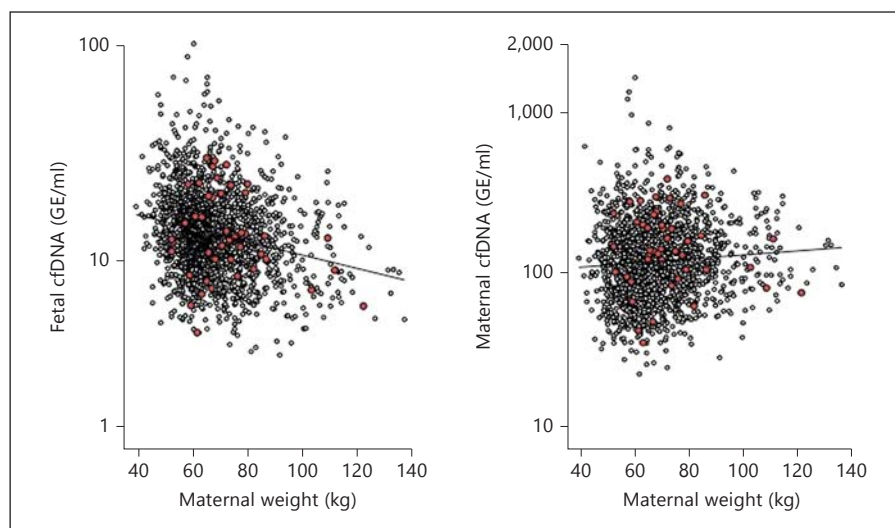
Univariate regression analysis demonstrated that  $\log_{10}$  fetal cfDNA was significantly associated with maternal weight, height, racial origin, smoking status, fetal CRL, fetal gender, trisomy 21 karyotype,  $\log_{10}$  PAPP-A,  $\log_{10}$  free  $\beta$ -hCG and  $\log_{10}$  uterine artery PI, but not method of conception, delta NT, trisomy 18 karyotype, PE, SGA or SPB (table 2). Multivariate regression analysis demonstrated that significant independent prediction of  $\log_{10}$  fetal cfDNA was provided by maternal weight, Afro-Caribbean and East Asian racial origin, smoking status,  $\log_{10}$  values of PAPP-A, free  $\beta$ -hCG and uterine artery PI, but not maternal height, fetal CRL, fetal gender or karyotype ( $R^2 = 0.318$ ; table 2). Fetal cfDNA concentration was 7% higher in women of Afro-Caribbean origin, 12% higher in East Asians and 14% lower in smokers. The correlations between fetal cfDNA with maternal weight and serum concentrations of PAPP-A and free  $\beta$ -hCG are illustrated in figures 1–3.

Univariate regression analysis demonstrated that  $\log_{10}$  maternal cfDNA was significantly associated with maternal weight, Afro-Caribbean, South and East Asian racial origin, smoking status,  $\log_{10}$  PAPP-A,  $\log_{10}$  free  $\beta$ -hCG and PE but not maternal height, method of conception, fetal CRL, fetal gender, karyotype, delta NT,  $\log_{10}$  uterine artery PI, SGA or SPB (table 2). Multivariate regression analysis demonstrated that significant independent prediction of  $\log_{10}$  maternal cfDNA was provided by maternal weight, racial origin, smoking status,  $\log_{10}$  values of PAPP-A and free  $\beta$ -hCG but not PE ( $R^2 = 0.120$ , table 3). Maternal cfDNA concentration was 25% higher in women of Afro-Caribbean origin, 17% higher in South Asians, 16% higher in East Asians and 22% lower in smokers. The correlations between maternal cfDNA with maternal weight and serum concentrations of PAPP-A and free  $\beta$ -hCG are illustrated in figures 1–3.

There was a significant association between fetal cfDNA and maternal cfDNA ( $r = 0.655$ ,  $p < 0.0001$ ; fig. 4).

## Discussion

The findings of this study demonstrate that there is a significant association between the level of fetal and maternal cfDNA in maternal plasma at 11–13 weeks' gesta-

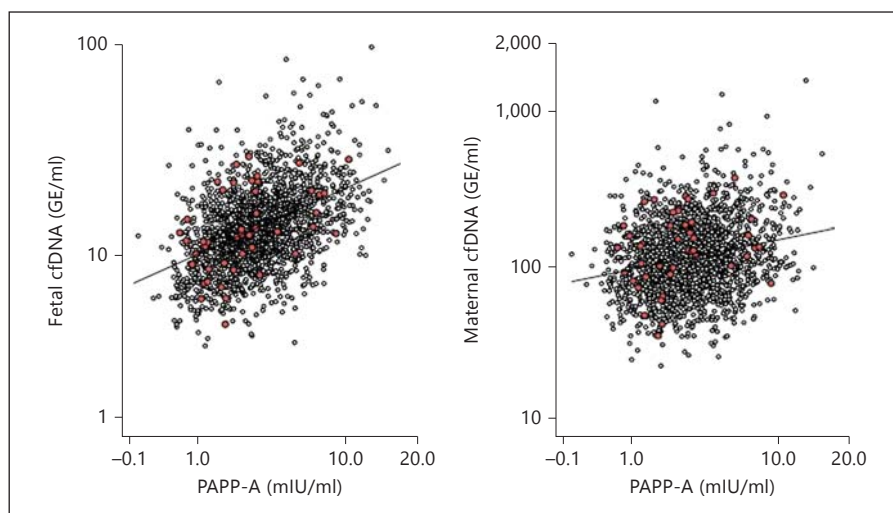


**Fig. 1.** Relationship of maternal weight with maternal plasma concentration of fetal (left) and maternal (right) cfDNA: PE (red circles) and unaffected (black circles).

**Table 1.** Maternal and fetal characteristics of the study population

Characteristics	Total (n = 1,949)	Normal (n = 1,805)	Aneuploidy (n = 10)	PE (n = 46)	SGA (n = 68)	SPB (n = 20)
Maternal age, years	31.8 (27.8–35.3)	31.9 (27.8–35.4)	39.6 (31.7–42.0)*	29.7 (24.8–33.6)	30.5 (26.6–34.7)	30.9 (26.3–34.0)
Maternal weight, kg	65.0 (58.3–75.9)	65.0 (58.4–75.0)	63.0 (60.0–80.2)	69.9 (63.0–77.9)*	60.8 (55.5–70.9)*	64.7 (58.0–74.4)
Maternal height, cm	164 (160–169)	164 (160–169)	167 (163–168)	165 (160–169)	162 (157–165)*	164 (162–170)
Racial origin						
Caucasian	1,377 (70.7)	1,303 (72.2)	7 (70.0)	20 (43.5)*	35 (51.5)*	12 (60.0)
Afro-Caribbean	390 (20.0)	340 (18.8)	3 (30.0)	21 (45.7)*	21 (30.9)	5 (25.0)
South Asian	77 (4.0)	66 (3.7)	0	4 (8.7)	6 (8.8)	1 (5.0)
East Asian	54 (2.8)	50 (2.8)	0	0	3 (4.4)	1 (5.0)
Mixed	51 (2.6)	46 (2.5)	0	1 (2.2)	3 (4.4)	1 (5.0)
Cigarette smoker	120 (6.2)	105 (5.8)	0	1 (2.2)	14 (20.6)*	0
Method of conception						
Spontaneous	1,910 (98.0)	1,771 (98.1)	10 (100.0)	44 (95.7)	67 (98.5)	18 (90.0)
Ovulation drugs	19 (1.0)	17 (0.9)	0	1 (2.2)	1 (1.5)	0
In vitro fertilization	20 (1.0)	17 (0.9)	0	1 (2.2)	0	2 (10.0)
Fetal CRL, mm	62.4 (57.3–67.4)	62.6 (57.5–67.5)	62.6 (57.7–67.8)	61.8 (56.8–65.8)	60.4 (56.6–66.1)	60.5 (57.6–66.6)
Fetal gender						
Male	1,010 (51.8)	944 (52.3)	5 (50.0)	23 (50.0)	30 (44.1)	8 (40.0)
Female	939 (48.2)	861 (47.7)	5 (50.0)	23 (50.0)	38 (55.9)	12 (60.0)
Delta NT	0.11 (–0.09–0.35)	0.11 (–0.08–0.35)	2.00 (0.99–4.44)*	0.07 (–0.15–0.30)	0.06 (–0.12–0.35)	0.06 (–0.12–0.30)
PAPP-A	2.85 (1.85–4.39)	2.87 (1.87–4.40)	1.14 (0.59–3.55)	2.47 (1.38–3.18)	2.76 (1.51–4.41)	2.61 (1.91–3.69)
Free $\beta$ -hCG	37.47 (24.86–58.01)	37.29 (24.85–57.11)	91.12 (22.19–191.21)	36.56 (24.1–57.87)	42.45 (26.13–61.00)	34.26 (25.30–67.56)
Uterine artery PI	1.70 (1.38–2.05)	1.69 (1.37–2.02)	1.66 (1.23–2.29)	1.96 (1.62–2.31)*	2.05 (1.62–2.40)*	1.88 (1.58–2.15)
cfDNA, GE/ml						
Fetal	13.30 (10.05–17.86)	13.29 (10.06–17.73)	18.20 (12.50–26.22)	12.87 (9.94–20.92)	15.47 (9.23–19.60)	12.80 (10.37–19.62)
Maternal	114.93 (80.53–164.01)	113.86 (80.16–163.81)	131.08 (98.61–154.84)	138.30 (98.80–200.07)*	111.16 (70.14–138.30)	136.38 (106.97–186.71)

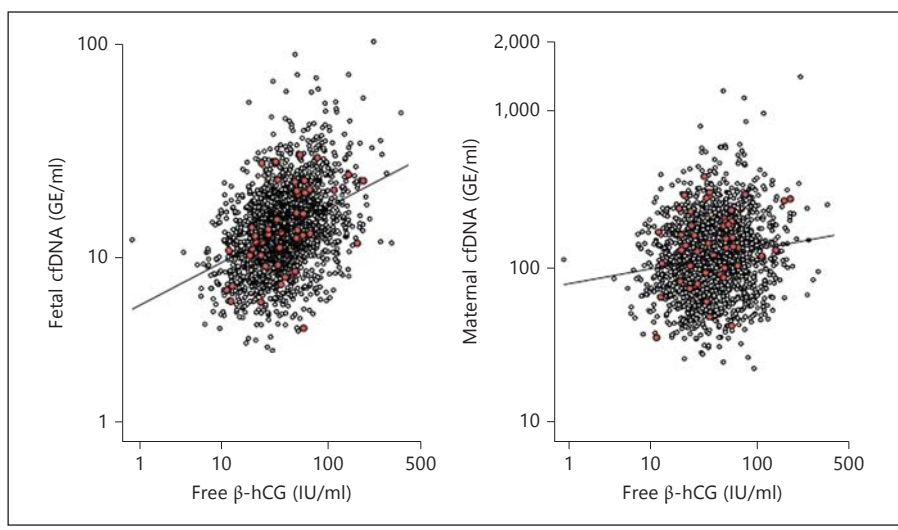
Values are either medians (interquartile range) or n (%). Comparisons between adverse outcome groups and normal are by Mann-Whitney U test for continuous variables and by  $\chi^2$  or Fisher's exact test for categorical variables, with post hoc Bonferroni correction with an adjusted p value of <0.0125 (\*).



**Fig. 2.** Relationship of maternal serum PAPP-A with maternal plasma concentration of fetal (left) and maternal (right) cfDNA: PE (red circles) and unaffected (black circles).

**Table 2.** Regression analysis for the prediction of  $\log_{10}$  fetal cfDNA ( $\log_{10}$  GE/ml)

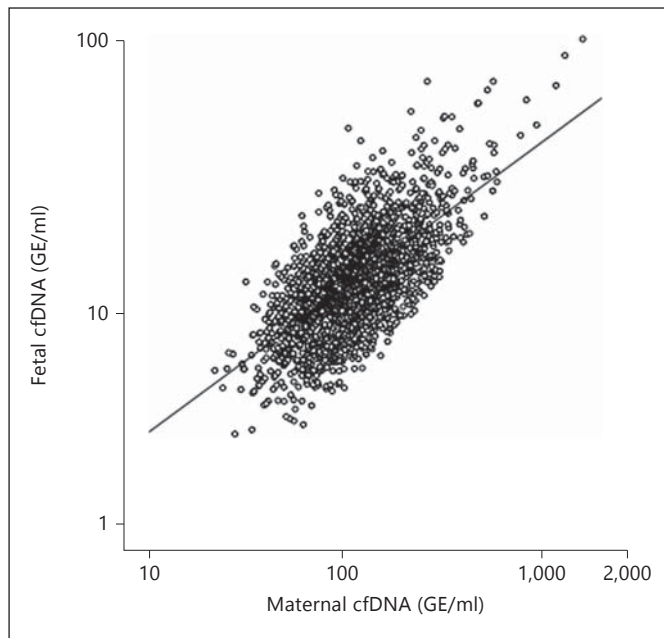
Independent variable	Univariate		Multivariate	
	regression coefficient (95% CI)	p value	regression coefficient (95% CI)	p value
Maternal weight (kg)	-0.003 (-0.004 to -0.003)	<0.0001*	-0.002 (-0.002 to -0.001)	<0.0001*
Maternal height (cm)	-0.003 (-0.004 to -0.001)	<0.0001*	-0.0002 (-0.001 to 0.001)	0.753
Racial origin				
Caucasian	0			
Afro-Caribbean	0.074 (0.052 to 0.096)	<0.0001*	0.031 (0.011 to 0.051)	0.003*
South Asian	0.065 (0.020 to 0.110)	0.005*	0.026 (-0.013 to 0.066)	0.187
East Asian	0.101 (0.048 to 0.154)	<0.0001*	0.048 (0.002 to 0.093)	0.040*
Mixed	-0.0001 (-0.055 to 0.055)	0.998	-	-
Smoking	-0.104 (-0.141 to -0.068)	<0.0001*	-0.064 (-0.096 to -0.032)	<0.0001*
Method of conception				
Spontaneous	0			
Ovulation drugs	0.013 (-0.077 to 0.103)	0.775	-	-
In vitro fertilization	0.057 (-0.031 to 0.144)	0.204	-	-
Fetal CRL (mm)	0.002 (0.0004 to 0.003)	0.007*	-0.001 (-0.002 to 0.0002)	0.183
Fetal gender				
Male	0		0	
Female	0.026 (0.008 to 0.044)	0.004*	0.005 (-0.010 to 0.020)	0.517
Karyotype				
Euploid	0			
Trisomy 21	0.175 (0.037 to 0.313)	0.013*	0.092 (-0.054 to 0.238)	0.215
Trisomy 18	-0.114 (-0.389 to 0.161)	0.417	-	-
Delta NT (mm)	-0.009 (-0.029 to 0.010)	0.354	-	-
$\log_{10}$ PAPP-A	0.317 (0.289 to 0.344)	<0.0001*	0.237 (0.207 to 0.266)	<0.0001*
$\log_{10}$ $\beta$ -hCG	0.276 (0.247 to 0.305)	<0.0001*	0.221 (0.194 to 0.249)	<0.0001*
$\log_{10}$ uterine artery PI	-0.121 (-0.193 to -0.049)	0.001*	-0.123 (-0.184 to -0.061)	<0.0001*
Adverse pregnancy outcome				
PE	-0.004 (-0.062 to 0.055)	0.904	-	-
SGA	0.017 (-0.031 to 0.065)	0.497	-	-
SPB	0.033 (-0.054 to 0.120)	0.460	-	-



**Fig. 3.** Relationship of maternal serum free  $\beta$ -hCG with maternal plasma concentration of fetal (left) and maternal (right) cfDNA: PE (red circles) and unaffected (black circles).

**Table 3.** Regression analysis for the prediction of  $\log_{10}$  maternal cfDNA ( $\log_{10}$  GE/ml)

Independent variable	Univariate		Multivariate	
	regression coefficient (95% CI)	p value	regression coefficient (95% CI)	p value
Maternal weight (kg)	0.001 (0.001 to 0.002)	0.001*	0.002 (0.001 to 0.003)	<0.0001*
Maternal height (cm)	-0.001 (-0.002 to 0.001)	0.492	-	-
Racial origin				
Caucasian	0			
Afro-Caribbean	0.140 (0.115 to 0.166)	<0.0001*	0.096 (0.070 to 0.123)	<0.0001*
South Asian	0.073 (0.021 to 0.126)	0.006*	0.067 (0.016 to 0.118)	0.010*
East Asian	0.077 (0.015 to 0.139)	0.015*	0.065 (0.005 to 0.126)	0.035*
Mixed	0.005 (-0.059 to 0.069)	0.874	-	-
Smoking	-0.143 (-0.186 to -0.100)	<0.0001*	-0.108 (-0.149 to -0.067)	<0.0001*
Method of conception				
Spontaneous	0			
Ovulation drugs	-0.0002 (-0.106 to 0.106)	0.998	-	-
In vitro fertilization	0.096 (-0.007 to 0.200)	0.068	-	-
Fetal CRL (mm)	0.001 (-0.001 to 0.002)	0.383	-	-
Fetal gender				
Male	0			
Female	0.019 (-0.002 to 0.040)	0.075	-	-
Karyotype				
Euploid	0			
Trisomy 21	0.053 (-0.110 to 0.216)	0.525	-	-
Trisomy 18	-0.025 (-0.351 to 0.301)	0.881	-	-
Delta NT (mm)	-0.002 (-0.025 to 0.021)	0.868	-	-
$\log_{10}$ PAPP-A	0.178 (0.142 to 0.215)	<0.0001*	0.133 (0.095 to 0.171)	<0.0001*
$\log_{10}$ $\beta$ -hCG	0.124 (0.088 to 0.161)	<0.0001*	0.110 (0.074 to 0.146)	<0.0001*
$\log_{10}$ uterine artery PI	-0.081 (-0.167 to 0.005)	0.064	-	-
Adverse pregnancy outcome				
PE	0.076 (0.007 to 0.145)	0.030*	0.043 (-0.022 to 0.108)	0.198
SGA	-0.015 (-0.072 to 0.042)	0.610	-	-
SPB	0.056 (-0.047 to 0.160)	0.285	-	-



**Fig. 4.** Relationship between fetal and maternal cfDNA.

tion. They both increase with serum PAPP-A and free  $\beta$ -hCG, are higher in women of Afro-Caribbean and East Asian racial origin than in Caucasians, and lower in smokers, but they are not significantly altered in pregnancies complicated by PE, SGA or SPB. Fetal cfDNA levels are inversely related to maternal weight and uterine artery PI and maternal cfDNA increases with maternal weight and is higher in women of South-Asian racial origin than in Caucasians.

A potential source of fetal cfDNA in maternal plasma is dying cells in the placenta and inevitably the number of apoptotic cells would be proportional to the placental mass, reflected in the serum concentration of free  $\beta$ -hCG and PAPP-A, which are also produced by the placenta [26]. The findings that Afro-Caribbean and East-Asian women, compared to Caucasians, have a higher concentration of fetal cfDNA can at least in part be explained by increased placental mass and/or activity in these racial groups reflected in the serum levels of free  $\beta$ -hCG and PAPP-A. The levels of free  $\beta$ -hCG and PAPP-A in Afro-Caribbean women, compared to Caucasians, are higher by 12 and 57%, respectively, and the respective values for East Asians are 8 and 9% [22]. In women of South Asian racial origin, the fetal cfDNA level is not significantly altered and serum PAPP-A is increased by 3%, but free  $\beta$ -hCG is decreased by 9% [22]. In smokers, where fetal cfDNA is decreased by 14%, serum free  $\beta$ -hCG and

PAPP-A are decreased by 4 and 17%, respectively [22]. Although in smokers fetal cfDNA would be expected to be elevated because the constituents of tobacco increase syncytiotrophoblastic necrosis [27, 28], the opposite is true, possibly reflecting the impaired placental development and decreased placental mass in such patients. Uterine artery PI provides an indirect measure of the physiological process whereby trophoblastic invasion and remodeling of spiral arteries converts them from high to low impedance vessels [29]. It would therefore be expected that there is an inverse association between uterine artery PI and placental mass and consequently plasma fetal cfDNA level.

In nonpregnant individuals, the most likely mechanism leading to the release of cfDNA into their circulation is apoptosis or some other form of cell death and the concentration of circulating cfDNA is increased in patients with malignant disorders or injuries [30–33]. If in pregnancy cell death is also the mechanism governing the concentration of plasma maternal cfDNA, our findings suggest that the fetoplacental unit, by some hitherto unknown mechanisms, influences such maternal apoptosis. This is reflected in the high association between maternal cfDNA and fetal cfDNA levels, as well as the finding that both maternal and fetal cfDNA levels increase with serum free  $\beta$ -hCG and PAPP-A, are higher in women of Afro-Caribbean and East Asian racial origin than in Caucasians, and lower in smokers. The only difference between fetal and maternal cfDNA levels is observed in relation to maternal weight. The decrease in fetal cfDNA with maternal weight is compatible with the plasma concentration of other fetoplacental products and can be attributed to a dilutional effect [22]. In contrast, maternal cfDNA levels increase with weight and recent evidence suggests that with increased weight there is active remodeling of adipose tissue with accelerated turnover of adipocytes [34].

We have previously reported that the fetal fraction is decreased in women of Afro-Caribbean racial origin and increased in smokers [11]. This study demonstrates that in women of Afro-Caribbean racial origin there is an increase in both fetal and maternal cfDNA, but more so for maternal cfDNA. In smokers there is a decrease in both fetal and maternal cfDNA, but more so for maternal cfDNA.

In pregnancies complicated by PE, SGA or SPB, the maternal plasma level of fetal and maternal cfDNA at 11–13 weeks' gestation was not significantly different from normal pregnancies. Previous studies reported that plasma cfDNA levels are increased in pregnancies with clini-

cally established PE [13–16], which has been attributed to placental ischemia, with release into the maternal circulation of necrotic or apoptotic syncytiotrophoblast fragments that contain fetal cfDNA [15, 35]. Some contradictory evidence suggests that in cases of early-onset PE the increase in cfDNA precedes the clinical onset of the disease and may be apparent from the first trimester of pregnancy [35–38]. In SGA without PE, one study reported increased plasma cfDNA [39], but in another study there was no significant difference from normal pregnancies [40]. Similarly, there is contradictory evidence as to whether any increase in fetal cfDNA in association with SGA precedes the clinical presentation of impaired fetal growth [36, 38].

There is some evidence that plasma fetal cfDNA is increased in pregnancies complicated by SPB with a suggested mechanism of early initiation of breakdown of the placental barrier in anticipation of labor [41]. Two studies examined women presenting with contractions and/or premature rupture of membranes and reported a correlation between fetal cfDNA levels and subsequent delivery [42, 43]. A cohort study of women undergoing routine fetal RHD genotyping at 25 weeks' gestation reported that if the fetal cfDNA levels were above the 95th percentile there was a 16-fold increase in risk for SPB before 34 weeks [44]. In contrast, a study examining women with short cervical length at 22–24 weeks reported no significant difference in the level of fetal cfDNA between those

that delivered before 37 weeks compared to those delivering at term [45]. Our study indicates that neither fetal nor maternal cfDNA levels at 11–13 weeks are altered in pregnancies with subsequent SPB.

In conclusion, the maternal plasma level of fetal cfDNA at 11–13 weeks' gestation increases with markers of placental size and decreases with maternal weight. There is a high association between fetal and maternal cfDNA levels suggesting that placental factors are also affecting maternal cell death. The levels of both fetal and maternal cfDNA at 11–13 weeks are not significantly altered in pregnancies that are complicated by PE, SGA and SPB. The implications of this finding are that (1) assessment of fetal and maternal cfDNA levels is unlikely to be useful in early prediction for these pregnancy complications, and (2) successful universal application of plasma cfDNA testing in screening for aneuploidies will not be hindered in pregnancies at high risk for PE, SGA or SPB.

## Disclosure Statement

T.M. and K.S. are paid employees of Ariosa Diagnostics. The study was supported by a grant from The Fetal Medicine Foundation (UK Charity No: 1037116). The cost of collection and analysis of the samples was covered by Ariosa Diagnostics Inc., San Jose, Calif., USA.

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