PRENATAL DIAGNOSIS
Prenat Diagn 2000; **20**: 411–416.

SHORT COMMUNICATION

Screening for trisomy 13 by fetal nuchal translucency and maternal serum free β-hCG and PAPP-A at 10–14 weeks of gestation

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In 42 cases of trisomy 13 at 10–14 weeks of gestation, compared with 947 controls, the median multiple of the median (MoM) of maternal serum free β-human chorionic gonadotrophin (β-hCG) and pregnancy associated plasma protein A (PAPP-A) was significantly decreased (0.506 MoM and 0.248 MoM respectively), whilst fetal nuchal translucency was increased (2.872 MoM). In 38% and 71% of cases of trisomy 13 maternal serum free β-hCG and PAPP-A was below the 5th centile of the appropriate normal range for gestation and in 62% of cases the nuchal translucency was above the 95th centile. When combined together in a multivariate algorithm with maternal age, 90% of cases of trisomy 13 could be detected at a 0.5% false positive rate or 84% at a 0.1% false positive rate. We conclude that specific trisomy 13 risks should be part of developing risk algorithms combining maternal serum biochemistry and nuchal translucency for use in first trimester screening alongside those for trisomy 21 and trisomy 18. Copyright © 2000 John Wiley & Sons, Ltd.

KEY WORDS: trisomy 13; biochemical screening; ultrasound screening; prenatal screening; nuchal translucency; free β-hCG; PAPP-A; first trimester

INTRODUCTION

Trisomy 21 pregnancies are associated with increased maternal age, increased fetal nuchal translucency (NT) thickness, increased maternal serum free β-human chorionic gonadotrophin (β-hCG) and decreased maternal serum pregnancy associated plasma protein A (PAPP-A). Screening for trisomy 21 at 10–14 weeks of gestation by a combination of maternal age, fetal NT and maternal serum β-hCG and PAPP-A, identifies about 90% of affected pregnancies for a screen positive rate of 5% (Spencer *et al*., 1999). Trisomy 18 is characterized by increased fetal NT and decreased maternal serum free β-hCG and PAPP-A (Sherod *et al*., 1997, Spencer *et al*., 1992, 1994; Brizot *et al*., 1994, 1995; Biagiotti *et al*., 1998). Furthermore screening by a combination of all three markers can identify 86–89% of affected pregnancies for a 0.5–1.0% false positive rate (Tul *et al*., 1999).

Trisomy 13 is the third most common autosomal trisomy and at 10–14 weeks of gestation the relative proportion of trisomy 21 to trisomy 13 is about eight to one (Snijders *et al*., 1995). Ultrasonographic features of trisomy 13 in the first trimester include increased NT, holoprosencephaly and exomphalos (Snijders *et al*., 1999). Studies of small number of cases have reported that trisomy 13 may be associated with a decrease in maternal serum free β-hCG and PAPP-A (Brizot *et al*., 1994, 1995; Spencer *et al*., 1997).

The aim of this study was to examine the effectiveness of screening for trisomy 13 by a combination of maternal age, fetal NT and maternal serum free β-hCG and PAPP-A at 10–14 weeks of gestation.

METHODS

Since 1994 maternal serum samples were collected at the Harris Birthright Centre from women prior to chorionic villus sampling (CVS) because of advanced maternal age or increased risk for chromosomal abnormality after NT measurement at 10–14 weeks. Serums were stored at −20°C. At the time of ultrasound examination crown–rump length (CRL) and NT were measured as previously described (Snijders *et al*., 1998). Maternal serum samples were available from 39 cases of trisomy 13. In addition, three cases of trisomy 13 were identified as part of prospective first trimester screening in the OSCAR clinic at Harold Wood. Maternal age, weight, duration of the pregnancy based on last menstrual period and all ultrasound findings were collected in a database. Outcome of pregnancy and fetal karyotypes were added as soon as available.

During the same period, serum from women attending the clinic for the assessment of risk for chromosomal abnormality or for CVS were also taken

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Received: 1 December 1999
Revised: 29 January 2000
Accepted: 2 February 2000

and the same data were entered in the database. From the stored sera 947 controls were selected with matching for maternal and gestational age. The inclusion criteria were normal karyotype at CVS or birth of a baby without abnormalities. These controls have been part of two previous studies (Spencer *et al.*, 1999; Tul *et al.*, 1999).

Maternal serum free β-hCG and PAPP-A were measured using the Kryptor analyser—a rapid random access immunoassay analyser using Time Resolved Amplified Cryptate Emmission (TRACE) technology and the CIS automated immunofluorescent assays (CIS UK Ltd., High Wycombe, Bucks, UK). The stored samples were measured over a period of five days. The between day precision of these assays has been previously reported (Spencer *et al.*, 1999). The three samples collected during prospective screening were analysed within 20 min of blood collection.

Statistical analysis

Regression analysis was carried out to derive the relationship between free β-hCG and PAPP-A with gestational age. All analyte measurements were converted to MoMs using the derived medians from normal pregnancies at the same gestation. Correction of each MoM for maternal weight was also performed using the reciprocal-linear regression weight correction procedure of Neveux *et al.* (1996). Assessment of the performance of various marker combinations as potential screening procedures was examined using standard statistical modeling techniques (Royston and Thompson, 1992). We used the measured parameters for PAPP-A and free β-hCG and the reported parameters for nuchal translucency from 95 476 normal control pregnancies (Snijders *et al.*, 1998; Nicolaides *et al.*, 1998) and the measured parameters for the trisomy 13 cases. Using these population parameters, a series of 15 000 random MoM values were selected for each marker from within the distributions of the affected and the unaffected pregnancies. These values were then used to calculate likelihood ratios for the various marker combinations. The likelihood ratios were then used together with the age-related risk for trisomy 13 in the first trimester (Snijders *et al.*, 1995) to calculate the expected detection rate of affected pregnancies, at a fixed false positive rate, in a population with the maternal age distribution of pregnancies in England and Wales (OPCS, 1986–1994).

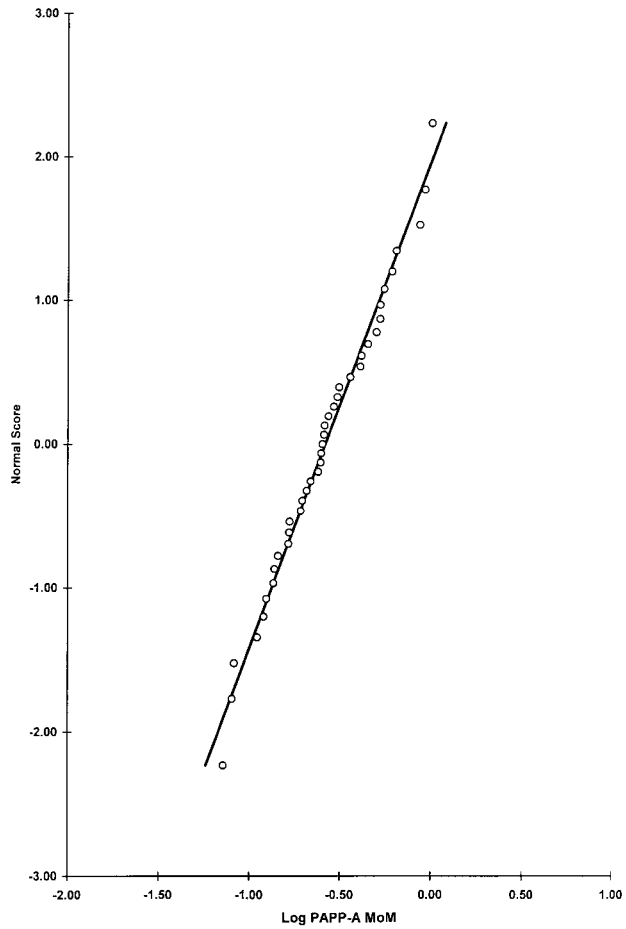

Figure 1—Probability plot of $\log_{10}$ PAPP-A MoM in cases of trisomy 13

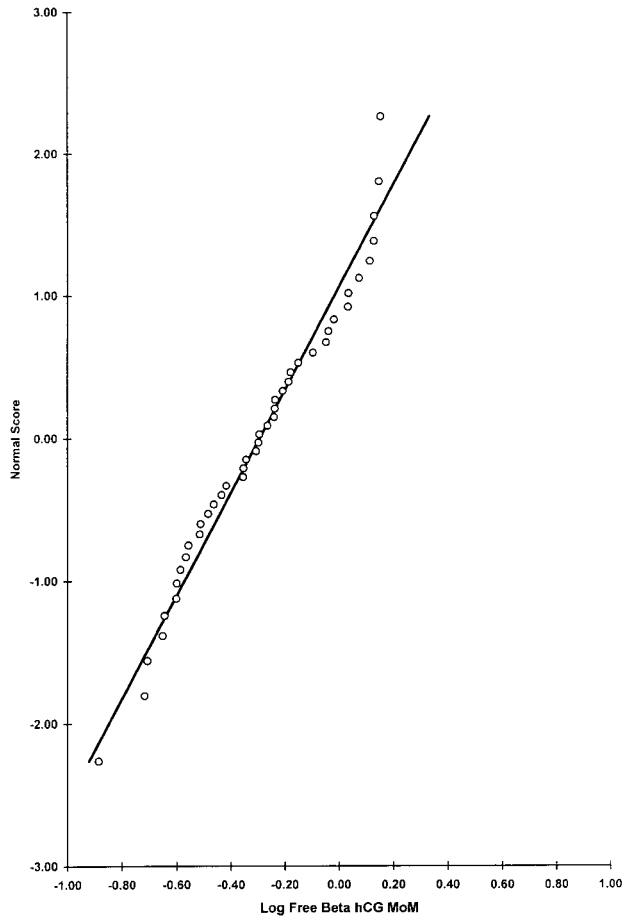

Figure 2—Probability plot of $\log_{10}$ free β-hCG MoM in cases of trisomy 13

Table 1—Maternal age, gestational age and crown–rump length for the trisomy 13 and control groups

	Trisomy 13 Mean (range)	Controls Mean (range)
Maternal age (years)	35.53 (26–45)	35.1 (15–47)
Gestational age (days)	84.9 (77–96)	85.1 (72–99)
Crown–rump length (mm)	56.3 (44–74.8)	60.4 (38–85)
Median length of storage (days)	1011 (0–2330)	546 (102–1811)
Number of cases	42	947

RESULTS

Table 1 summarizes the data of the trisomy 13 and the control groups. The mean maternal age was 35.5 years for the trisomy 13 group and 35.1 years for the controls; the gestational age based on CRL was 84.9 days for the trisomy 13 group and 85.1 for the controls. These differences were not statistically significant.

Free β-hCG, PAPP-A and NT all fitted a gaussian distribution after $\log_{10}$ transformation in both the control group (Spencer *et al.*, 1999; Nicolaides *et al.*, 1998) and the trisomy 13 group, with Kolmogorov–Smirnov and Anderson Darling tests showing linearity at the 0.01 probability levels after exclusion of three outliers for PAPP-A (exclusion criteria outside—three SD's). Figures 1, 2 and 3 show the normal probability plots for the markers in the trisomy 13 group.

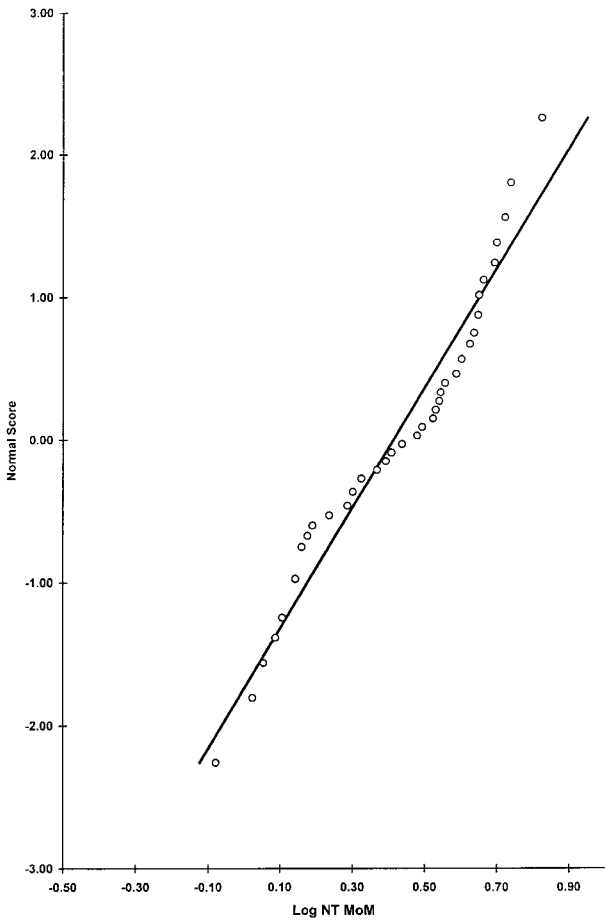

Figure 3—Probability plot of $\log_{10}$ NT MoM in cases of trisomy 13

In cases of trisomy 13, the median MoM was significantly lower than the controls for the markers free β-hCG and PAPP-A with medians of 0.506 and 0.248 respectively. For NT the median MoM was significantly higher (2.872 MoM) than in the controls. The standard deviation of $\log_{10}$ free β-hCG and PAPP-A MoM in the trisomy 13 group was 0.277 and 0.296 respectively, whilst that for $\log_{10}$ NT MoM was 0.238. These values are similar to those observed in a large series of trisomy 21 cases (Spencer *et al.*, 1999). As reported in our previous study (Spencer *et al.*, 1999), in the control group no significant correlation was found between maternal age and $\log_{10}$ MoM of the markers NT, free β-hCG and PAPP-A (correlation coefficients—0.010, 0.036, 0.036, respectively) or between NT and free β-hCG and PAPP-A (correlation coefficients—0.057 and 0.000). There was a small significant correlation between free β-hCG and PAPP-A ($r = 0.160$).

In the trisomy 13 group no significant correlation was found between $\log_{10}$ MoM of the markers free β-hCG and PAPP-A (0.1386), NT and PAPP-A (0.0964) and NT and free β-hCG (0.0095). Table 2 summarizes the distribution parameters for the control population from our previous study (Spencer *et al.*, 1999) and for the trisomy 13 cases from this study. Figures 4, 5 and 6 show the individual cases of trisomy 13 plotted against gestational age for each of the markers. Free β-hCG MoM , was below the 5th centile (0.397 MoM) in 16 (38%) of the cases with trisomy 18. PAPP-A was below

Table 2—Distribution parameters for the trisomy 13 and control populations

	Free β-hCG	PAPP-A	NT
Log_{10} mean controls	0.004	−0.004	0.000
Log_{10} SD controls	0.2558	0.2431	0.120
Log_{10} mean affected	−0.2943	−0.5817	0.4130
Log_{10} SD affected	0.277	0.296	0.238
10th centile controls (MoM)	0.47	0.48	0.69
50th centile controls (MoM)	1.00	1.00	1.00
90th centile controls (MoM)	2.16	1.98	1.40
10th centile affected (MoM)	0.23	0.08	1.28
50th centile affected (MoM)	0.506	0.248	2.872
90th centile affected (MoM)	1.30	0.61	4.93

NT data for control population from Nicoladies *et al.* (1998).

Table 3—Trisomy 13 detection rates at various false positive rates (FPR) for different marker combinations modelled against the age distribution of pregnancies in England and Wales

Marker combination	Detection at a FPR of 0.1%	Detection at a FPR of 0.5%
MA + free β-hCG	32%	40%
MA + PAPP-A	52%	64%
MA + NT	68%	79%
MA + NT + free β-hCG	70%	79%
MA + NT + PAPP-A	84%	90%
MA + free β-hCG + PAPP-A	58%	69%
MA + NT + free β-hCG + PAPP-A	84%	90%

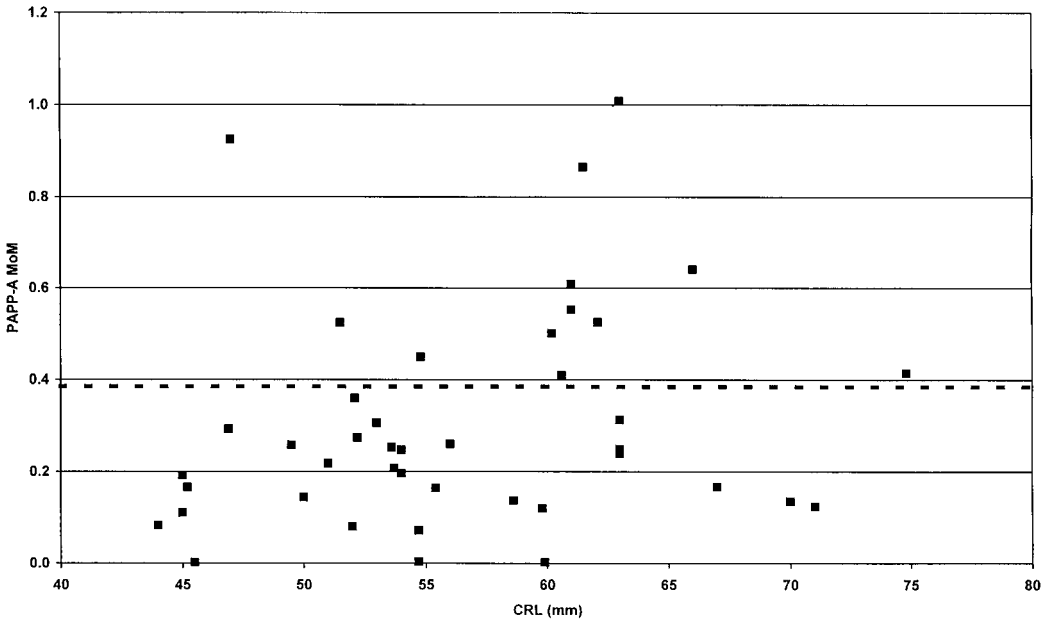

Figure 4—PAPP-A MoM in 42 cases of trisomy 13 in the first trimester. Dotted line is the 5th centile of normal

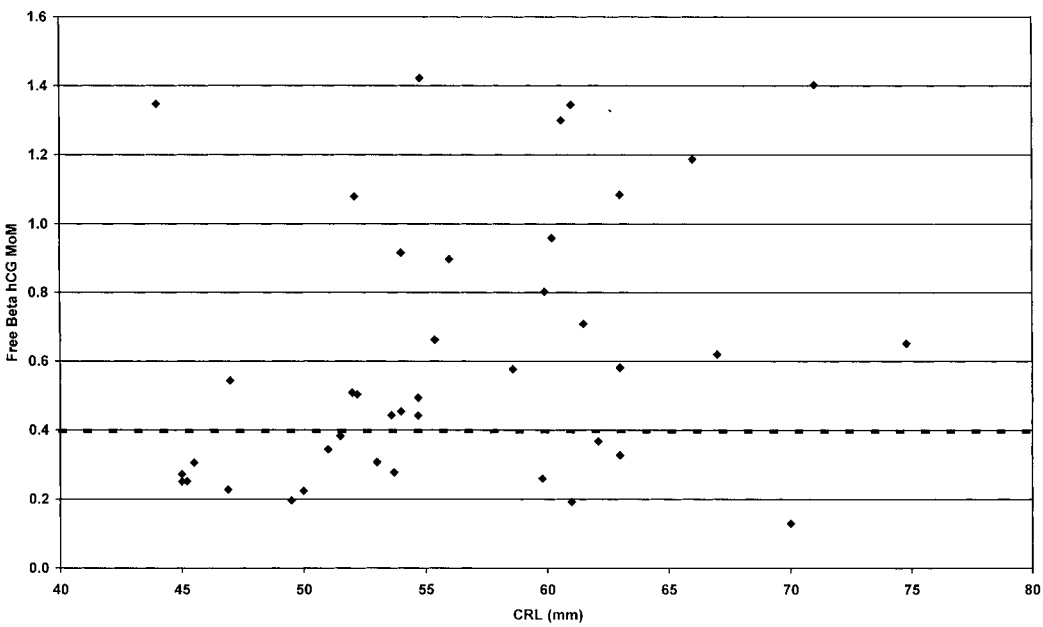

Figure 5—Free β-hCG MoM in 42 cases of trisomy 13 in the first trimester. Dotted line is the 5th centile of normal

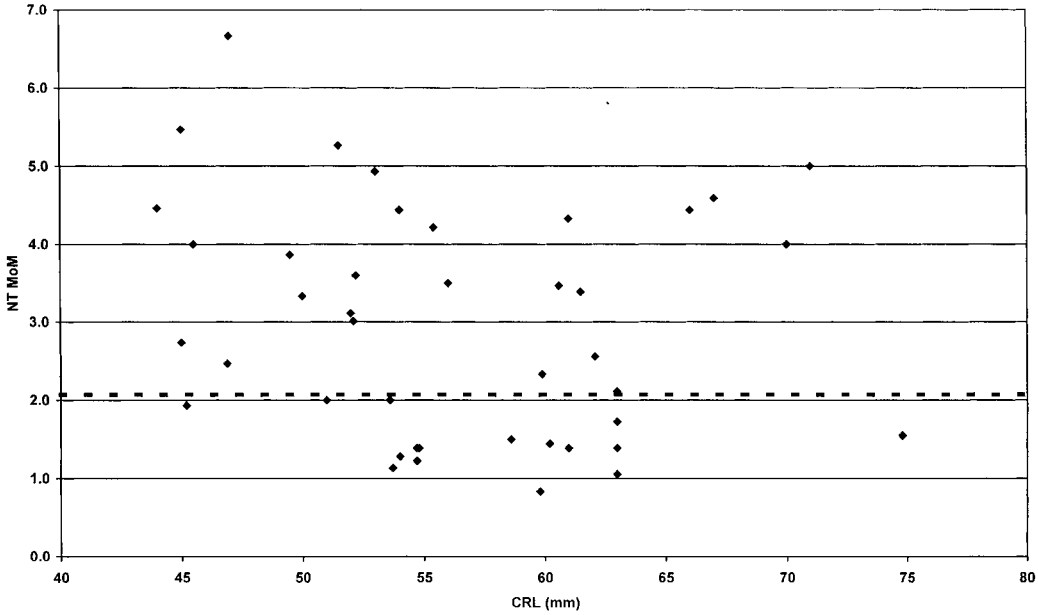

Figure 6—NT MoM in 42 cases of trisomy 13 in the first trimester. Dotted line is the 95th centile of normal

the 5th centile (0.385 MoM) in 30 (71%) cases and NT was above the 95th centile (1.57 MoM) in 26 (62%) cases.

When the observed statistical parameters were used in the mathematical model of a population with the maternal age distribution of pregnancies in England and Wales, the estimated detection rates using various marker combinations with maternal age at a fixed false positive rate of 0.1% varied from 32% with maternal age and free β-hCG to 90% with maternal age, NT, PAPP-A and free β-hCG (Table 3).

DISCUSSION

The findings of this study demonstrate that in trisomy 13 pregnancies at 10–14 weeks of gestation both maternal serum free β-hCG and PAPP-A are decreased (0.506 MoM and 0.248 MoM respectively) and that fetal NT is increased. These findings are compatible with the results of previous observations. In a small series of nine cases, Pandya *et al.* (1995) showed increased NT in cases of trisomy 13 and Snijders *et al.* (1998) showed that 72% of cases (33/46) were above the 95th centile for NT thickness. For free β-hCG Brizot *et al.* (1995) showed a median MoM of 0.3 from eight cases, Spencer *et al.* (1997) showed a median MoM of 0.64 from five cases and Brambati *et al.* (1997) showed a median MoM of 0.19 from three cases. Similarly for PAPP-A, Brizot *et al.* (1994) showed a median MoM of 0.25 from eight cases and Brambati *et al.* (1997) showed a median MoM of 0.67 from three cases. Therefore, trisomy 13 pregnancies present with a similar pattern of markers (low maternal serum free β-hCG and PAPP-A and high fetal NT) as trisomy 18 pregnancies (Tul *et al.*, 1999). Specific risks for trisomy 13 can now be calculated from our observed distributions of the markers and the *apriori* maternal age and gestation-related risks (Snijders *et al.*, 1995). This is unlike the situation in the second trimester when it has been suggested that specific risk algorithms cannot be constructed for trisomy 13 (Saller *et al.*, 1999).

The use of rapid immunodiagnostic technology for the measurement of the biochemical markers has allowed the introduction of a one-stop clinic for assessment of risk for fetal abnormalities (OSCAR) in which during a 1 h visit the patient undergoes pre-test counselling, biochemical and ultrasound assessment with combined risk estimation, prior to receiving post-test counselling. Screening for trisomy 21 in such a programme identifies about 90% of affected pregnancies for a screen positive rate of 5% (Spencer *et al.*, 1999). In such a system it is also possible to identify 86% of trisomy 18 pregnancies for a 0.5% false positive rate (Tul *et al.*, 1999) and as suggested by this study, about 84% of trisomy 13 pregnancies for a 0.1% false positive rate.

ACKNOWLEDGEMENTS

We acknowledge the support of CIS in providing the instrument and reagents for this study. The work at the Harris Birthright Centre is supported by a grant from the Fetal Medicine Foundation (Charity no. 1037116).

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