



Fetal fraction in maternal plasma cell-free DNA at 11–13 weeks' gestation: relation to maternal and fetal characteristics

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KEYWORDS: cell-free DNA; fetal fraction; first-trimester screening; non-invasive prenatal testing; trisomy 21

ABSTRACT

Objective To examine the possible effects of maternal and fetal characteristics on the fetal fraction in maternal plasma cell-free (cf) DNA at 11–13 weeks' gestation and estimate the proportion of pregnancies at high risk of non-invasive prenatal testing (NIPT) failure because the fetal fraction is less than 4%.

Methods In 1949 singleton pregnancies at 11–13 weeks' gestation cf-DNA was extracted from maternal plasma. Chromosome-selective sequencing of non-polymorphic and polymorphic loci, where fetal alleles differ from maternal alleles, was used to determine the proportion of cf-DNA that was of fetal origin. Multivariable regression analysis was used to determine significant predictors of the fetal fraction among maternal and fetal characteristics.

Results The fetal fraction decreased with increased maternal weight, it was lower in women of Afro-Caribbean origin than in Caucasians and increased with fetal crown–rump length, serum pregnancy-associated plasma protein-A, serum free β -human chorionic gonadotropin, smoking and trisomy 21 karyotype. The median fetal fraction was 10.0% (interquartile range, 7.8–13.0%) and this decreased with maternal weight from 11.7% at 60 kg to 3.9% at 160 kg. The estimated proportion with fetal fraction below 4% increased with maternal weight from 0.7% at 60 kg to 7.1% at 100 kg and 51.1% at 160 kg.

Conclusions Fetal fraction in maternal plasma cf-DNA is affected by maternal and fetal characteristics. Copyright © 2012 ISUOG. Published by John Wiley & Sons, Ltd.

INTRODUCTION

Non-invasive prenatal testing (NIPT) by analysis of cell-free DNA (cf-DNA) in maternal blood is highly accurate in the detection of trisomies 21 and 18 and to a lesser degree trisomy 13. Several clinical studies in high-risk pregnancies^{1–10} and a recent study in a population undergoing routine first-trimester aneuploidy screening¹¹ have demonstrated that the performance of NIPT for trisomies 21 and 18, with a detection rate of > 99% and false-positive rate of < 1%, is far superior to that of all other currently available screening methods¹². Consequently, NIPT can potentially be used in universal screening for these trisomies in all pregnant women, the main limiting factor for such widespread application of the test at present being the associated high cost.

A potential issue with NIPT as a universal screening test is the failure rate in providing a result, which primarily depends on the relative proportion of fetal to maternal origin of the cf-DNA in maternal plasma. In trisomic pregnancies DNA derived from the extra fetal chromosome results in a higher proportion of fetal DNA 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. However, if the fetal fraction is below 4%, NIPT fails to provide a result^{3,4,8}. A nested case–control study of maternal plasma cf-DNA in high-risk pregnancies at 11–13 weeks' gestation, including 300 euploid, 50 trisomy 21 and 50 trisomy 18 fetuses, reported that fetal fraction decreases with maternal weight and increases with serum pregnancy-associated plasma protein-A (PAPP-A) and free β -human chorionic

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Accepted: 18 October 2012

gonadotropin (β -hCG)¹³. Several other maternal and fetal characteristics demonstrated non-significant trends.

The aims of this cohort study of about 2000 women undergoing routine screening for aneuploidies at 11–13 weeks' gestation were firstly, to examine the possible effects of maternal and fetal characteristics on the fetal fraction in maternal plasma cf-DNA and secondly, to estimate the proportion of pregnancies at high risk of NIPT failure because the fetal fraction was < 4%.

PATIENTS AND 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 pregnancy¹⁴. Venous blood was obtained from women who gave written informed consent to provide samples for research into the early prediction of pregnancy complications, which was approved by the National Health Service's National Research Ethics Service. Blood samples were collected in ethylenediaminetetraacetic acid BD vacutainerTM tubes (Becton Dickinson UK Ltd, Oxfordshire, UK), and within 15 min of collection they were centrifuged at 2000 *g* for 10 min and subsequently at 16 000 *g* for 10 min. Plasma samples were then stored at –80 °C until subsequent analysis.

At 11 + 0 to 13 + 6 weeks' gestation, we recorded maternal characteristics and medical history, including maternal age, 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 maternal weight and height. We then performed an ultrasound scan to determine gestational age from measurement of fetal crown–rump length (CRL), to diagnose any major fetal abnormalities and to measure fetal nuchal translucency (NT) thickness^{15–17}. In addition, maternal serum PAPP-A and free β -hCG were determined within 10 min of blood collection using automated machines (DELFIAXpress system, PerkinElmer Life and Analytical Sciences, Waltham, USA). The measured NT was expressed as a difference from the expected normal mean for gestation (delta value)¹⁶. Similarly, the measured free β -hCG and PAPP-A were converted into multiples of the median (MoMs) for gestational age adjusted for maternal weight, racial origin, smoking status, method of conception, parity and machine used for the assays¹⁸. Biophysical and biochemical markers were combined to estimate the patient-specific risk for trisomies 21, 18 and 13. Women were given their estimated individual risk for these trisomies, and those considering their risk to be high were offered chorionic villus sampling or amniocentesis for fetal karyotyping.

Demographic characteristics, ultrasonographic measurements and biochemical results were recorded in computer databases. Karyotype results (obtained from

genetics laboratories) and details on pregnancy outcomes (obtained from the maternity computerized records or the women's general medical practitioners), were added to the database as soon as they became available.

The study included 1949 pregnancies examined between October 2010 and January 2011 in which cf-DNA analysis was carried out in stored 2-mL samples of maternal plasma (HarmonyTM Prenatal Test, Ariosa Diagnostics, Inc., San Jose, CA, USA)^{10,19}. This is the same group of women that participated in a previous study in which sensitivity and specificity for trisomy detection were determined¹¹.

Statistical analysis

Descriptive data are presented as median and interquartile range (IQR) for continuous variables and as *n* (%) for categorical variables. The measured fetal fraction was square root ($\sqrt{}$) transformed to make the distribution Gaussian and normality of distribution was assessed using probability plot. Regression analysis was used to determine which of the factors among maternal age, weight, height, racial origin, smoking status, method of conception, $\log_{10}$ PAPP-A-MoM, $\log_{10}\beta$ -hCG-MoM, fetal CRL, delta NT, fetal gender and karyotype were significant predictors of $\sqrt{\text{fetal fraction}}$.

The main determinants of fetal fraction were maternal weight, racial origin and fetal CRL. The regression equation combining these factors was used to derive the estimated median fetal fraction according to maternal weight in Caucasian women at 12 weeks' gestation (CRL, 65 mm). The difference between the estimated median fetal fraction and 4% for each maternal weight was then expressed as a Z-score (number of SDs) and this was used to derive the proportion of cases with fetal fraction below 4%. Similarly, we estimated the proportion of cases with fetal fraction below 4% for combinations of maternal weight, racial origin and fetal CRL. The statistical software package SPSS 20.0 (SPSS Inc., Chicago, IL, USA) was used for data analysis.

RESULTS

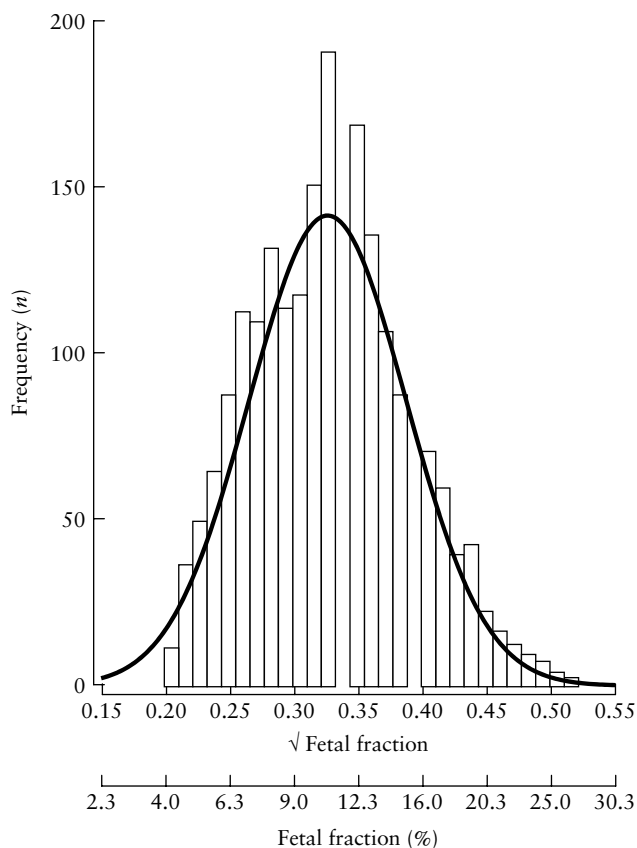
Maternal and fetal characteristics of the study population are presented in Table 1 and the frequency distribution of $\sqrt{\text{fetal fraction}}$ is shown in Figure 1. Univariable regression analysis demonstrated that $\sqrt{\text{fetal fraction}}$ was significantly associated with maternal weight, height, Afro-Caribbean origin, cigarette smoking, fetal CRL, trisomy 21 karyotype, $\log_{10}$ PAPP-A-MoM and $\log_{10}\beta$ -hCG-MoM, but not maternal age, method of conception, fetal gender, trisomy 18 karyotype or delta NT (Table 2).

Multivariable regression analysis demonstrated that significant independent prediction of $\sqrt{\text{fetal fraction}}$ was provided by maternal weight (regression coefficient = –0.015, $P < 0.0001$), Afro-Caribbean origin (regression coefficient = –0.130, $P < 0.0001$), smoking (regression coefficient = 0.122, $P = 0.020$), fetal CRL (regression coefficient = 0.005, $P = 0.001$), trisomy 21

Table 1 Maternal and fetal characteristics of the study population ($n = 1949$)

Characteristic	Value
Maternal age (years)	31.8 (27.8 to 35.3)
Maternal weight (kg)	65.0 (58.3 to 75.9)
Maternal height (cm)	164 (160 to 169)
Racial origin	
Caucasian	1377 (70.7)
Afro-Caribbean	390 (20.0)
South Asian	77 (4.0)
East Asian	54 (2.8)
Mixed	51 (2.6)
Cigarette smoker	120 (6.2)
Method of conception	
Spontaneous	1910 (98.0)
Ovulation drugs	19 (1.0)
In-vitro fertilization	20 (1.0)
Fetal CRL (mm)	62.4 (57.3 to 67.4)
Fetal gender	
Male	1010 (51.8)
Female	939 (48.2)
Delta NT	0.11 (−0.09 to 0.35)
PAPP-A-MoM	1.00 (0.69 to 1.41)
Free β -hCG-MoM	1.00 (0.68 to 1.50)
Fetal fraction (%)	10.0 (7.8 to 13.0)

Data given as median (interquartile range) or n (%). β -hCG, β -human chorionic gonadotropin; CRL, crown–rump length; MoM, multiples of the median; NT, nuchal translucency; PAPP-A, pregnancy-associated plasma protein-A.

**Figure 1** Frequency distribution of square root ($\sqrt{}$) of fetal fraction in maternal plasma cell-free DNA.

karyotype (regression coefficient = 0.394, $P = 0.047$), $\log_{10}$ PAPP-A-MoM (regression coefficient = 0.346, $P < 0.0001$) and $\log_{10}\beta$ -hCG-MoM (regression coefficient = 0.363, $P < 0.0001$), but not maternal height (regression coefficient = 0.002, $P = 0.302$; Table 2).

In the estimation of MoM values for free β -hCG and PAPP-A, the measured values are corrected for gestational age, maternal weight, racial origin and smoking status. We therefore conducted a separate analysis for the prediction of $\sqrt{\text{fetal fraction}}$ in which the measured rather than MoM values of free β -hCG and PAPP-A were considered. Multi-variable regression analysis demonstrated that significant independent predictors for $\sqrt{\text{fetal fraction}}$ were maternal weight (regression coefficient = -0.011 , $P < 0.0001$), Afro-Caribbean origin (regression coefficient = -0.217 , $P < 0.0001$), smoking (regression coefficient = 0.160, $P = 0.002$), trisomy 21 karyotype (regression coefficient = 0.418, $P = 0.035$), $\log_{10}$ PAPP-A (regression coefficient = 0.383, $P < 0.0001$) and $\log_{10}\beta$ -hCG (regression coefficient = 0.346, $P < 0.0001$) but not maternal height (regression coefficient = 0.002, $P = 0.247$) or fetal CRL (regression coefficient = 0.003, $P = 0.104$).

The median fetal fraction in the total population was 10.0% (IQR, 7.8–13.0%) and it decreased with increasing maternal weight. In Caucasian women, at 12 weeks' gestation (CRL, 65 mm), the median fetal fraction decreased with maternal weight from 11.7% at 60 kg to 3.9% at 160 kg (Figure 2), and the estimated proportion with fetal fraction below 4% increased from 0.7% at 60 kg to 51.1% at 160 kg (Table 3). The median fetal fraction increased by 7.5% in smokers and by 25.0% in pregnancies with fetal trisomy 21.

The median fetal fraction increased with fetal CRL and was lower in women of Afro-Caribbean origin than in Caucasians. The estimated proportions of cases with fetal fraction below 4% at a given maternal weight and fetal CRL for women of Caucasian and Afro-Caribbean origin are given in Table 4.

DISCUSSION

In this study we examined women undergoing first-trimester screening for aneuploidies as part of their routine antenatal care. The results are particularly relevant because over the last decade there has been a major shift from second- to first-trimester screening and diagnosis of aneuploidies¹². It is probable that NIPT screening will be applied at 11–13 weeks' gestation. Additionally, assessment at 11–13 weeks is emerging as a vital test not only in screening for aneuploidies but also for the prediction of many pregnancy complications, including preterm birth, pre-eclampsia and fetal growth restriction¹⁴.

At 11–13 weeks the median fraction of fetal cf-DNA in maternal plasma is 10%. Fetal fraction decreases with maternal weight, increases with fetal CRL, maternal serum level of free β -hCG and PAPP-A and is higher in smokers and in the presence of fetal trisomy 21 and lower in women of Afro-Caribbean origin than in Caucasians;

Table 2 Regression analysis for prediction of square root-fetal fraction in maternal plasma cell-free DNA

Independent variable	Univariable		Multivariable	
	Regression coefficient (95% CI)	P	Regression coefficient (95% CI)	P
Maternal age (years)	0.002 (−0.003 to 0.007)	0.509	—	—
Maternal weight (kg)	−0.015 (−0.017 to −0.013)	< 0.0001*	−0.015 (−0.017 to −0.013)	< 0.0001*
Maternal height (cm)	−0.007 (−0.011 to −0.003)	0.001*	0.002 (−0.002 to 0.006)	0.302
Racial origin				
Caucasian	0			
Afro-Caribbean	−0.214 (−0.282 to −0.145)	< 0.0001*	−0.130 (−0.193 to −0.067)	< 0.0001*
South Asian	−0.026 (−0.165 to 0.113)	0.716	—	—
East Asian	0.086 (−0.079 to 0.251)	0.308	—	—
Mixed	−0.016 (−0.185 to 0.154)	0.856	—	—
Smoker	0.127 (0.015 to 0.240)	0.027*	0.122 (0.020 to 0.225)	0.020*
Method of conception				
Spontaneous	0			
Ovulation drugs	0.037 (−0.240 to 0.314)	0.792	—	—
In-vitro fertilization	−0.122 (−0.392 to 0.148)	0.376	—	—
Fetal crown–rump length (mm)	0.003 (0.0002 to 0.007)	0.049*	0.005 (0.002 to 0.009)	0.001*
Fetal gender				
Male	1		—	—
Female	0.028 (−0.026 to 0.083)	0.305	—	—
Karyotype				
Euploid	0			
Trisomy 21	0.429 (0.004 to 0.854)	0.048*	0.394 (0.005 to 0.783)	0.047*
Trisomy 18	−0.275 (−1.123 to 0.573)	0.525	—	—
Delta NT (mm)	−0.026 (−0.086 to 0.034)	0.398	—	—
Log ₁₀ PAPP-A-MoM	0.384 (0.272 to 0.496)	< 0.0001*	0.346 (0.241 to 0.452)	< 0.0001*
Log ₁₀ β-hCG-MoM	0.424 (0.326 to 0.523)	< 0.0001*	0.363 (0.269 to 0.457)	< 0.0001*

*Statistically significant. β-hCG, β-human chorionic gonadotropin; MoM, multiples of the median; NT, nuchal translucency; PAPP-A, pregnancy-associated plasma protein-A.

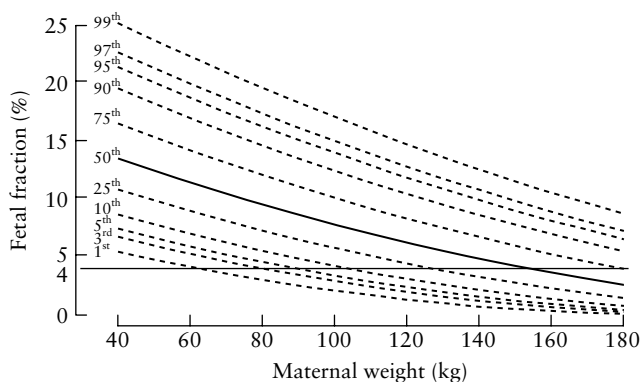


Figure 2 Association between fetal fraction in maternal plasma cell-free DNA and maternal weight. The proportion of pregnancies with fetal fraction below 4% (—) increases with maternal weight.

the greatest contribution to fetal fraction is provided by maternal weight.

The inverse association between fetal fraction and maternal weight, which could be attributed to a dilutional effect, is compatible with the results of previous NIPT studies in high-risk pregnancies^{4,13} and has also been reported for other fetoplacental products in the maternal circulation^{18,20,21}. Another possible explanation for this association is that in obese women there is an accelerated turnover of adipocytes, which releases an increased amount of cf-DNA of maternal origin into the circulation, resulting in a lower proportion of fetal cf-DNA²².

The linear association between fetal fraction and serum free β-hCG and PAPP-A is compatible with the results of a recently published NIPT study in high-risk pregnancies¹³. We have previously suggested that since these metabolites are produced by the placenta, their maternal serum concentration provides an indirect measure of placental mass¹³. A potential source of fetal cf-DNA in maternal plasma is dying cells in the placenta, and inevitably the number of apoptotic cells would be proportional to the placental mass. In cigarette smokers there is impaired placental development, reflected in decreased levels of serum PAPP-A and free β-hCG, which would be expected to decrease levels of fetal cf-DNA in maternal plasma, although in fact the opposite is the case, presumably because of the effect of constituents of tobacco in increasing syncytiotrophoblastic necrosis^{18,23,24}.

In this study, unlike our previous one of high-risk pregnancies¹³, there was a small but significant additional contribution to fetal fraction from fetal CRL. A previous NIPT study in high-risk pregnancies at 8–22 (average 15) weeks' gestation found no significant association between fetal fraction and gestational age⁴.

Maternal circulating concentrations of several fetoplacental products at 11–13 weeks' gestation – including PAPP-A, free β-hCG, placental growth factor and alpha-fetoprotein – are higher in women of Afro-Caribbean origin than in Caucasian women, with increases of about 60, 10, 40 and 25%, respectively^{18,20,21}. It would therefore be expected that in women of Afro-Caribbean origin,

Table 3 Estimated median fetal fraction in maternal plasma cell-free DNA according to maternal weight in Caucasian women at 12 weeks' gestation (fetal crown–rump length, 65 mm) and estimated proportion of women with fetal fraction below 4%

Weight (kg)	Estimated median fetal fraction (%)	4% – expected median*	Z-score	Estimated proportion (%) with fetal fraction < 4%
40	13.7	–1.7	–3.0	0.2
50	12.6	–1.6	–2.7	0.3
60	11.7	–1.4	–2.5	0.7
70	10.7	–1.3	–2.2	1.3
80	9.8	–1.1	–2.0	2.4
90	8.9	–1.0	–1.7	4.3
100	8.1	–0.8	–1.5	7.1
110	7.3	–0.7	–1.2	11.1
120	6.5	–0.6	–1.0	16.6
130	5.8	–0.4	–0.7	23.5
140	5.2	–0.3	–0.5	31.9
150	4.5	–0.1	–0.2	41.2
160	3.9	0.0	0.0	51.1
170	3.4	0.2	0.3	60.9
180	2.9	0.3	0.5	70.1

*Square root of 4 minus square root of expected median fetal fraction.

Table 4 Estimated proportion of cases with fetal fraction in maternal plasma cell-free DNA below 4% at a given maternal weight and fetal crown–rump length for women of Caucasian and Afro-Caribbean origin

Weight (kg)	Racial origin	Estimated proportion (%) with fetal fraction < 4% at crown–rump length of:						
		25 mm	35 mm	45 mm	55 mm	65 mm	75 mm	85 mm
40	Caucasian	0.5	0.4	0.3	0.2	0.2	0.1	0.1
	Afro-Caribbean	1.0	0.7	0.5	0.4	0.3	0.2	0.2
50	Caucasian	1.0	0.8	0.6	0.4	0.3	0.2	0.2
	Afro-Caribbean	1.8	1.4	1.1	0.8	0.6	0.5	0.4
60	Caucasian	1.9	1.5	1.2	0.9	0.7	0.5	0.4
	Afro-Caribbean	3.3	2.6	2.0	1.6	1.2	1.0	0.7
70	Caucasian	3.4	2.7	2.2	1.7	1.3	1.0	0.8
	Afro-Caribbean	5.5	4.5	3.6	2.9	2.3	1.8	1.4
80	Caucasian	5.8	4.7	3.8	3.1	2.4	1.9	1.5
	Afro-Caribbean	8.9	7.4	6.1	5.0	4.1	3.3	2.6
90	Caucasian	9.3	7.8	6.4	5.3	4.3	3.4	2.8
	Afro-Caribbean	13.6	11.6	9.8	8.2	6.8	5.6	4.5
100	Caucasian	14.2	12.1	10.2	8.5	7.1	5.8	4.8
	Afro-Caribbean	19.9	17.2	14.8	12.6	10.7	8.9	7.4
110	Caucasian	20.5	17.8	15.4	13.1	11.1	9.3	7.8
	Afro-Caribbean	27.5	24.3	21.3	18.5	16.0	13.7	11.6
120	Caucasian	28.3	25.1	22.0	19.2	16.6	14.2	12.1
	Afro-Caribbean	36.4	32.7	29.3	25.9	22.8	19.9	17.3
130	Caucasian	37.3	33.6	30.1	26.7	23.5	20.6	17.9
	Afro-Caribbean	46.1	42.2	38.3	34.6	31.0	27.6	24.4
140	Caucasian	47.1	43.1	39.3	35.5	31.9	28.4	25.1
	Afro-Caribbean	56.0	52.1	48.1	44.2	40.3	36.5	32.8
150	Caucasian	57.0	53.0	49.1	45.1	41.2	37.4	33.7
	Afro-Caribbean	65.6	61.9	58.0	54.1	50.1	46.2	42.3
160	Caucasian	66.5	62.8	59.0	55.1	51.1	47.1	43.2
	Afro-Caribbean	74.2	70.9	67.4	63.8	60.0	56.1	52.2
170	Caucasian	75.0	71.8	68.3	64.7	60.9	57.1	53.1
	Afro-Caribbean	81.6	78.8	75.8	72.6	69.2	65.7	61.9
180	Caucasian	82.2	79.5	76.6	73.4	70.1	66.5	62.9
	Afro-Caribbean	87.5	85.3	82.9	80.3	77.4	74.3	71.0

fetal cf-DNA levels in maternal plasma would be higher than in Caucasians. The finding of reduced fetal fraction may be a consequence of reduced fetal cf-DNA and/or increased maternal cf-DNA, but this requires further investigation.

In trisomy 21 pregnancies, compared with euploid ones, there was a small but significant increase in fetal fraction. In our previous NIPT study in high-risk pregnancies this increase was masked, presumably because of the associated abnormal serum biochemistry, with high free

β -hCG and low PAPP-A, not only in trisomic but also in euploid pregnancies¹³.

The lack of a significant contribution to fetal fraction from maternal age, fetal gender and NT thickness is compatible with the results of our NIPT study in high-risk pregnancies¹³. Additionally, in this study we found no significant contribution from method of conception.

Implications for practice

Current NIPT methods require a fetal fraction of at least 4%. On the basis of the results of this study the greatest risk factor for low fetal fraction is obesity, with a small contribution from Afro-Caribbean origin and early gestational age. The estimated proportion of pregnancies with fetal fraction below 4% increased with maternal weight from <1% at 60 kg to >50% at 160 kg. The low fetal fraction in heavier women may be challenging to overcome by currently available NIPT techniques, therefore further studies are needed to investigate the optimal method of aneuploidy screening and the role of NIPT in obese women.

The optimal gestational age for the first-trimester combined test in screening for aneuploidies is 12 weeks¹². Biochemical testing can be undertaken with the use of automated machines, which provide results within 30 min so that assessment of risk and counseling can be undertaken in the same hospital visit. The alternative strategy of collecting blood at the time of the scan and sending this to another laboratory for testing is less satisfactory because the opportunity for testing and counseling in the same visit is missed. Another option for combined screening is to perform biochemical testing prior to the scan, allowing for the results of both to be available at the same visit. In women identified by screening as being at high risk, diagnostic testing by chorionic villus sampling provides the option for first-rather than second-trimester termination of pregnancy, should the fetus be found to be affected by a major abnormality.

The performance of NIPT in screening for trisomies 21 and 18 is far superior to that of currently available screening methods, with a substantial increase in detection rates and decrease in false-positive rates^{11,12}. One limiting factor in the application of NIPT in universal screening for aneuploidies is the economic cost, but this is likely to come down with widespread uptake of the test. Another limiting factor relates to the delay of 1–2 weeks between sampling and obtaining results. **This problem can be overcome by taking the blood sample 1–2 weeks before the scheduled first-trimester ultrasound examination at 12 weeks. At this 12-week visit, based on the results of NIPT and the ultrasound findings, the parents can be counseled concerning the option of invasive testing. In the few cases where NIPT failed to provide a result the parents would still have the option of the first-trimester combined test.** However, such a two-stage strategy in first-trimester aneuploidy screening would actually tend to exaggerate the problem of low fetal fraction. For example,

the estimated proportion with fetal fraction below 4% in Caucasian women weighing 100 kg is 14.2% at 9 weeks' gestation (CRL, 25 mm) and 7.1% at 12 weeks (CRL, 65 mm).

At the present time screening for aneuploidies by cf-DNA testing requires that the minimum fetal fraction is 4%. Future improvements in the technology may make it possible to obtain results at lower fetal fractions. In the meantime, the findings of this study can form the basis for counseling parents concerning the likelihood of failure to obtain a result from NIPT. Further research is needed to define the biological variation in fetal fraction and identify factors that could potentially increase it in obese women.

ACKNOWLEDGMENTS

This 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, CA, USA.

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