Ultrasound Obstet Gynecol 2008; **31**: 493–502
Published online in Wiley InterScience (www.interscience.wiley.com). **DOI:** 10.1002/uog.5332

First-trimester screening for trisomy 21 by free beta-human chorionic gonadotropin and pregnancy-associated plasma protein-A: impact of maternal and pregnancy characteristics

K. O. KAGAN*†, D. WRIGHT‡, K. SPENCER*§, F. S. MOLINA*¶ and K. H. NICOLAIDES*

**Harris Birthright Research Centre for Fetal Medicine, King's College Hospital, London, ‡Department of Mathematics and Statistics, University of Plymouth, Plymouth, §Prenatal Screening Unit, Clinical Biochemistry Department, King George Hospital, Goodmayes, UK, †Department of Obstetrics and Gynecology, University of Tuebingen, Tuebingen, Germany and ¶Hospital Universitario Virgen de las Nievas, Granada, Spain*

KEYWORDS: first-trimester screening; free β-hCG; PAPP-A; trisomy 21

ABSTRACT

Objectives *To use multiple regression analysis to define the contribution of maternal variables that influence the measured concentration of free beta-human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein-A (PAPP-A), and the interaction between these covariates, in first-trimester biochemical screening for trisomy 21.*

Methods *This was a multicenter study of prospective screening for trisomy 21 by a combination of fetal nuchal translucency thickness, and maternal serum free β-hCG and PAPP-A at 11 + 0 to 13 + 6 weeks of gestation. In the pregnancies subsequently found to have trisomy 21 and in those with no obvious chromosomal abnormality, we used multiple regression analysis to account for pregnancy characteristics that influence the measured concentrations of free β-hCG and PAPP-A. We fitted Gaussian distributions to the distribution of log multiples of the median (MoM) values in trisomy 21 and in unaffected pregnancies.*

Results *There were 491 cases of trisomy 21 and 96 803 chromosomally normal pregnancies. Compared with values in Caucasian women, those who were parous, non-smokers and those who conceived spontaneously, PAPP-A was 57% higher in women of Afro-Caribbean origin, 3% higher in South Asians, 9% higher in East Asians, 2% higher in nulliparous women, 17% lower in smokers and 10% lower in those conceiving by* in-vitro *fertilization (IVF). Free β-hCG was 12% higher in women of Afro-Caribbean origin, 9% lower in South Asians, 8% higher in East Asians, 2% higher in nulliparous women, 4% lower in smokers and 9% higher in those conceiving by IVF. In screening for trisomy 21 by maternal age and serum free β-hCG and PAPP-A the estimated detection rate was 65% for a false-positive rate of 5%.*

Conclusions *In first-trimester biochemical screening for trisomy 21 it is essential to adjust the measured values of free β-hCG and PAPP-A for maternal and pregnancy characteristics. Copyright © 2008 ISUOG. Published by John Wiley & Sons, Ltd.*

INTRODUCTION

Screening for trisomy 21 by fetal nuchal translucency (NT) thickness and maternal serum free beta-human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein-A (PAPP-A) at 11 + 0 to 13 + 6 weeks of gestation detects about 90% of affected pregnancies for a false-positive rate of 5%[1–4].

In trisomy 21 pregnancies maternal serum free β-hCG is about twice as high and PAPP-A is reduced to about half compared with values in chromosomally normal pregnancies. In the development of risk algorithms for combined screening the estimation of accurate patient-specific risks necessitates adjustments in the measured free β-hCG and PAPP-A to take into account their association with gestational age, maternal weight, ethnicity, smoking status and method of conception[1,5–11]. Essentially, each measured level is first converted to a multiple of the expected normal median (MoM) specific to a pregnancy of the same gestational age, maternal weight, smoking

Correspondence to: Prof. K. H. Nicolaides, Harris Birthright Research Centre for Fetal Medicine, King's College Hospital Medical School, Denmark Hill, London SE5 8RX, UK (e-mail: fmf@fetalmedicine.com)

Accepted: 7 March 2008

status, ethnicity and method of conception. The MoM distribution of each metabolite in unaffected and trisomy 21 pregnancies is assumed to be Gaussian, specified in terms of means, SD and correlations.

In this study of 491 pregnancies with trisomy 21 and 96 803 unaffected pregnancies we used multiple regression analysis, instead of a sequential approach, to take into account the pregnancy characteristics that influence the measured concentration of free β-hCG and PAPP-A. The multiple regression model was then used to estimate likelihood ratios for the biochemical markers that can be combined with maternal age to produce patient-specific risks for each case.

METHODS

The data for this study were derived from prospective assessment of risk for trisomy 21 by a combination of maternal age, fetal NT thickness, and maternal serum PAPP-A and free β-hCG at 11 + 0 to 13 + 6 weeks of gestation[3]. Two groups of UK hospitals were included. In Group A (Fetal Medicine Centre, London; King's College Hospital, London) the ultrasound and biochemical measurements and assessment of risk were carried out in the same hospital visit. In Group B (Harold Wood Hospital, Romford; King George Hospital, Goodmayes; Canterbury Hospital, Canterbury; William Harvey Hospital, Ashford; Queen Elizabeth The Queen Mother's Hospital, Margate), the biochemical measurements and assessment of risk were carried out either in the same hospital visit, on the same day, or 1 day before or after the ultrasound scan.

Transabdominal ultrasound examination was performed to diagnose any major fetal defects and for measurement of the crown–rump length (CRL) and fetal NT thickness[12]. Measurements of PAPP-A and free β-hCG were carried out by an automated machine that provides reproducible results within 30 min (Kryptor system, Brahms AG, Berlin, Germany; or Delfia Express system, Perkin Elmer, Waltham, MA, USA). Gestational age was based on the CRL at the time of the screening, and was calculated using the formula obtained from Robinson and Fleming[13].

Maternal weight, measured at the time of the scan, demographic characteristics, ultrasonographic measurements and biochemical results were recorded in computer databases. Ethnic origin was assessed by self-classification and included Caucasian, South Asian (Indian, Pakistani, Bangladeshi), Afro-Caribbean, East Asian (Chinese, Korean, Japanese), and mixed ethnicity, which mainly consisted of those of Caucasian and Afro-Caribbean origin. Smoking status was similarly based on a self-completed questionnaire, and each woman was classified as either a non-smoker or smoker irrespective of the individual cigarette consumption. Women were grouped as parous if they had previous deliveries beyond 23 weeks and nulliparous if they had no pregnancies resulting in delivery beyond 23 weeks. Mode of conception was either spontaneous, including those receiving ovulation induction drugs, or assisted reproduction by *in-vitro* fertilization (IVF). Karyotype results and details of pregnancy outcomes were added to the databases as soon as they became available.

A search of the databases was done to identify all singleton pregnancies in which first-trimester screening by NT, PAPP-A and free β-hCG was carried out between June 1999 and December 2006.

Statistical analysis

The statistical analysis was performed on the separate datasets A and B, and on the combined data. Forest plots showing point-wise 95% CI for effects from the two sources and the combined source were produced.

Multiple regression analysis of log-transformed marker values was carried out to provide estimates of parameters required to produce log MoM values for PAPP-A and free β-hCG[14]. Quadratic terms were used to model the effect of gestational age and maternal weight. Effects of the machine used for biochemical analysis, maternal ethnic origin, smoking status and method of conception were included as factors in the multiple regression models. Parity was analyzed separately owing to incomplete data on this factor. The mean log MoM in trisomy 21 was represented as a linear function of gestational age. Bivariate Gaussian models were fitted to the distribution of log MoM PAPP-A and log MoM free β-hCG in trisomy 21. In the analyses observations falling outside the 99.99th contour were considered as outliers and removed. For the analysis of the pooled dataset, a term was used to model the difference between sources.

Likelihood ratios were computed from the fitted distribution and used with maternal age to produce patient-specific risks for each case. Crude detection rates and false-positive rates were calculated by taking the proportions with risks above a given risk threshold. Maternal age-specific detection and false-positive rates were then produced, and adjusted according to the maternal age distribution of pregnancies in England and Wales in 2000–2002[15]. These standardized rates were compared with detection and false-positive rates estimated using Monte Carlo methods to sample from the modeled Gaussian distributions.

RESULTS

Data description

In Group A, the search of the databases identified 45 668 singleton pregnancies in which first-trimester combined screening was carried out, and data on maternal weight, ethnic origin, smoking status and mode of conception were available. We excluded 1869 (4.1%) cases from further analysis because there were no data on fetal karyotype or pregnancy outcome ($n = 1605$) or there was an abnormal karyotype other than trisomy 21 ($n = 264$). The remaining data included 43 478 unaffected cases and 321 cases with trisomy 21. In Group B, the search of

the database identified 55 482 singleton pregnancies in which first-trimester combined screening was carried out, and data on maternal weight, ethnic origin, smoking status and mode of conception were available. We excluded 1987 (3.6%) cases from further analysis because there were no data on fetal karyotype or pregnancy outcome ($n = 1866$) or there was an abnormal karyotype other than trisomy 21 ($n = 121$). The remaining data included 53 325 unaffected pregnancies and 170 cases with trisomy 21. In summary, the pooled dataset consisted of 96 803 unaffected pregnancies and 491 cases with trisomy 21.

In the pooled dataset the median maternal age was 32.8 (range, 14–53; mean (SD), 32.1 (5.7)) years, the median gestational age at screening was 12 + 5 (range, 11 + 0 to 13 + 6) weeks and the median CRL was 62.8 (range, 45.0–84.0) mm (Table 1). The median maternal age of pregnancies in England and Wales in 2000–2002 was 29.0 years.

Distribution of log MoM in unaffected and trisomy 21 pregnancies

Free β-hCG and PAPP-A values were outside the 99.99th contours for unaffected and trisomy 21 pregnancies in 84/96 803 (0.09%) and 1/491 (0.2%) cases, respectively and these were excluded from further analysis.

In the unaffected pregnancies (pooled dataset) multiple regression analysis demonstrated that, compared with values in Caucasian women who were parous, non-smokers and conceived spontaneously, PAPP-A MoM was 57% higher in women of Afro-Caribbean origin, 3% higher in South Asians, 9% higher in East Asians, 2%

Table 2 Adjustment factors for multiples of the median values of pregnancy-associated plasma protein-A (PAPP-A) and serum free beta-human chorionic gonadotropin (β-hCG) derived from the pooled dataset

	Coefficient			
	Log_{10} *scale*		*Original scale*	
Parameter	*Estimate*	*Standard error*	*Estimate*	*95% CI*
PAPP-A				
Afro-Caribbean	0.195	0.004	1.566	1.540–1.592
South Asian	0.012	0.003	1.028	1.014–1.042
Mixed	0.036	0.010	1.085	1.038–1.135
East Asian	0.039	0.009	1.093	1.048–1.140
Smoker	−0.082	0.002	0.828	0.819–0.836
Conception by IVF	−0.047	0.005	0.897	0.878–0.918
Nulliparous	0.009	0.002	1.020	1.012–1.028
Delfia Express	−0.123	0.003	0.753	0.743–0.763
Group B effect	0.006	0.002	1.015	1.007–1.022
Free β-hCG				
Afro-Caribbean	0.049	0.004	1.121	1.099–1.143
South Asian	−0.043	0.004	0.905	0.891–0.920
Mixed	−0.025	0.011	0.945	0.898–0.994
East Asian	0.032	0.011	1.076	1.024–1.129
Smoker	−0.018	0.003	0.959	0.948–0.971
Conception by IVF	0.037	0.006	1.088	1.061–1.116
Nulliparous	0.007	0.002	1.016	1.007–1.026
Delfia Express	0.022	0.003	1.052	1.036–1.068
Group B effect	0.006	0.002	1.013	1.004–1.021

Adjustments were for other races relative to Caucasian, smokers relative to non-smokers, *in-vitro* fertilization (IVF) relative to spontaneous conception, nulliparous relative to parous, Delfia Express relative to Kryptor system, and Group B relative to Group A.

Table 1 Characteristics of the study population

Parameter	*Group A* (n = *43 799*)	*Group B* (n = *53 495*)
Maternal characteristics		
Age (years)	35.4 (16.5–52.1)	30.5 (13.5–53.0)
Weight (kg)	63.6 (34.0–150.0)	65.4 (29.6–192.0)
Spontaneous conception	41 879 (95.6)	53 300 (99.6)
Smoker	2044 (4.7)	9288 (17.4)
Nulliparous	19 419 (44.3)	7596 (14.2)
Ethnicity		
Caucasian	39 661 (90.6)	46 803 (87.5)
Afro-Caribbean	1614 (3.7)	2319 (4.3)
East Asian	471 (1.1)	91 (0.2)
South Asian	1538 (3.5)	4282 (8.0)
Mixed	515 (1.2)	0 (0)
Gestational age		
11 + 0 to 11 + 6 weeks	4628 (10.6)	6752 (12.6)
12 + 0 to 12 + 6 weeks	24 303 (55.5)	29 098 (54.4)
13 + 0 to 13 + 6 weeks	14 868 (33.9)	17 645 (33.0)
Crown–rump length (mm)	62.9 (45.0–84.0)	62.7 (45.0–84.0)
Karyotype		
Normal	43 478 (99.3)	53 325 (99.7)
Trisomy 21	321 (0.7)	170 (0.3)

Values are median (range) or *n* (%). Group A, Fetal Medicine Centre and King's College Hospital; Group B, Harold Wood Hospital, King George Hospital, Canterbury Hospital, William Harvey Hospital and Queen Elizabeth The Queen Mother's Hospital.

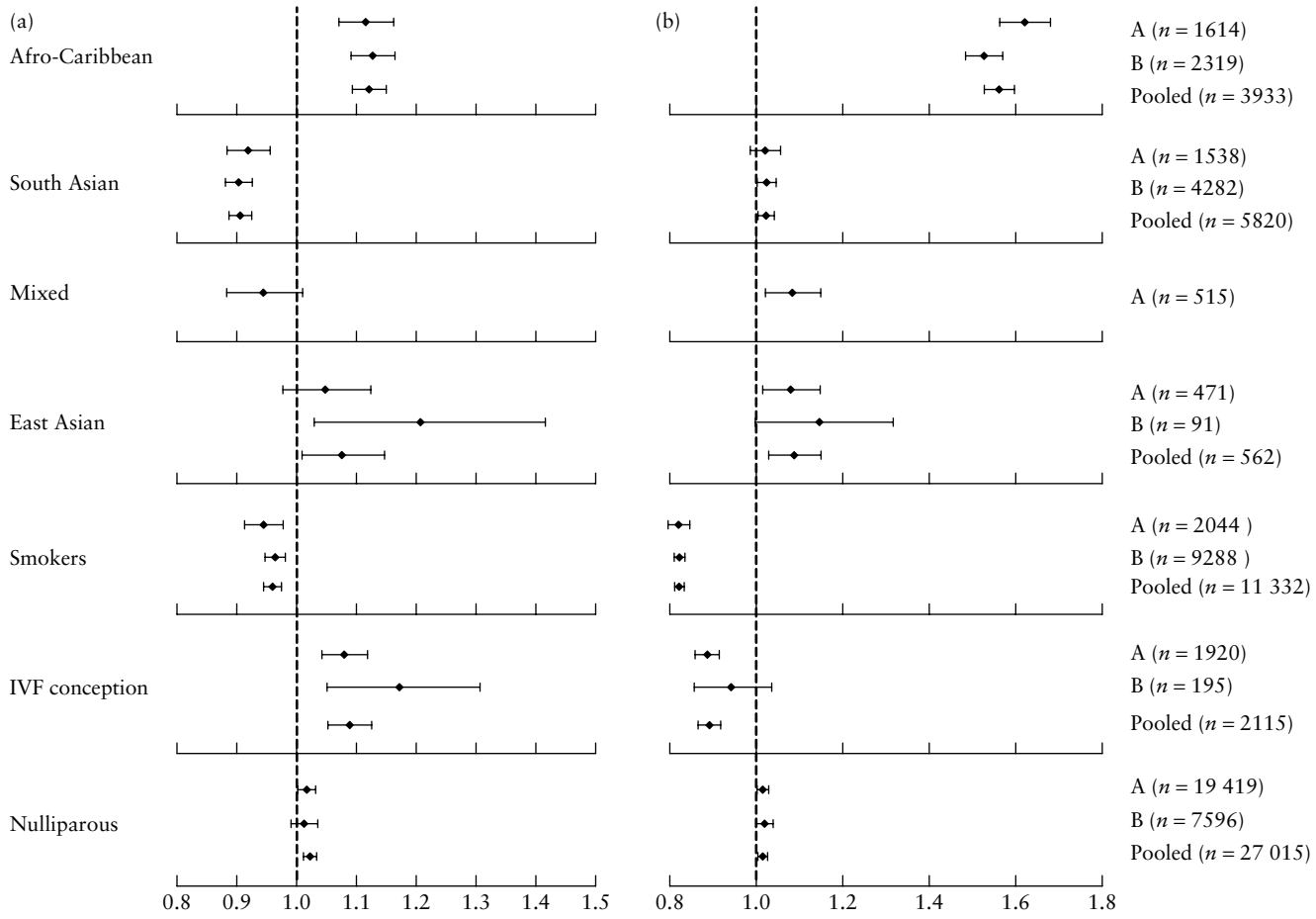

Figure 1 Estimated effects on free beta-human chorionic gonadotropin (a) and pregnancy-associated plasma protein-A (b) of different ethnic groups relative to Caucasian, of smokers relative to non-smokers, of *in-vitro* fertilization (IVF) relative to spontaneous conception, and of nulliparous relative to parous women. Effects are shown with 95% CI.

Table 3 Parameter estimates and correlation for unaffected and trisomy 21 cases

Parameter	*Karyotype*	n	*Estimate*	*95% CI*
SD of log MoM PAPP-A	Normal	96 719	0.2203	0.2190–0.2216
	Trisomy 21	490	0.2359	0.2179–0.2570
SD of log MoM free β-hCG	Normal	96 719	0.2544	0.2529–0.2559
	Trisomy 21	490	0.2699	0.2493–0.2940
Correlation	Normal	96 719	0.2143	0.2064–0.2222
	Trisomy 21	490	0.0821	−0.0344 to 0.1964

β-hCG, beta-human chorionic gonadotropin; MoM, multiples of the median; PAPP-A, pregnancy-associated plasma protein-A.

higher in nulliparous women, 17% lower in smokers and 10% lower in those conceiving by IVF. Free β-hCG MoM was 12% higher in women of Afro-Caribbean origin, 9% lower in South Asians, 8% higher in East Asians, 2% higher in nulliparous women, 4% lower in smokers and 9% higher in those conceiving by IVF. The results are shown in Figure 1 and Table 2. Parameter estimates for the fitted Gaussian distributions are shown in Figure 2 and Table 3.

The fitted equation for estimating median log metabolite values in chromosomally normal pregnancies from gestation (in days) and weight (in kg) were:

$$\begin{aligned}\text{Median } \log_{10} \text{ free } \beta\text{-hCG} &= 1.64931 - 0.0057856 \times (\text{gestation} - 77) \\ &\quad - 0.00023901 \times (\text{gestation} - 77)^2 - 0.0045501 \\ &\quad \times (\text{weight} - 69) + 0.000028909 \times (\text{weight} - 69)^2\end{aligned}$$

$$\begin{aligned}\text{Median } \log_{10} \text{ PAPP-A} &= 0.18992 + 0.026102 \times (\text{gestation} - 77) - 0.0074642 \\ &\quad \times (\text{weight} - 69) + 0.000030669 \times (\text{weight} - 69)^2.\end{aligned}$$

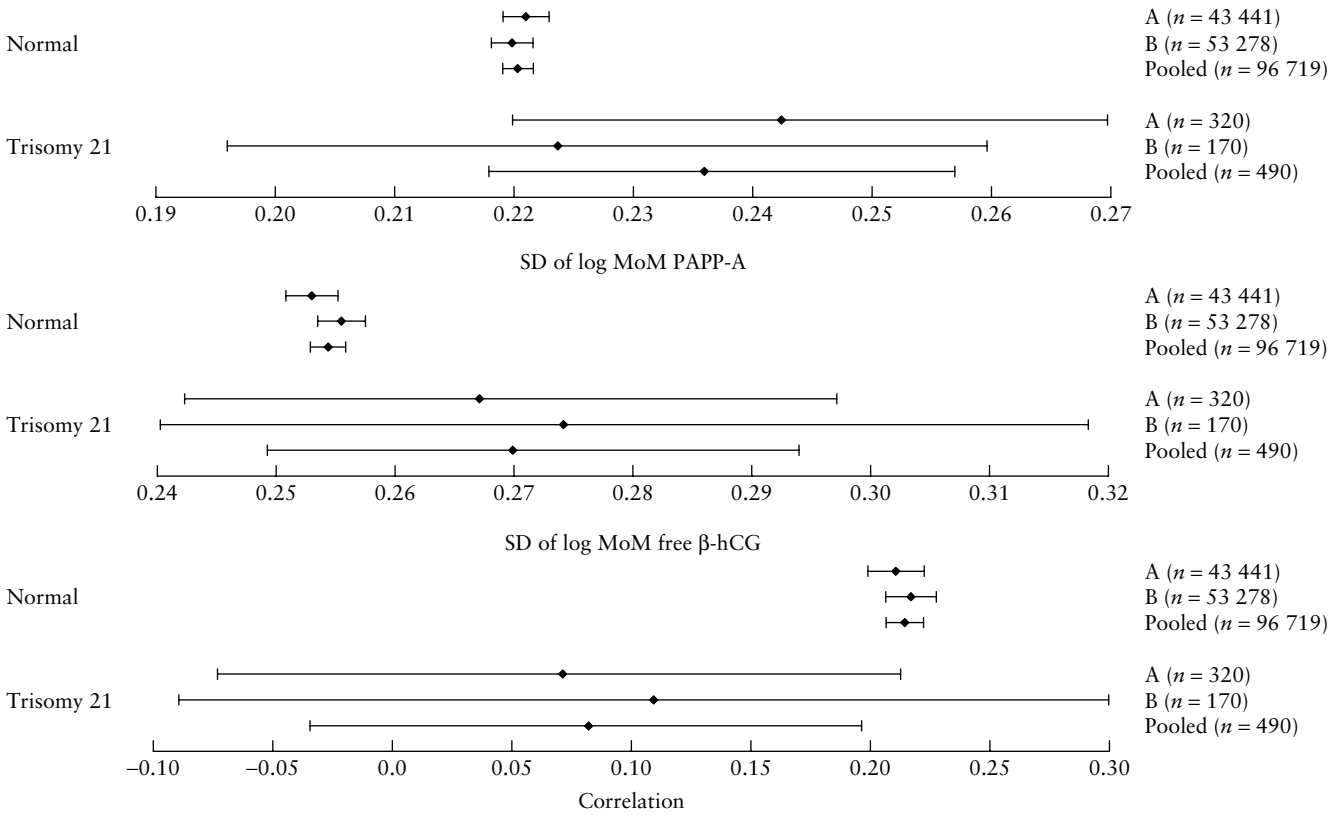

Figure 2 Parameter estimated SDs and correlations with 95% CIs for trisomy 21 and unaffected pregnancies. β-hCG, beta-human chorionic gonadotropin; MoM, multiple of the median; PAPP-A, pregnancy-associated plasma protein-A.

Table 4 Detection rates of trisomy 21 for given false-positive rates (FPR)

	Detection rate (%)											
	Overall (n = *491)*			*11 weeks* (n = *52)*			*12 weeks* (n = *273)*			*13 weeks* (n = *166)*		
FPR (%)	*Crude*	*Standardized*	*Modeled*	*Crude*	*Standardized*	*Modeled*	*Crude*	*Standardized*	*Modeled*	*Crude*	*Standardized*	*Modeled*
1	40	37	45	37	38	54	43	43	46	34	33	40
2	50	51	55	52	69	64	54	56	56	43	44	49
3	58	57	61	67	79	70	60	61	62	52	49	55
4	63	60	65	73	79	74	65	64	66	54	53	60
5	66	65	68	73	86	77	70	68	69	58	57	62
10	78	79	78	90	98	85	79	80	79	70	79	74

The crude rates are those observed in our population, the standardized rates are rates after adjustments for the maternal age distribution of pregnancies in England and Wales in 2000–2002, and the modeled rates are the standardized rates predicted from the fitted Gaussian model in this study.

Table 5 Accuracy of estimated risk for trisomy 21 by a combination of maternal age, and serum free beta-human chorionic gonadotropin and pregnancy-associated plasma protein-A

Estimated risk				
Range	*Median*	*Trisomy 21* (n *(%))*	*Chromosomally normal* (n *(%))*	*Observed risk*
1 in 10 or higher	1 in 5	158 (32.2)	757 (0.8)	1 in 6
1 in 10 to 1 in 100	1 in 52	185 (37.7)	5711 (5.9)	1 in 32
1 in 100 to 1 in 250	1 in 169	68 (13.8)	7105 (7.3)	1 in 105
1 in 250 to 1 in 1000	1 in 555	56 (11.4)	21 589 (22.3)	1 in 387
1 in 1000 to 1 in 5000	1 in 2249	20 (4.1)	35 102 (36.3)	1 in 1756
1 in 5000 or lower	1 in 10 628	4 (0.8)	26 539 (27.4)	1 in 6636

These apply to non-smoking, parous, Caucasian women who conceived spontaneously and whose assays were performed using the Kryptor machine. Adjustment factors for other groups are given in Table 2. Forest plots for the effects of ethnicity, smoking, IVF and parity are shown in Figure 1.

The distributions of log MoM values in unaffected and trisomy 21 pregnancies were well approximated by a bivariate Gaussian model with a mean of zero for unaffected pregnancies. For trisomy 21 pregnancies the mean log MoM depended on gestation according to the fitted regression models:

$$\text{Free } \beta\text{-hCG}(\log_{10}\text{MoM}) = 0.2468 + 0.004267 \times (\text{gestation} - 77)$$

$$\text{PAPP-A}(\log_{10}\text{MoM} = -0.4668 + 0.01642 \times (\text{gestation} - 77)$$

The fitted regression lines with 95% confidence limits are shown with the log MoM values in Figure 3. SD values and correlations for the fitted bivariate Gaussian distributions are given in Table 3. Gaussian probability plots for log MoM values of free β-hCG and PAPP-A are shown in Figure 4.

Empirical and model-based predictions

Table 4 shows the crude, standardized (for the maternal age distribution in England and Wales in 2000–2002) and modeled false-positive and detection rates. The observed prevalence of trisomy 21 according to the predicted risk, based on maternal age and serum free β-hCG and PAPP-A, is shown in Table 5.

Effect of maternal weight and fetal crown–rump length on patient-specific risk

The effect of changes in weight on computed MoM values and trisomy 21 risk is shown in Figure 5. In the example it is assumed that the pregnant woman is 35 years old, Caucasian, parous and a non-smoker who conceived spontaneously, with a fetal CRL of 65 mm and serum concentrations of PAPP-A and free β-hCG of 3.7 U/L and 40 U/L respectively measured by the Kryptor system at 12 weeks of gestation. The maternal serum concentrations of PAPP-A and free β-hCG expressed as MoMs increase with maternal weight and the patient-specific risk of trisomy 21 decreases with maternal weight.

The effect of fetal CRL on patient-specific risk for trisomy 21 is illustrated in Figure 6. The assumptions are the same as in the example in Figure 5 but, in addition, the maternal weight is 65 kg. The maternal serum concentrations of PAPP-A and free β-hCG expressed as MoMs decrease and increase respectively with fetal CRL, and the patient-specific risk of trisomy 21 increases with fetal CRL.

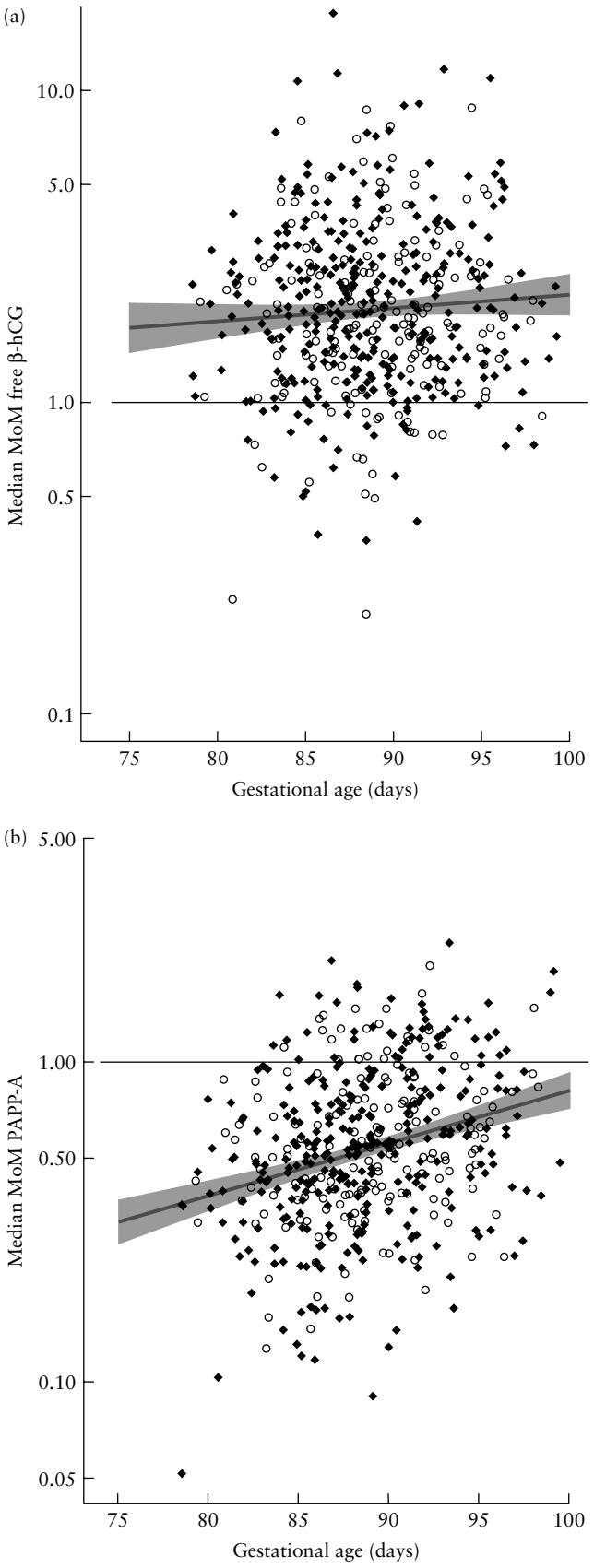

Figure 3 Multiples of the median (MoM) values of free beta-human chorionic gonadotropin (β-hCG) (a) and pregnancy-associated plasma protein-A (PAPP-A) (b) for trisomy 21 pregnancies, with fitted regression line and 95% CI (shaded area). ♦, Group A; ○, Group B.

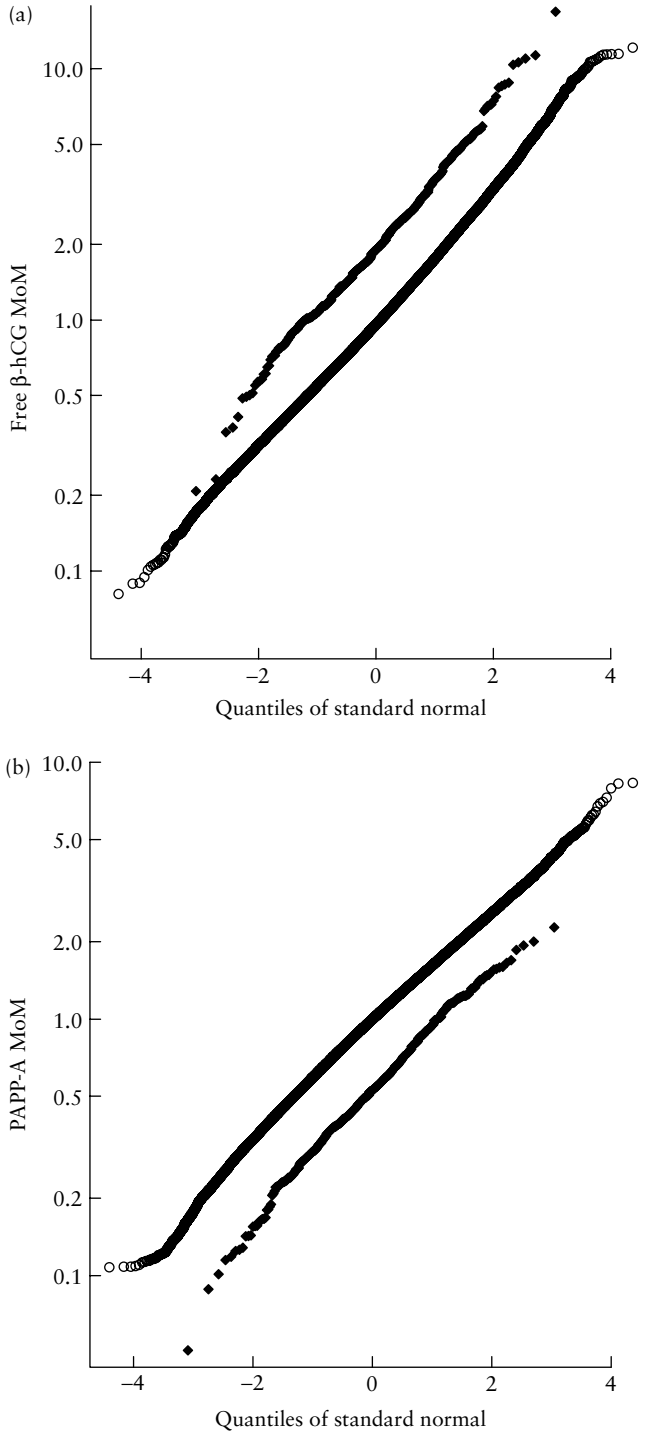

Figure 4 Gaussian probability plots for log multiples of the median (MoM) values of free beta-human chorionic gonadotropin (β-hCG) (a) and pregnancy-associated plasma protein-A (PAPP-A) (b), showing unaffected cases (○) and trisomy 21 cases (♦).

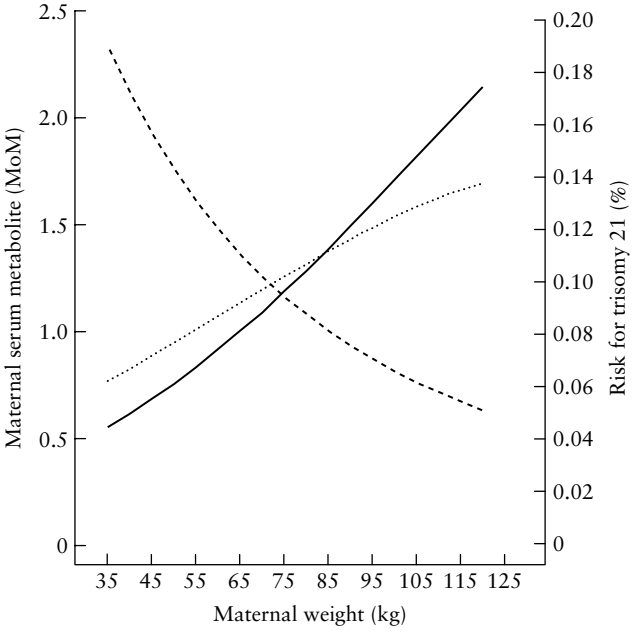

Figure 5 Effect of maternal weight on computed multiples of the median (MoM) values for pregnancy-associated plasma protein-A (PAPP-A) (———) and free beta-human chorionic gonadotropin (β-hCG) (·····) and on computed risk for trisomy 21 (- - - - - - -). These results apply to a pregnancy in a 35-year-old woman, with fetal crown–rump length 65 mm and with concentrations of PAPP-A and free β-hCG of 3.7 U/L and 40 U/L, respectively.

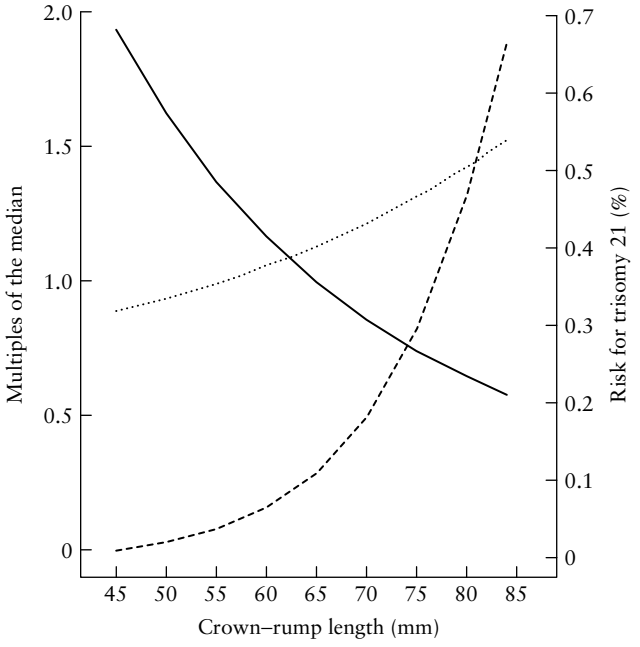

Figure 6 Effect of crown–rump length on computed multiples of the median (MoM) values for pregnancy-associated plasma protein-A (PAPP-A) (———) and free beta-human chorionic gonadotropin (β-hCG) (·····) and on computed risk for trisomy 21 (- - - - - - -). These results apply to a pregnancy in a 35-year-old woman with maternal weight 65 kg and with concentrations of PAPP-A and free β-hCG of 3.7 U/L and 40 U/L, respectively.

Failure to adjust for maternal characteristics

Table 6 shows the false-positive and detection rates of screening by maternal serum biochemistry for trisomy 21 if no adjustments are made for maternal characteristics, other than maternal weight and gestation. At a risk cut-off of 1 in 100 at 12 weeks of gestation in a reference group of Caucasian, parous, non-smoking women who conceived spontaneously, and with measurement of analytes by the Kryptor system, the false-positive and detection rates would be 4.6% and 68%, respectively. If the women were of Afro-Caribbean origin and the appropriate adjustments

Table 6 False-positive and detection rates of trisomy 21 at a risk cut-off of 1 in 100 in screening by a combination of maternal age, and maternal serum free beta-human chorionic gonadotropin and pregnancy-associated plasma protein-A at 12 weeks of gestation

Group	*False-positive rate (%)*	*Detection rate (%)*
Reference group	4.6	68
Afro-Caribbean	1.4	51
South Asian	3.3	63
East Asian	4.2	66
Smoker	7.0	74
IVF conception	7.6	75
Nulliparous	4.5	68
Delfia Express	11	80

These are modeled performance measures under the assumption that no adjustments are made for maternal characteristics. The reference group comprises Caucasian, parous, non-smoking women with spontaneous conception and measurement of analytes by the Kryptor system. IVF, *in-vitro* fertilization.

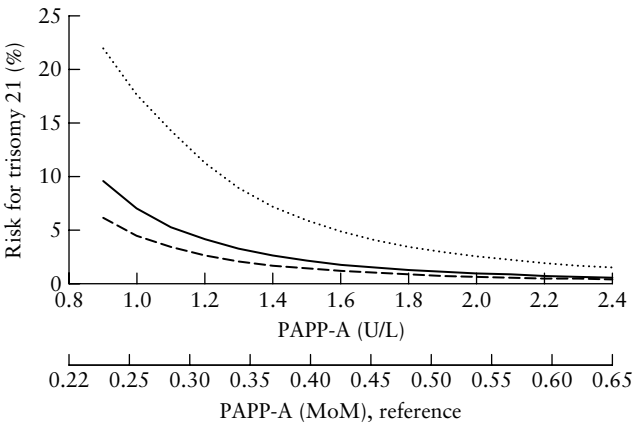

Figure 7 Patient-specific risk for trisomy 21 in a reference woman (———), an Afro-Caribbean woman (·····) and a cigarette smoker (- - - - - - -) in relation to the measured maternal serum concentration of pregnancy-associated plasma protein-A (PAPP-A). The reference woman is a 35-year-old Caucasian, who is a non-smoker, parous, weighs 65 kg and conceived spontaneously; the fetal crown–rump length is 65 mm, and maternal serum free beta-human chorionic gonadotropin (β-hCG) and PAPP-A were measured with the Kryptor analyzer. The free β-hCG concentration was 53 U/L, which corresponds to 1.5 multiples of the median (MoM) in the reference woman, 1.34 MoM in the Afro-Caribbean woman and 1.56 MoM in the cigarette smoker. The lower *x*-axis shows the PAPP-A MoM values corresponding to the measured concentration in the reference woman.

were made, at the same risk cut-off of 1 in 100, the false-positive and detection rates would also be 4.6% and 68%, but without adjustments the respective values would be 1.4% and 51%. Similarly, without appropriate adjustments the false-positive rates for cigarette smokers and women conceiving by IVF would be 7.0% and 7.6%, with respective detection rates of 74% and 75%.

Table 7 illustrates the effect of maternal characteristics on the patient-specific estimated risk of trisomy 21. For example, an Afro-Caribbean woman with a risk estimate of 1 in 40 would have been given a risk of 1 in 100 if her ethnic origin was not taken into account.

The correct estimated patient-specific risk for trisomy 21 for the same measured maternal serum concentrations of free β-hCG and PAPP-A depends on the maternal characteristics. This is illustrated in Figure 7, which shows the patient-specific risk in a reference woman, an Afro-Caribbean and a cigarette smoker in relation to various serum concentrations of PAPP-A and a fixed concentration of free β-hCG.

DISCUSSION

This study confirms that in trisomy 21 pregnancies at 11 to 13 + 6 weeks' gestation the level of free β-hCG is higher and that of PAPP-A is lower than in unaffected pregnancies. It has also demonstrated that assessment of accurate patient-specific risks necessitates making adjustments in the measured maternal serum

Table 7 Differences in multiples of the median (MoM) values and risks for trisomy 21 in women with different maternal characteristics

Case	*Characteristics*	*β-hCG (MoM)*	*PAPP-A (MoM)*	*Risk*
1	Caucasian, parous, non-smoker, weight 85 kg, aged 38 years, spontaneous conception, crown–rump length 65 mm	1.20	0.56	1 in 100
2	Same as Case 1 but crown–rump length 55 mm	1.05	0.77	1 in 347
3	Same as Case 1 but maternal weight 50 kg	0.83	0.31	1 in 48
4	Same as Case 1 but Afro-Caribbean	1.07	0.36	1 in 40
5	Same as Case 1 but East Asian	1.12	0.51	1 in 93
6	Same as Case 1 but South Asian	1.33	0.55	1 in 73
7	Same as Case 1 but smoker	1.25	0.68	1 in 145
8	Same as Case 1 but nulliparous	1.18	0.55	1 in 99
9	Same as Case 1 but conception by IVF	1.10	0.63	1 in 158
10	Same as Case 1 but analytes measured by Delfia Express	1.14	0.75	1 in 224
11	Same as Case 1 but Afro-Caribbean, weight 50 kg, smoker	0.77	0.24	1 in 29

Case 1 is a Caucasian woman, who is 38 years old, parous, a non-smoker, weighs 85 kg and conceived spontaneously, with a maternal serum free beta-human chorionic gonadotropin (β-hCG) level of 35 U/mL and pregnancy-associated plasma protein-A (PAPP-A) level of 1.5 U/mL measured by the Kryptor system. If the fetal crown–rump length is 65 mm, the free β-hCG and PAPP-A correspond to 1.20 MoM and 0.56 MoM respectively, and the estimated maternal and gestational age-related risk for trisomy 21 is 1 in 136. Cases 2–11 are similar to Case 1 but with variations in some of the characteristics.

concentration of free β-hCG and PAPP-A to correct for gestational age, maternal weight, ethnicity, smoking status, method of conception and parity, as well as the machine and reagents used for the assays.

The advantages of our study are the large number of cases examined (491 cases of trisomy 21 and 96 803 unaffected pregnancies) and the use of multiple regression analysis to define the contribution of maternal variables that influence the measured concentration of free β-hCG and PAPP-A, and the interaction between these covariates. The alternative method of sequential adjustment for each individual parameter fails to take into account the interaction between the covariates[14].

The levels of both PAPP-A and β-hCG were influenced by the ethnic origin of the women. The most striking finding was that there was a 57% increase in the levels of PAPP-A and a 12% increase in β-hCG in women of Afro-Caribbean origin compared with Caucasians. In biochemical screening of women of Afro-Caribbean origin failure to take into account ethnic origin would result in a substantial underestimate of the true risk of trisomy 21. As illustrated in Table 7, an Afro-Caribbean woman with a risk estimate of 1 in 40 would have been given a risk of 1 in 100 if her ethnic origin was not taken into account. Similarly, erroneous risks would be given to women who smoke and those conceiving by IVF because the associated decrease in serum PAPP-A could be misinterpreted as an increased risk for trisomy 21 and a substantial increase in false-positive rate. Another important factor in biochemical screening highlighted by our results is the need to make adjustments for the reagents and machines used for the measurement of free β-hCG and PAPP-A.

Likelihood ratios were established from the Gaussian distributions in trisomy 21 and unaffected pregnancies, and these were then used together with maternal age to produce patient-specific risks and to calculate detection and false-positive rates. The modeled performance of first-trimester biochemical screening was similar to that observed and, for a false-positive rate of 5%, the detection rate of trisomy 21 was 68%.

The overall performance of screening by maternal age and serum free β-hCG and PAPP-A was better at 11 weeks than at 13 weeks, with a greater relative contribution from PAPP-A at 11 weeks and from free β-hCG at 13 weeks. In trisomy 21 pregnancies the median MoM free β-hCG increased from 1.80 at 11 weeks to 2.09 at 13 weeks, and the respective values for PAPP-A were 0.38 and 0.65 MoMs. These values derived from our large prospective study, in which the biochemical tests were carried out in all 96 803 cases, are different from those reported in two previous multicenter studies in the UK and USA[16,17]. The Serum, Urine and Ultrasound Screening Study (SURUSS) recruited 47 053 pregnancies, including 101 with trisomy 21, but measured free β-hCG and PAPP-A retrospectively in stored samples from 98 trisomy 21 pregnancies and 1090 matched unaffected controls[16]. Similarly, 38 167 pregnancies were recruited in the First- and Second-Trimester Evaluation of Risk (FASTER) study, including 117 with trisomy 21, and free β-hCG and PAPP-A were measured retrospectively in stored samples from 79 trisomy 21 pregnancies and 395 matched unaffected controls[17]. Compared with our findings, the difference between trisomy 21 and unaffected pregnancies in free β-hCG in the SURUSS was overestimated by 8% at 11 weeks and by 19% at 13 weeks, and in the FASTER study it was underestimated by 34% and 19%, respectively[16,17]. The difference in PAPP-A in both previous studies was underestimated by 11% at 11 weeks and overestimated by 18% at 13 weeks[16,17]. In our study, the modeled SD of PAPP-A in unaffected pregnancies was 3–12% lower and in trisomy 21 cases it was 16–20% lower than in previous reports[16,17]. The respective values for free β-hCG in our study were lower by 4–17% in unaffected pregnancies. The SD for free β-hCG in the trisomy 21 pregnancies in our study was 26% lower than in the FASTER study and similar to that in the SURUSS[16,17].

Our findings have two implications. First, in first-trimester biochemical screening for calculation of accurate patient-specific risks for trisomy 21, it is essential to take into account gestational age, maternal weight, ethnicity, smoking status, method of conception and machine used for the assays. Second, the performance of the test is substantially better at 11–12 weeks than at 13 weeks.

ACKNOWLEDGMENT

This study was supported by a grant from The Fetal Medicine Foundation (charity number 1037116).

REFERENCES

1. Spencer K, Souter V, Tul N, Snijders R, Nicolaides KH. A screening program for trisomy 21 at 10–14 weeks using fetal nuchal translucency, maternal serum free beta-human chorionic gonadotropin and pregnancy-associated plasma protein-A. *Ultrasound Obstet Gynecol* 1999; **13**: 231–237.
2. Spencer K, Spencer CE, Power M, Dawson C, Nicolaides KH. Screening for chromosomal abnormalities in the first trimester using ultrasound and maternal serum biochemistry in a one stop clinic: a review of three years prospective experience. *BJOG* 2003; **110**: 281–286.
3. Avgidou K, Papageorghiou A, Bindra R, Spencer K, Nicolaides KH. Prospective first-trimester screening for trisomy 21 in 30 564 pregnancies. *Am J Obstet Gynecol* 2005; **192**: 1761–1767.
4. Nicolaides KH, Spencer K, Avgidou K, Faiola S, Falcon O. Multicenter study of first-trimester screening for trisomy 21 in 75 821 pregnancies: results and estimation of the potential impact of individual risk-orientated two-stage first-trimester screening. *Ultrasound Obstet Gynecol* 2005; **25**: 221–226.
5. Spencer K, Bindra R, Nicolaides KH. Maternal weight correction of maternal serum PAPP-A and free beta-hCG MoM when screening for trisomy 21 in the first trimester of pregnancy. *Prenat Diagn* 2003; **23**: 851–855.
6. Spencer K, Crossley JA, Aitken DA, Nix AB, Dunstan FD, Williams K. Temporal changes in maternal serum biochemical markers of trisomy 21 across the first and second trimester of pregnancy. *Ann Clin Biochem* 2002; **39**: 567–568.
7. Spencer K, Ong CY, Liao AW, Nicolaides KH. The influence of ethnic origin on first trimester biochemical markers of chromosomal abnormalities. *Prenat Diagn* 2000; **20**: 491–494.
8. Spencer K, Heath V, El-Sheikhah A, Ong CYT, Nicolaides KH. Ethnicity and the need for correction of biochemical and ultrasound markers of chromosomal anomalies in the first

trimester: a study of Oriental, Asian and Afro-Caribbean populations. *Prenat Diagn* 2005; **25**: 365–369.
9. Spencer K, Bindra R, Cacho AM, Nicolaides KH. The impact of correcting for smoking status when screening for chromosomal anomalies using maternal serum biochemistry and fetal nuchal translucency thickness in the first trimester of pregnancy. *Prenat Diagn* 2004; **24**: 169–173.
10. Kagan KO, Frisova V, Nicolaides KH, Spencer K. Dose dependency between cigarette consumption and reduced maternal serum PAPP-A levels at 11–13 + 6 weeks of gestation. *Prenat Diagn* 2007; **27**: 849–853.
11. Liao AW, Heath V, Kametas N, Spencer K, Nicolaides KH. First-trimester screening for trisomy 21 in singleton pregnancies achieved by assisted reproduction. *Hum Reprod* 2001; **16**: 1501–1504.
12. Snijders RJM, Noble P, Sebire N, Souka A, Nicolaides KH. UK multicentre project on assessment of risk of trisomy 21 by maternal age and fetal nuchal-translucency thickness at 10–14 weeks of gestation. Fetal Medicine Foundation First Trimester Screening Group. *Lancet* 1998; **352**: 343–346.
13. Robinson HP, Fleming JE. A critical evaluation of sonar crown–rump length measurements. *Br J Obstet Gynaecol* 1975; **82**: 702–710.
14. Draper N, Smith R. *Applied Regression Analysis* (3rd edn). Wiley: New York, NY, 1998.
15. Office for National Statistics. *Birth Statistics. Review of the Registrar General on births and patterns of family building in England and Wales.* Series FM1, Nos 29–31. Stationary Office: London, 2002.
16. Wald NJ, Rodeck C, Hackshaw AK, Walters J, Chitty L, Mackinson AM. First and second trimester antenatal screening for Down's syndrome: the results of the Serum, Urine and Ultrasound Screening Study (SURUSS). *J Med Screen* 2003; **10**: 56–104.
17. Canick JA, Lambert-Messerlian GM, Palomaki GE, Neveux LM, Malone FD, Ball RH, Nyberg DA, Comstock CH, Bukowski R, Saade GR, Berkowitz RL, Dar P, Dugoff L, Craigo SD, Timor-Tritsch IE, Carr SR, Wolfe HM, D'Alton ME. Comparison of serum markers in first-trimester Down syndrome screening. *Obstet Gynecol* 2006; **108**: 1192–1199.

