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IONA test for first-trimester detection of trisomies 21, 18 and 13

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ABSTRACT

Objective *To assess the potential performance of screening for fetal trisomies 21, 18 and 13 by cell-free DNA (cfDNA) analysis of maternal blood using the IONA® test.*

Methods *This was a nested case–control study of cfDNA analysis of maternal plasma using the IONA test. Samples were obtained at 11–13 weeks' gestation, before chorionic villus sampling, from 201 euploid pregnancies, 35 with trisomy 21, four with trisomy 18 and two with trisomy 13. Laboratory personnel were blinded to the fetal karyotype.*

Results *Probability scores for trisomies 21, 18 and 13 were given for 241/242 samples analyzed. No probability score was provided for one (0.5%) euploid pregnancy because of low fetal fraction. In all 35 cases of trisomy 21 the probability score for trisomy 21 was > 95% and the scores for trisomies 18 and 13 were ≤ 0.0001%. In all four cases of trisomy 18, the probability score for trisomy 18 was > 77% and the scores for trisomies 21 and 13 were ≤ 0.0001%. In the two cases of trisomy 13, the probability score for trisomy 13 was > 59% and the scores for trisomies 21 and 18 were ≤ 0.0001%. In the 200 euploid pregnancies with a test result, the probability score was < 0.08% for trisomy 21, < 0.001% for trisomy 18 and < 0.002% for trisomy 13. Therefore, the IONA test detected 100% of all three trisomies, with a false-positive rate of 0%.*

Conclusion *The IONA test successfully differentiated all cases of trisomies 21, 18 and 13 from euploid pregnancies.*

INTRODUCTION

Diagnosis of fetal aneuploidy relies on invasive testing by chorionic villus sampling (CVS) or amniocentesis in pregnancies that are identified by screening to be at high risk for such aneuploidies[1]. In many developed countries the most widely accepted method of screening at present is one that is based on a combination of maternal age, fetal nuchal translucency (NT) thickness, maternal serum free beta-human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein-A (PAPP-A), that could identify approximately 90% of fetuses with trisomies 21, 18 or 13 at a false-positive rate (FPR) of 5%[2]. A new method of screening for fetal trisomy relies on the examination of cell-free DNA (cfDNA) in maternal plasma[3]. Several studies in the last 4 years have reported the clinical validation and/or implementation of cfDNA testing and a recent meta-analysis of such studies weighted pooled detection rates (DR) and FPR in singleton pregnancies as 99.2% and 0.09%, respectively, for trisomy 21, 96.3% and 0.13% for trisomy 18, and 91.0% and 0.13% for trisomy 13[4].

The IONA® test and IONA software (Premaitha Health plc, Manchester, UK) is a cfDNA test developed recently which uses the Ion Proton™ sequencing platform (Thermo Fisher Scientific, Waltham, MA, USA) and an algorithm that determines the relative number of chromosomal copies, enabling the detection of fetal trisomies[5]. The objective of this study was to assess the potential performance of the IONA test and IONA software in screening for trisomies 21, 18 and 13 at 11–13 weeks' gestation.

METHODS

This was a nested case–control study of stored maternal plasma from 242 singleton pregnancies at 11–13 weeks' gestation, including 201 with euploid fetuses, 35 with trisomy 21, four with trisomy 18 and two with trisomy 13. In all cases fetal karyotyping was performed following CVS in our tertiary referral center because screening by the combined test had demonstrated an increased risk for trisomies 21, 18 or 13. Gestational age was determined from the measurement of fetal crown–rump length (CRL)[6].

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Maternal venous blood (10 mL) was collected before CVS in ethylenediaminetetraacetic acid vacutainer tubes (Becton Dickinson UK Limited, Oxfordshire, UK) and was processed within 15 min of collection. Samples were centrifuged at 2000 *g* for 10 min to separate the plasma from packed cells and buffy coat (plasma 1) and again at 16 000 *g* for 10 min to further separate cell debris (plasma 2). Plasma 1 and 2 (2 mL each) were divided into 0.5-mL aliquots in separate Eppendorf tubes that were labeled with a unique patient identifier and stored at −80°C until subsequent analysis. Written informed consent was obtained from the women who agreed to participate in the study, which was approved by the King's College Hospital Ethics Committee.

We searched our database and selected 35 consecutive cases of trisomy 21, four cases of trisomy 18 and two cases of trisomy 13 that had 2 mL of stored plasma 2 available. Two hundred and one euploid control subjects were selected; none of their samples was previously thawed and refrozen. Maternal blood was collected between April 2007 and June 2012.

Laboratory analysis

Plasma samples (four tubes of 0.5 mL per patient) from selected cases were sent from London to the laboratory of Premaitha Health plc in Manchester, UK. The following information was provided to Premaitha for each case: patient-unique identifier, maternal age, weight and height, racial origin, method of conception, smoking habit, gestational age in weeks, fetal CRL, and date of blood collection. Before evaluation for fetal trisomy, Premaitha assessed each sample for volume, adequacy of labeling, and risk of contamination or sample mixing and informed us that all samples met their acceptance criteria. The 242 samples were then analyzed using the IONA® test[5]. Results were provided for the risk of trisomies 21, 18 and 13 in each case and the correlation between the assay results and the fetal karyotype was determined.

Statistical analysis

Descriptive statistics are presented as median (interquartile range (IQR)) for continuous variables and *n* (%) for categorical variables. Probability scores for each trisomy are presented in scatterplots. DR and FPR are reported based on a predefined cut-off for probability. An age-adjusted probability of trisomy calculated to be ≥ 1 in 150 was considered a positive result, as used in the UK National Health Service (NHS)[7]. Point estimates with 95% CI have been provided.

RESULTS

All 242 samples were processed with the IONA test, however, one of these did not meet the validity criteria applied by the IONA software owing to to a low fetal fraction and was excluded from subsequent analysis. In this case, sampling was performed at 13 weeks' gestation, the maternal weight was 86 kg and the serum PAPP-A multiples of the median (MoM) value was 1.316 and there were no obvious reasons for why the fetal fraction was low. Results were generated by the IONA software for the remaining 241 samples.

The characteristics of the euploid and aneuploid pregnancies are summarized in Table 1. In all 35 cases of trisomy 21, the probability score for trisomy 21

Table 1 Characteristics of study population of women with a singleton pregnancy who underwent cell-free DNA analysis using the IONA® test, grouped according to karyotype obtained following chorionic villus sampling

Characteristic	*Euploid* (n = *200*)	*Trisomy 21* (n = *35*)	*Trisomy 18* (n = *4*)	*Trisomy 13* (n = *2*)
Maternal age (years)	32.9 (29.2–36.9)	38.0 (35.5–40.5)	37.7 (30.0–40.1)	32.1 (31.3–32.1)
Maternal weight (kg)	67.0 (60.0–76.0)	63.6 (57.2–74.5)	68.5 (60.9–72.0)	53.6 (50.8–53.6)
Maternal height (cm)	165 (160–170)	165 (160–167)	172 (166–173)	163 (162–163)
GA at testing (weeks)	12.7 (12.2–13.1)	13.0 (12.4–13.6)	11.9 (11.4–13.3)	12.8 (12.0–12.8)
Racial origin				
Caucasian	146 (73.0)	31 (88.6)	2 (50.0)	2 (100.0)
Afro-Caribbean	38 (19.0)	1 (2.9)	1 (25.0)	0 (0)
South Asian	5 (2.5)	3 (8.6)	0 (0)	0 (0)
East Asian	3 (1.5)	0 (0)	0 (0)	0 (0)
Mixed	8 (4.0)	0 (0)	1 (25.0)	0 (0)
Cigarette smoker	10 (5.0)	3 (8.6)	1 (25.0)	0 (0)
Mode of conception				
Spontaneous	189 (94.5)	33 (94.3)	2 (50.0)	2 (100.0)
Ovulation drugs	2 (1.0)	2 (5.7)	0 (0)	0 (0)
In-vitro fertilization	9 (4.5)	0 (0)	2 (50.0)	0 (0)
Fetal NT (mm)	1.8 (1.5–2.2)	4.2 (3.1–5.3)	2.5 (1.6–6.8)	5.9 (2.8–5.9)
Free β-hCG MoM	1.232 (0.821–1.950)	2.551 (1.696–3.615)	0.352 (0.224–0.901)	1.136 (0.515–1.136)
PAPP-A MoM	1.144 (0.736–1.612)	0.708 (0.500–1.157)	0.232 (0.145–0.288)	0.621 (0.146–0.621)

Data are given as median (interquartile range) or *n* (%). β-hCG, beta-human chorionic gonadotropin; GA, gestational age; MoM, multiples of the median; NT, nuchal translucency; PAPP-A, pregnancy-associated plasma protein-A.

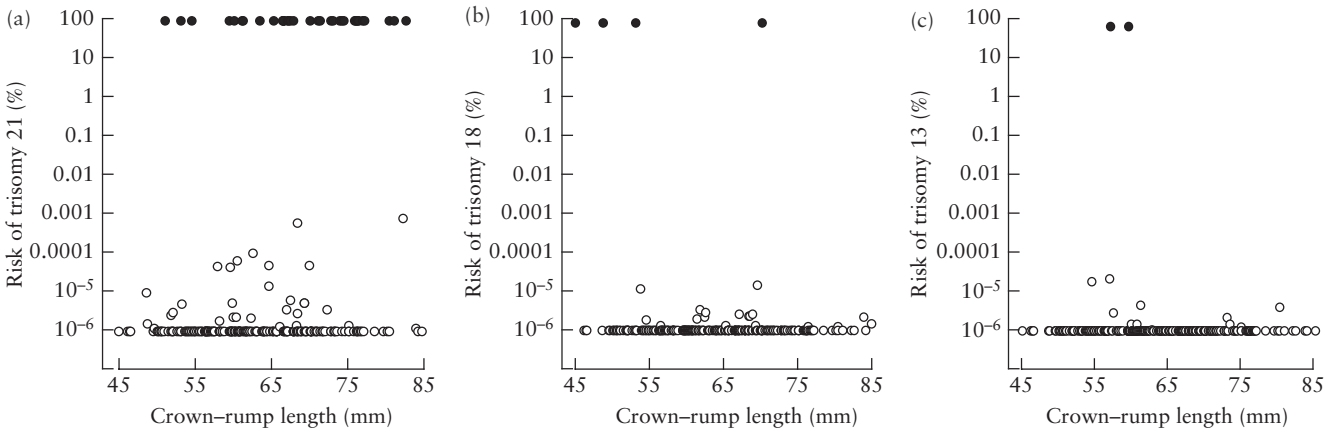

Figure 1 Probability scores for trisomy 21 (a), trisomy 18 (b) and trisomy 13 (c) in singleton pregnancies with trisomic (●) or euploid (○) fetuses that underwent cell-free DNA analysis using the IONA® test.

was > 95% and the scores for trisomies 18 and 13 were ≤ 0.0001% (Figure 1). In all four cases of trisomy 18, the probability score for trisomy 18 was > 77% and the scores for trisomies 21 and 13 were ≤ 0.0001%. In the two cases of trisomy 13, the probability score for trisomy 13 was > 59% and the scores for trisomies 21 and 18 were ≤ 0.0001%. In the 200 euploid pregnancies with a test result, the probability score was < 0.08% for trisomy 21, < 0.001% for trisomy 18 and < 0.002% for trisomy 13. Therefore, the DR for trisomy 21 was 100% (95% CI, 90.1–100.0%; 35/35 cases), the DR for trisomy 18 was 100% (95% CI, 51.0–100.0%; 4/4 cases) and the DR for trisomy 13 was 100% (95% CI, 34.2–100.0%; 2/2 cases), with a FPR of 0% (95% CI, 0.0–1.9%; 200/200 cases).

DISCUSSION

Main findings of the study

This nested case–control study has demonstrated that, in pregnancies considered by the combined test to be at high risk for trisomies 21, 18 or 13, cfDNA testing of maternal plasma at 11–13 weeks' gestation using the IONA test identified correctly all trisomic pregnancies, at a FPR of 0%.

Comparison with findings from previous studies

The performance of the IONA test in the detection of trisomy 21 is comparable to the reported performance of cfDNA analysis in a recent meta-analysis[4]. Although the number of cases of trisomies 18 and 13 is too small for definitive conclusions to be drawn, the performance of the test may be higher than in other studies[8–30]. In this study, compared with most previous publications on cfDNA testing, we used samples that were obtained at 11–13 weeks exclusively. This is important because, in the last decade, there has been a major shift from second- to first-trimester screening and diagnosis of aneuploidies.

Strengths and limitations of the study

The study has several strengths, including a large number of pregnancies affected by trisomy 21, laboratory staff blinded to the fetal karyotype or outcome at the time of cfDNA testing, and complete outcome data. The study examined the application of a novel technique and software. The main limitations include its retrospective design, inclusion of only high-risk pregnancies and a small number of pregnancies affected by trisomies 18 or 13. However, the reported performance of cfDNA testing is similar in studies in both high- and low-risk populations[11,22,23,25,29,31–34], we therefore believe that the results of the study can be applied to a general population but in an ideal situation such results should be validated in a prospective study.

Conclusion

In this study, the IONA test differentiated all cases of trisomy 21, 18 and 13 from euploid pregnancies.

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