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

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In 25 cases of triploidy at 10–14 weeks of gestation, compared with 947 controls, the median multiple of the median (MoM) fetal nuchal translucency (NT) thickness was significantly increased (1.89 MoM), and maternal serum total and free β-human chorionic gonadotrophin (hCG) were increased (3.13 MoM and 4.59 MoM respectively), alpha fetoprotein (AFP) was increased (2.14 MoM), and pregnancy associated plasma protein A (PAPP-A) was decreased (0.12 MoM). There are two types of triploidy. In type I, where the additional chromosome set is of paternal origin, the placenta is partially molar and the fetus is relatively well-grown. Type II, where the extra chromosome set is of maternal origin, is characterized by a small normal looking placenta and severe asymmetrical fetal growth restriction. In type I triploidy there was increased fetal NT (2.76 MoM), maternal serum total hCG (4.91 MoM), free β-hCG (8.04 MoM), and AFP (3.22 MoM), and mildly decreased PAPP-A (0.75 MoM). In type II triploidy fetal NT was not increased (0.88 MoM), and there was a decrease in maternal serum total hCG (0.16 MoM), free β-hCG (0.18 MoM), PAPP-A (0.06 MoM) and AFP (0.77 MoM). We conclude that a large proportion of triploidy cases of both phenotypes could be identified in the first trimester using NT, maternal serum free β-hCG and PAPP-A with a combination of trisomy 21 risk and an atypicality approach. Copyright © 2000 John Wiley & Sons, Ltd.

KEY WORDS: triploidy; 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; 1993; 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 associated with increased NT and reduced levels of maternal serum free β-hCG and PAPP-A; screening by a combination of all three markers can identify 84–90% of affected pregnancies for a 0.1–0.5% false positive rate (Spencer *et al*., 2000a). In cases of sex chromosomal anomalies, particularly Turner's syndrome, the pattern of increased NT, normal maternal serum free β-hCG and low PAPP-A can also be utilized to identify over 90% of cases (Spencer *et al*., 2000b).

Triploidy is estimated to occur in 1% of all conceptions (Jacobs *et al*., 1978). Most fetuses die during the first trimester and the prevalence of triploidy at 12 weeks is estimated to be 1 in 3500 compared with 1 in 30 000 at 16 weeks (Snijders *et al*., 1995). Triploidy can be classified into two phenotypes based on placental and ultrasound findings (McFadden and Kalousek, 1991; Jauniaux *et al*., 1996a). In type I the placenta is enlarged and partially multicystic (molar) whereas the fetus is relatively well-grown with either proportionate head size or slight microcephaly. Type II, which is the most common, is characterized by a small normal looking placenta and severely growth restricted fetus with pronounced wasting of the body and sparing of the head (Figure 1). The two phenotypes have been shown to be dependent upon the parental origin of the extra chromosome set. In type I the additional chromosome set is of paternal origin (diandric) and in type II it is of maternal origin (digynic) (McFadden *et al*., 1993; Dietzsch *et al*., 1995). Unlike other common chromosomal abnormalities, triploidy may also affect the mother with varying degrees of pre-eclampsia (Jauniaux *et al*., 1996a; Rijhsinghani *et al*., 1997) or persistent trophoblastic disease (Goldstein and Berkowitz, 1994). This study examines the effectiveness of screening for triploidy by a combination of fetal NT and maternal serum free β-hCG and PAPP-A at 10–14 weeks of gestation.

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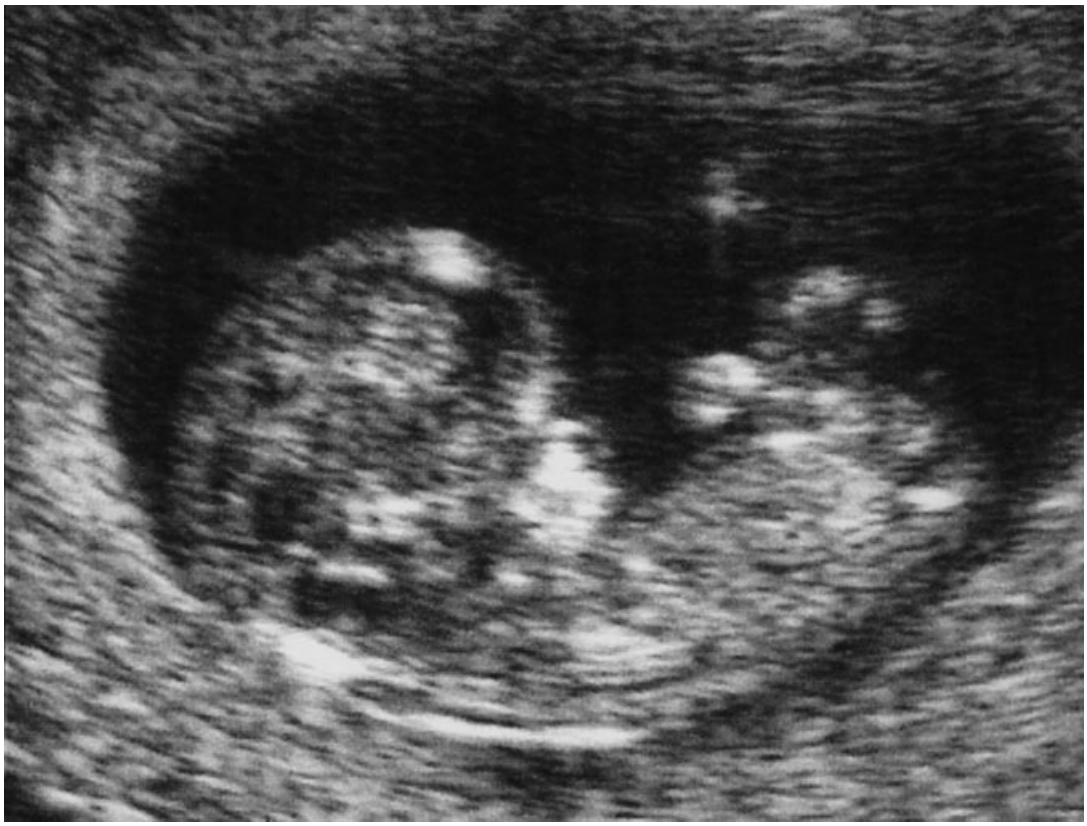

Figure 1—Severe asymmetrical growth restriction in a 13 week fetus with triploidy. The placenta looks normal

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. Serum was 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 22 cases of triploidy. In addition, three cases of triploidy were identified as part of prospective first trimester screening in the OSCAR clinic at Harold Wood Hospital (Spencer, 1999). 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 Harris Birthright Centre for the assessment of risk for chromosomal abnormality or for CVS were also taken 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 previous studies (Spencer *et al.*, 1999; 2000a; 2000b; Tul *et al.*, 1999).

Maternal serum free β-hCG, PAPP-A, AFP and total hCG 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; 2000c). 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 marker levels and gestational age. All marker measurements were converted to MoMs using the derived medians from normal pregnancies at the same gestation as assessed by CRL. Correction of each MoM for maternal weight was also performed using the reciprocal-linear regression weight correction procedure of Neveux *et al.* (1996). Statistical analysis of data was performed using Microsoft Excel 97 and Analyse-It (Smart Software, Leeds, U.K.), a statistical software add-in.

RESULTS

There were no significant differences between the triploid and control pregnancies in maternal age, gestational age based on fetal CRL, and sample

Table 1—Mean (range) maternal age, gestational age, crown–rump lenth and length of storage of the samples from the triploidy ($n=25$) and normal ($n=947$) pregnancies

	Triploidy	Controls
Maternal age (years)	33.4 (23–41)	35.1 (15–47)
Gestational age (days)	86.2 (73–97)	85.1 (72–99)
Crown–rump length (mm)	49.4 (39–73.8)	60.4 (38–85)
Median length of storage (days)	557 (0–305)	546 (102–1811)

storage time (Table 1). In the triploidy pregnancies, compared to the controls, the median fetal NT (1.89 MoM), and maternal serum free β-hCG (4.59 MoM), total hCG (3.13 MoM) and AFP (2.14 MoM) were significantly increased ($p<0.0001$) and PAPP-A (0.12 MoM) was decreased ($p<0.0001$). In 15 cases the placentas were enlarged with molar changes and in 7 they appeared normal on ultrasound (in 3 cases no placental morphology was recorded). In the patients with regular menstrual dates and certain dates of their last period, the difference in gestation estimated by dates and from fetal crown-rump length was calculated as an index of fetal growth restriction. In the group with a molar placenta the deficit in fetal growth (median = 8, range 4–12 days) was smaller than in those with a normal looking placenta (median = 19, range 12–25). The individual marker values in the 25 cases of triploidy are shown in Table 2 along with the method by which each case was originally identified and the two phenotypes are compared in Table 3.

All 15 cases of type I triploidy would have been identified using an algorithm for trisomy 21 (Spencer *et al*., 1999) and a risk cut off of 1 in 300. In the 10 cases of type II triploidy seven of the cases would have been identified using an algorithm for trisomy 21 (Spencer *et al*., 1999), primarily as a result of the low PAPP-A and the advanced maternal age (median age = 36 years). If a non age related procedure, such as the method of atypicality (Wright *et al*., 1993), had been used and the atypicality threshold set to identify 1% of unaffected cases (mahalanobis distance of 11.3 for a three marker system), all 10 of our cases would have been identified.

DISCUSSION

The findings of this study indicate that the two phenotypic types of triploidy demonstrate striking differences both in fetal NT and maternal serum total

Table 2—Individual maker levels (as MoM) in 25 cases of triploidy classified by phenotype and the method of original identification

Case	NT MoM	Free β-hCG MoM	PAPP-A MoM	AFP MoM	Total hCG MoM	Phenotype	Identification
1	1.89	3.68	0.01	3.19	6.09	1	US & placenta
2	5.73	4.13	1.58	1.41	3.13	1	US & placenta
3	1.61	5.35	1.44	3.06	4.12	1	US & placenta
4	2.83	5.69	0.38	4.26	4.15	1	US & biochem
5	2.05	6.54	1.04	16.18	2.47	1	US & placenta
6	2.22	8.04	0.12	1.89	4.91	1	US & placenta
7	3.27	9.48	0.93	2.14	5.11	1	US & placenta
8	5.05	10.12	0.83	4.61	7.58	1	US & placenta
9	1.45	14.41	0.31	17.82	5.37	1	US & placenta
10	3.93	16.11	0.36	2.72	5.65	1	US & placenta
11	1.90	20.07	0.01	6.73	12.66	1	US & placenta
12	2.56	12.60	0.75	3.56	10.27	1	US & biochem
13	3.85	8.25	1.03	3.22	4.59	1	US
14	2.91	4.59	0.50	4.26	2.56	1	US
15	2.76	6.64	0.95	2.44	4.31	1	US
16	0.93	0.04	0.08	0.78	0.05	2	US & IUGR
17	0.87	0.06	0.05	0.51	0.15	2	T18 risk biochem
18	0.50	0.07	0.01	0.13	0.07	2	US & IUGR
19	0.70	0.10	0.01	1.89	0.09	2	US & IUGR
20	1.13	0.36	0.06	0.57	0.21	2	US & IUGR
21	1.15	0.47	0.02	0.58	0.19	2	US & IUGR
22	0.61	0.48	0.09	0.87	0.16	2	T18 risk biochem
23	0.79	0.25	0.11	0.97	0.23	2	?
24	0.99	0.11	0.06	0.75	0.25	2	?
25	0.89	0.31	0.12	1.06	0.15	2	?

US, ultrasound; IUGR, intrauterine growth restriction.

Table 3—Median (95% confidence interval) fetal nuchal translucency thickness and maternal serum markers in the two phenotypes of triploidy at 10–14 weeks of gestation

Parameter	Type I	Type II	Comparison
NT MoM	2.76 (1.90 to 3.85)	0.88 (0.61 to 1.13)	$p<0.0001$
Free β-hCG MoM	8.04 (4.59 to 12.60)	0.18 (0.06 to 0.47)	$P<0.0001$
Total hCG MoM	4.91 (4.12 to 6.09)	0.16 (0.08 to 0.23)	$P<0.0001$
PAPP-A MoM	0.75 (0.31 to 1.03)	0.06 (0.01 to 0.11)	$p<0.0001$
AFP MoM	3.22 (2.44 to 4.60)	0.77 (0.51 to 1.06)	$p<0.0001$

and free β-hCG, PAPP-A and AFP at 10–14 weeks of gestation. In type I, where the placenta is partially molar and the fetus is relatively well-grown, there is increased fetal NT and maternal serum total hCG, free β-hCG and AFP with mildly decreased PAPP-A. Type II, characterized by a small normal looking placenta and severe asymmetrical fetal growth restriction, is associated with normal fetal NT and markedly decreased maternal serum total hCG, free β-hCG and PAPP-A with mildly decreased AFP.

Previous biochemical studies in triploid pregnancies during the second trimester have also shown that maternal serum hCG can either be low or high. Thus, five studies on a total of 10 triploid pregnancies reported very low levels of total hCG and unconjugated oestriol (Bogart *et al*., 1989; Kohn *et al*., 1991; Mason *et al*., 1992; Fejgin *et al*., 1992; 1993). Two studies on a total of seven cases of triploidy reported that in some pregnancies total hCG can be very low and in others it can be very high (Oyer and Canick, 1992; Muller *et al*., 1993). Jacobs *et al*. (1982) reported that in triploidy with partial molar placentas and elevated maternal serum hCG the extra haploid set of chromosomes is paternally derived; and Schmidt *et al*. (1994) reported that in triploidy with low maternal serum total hCG and oestriol the extra haploid set of chromosomes is maternally derived. These findings represent an example of genomic imprinting in humans with the extra paternal set of chromosomes over expressing hCG (McFadden and Kalousek, 1991; McFadden *et al*., 1993; 1998; Dietzsch *et al.,* 1995; Goshen, 1994).

In the second trimester ultrasound examination revealing partial molar changes in the placenta, or severe asymmetrical fetal growth restriction in the presence of an apparently normal placenta, has been suggested as an indicator of triploidy (Jaunieux *et al.,* 1996a). However, molar changes in the placenta are less easily detectable in the first than in the second or third trimesters (Jaunieux *et al.,* 1996b). As for fetal NT, in a series of 18 fetuses with triploidy at 10–14 weeks the NT was above the 95th centile in 67% of cases; although molar changes in the placenta were noted in only 33% of cases, 85% had elevated maternal serum total hCG (Jaunieux *et al.,* 1997). In an extension of this series, fetal NT was above the 95th centile in 59% of 32 cases of triploidy (Snijders *et al*., 1998).

In first trimester screening for chromosomal defects by a combination of fetal NT and maternal serum free β-hCG and PAPP-A, type I triploidy will be identified since the marker patterns are very similar to those seen in trisomy 21 (Spencer *et al*., 1999). However, in type II, despite the biochemical pattern being very similar to that in trisomy 18 and trisomy 13 (Tul *et al*., 1999; Spencer *et al*., 2000a), the near normal NT would negate their detection. However, this problem could be overcome by paying attention to fetal symmetry during the first trimester scan, especially in cases of very low free β-hCG and PAPP-A, since type II fetuses demonstrate severe asymmetrical growth restriction (Figure 1). Another possible way to increase the chances of detection of type II triploidy cases would be to use the concept of atypicality, which has been proposed for use in second trimester screening (Wright *et al*., 1993). This concept assesses the probability of a pattern of results having arisen from the trisomy 21 population or the normal population. If the probability of arising from either group is low the pattern of results are considered atypical (i.e. they are neither normal or trisomy 21 like). The function defining atypicality (the Mahalanobis distance) is already calculated in conventional trisomy 21 risk algorithms. Setting the atypicality threshold to identify 1% of unaffected cases (Mahalanobis distance of 11.3 for a three marker system) in our series of type II triploidy all 10 cases would have been identified. Another alternative approach would be to devise specific algorithms for type II triploidy; however this approach would require considerably more data on affected cases.

Screening for trisomy 21 at 10–14 weeks of gestation by a combination of maternal age, fetal NT and maternal serum free β-hCG and PAPP-A, identifies about 90% of pregnancies with trisomy 21, trisomy 18, trisomy 13 and sex chromosome abnormalities for a screen positive rate of about 6% (Spencer *et al*., 1999; 2000a; 2000b; Tul *et al*., 1999). The findings of this study indicate that the same method of screening can identify more than 90% of fetuses with triploidy of both phenotypes.

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