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Screening for trisomies by cell-free DNA testing of maternal blood: consequences of failed result

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Running head: Failed cell-free DNA test

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Abstract

Objectives: First, to report the distribution of fetal fraction and the rate of failed result in trisomies 21, 18 and 13, by comparison with pregnancies unaffected by these trisomies, secondly, examine the possible effects of maternal and fetal characteristics on the fetal fraction and thirdly, consider the options for the further management of pregnancies with failed cfDNA result.

Methods: This was a cohort study of 10,698 singleton pregnancies undergoing screening for fetal trisomies 21, 18 and 13 by cfDNA testing at 10-14 weeks' gestation. There were 160 cases of trisomy 21, 50 of trisomy 18, 16 of trisomy 13 and 10,472 unaffected by these trisomies. Multivariate regression analysis was used to determine significant predictors of fetal fraction and failed result amongst maternal and fetal characteristics.

Results: Fetal fraction decreased with increasing body mass index and maternal age, was lower in women of South Asian racial origin than in Caucasians and in assisted than natural conceptions, and increased with fetal crown-rump length, serum PAPP-A and free β-hCG. The median fetal fraction was 11.0% (IQR 8.3-14.4%) in the unaffected group, 10.7% (IQR 7.8-14.3%) in trisomy 21, 8.6% (IQR 5.0-10.2%) in trisomy 18 and 7.0% (IQR 6.0-9.4%) in trisomy 13. There was a failed result from cfDNA testing after first sampling in 2.9% of the unaffected group, 1.9% of trisomy 21, 8.0% of trisomy 18 and 6.3% of trisomy 13. In the cases of failed result, 7% of women had invasive testing, mainly because of high-risk from the combined test and/or presence of sonographic features suggestive of trisomies 18 and 13. All cases of trisomies were detected prenatally.

Conclusions: In cases of failed cfDNA test the rate of trisomies 18 and 13, but not trisomy 21, are higher than in those with a successful test. In the management of such cases, the decision in favor of invasive testing should depend on the risk of *prior* screening and the results of detailed ultrasound examination.

INTRODUCTION

Cell-free (cf) DNA analysis of maternal blood provides effective screening for fetal trisomies 21, 18 and 13 with reported detection rates (DR) of 99%, 96% and 91%, respectively, at overall false positive rate (FPR) of 0.35%.[1] However, the test fails to provide a result in up to 8% of cases and the most common reason for such failure is low fetal fraction.[2] There is some limited data that in the pregnancies with failed results fetal chromosomal abnormalities are over-represented [2] and this has led to a recommendation by the American College of Obstetricians and Gynecologists (ACOG) that in cases of failed result women should be offered diagnostic testing [3].

The objectives of this cohort study of 10,698 singleton pregnancies undergoing screening for fetal trisomies 21, 18 and 13 by cfDNA testing at 10-14 weeks' gestation are to firstly, report the distribution of fetal fraction and the rate of failed result in each of the trisomies, by comparison with pregnancies unaffected by these trisomies, secondly, examine the possible effects of maternal and fetal characteristics on the fetal fraction and thirdly, consider the options for the further management of pregnancies with failed cfDNA result.

METHODS

The data for this study were derived from first, cfDNA testing as an option following first-trimester combined testing in women with singleton pregnancies attending for routine care at 11^{+0}-13^{+6} weeks' gestation in one of two National Health Service (NHS) hospitals in England [4] and second, cfDNA testing as part of routine screening in women with singleton pregnancies at 10^{+0}-13^{+6} weeks attending the Fetal Medicine Centre in London, which is a private clinic [5]. The patients were examined between October 2012 and August 2015.

We recorded maternal characteristics and medical history, including maternal age, racial origin (Caucasian, African, South Asian, East Asian and mixed), method of conception (natural or assisted conception requiring the use of ovulation drugs or *in-vitro* fertilization), cigarette smoking during pregnancy (yes or no) and parity (parous or nulliparous if no previous pregnancy at or after 24 weeks' gestation). We also measured maternal weight and height. In all cases free ß-hCG and PAPP-A were measured within 10 minutes of blood collection at 10^{+0}-13^{+6} weeks (DELFIA Xpress system, PerkinElmer Life and Analytical Sciences, Waltham, USA, or Kryptor, Thermo Scientific, Berlin, Germany). An ultrasound scan was carried out at 11^{+0}-13^{+6} weeks to determine gestational age from the measurement of the fetal crown-rump length (CRL) [6], diagnose any major fetal abnormalities and measure fetal nuchal translucency (NT) thickness. The measured NT was expressed as a difference from the expected normal mean for gestation (delta value) [7]. Similarly, the measured free ß-hCG and PAPP-A were converted into multiple of the median (MoM) for gestational age adjusted for maternal weight, racial origin, smoking status, method of conception, parity and machine for the assays [8]. Biophysical and biochemical markers were combined to estimate the patient-specific risk for trisomies 21, 18 and 13.

Women provided written informed consent and maternal blood (20 mL) was sent via courier to the USA for cfDNA testing (Harmony™ Prenatal Test, Ariosa Diagnostics, Inc., San Jose, CA) [9-13]. Chromosome-selective sequencing, referred to as digital analysis of selected regions (DANSR), and fetal-fraction optimized risk of trisomy evaluation (FORTE) were used to assay non-polymorphic and polymorphic loci, where fetal alleles differ from maternal alleles, enabling simultaneous determination of chromosome proportion and fetal fraction. The results from cfDNA testing were presented as risk scores for trisomy 21, 18, and 13 which in most cases were either >99% or

<1:10,000. In cases where the cfDNA test did not provide results the parents were offered repeat testing or to rely on the results of the combined test in deciding whether to have an invasive test or not. In cases with a high-risk result from the cfDNA test, the parents were advised to consider having invasive fetal karyotyping before deciding on the further management of their pregnancy.

Patient characteristics and results of the investigations were recorded in a fetal database. Results from invasive testing, obtained from laboratories, and pregnancy outcome, obtained from obstetricians, general practitioners or the patients, were recorded in the same database. The outcomes were divided into firstly, trisomy 21, 18 or 13 if the karyotype of chorionic villi, amniotic fluid or neonatal blood demonstrated the relevant trisomy, secondly, no trisomy 21, 18 or 13 if the karyotype of chorionic villi, amniotic fluid or neonatal blood was normal or the neonate was phenotypically normal, thirdly, no known karyotype because the pregnancies resulted in miscarriage or stillbirth and no karyotyping of fetal tissue was carried out, and fourthly, outcome unknown because the pregnancies were lost to follow up.

Statistical analyses

Descriptive data were presented in median and interquartile range (IQR) for continuous variables and in numbers and percentages for categorical variables. The measured fetal fraction was $\log_{10}$ transformed to make the distribution Gaussian, which was assessed using histograms and probability plots. Univariate and multivariate regression analysis were used to determine which of the factors amongst maternal age, body mass index, racial origin, smoking status, method of conception, fetal CRL, serum PAPP-A and free ß-hCG, fetal NT and fetal karyotype were significant predictors of $\log_{10}$ fetal fraction. In each trisomic and unaffected pregnancy the $\log_{10}$ fetal fraction was expressed as a MoM after adjusting for the maternal variables found to be significant in the multivariate regression analysis. Logistic regression analysis was undertaken to examine the maternal and pregnancy characteristics providing significant contribution to prediction of failed cfDNA test result.

The statistical software package SPSS 21.0 (SPSS Inc.,Chicago, IL) was used for data analyses.

Role of the funding source

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 for the cell-free DNA test in the NHS hospitals was covered by Ariosa Diagnostics, Inc. San Jose, CA, USA. These organizations had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

RESULTS

Characteristics of study population

A total of 10,963 women had cfDNA testing and combined screening for trisomies, but 265 (2.4%) of these were excluded from further analysis either because the pregnancies ended in termination, miscarriage or stillbirth with no known karyotype (n=155), they were lost to follow up (n=85) or they had chromosomal abnormalities other than trisomies 21, 18 or 13 (n=25).

Maternal and pregnancy characteristics in the 10,698 cases with known outcome are summarized in Table 1. There were 160 cases of trisomy 21, 50 of trisomy 18, 16 of trisomy 13 and 10,472 unaffected by these trisomies. Results from cfDNA testing were provided after first sampling for 97.0% (10,382/10,698) cases, including 97.1% in the unaffected group, 98.1% in trisomy 21, 92.0% in trisomy 18 and 93.7% in trisomy 13. The reasons for failure to provide a result were low fetal fraction in 219 (69.3%) cases and laboratory processing problems in 97 (30.7%) cases.

Factors affecting fetal fraction

At first sampling, the median fetal fraction was 11.0% (IQR 8.3-14.4%) in the unaffected group, 10.7% (IQR 7.8-14.3%) in trisomy 21, 8.6% (IQR 5.0-10.2%) in trisomy 18 and 7.0% (IQR 6.0-9.4%) in trisomy 13; in these calculations, it was assumed that in the cases of failed result because of low fetal fraction, the fetal fraction was 3%.

Log_{10} fetal fraction from first sampling had a Gaussian distribution (Figure 1). Univariate regression analysis demonstrated that significant independent prediction of log_{10} fetal fraction was provided by maternal age, body mass index, African, South Asian and East Asian racial origin, assisted conception, fetal CRL, PAPP-A and free β-hCG MoM and trisomies 18 and 13 (Table 2). In the multivariate regression analysis significant contribution was provided by maternal age, body mass index, South Asian racial origin, assisted conception, fetal CRL and PAPP-A and free β-hCG MoM, but not trisomies 18 or 13 (Adjusted R^2=0.251, p<0.0001). If in the multivariate regression analysis we excluded PAPP-A and free β-hCG MoM, significant contribution to log_{10} fetal fraction was provided by maternal age, body mass index, South Asian racial origin, assisted conception, fetal CRL and trisomies 18 and 13 (Adjusted R^2=0.174, p<0.0001).

We used the coefficients of the maternal characteristics with significant contribution to log_{10} fetal fraction in the multivariate regression analysis to derive a model for calculation of MoMs. The median fetal fraction MoM in unaffected pregnancies was 1.03 (IQR 0.79-1.32). Compared to unaffected pregnancies, the fetal fraction MoM was not significantly different in trisomy 21 (0.99, IQR 0.77-1.29, p=0.527), but it was lower in those with trisomy 18 (0.80, IQR 0.49-1.05, p<0.0001) or trisomy 13 (0.71, IQR 0.54-0.90, p<0.0001) (Figure 2). If in the estimation of fetal fraction MoM we included the coefficients for PAPP-A and free β-hCG MoM, there were no significant differences between the unaffected pregnancies (1.03, IQR 0.81-1.30) and those with trisomy 21 (1.05, IQR 0.76-1.29; p=0.894), trisomy 18 (1.10, IQR 077-1.52; p=0.198) or trisomy 13 (0.81, IQR 0.66-1.14; p=0.051).

Multivariate logistic regression analysis demonstrated that the risk of test failure increased with increasing maternal age and body mass index, decreased with increasing PAPP-A and free β-hCG MoM and it was higher in women of South Asian than Caucasian racial origin and in pregnancies achieved by assisted conception than naturally (Adjusted R^2=0.212, p<0.0001).

Management of pregnancies with cfDNA test failure

There was a failed cfDNA result after first sampling in 2.9% (308/10,472) cases in the unaffected group, 1.9% (3/160) in trisomy 21, 8.0% (4/50) in trisomy 18 and 6.3% (1/16) in trisomy 13 (Table 3).

In the 308 unaffected cases with failed cfDNA result, 234 (76.0%) chose repeat cfDNA testing, 8 (2.6%) had invasive testing and 66 (21.4%) opted for no further investigations. Repeat cfDNA

testing provided a result in 147 (62.8%) of the 234 cases; 7 (8.0%) of the 87 with a failed second cfDNA test had invasive testing and 80 (92.0%) opted for no further investigations. In total, 15 (4.9%) of the 308 with failed cfDNA result ended up having an invasive test and in 13 (86.7%) of the 15 cases in this group the estimated risk for trisomies from the combined test was ≥1 in 100. In contrast, 213 of the 308 cases did not have a result from the cfDNA test, either after first or repeat testing; in this group no invasive test was carried out and only 28 (13.1%) had an estimated risk for trisomies from the combined test of ≥1 in 100. In contrast, in the 146 cases with no result from first or repeat cfDNA testing where the women decided against invasive testing, only 28 (13.1%) had an estimated risk for trisomies from the combined test of ≥1 in 100.

In two of the three cases of trisomy 21 with failed cfDNA result, invasive testing was carried out because the estimated risk from the combined test was ≥1 in 3; in the third case with estimated risk of 1 in 13, the cfDNA test was repeated and this gave a result. In the four cases of trisomy 18, invasive testing was carried out because in all cases the estimated risk from the combined test was ≥1 in 5, serum PAPP-A and free ß-hCG was ≤0.3 MoM and there were sonographic features suggestive of this trisomy, including clenched hands, cardiac defect and / or exomphalos. In the case of trisomy 13, invasive testing was carried out because the estimated risk from the combined test was 1 in 2, the fetal NT was 4.1 mm and the fetal heart rate was 200 bpm.

DISCUSSION

Principal findings of this study

In this study selective sequencing was used for cfDNA analysis of maternal blood in screening for fetal trisomies 21, 18 and 13 at 10-14 weeks' gestation. The median fetal fraction was 11.0% and this decreased with increasing maternal age and body mass index, increased with fetal CRL and maternal serum free β-hCG and PAPP-A and it was lower in women of South Asian racial origin than in Caucasians and in pregnancies conceived by assisted reproduction techniques than in natural conceptions.

The median fetal fraction in pregnancies with fetal trisomy 21 was not significantly different from unaffected pregnancies. In trisomies 18 and 13 the fetal fraction was significantly reduced and this decrease could be explained by the association of these trisomies with low serum PAPP-A and free ß-hCG, reflecting the smaller placental source of fetal cfDNA in maternal blood.

In 3% of pregnancies the cfDNA test failed to provide a result after first sampling and the main reason for such failure was low fetal fraction <4%. The rate of failed result was similar in unaffected and trisomy 21 cases, but this was increased in trisomy 18 and 13 pregnancies. Logistic regression analysis demonstrated that the risk of failed cfDNA test was inevitably affected by the same factors as those affecting fetal fraction. Thus, the rate of failed result increased with increasing maternal age and body mass index, decreased with increasing maternal serum level of free β-hCG and PAPP-A and it was higher in women of South Asian racial origin than in Caucasians and in pregnancies conceived by assisted reproduction techniques than in natural conceptions.

The options for the management of pregnancies with failed cfDNA test after first sampling include repeat cfDNA testing, invasive testing and no further investigations; similarly, the options for women with a second failed cfDNA test are invasive testing or no further investigations. An

important determinant for selecting the appropriate management option is the estimated risk for trisomies from the combined test and the presence of sonographic features suggestive of trisomies 18 and 13. In this study only 7% (22/316) with failed cfDNA test chose to have invasive testing and in 91% of these cases the estimated risk for trisomies from the combined test was ≥1 in 100. In contrast, in the patients with failed cfDNA test, either after first or repeat testing, that decided against invasive testing only 13% had an estimated risk for trisomies from the combined test of ≥1 in 100.

Comparison of findings to those in previous studies

The inverse association between fetal fraction and maternal body mass index, which could be attributed to a dilutional effect, but also increase in maternal cfDNA with increasing weight, is compatible with the results of previous cfDNA studies [12-15]. Similarly our finding of a linear association between fetal fraction and serum free β-hCG and PAPP-A provides further support to our suggestion that since all three are produced by the placenta, their maternal serum levels provide an indirect measure of placental mass [12-14]. A 3-dimensional ultrasound study reported that in trisomy 21 pregnancies, placental volume at 11–13 weeks' gestation was not significantly different from euploid pregnancies, but in trisomies 18 and 13 placental volume was decreased [16].

In previous studies we found that the fetal fraction is decreased in women of African racial origin, primarily because of an increase in maternal cfDNA level rather than decrease in fetal cfDNA in maternal blood [12-14]. In this larger study, we found in the univariate analysis that in women of African racial origin the fetal fraction was significantly lower than in Caucasians, but in the multrivariate analysis this significance was lost. In contrast, in women of South Asian racial origin the fetal fraction was significantly reduced both in the univariate and multivariate analysis and this decrease may be a consequence of an increase in maternal cfDNA level, rather than decrease in fetal cfDNA in maternal blood [14].

The finding that in pregnancies conceived by assisted reproduction techniques the fetal fraction is lower than in natural conceptions is compatible with a previous report in multiple pregnancies [17] and this may be the consequence of a degree of impaired placentation which can also explain the higher incidence of associated pregnancy complications, such as preeclampsia [18].

A few studies compared fetal fraction in pregnancies with fetal trisomies 21, 18 and 13 with that in euploid pregnancies. Rava *et al.*, examined high-risk pregnancies undergoing invasive testing at 10-23 weeks' gestation, including 160 euploid pregnancies and 90, 38 and 16 with trisomies 21, 18 and 13, respectively [19]. The mean fetal fraction in trisomy 21 (13.5%) was significantly higher and in trisomies 18 (8.9%) and 13 (9.0%) was lower than in euploid pregnancies (12.6%). Dar *et al.*, reported the results of screening at a median gestational age of 13 (range 9-41) weeks, in 17,885 pregnancies, including 140 with trisomy 21, 27 with trisomy 18 and 8 with trisomy 13 [20]. The median fetal fraction was 10.1% and this increased with gestational age and decreased with maternal weight; after adjustment for these variables the median MoM fetal fraction in trisomy 21 (1.05 MoM) was significantly higher and in trisomies 18 (0.92 MoM) and 13 (0.76 MoM) was lower than in euploid pregnancies (1.0 MoM). Palomaki *et al.*, reported the results of a case-control study at a median gestational age of 15 (range 8-22) weeks, in 2,157 pregnancies, including 212 with trisomy 21, 62 with trisomy 18 and 12 with trisomy 13 [21]. The median fetal fraction in trisomy 21 (15.5%) was higher and in trisomy 18 (9.4%) was lower than in euploid pregnancies (13.3%); the value in trisomy 13 was 13.6%.

Kinnings *et al.*, reported the results of screening at a median gestational age of 13 (range 10-40) weeks, in 140,377 pregnancies, including 2,214 with trisomy 21, 835 with trisomy 18 and 432 with trisomy 13 [22]. The median fetal fraction increased with gestational age, decreased with maternal weight and was affected by the fetal aneuploidy status; the median value was 9.6% for euploid and trisomy 21 pregnancies, 8.2% for trisomy 18 and 8.7% for trisomy 13. The study also demonstrated that the fetal fraction in trisomic, compared to euploid pregnancies, changed with gestational age; the fetal fraction was initially lower for all three trisomies but then became higher than in euploid pregnancies after 16, 21 and 18 weeks for trisomies 21, 18 and 13, respectively [22].

Implications for practice

On the basis of results from this and previous studies it can be concluded that in trisomies 18 and 13, but not in trisomy 21, the fetal fraction is lower and the rate of failed cfDNA test is higher than in unaffected pregnancies. Consequently, pregnancies with a failed test can be considered as being at increased risk for trisomies 18 and 13, but not for trisomy 21. However, the results of the study of Kinnings *et al.*, suggest that this problem of over-representation of trisomies 18 and 13 in the pregnancies with failed cfDNA test is confined to pregnancies in the first half of pregnancy [22].

The management of pregnancies with failed cfDNA test should essentially depend on the reason for carrying out such test in the first place, as well as the cost of the cfDNA test; however, most companies accept to repeat the test at no additional cost. If there was prior screening with a low-risk result, the preferred option would be to repeat the cfDNA test and explain to the parents that such testing would provide a result in >60% of cases. Some patients would prefer to avoid any further testing because of the associated anxiety; in these patients and in those with a failed second cfDNA test it would be advisable to carry out a detailed ultrasound scan for features of trisomies 18 and 13 and in the presence of such features invasive testing should be considered. If prior screening had provided a high-risk result but there are no ultrasound features of an aneuploidy, most patients would prefer repeat cfDNA testing but a few would select to have invasive testing.

Conclusions

In cases of failed cfDNA test fetal trisomies 18 and 13, but not trisomy 21, are over-represented. In the management of such cases, the decision in favor of invasive testing should depend on the risk from prior screening and the results of detailed ultrasound examination.

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Table 1. Maternal characteristics of the study population.

Maternal and pregnancy characteristic	**Study population (n=10,698)**
Maternal age in years, median (IQR)	36.3 (33.2-39.3)
Maternal weight in kg, median (IQR)	64.0 (57.9-73.0)
Maternal height in cm, median (IQR)	165 (161-170)
Racial origin	
Caucasian, n (%)	8,751 (81.8)
African, n (%)	698 (6.5)
South Asian, n (%)	663 (6.2)
East Asian, n (%)	386 (3.6)
Mixed, n (%)	200 (1.9)
Parity	
Nulliparous, n (%)	4,760 (44.5)
Cigarette smoker, n (%)	263 (2.5)
Conception	
Natural, n (%)	9,515 (88.9)
Assisted, n (%)	1,183 (11.1)
Crown-rump length in mm, median (IQR)	53.7 (38.5-65.7)

CRL = crown-rump length, NT = nuchal translucency, MoM = multiple of median, PAPP-A = pregnancy associated plasma protein-A, β-hCG = β-human chorionic gonadotropin, IQR = interquartile range

Table 2. Univariate and multivariate regression analysis demonstrating factors from maternal and pregnancy characteristics providing significant contribution to prediction of log_{10} transformed fetal fraction

Independent variable	Univariate analysis		Multivariate analysis	
	Regression coefficient (95% CI)	**P value**	**Regression coefficient (95% CI)**	**P value**
Intercept			1.451 (1.419 to 1.483)	<0.0001
Age in years	-0.003 (-0.004 to -0.002)	<0.0001	-0.002 (-0.003 to -0.001)	<0.0001
Body mass index, kg/m^2	-0.016 (-0.016 to -0.015)	<0.0001	-0.016 (-0.017 to -0.015)	<0.0001
Race origin				
Caucasian	0.000 (reference)			
African	-0.055 (-0.070 to -0.039)	<0.0001		
South Asian	-0.021 (-0.036 to -0.005)	0.011	-0.019 (-0.032 to -0.005)	0.008
East Asian	0.029 (0.009 to 0.050)	0.005		
Mixed	0.022 (-0.006 to 0.051)	0.123		
Smoking	0.013 (-0.011 to 0.038)	0.293		
Assisted conception	-0.089 (-0.101 to -0.077)	<0.0001	-0.086 (-0.097 to -0.075)	<0.0001
Fetal CRL in mm	$1.1e^{-04}$ ($1.1\ e^{-04}$ to $3.2e^{-04}$)	0.412	0.001 ($4.7e^{-04}$ to 0.001)	<0.0001
Log_{10} PAPP-A MoM	0.166 (0.152 to 0.180)	<0.0001	0.133 (0.119 to 0.146)	<0.0001
Log_{10} free β-hCG MoM	0.171 (0.158 to 0.184)	<0.0001	0.140 (0.128 to 0.152)	<0.0001
Delta NT	-0.003 (-0.009 to 0.002)	0.251		
Trisomy 21	0.003 (-0.028 to 0.035)	0.837		
Trisomy 18	-0.142 (-0.198 to -0.085)	<0.0001		
Trisomy 13	-0.161 (-0.260 to -0.063)	<0.0001		

CI = confidence interval; CRL = crown-rump length; MoM = multiple of the median; NT = nuchal translucency

Table 3. Results from cfDNA testing and further management of pregnancy according to trisomic status of the fetus.

	Non-trisomy	Trisomy 21	Trisomy 18	Trisomy 13
Number of cases	10,472	160	50	16
Median fetal fraction				
Percentage, median (IQR)	11.0	10.7	8.6	7.0
MoM, median (IQR)	1.03 (0.79-1.32)	0.99 (0.77-1.29	0.80 (0.49-1.05)	0.71 (0.54-0.90
Failed result	308 (2.9)	3 (1.9)	4 (8.0)	1 (6.3)
Low fetal fraction	214	2	2	1
Laboratory processing	94	1	2	
Response to failed result				
Invasive testing	8/308 (2.6)	2/3 (66.7)	4/4 (100)	1/1 (100)
No further test	66/308 (21.4)			
Repeat testing	234/308 (76.0)	1/3 (33.3)		
Result	147/234 (62.8)	1/1 (100)		
Failed result	87 (37.2)			
Invasive testing	7/87 (8.0)			
No further test	80/87 (92.0)			

Figure legends

Figure 1. Frequency distribution of $\log_{10}$ fetal fraction in maternal blood cfDNA.

Figure 2. Box and whisker plot of fetal fraction and fetal fraction multiple of median (MoM) in fetal trisomies and unaffected pregnancies. The MoMs were calculated from coefficients in Table 2