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Trisomy 13 detection in the first trimester of pregnancy using a chromosome-selective cell-free DNA analysis method

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KEYWORDS: first trimester; non-invasive prenatal testing; trisomy 13

ABSTRACT

Objective *To assess the performance of chromosome-selective sequencing of maternal plasma cell-free DNA (cfDNA) in non-invasive prenatal testing for trisomy 13.*

Methods *Two-phase case–control study on a single plasma sample per case. The first phase was used to optimize the trisomy 13 algorithm, which was then applied to a second dataset to determine the risk score for trisomy 13 by laboratory personnel who were blinded to the fetal karyotype.*

Results *In the first phase, trisomy 13 risk scores were given for 11 cases of trisomy 13 and 145 euploid cases at 11–13 weeks' gestation. The test identified seven (63.6%) cases of trisomy 13 with no false positives. The trisomy 13 algorithm was subsequently modified and the trisomy 13 risk score was > 99% in all 11 cases of trisomy 13 and < 0.01% in all 145 euploid cases. In the second phase, the new algorithm was used to generate trisomy 13 risk scores for 10 cases of trisomy 13 and 1939 euploid cases. The trisomy 13 risk scores were > 99% in eight (80.0% (95% confidence interval (CI), 49.0–94.3%)) cases of trisomy 13. In the 1939 euploid cases the risk score for trisomy 13 was < 0.01% in 1937 (99.9%), 0.79% in one, and > 99% in one. Therefore, at the predefined risk cut-off of 1% for classifying a sample as high or low risk, the false-positive rate (FPR) was 0.05% (95% CI, 0.0–0.3%).*

Conclusions *Chromosome-selective sequencing of cfDNA can detect the majority of cases of trisomy 13 at an FPR of less than 0.1%. Copyright © 2012 ISUOG. Published by John Wiley & Sons, Ltd.*

INTRODUCTION

Chromosome-selective sequencing of loci from chromosomes 21 and 18 in maternal plasma cell-free DNA (cfDNA) has been successfully applied in non-invasive prenatal testing (NIPT) for fetal trisomies 21 and 18[1–5]. In pregnancies with a trisomic fetus, the extra cfDNA molecules derived from the extra fetal chromosome can be detected, given the higher proportion of molecules relative to a reference disomic chromosome.

Sparks *et al.*[2] used a training set of euploid and trisomic pregnancies to perform selective sequencing of cfDNA and develop a novel algorithm for the estimation of individualized trisomy risk. They subsequently applied this approach for assessment of a blinded validation set and correctly discriminated the 36 cases of trisomy 21 and eight cases of trisomy 18 from the 123 euploid cases. Ashoor *et al.*[3] performed a nested case–control study of cfDNA in plasma obtained at 11–13 weeks' gestation before chorionic villus sampling (CVS) from 300 euploid pregnancies, 50 pregnancies with trisomy 21 and 50 pregnancies with trisomy 18. Chromosome-selective sequencing correctly detected all cases of trisomy 21 and 49 (98.0%) of the cases of trisomy 18, with a false-positive rate (FPR) of 0%[3]. Norton *et al.*[4] performed chromosome-selective sequencing on chromosomes 21 and 18 in a multicenter cohort of high-risk pregnancies at 10–39 (mean, 17) weeks' gestation. They correctly detected all 81 cases of trisomy 21 with an FPR of 0.03% (1/2888 normal cases) and detected 37 (97.4%) of the 38 cases of trisomy 18 with an FPR of 0.07% (2/2888). More recently, the chromosome-selective sequencing approach was applied for NIPT in pregnant women undergoing routine screening for aneuploidies at 11–13 weeks'

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gestation[5]. All eight cases of trisomy 21, and two cases of trisomy 18, had a trisomy risk score of > 99%, whereas the risk score for trisomy 21 was < 1% in all 1939 euploid pregnancies (FPR, 0%) and the risk score for trisomy 18 was < 1% in 1937 (FPR, 0.1%).

The objective of this study was to assess the performance of selective sequencing of cfDNA in maternal plasma for the prenatal detection of fetal trisomy 13.

SUBJECTS AND METHODS

Study population

Two groups of women with singleton pregnancies were examined. In Group 1, all women had fetal karyotyping carried out following CVS because screening by maternal age, serum free β-human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein-A (PAPP-A), and ultrasound examination at 11–13 weeks' gestation, demonstrated that there was a high risk for aneuploidies[6,7]. Maternal blood samples for research were obtained before CVS and the plasma stored at −80°C. This group included 150 euploid and 15 trisomy 13 cases, all of which had been recruited at King's College Hospital, London, UK. Essentially, we searched our database to identify cases of trisomy 13 with a minimum of 2 mL available stored plasma, and each trisomy 13 case was matched with 10 euploid controls for length of storage of their plasma samples. Maternal blood was collected between April 2006 and February 2011.

Group 2 consisted of 1992 euploid pregnancies and 10 confirmed trisomy 13 pregnancies. The euploid pregnancies underwent routine first-trimester combined screening (between October 2010 and January 2011) and subsequently delivered phenotypically normal neonates at King's College Hospital. Maternal blood samples for research were collected at the time of screening and the plasma stored at −80°C; the findings of a study on NIPT for trisomies 21 and 18 using this cohort of patients have already been reported[5]. The confirmed trisomy 13 cases were recruited from US centers. Maternal blood samples for research were collected at 13–26 weeks' gestation post confirmation of trisomy as determined following CVS or amniocentesis, and the plasma stored at −80°C.

All women who participated in the study provided written informed consent under protocols approved by local ethics committees.

Sample collection and processing

Maternal venous blood was collected in ethylene diamine tetraacetic acid BD vacutainer™ tubes (Becton Dickinson UK limited, Oxfordshire, UK) or Cell-free™ BCT tubes (Streck, Omaha, NE, USA). Plasma was isolated from each sample via a double centrifugation protocol of 2000 *g* for 10 min followed by 16 000 *g* for 10 min, after a tube transfer following the first spin. cfDNA was isolated from plasma using the Dynabeads® Viral NA DNA purification kit (Dynal, Life Technologies Ltd, Paisley, PA, USA) protocol, with minor modifications as previously described[1,2].

Trisomy analysis

A single aliquot of plasma for each case that met the minimum fetal fraction concentration of 4% was analyzed at Ariosa Diagnostics by laboratory personnel who were blinded to trisomy status. Fetal fraction was determined as previously described[2]. Samples were analyzed with the digital analysis of selected regions (DANSR™) assay and the fetal-fraction optimized risk of trisomy evaluation (FORTE™) algorithm, as previously described, with the addition of 576 non-polymorphic loci on chromosome 13 to the existing non-polymorphic loci on chromosomes 18 and 21 as part of the DANSR assay[1,2]. Briefly, this method uses ligation of locus-specific oligonucleotides to produce a sequencing template only from selected genomic loci, thus reducing the amount of DNA sequencing needed. The FORTE algorithm was used to estimate the risk of aneuploidy for chromosome 13 in each sample. Proportion metrics for chromosomes 13, 18 and 21 are determined by computing the mean cfDNA counts of the loci for each chromosome and then dividing the mean count of each chromosome by the sum of all three means. The FORTE risk score is determined by calculating the odds ratio for trisomy based on chromosomal cfDNA counts (proportion metrics) and fraction of fetal cfDNA in the sample, then applying this as a likelihood ratio to the *a priori* trisomy risk based on maternal age and gestational age. A predefined cut-off value of 1 in 100 (1%) was designated as the threshold for classifying a sample as high or low risk. Results were provided on the risk of trisomy 13.

Group 1 samples were used in the first phase of the study. 384 loci on chromosome 13 were selected based primarily on previously studied euploid pregnancies. Of the 165 samples that were analyzed, nine failed amplification and sequencing and no risk score could be provided. The cases for which a risk score was ascertained were then unblinded and these samples were used to train and improve the trisomy 13 algorithm by selection of a new set of 384 loci from chromosome 13, which were able to best discriminate trisomy 13 from euploid samples using the FORTE algorithm.

In the second phase of the study, the new set of 384 loci from chromosome 13 was used to analyze the samples of Group 2. Of the 2002 samples analyzed, 53 of the euploid samples failed amplification and sequencing and no risk score could be provided.

Statistical analysis

Descriptive data are presented as median ± SD for continuous variables and as *n* (%) for categorical variables (Table 1). Comparison between the outcome groups was by Fisher's exact test for categorical variables and the Mann–Whitney *U*-test for continuous variables. The statistical software R version 2.15.1 was used for data analysis.

RESULTS

First phase of the study: Group 1

In the first phase of the study, trisomy 13 risk scores were given for 156 of the 165 samples in Group 1, including 145 of the 150 euploid and 11 of the 15 trisomy 13 cases. The characteristics of this population of 156 pregnant women are summarized in Table 1.

The risk score for trisomy 13 was > 1% in seven (63.6% (95% confidence interval (CI), 35.4–84.8%)) of the 11 cases of trisomy 13 and the score was < 0.01% in all 145 euploid cases (FPR 0.0% (95% CI, 0–2.6%)) (Figure 1). After unblinding and modification of the trisomy 13 algorithm, the trisomy 13 risk score was > 99% in all 11 cases of trisomy 13 and < 0.01% in all 145 euploid cases.

Second phase of the study: Group 2

In the second phase of the study, trisomy 13 risk scores were given for 1949 of the 2002 samples in Group 2, including 1939 of the 1992 euploid and all 10 trisomy 13 cases. The characteristics of these cases are summarized in Table 1.

In the 10 cases of trisomy 13, the estimated trisomy 13 risk score was > 99% in eight (80.0%) and < 0.01% in two (Figure 2). In the 1939 euploid cases, the estimated trisomy 13 risk score was < 0.01% in 1937 (99.9%), 0.79% in one and > 99% in one. Therefore, at the predefined risk cut-off of 1% for classifying a sample as high or low risk, the detection rate of trisomy 13 was 80.0% (95% CI, 49.0–94.3%) with an FPR of 0.05% (95% CI, 0–0.3%).

DISCUSSION

This two-phase case–control study shows that chromosome-selective sequencing of cfDNA in maternal plasma can detect the majority of trisomy 13 pregnancies with an FPR of 0.05%. The first phase of the study was used to optimize the trisomy 13 algorithm, which was then applied via a blinded analysis to a second set of cases.

Table 1 Characteristics of cases and controls for the subjects in the two phases of the study

	Phase 1		*Phase 2*	
Characteristic	*Euploid* (n = *145*)	*Trisomy 13* (n = *11*)	*Euploid* (n = *1939*)	*Trisomy 13* (n = *10*)
Maternal age (years, median ± SD)	37.2 ± 6.0	32.8 ± 4.2	31.8 ± 5.6	37.5 ± 5.3*
Gestational age (weeks, median ± SD)	13.3 ± 0.8	12.0 ± 0.6	12.6 ± 0.56	20.9 ± 3.88*
Racial origin (*n* (%))				
Caucasian	128 (88.3)	9 (81.8)	1370 (70.7)	8 (80.0)
African	7 (4.8)	2 (18.2)	387 (20.0)	2 (20.0)
Asian	8 (5.5)	—	131 (6.8)	—
Mixed	2 (1.4)	—	51 (2.6)	—
Fetal fraction (%, median (range))	11.5 (4.6–25.5)	7.6 (6.0–11.8)	10.0 (4.1–31.0)	14.0 (6.1–24.0)

Comparisons between trisomy 13 and euploid pregnancies were by Mann–Whitney *U*-test for continuous variables and by Fisher's exact test for categorical variables. $^{**}P < 0.05$ between trisomy 13 and euploid cases.

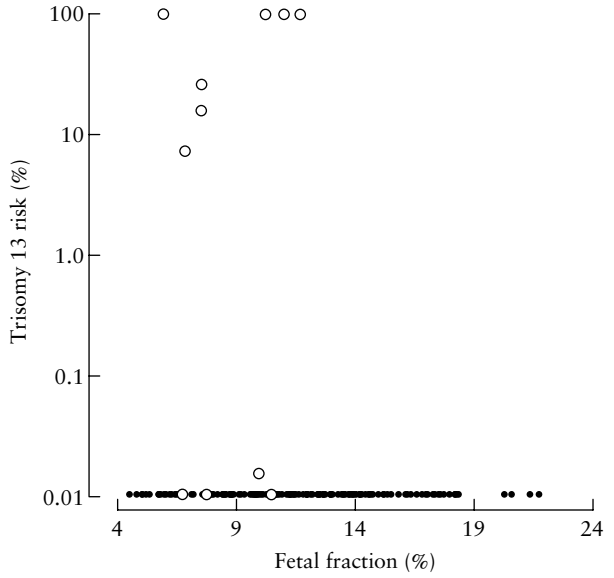

Figure 1 Phase 1: estimated risk score for trisomy 13 in trisomy 13 (○, $n = 11$) and euploid (●, $n = 145$) pregnancies plotted in relation to fetal fraction.

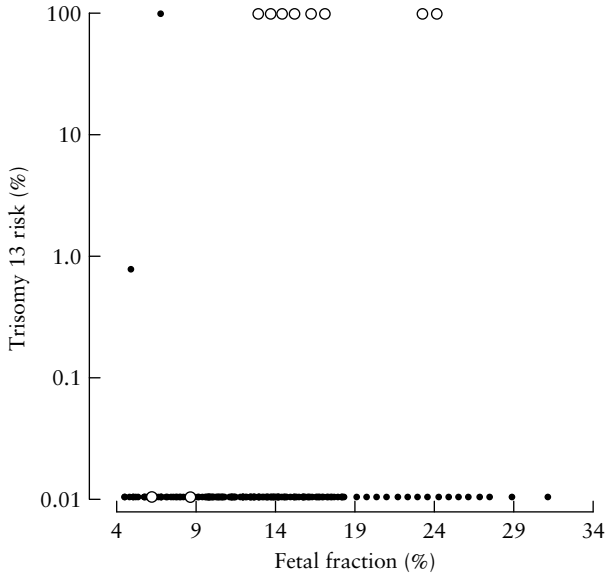

Figure 2 Phase 2: estimated risk score for trisomy 13 in trisomy 13 (○, $n = 10$) and euploid (●, $n = 1939$) pregnancies plotted in relation to fetal fraction.

In eight of the 10 cases of trisomy 13, the estimated risk for this aneuploidy was > 99%, whereas in 99.9% of the euploid cases the risk score for trisomy 13 was ≤ 0.01%.

The detection rate of trisomy 13 by chromosome-selective sequencing of maternal plasma cfDNA is lower than that for the detection of trisomies 21 and 18. Previous studies utilizing DANSR and FORTE, both in high-risk and routinely screened populations, reported that the detection rates of trisomy 21 and 18 were 100 and 97–98%, respectively, with FPRs of 0.1% or less[2–5].

Three previous studies, utilizing non-selective massively parallel shotgun sequencing, have examined NIPT for trisomy 13 from analysis of cfDNA in maternal plasma. Chen *et al.*[8] examined 25 trisomy 13 pregnancies and reported that with the use of a previously standardized *z*-score method developed for trisomy 21, they detected nine (36.0%) of the cases of trisomy 13 at an FPR of 7.6%. After adjustments, including removal of repeat-masking and correction for the GC content bias in the sequencing data, the detection rate increased to 100% and the FPR decreased to 1.1%. Palomaki *et al.*[9] used a similar approach and detected 11 of 12 (91.7%) cases of trisomy 13 at an FPR of 0.9%. Bianchi *et al.*[10] examined 16 cases of trisomy 13 and with a *z*-score cut-off of 2.5 the detection rate and FPR were 81.2% (13/16) and 0%, respectively, whereas at a *z*-score cut-off of 4.0 the detection rate was 68.8% (11/16) at an FPR of 0%[10,11].

One possible explanation for the observed lower detection rate of trisomy 13 with NIPT, compared with trisomy 21, is that in pregnancies with trisomy 13 fetuses there is placenta-confined mosaicism with a high proportion of cells being disomic, whereas in trisomy 21 all placental cells are invariably trisomic[12]. As the primary source of cfDNA in the maternal circulation is thought to be the placenta[13], a mosaic placenta that is predominantly disomic would lead to a false-negative result when using NIPT for the detection of trisomy 13. Likewise, a mosaic placenta that is predominantly trisomic in which the fetus is euploid could lead to a false-positive result.

At 11–13 weeks' gestation, the relative prevalences of trisomies 18 and 13 to trisomy 21 are 1:3 and 1:7, respectively[14–16]. However, in trisomies 18 and 13 the rate of spontaneous abortion or fetal death between 12 and 40 weeks' gestation is about 80%, therefore the relative prevalence of these trisomies to trisomy 21 at birth are 1:12 and 1:28, respectively.

Effective first-trimester screening for trisomy 21 is provided by a combination of maternal age, fetal nuchal translucency (NT) thickness and maternal serum free β-hCG and PAPP-A, with a detection rate of more than 90% for an FPR of 5%[6]. A beneficial consequence of screening for trisomy 21 is the early diagnosis of about 75% of cases of trisomies 18 and 13, which are the second and third most common chromosomal abnormalities. All three trisomies are associated with increased maternal age, increased fetal NT and decreased maternal serum PAPP-A, but in trisomy 21 serum free β-hCG is increased, while in trisomies 18 and 13 it is decreased[6,7,17–19]. When specific algorithms for trisomies 18 and 13 in addition to the one for trisomy 21 are also used, about 90% of fetuses with trisomy 21 and 95% of those with trisomies 13 and 18 can be detected for an overall increase in FPR of only 0.1%[7]. This increasing FPR has generally been considered acceptable despite the low prevalence of trisomies 13 and 18.

Trisomy 13 is often associated with abnormalities that can be readily identified by ultrasonography, not only in the second but also in the first trimester[20,21]. A study of 181 fetuses with trisomy 13 reported that at the 11–13-week scan 92 (50.8%) had holoprosencephaly, exomphalos and/or megacystis and in 129 (71.3%) the heart rate was above the 95th percentile[20]. Another study of holoprosencephaly, exomphalos and megacystis detected at 11–13 weeks reported that these defects are associated with a high incidence of chromosomal abnormalities, mainly trisomies 18 and 13, found in about 65, 55 and 30% of cases, respectively[21].

The performance of NIPT in screening for both trisomies 21 and 18 is far superior to that of currently available screening methods, with a substantial increase in detection rate and decrease in FPR[5,6]. A study of NIPT with a chromosome-selective sequencing approach in pregnant women undergoing routine screening for aneuploidies at 11–13 weeks' gestation reported that the estimated trisomy risk score was > 99% in all cases of trisomy 21 and trisomy 18 and < 1% in 99.9% of the euploid cases[5].

This study has shown that NIPT is also useful in screening for trisomy 13. Although the total number of cases of trisomy 13 examined is too small for accurate assessment of the detection rate, the FPR was only 0.05%. Consequently, if the NIPT result is positive for trisomy 13 there is a 1600-fold (80/0.05) increase in risk for this trisomy, therefore such patients should be offered the option of invasive diagnostic testing. If the NIPT result is negative for trisomy 13 there is a 5-fold (99.95/20) decrease in the *a-priori* risk. In NIPT there is a delay of 1–2 weeks between sampling and obtaining results. The blood sample could be collected at 9–10 weeks' gestation so that the results would be available by 12 weeks, which is the best time to carry out the first-trimester ultrasound examination[6]. If the 12-week scan demonstrates holoprosencephaly, exomphalos or megacystis, where the risk for aneuploidies is very high, the 5-fold reduction in risk following a negative NIPT test is unlikely to reassure the parents, and they should still be offered invasive testing. Since the prevalence of these defects is less than 0.1%, the effect on the overall proportion of pregnancies requiring an invasive test would be minimal.

The sensitivity and specificity of NIPT for trisomies 21, 18 and 13 is not 100%, therefore NIPT should not be considered a diagnostic test to replace invasive testing in high-risk pregnancies. It is a new high-performance screening test that identifies a high-risk group requiring further investigation by invasive testing. Similarly, the introduction of NIPT in universal screening for trisomies 21, 18 and 13 will complement rather than replace the 11–13-week scan. Not only is the latter useful in

screening for aneuploidies but it is also a diagnostic test for many major fetal defects, some of which, such as holoprosencephaly, require further investigation by invasive testing; additionally, in combination with biochemical and other biophysical markers, the scan can provide effective early screening for pregnancy complications, including pre-eclampsia and preterm birth[22–25].

Trisomy 13 is associated with a high rate of fetal death, and the prevalence in live births is about 30 times lower than that of trisomy 21. Additionally, unlike individuals with trisomy 21, who can survive for more than 60 years, individuals with trisomy 13 rarely survive beyond the first few months following birth. Chromosome-selective sequencing of cfDNA can detect the majority of cases of trisomy 13 with an FPR of less than 0.1%, but the detection rate is lower than that reported for trisomies 21 and 18.

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Conflict of interest

E.W., C.S., A.O. and K.S. are paid employees of Ariosa Diagnostics.

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