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# Maternal thyroid function at 11–13 weeks of gestation in fetal trisomies 21 and 18

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**Objective** To examine the association between maternal serum levels of thyroid stimulating hormone (TSH) and free $\beta$-human chorionic gonadotrophin (free $\beta$-hCG) in trisomy 21, trisomy 18 and euploid pregnancies at 11–13 weeks and investigate the potential value of TSH in first-trimester screening for aneuploidies.

**Methods** Maternal serum TSH and free $\beta$-hCG levels at 11–13 weeks in 25 trisomy 21 and 25 trisomy 18 pregnancies were compared with levels in 3592 unaffected pregnancies. Only women with no history of thyroid disease and negative for antithyroid antibodies were included.

**Results** Serum TSH in the trisomy 21 pregnancies was lower [0.76 multiples of the normal median (MoM), interquartile range (IQR) 0.46–1.09 MoM] and in trisomy 18 it was higher (1.25 MoM, IQR 0.88–1.98 MoM) than in unaffected pregnancies (1.01 MoM, IQR 0.61–1.51 MoM). There were significant associations between TSH and free $\beta$-hCG in the unaffected pregnancies ($r = -0.214$, $p < 0.0001$), but not in those with trisomy 21 ($r = -0.157$, $p = 0.452$) or trisomy 18 ($r = -0.176$, $p = 0.401$).

**Conclusions** hCG, rather than TSH, may be the primary thyrotropic factor in early pregnancy. Measurement of TSH does not improve the performance of screening for trisomies 21 and 18 provided by nuchal translucency, free $\beta$-hCG and pregnancy-associated plasma protein-A. Copyright © 2011 John Wiley & Sons, Ltd.

KEY WORDS: thyroid function; trisomy 21; trisomy 18; free $\beta$-hCG; first-trimester screening

## INTRODUCTION

Human chorionic gonadotrophin (hCG), which has an $\alpha$-subunit and structurally similar $\beta$-subunit identical to those of the thyroid stimulating hormone (TSH), has thyrotropic properties and in early pregnancy there is an inverse association between maternal serum levels of TSH and hCG (Braunstein and Hershman, 1976; Yoshikawa *et al*., 1989; Glinoer *et al*., 1990; Ballabio *et al*., 1991; Yoshimura and Hershman, 1995). Free $\beta$-hCG is an established screening marker for trisomy 21 and we demonstrated that $\beta$-hCG mRNA correlates with serum hCG levels (Banerjee *et al*., 2005).

In pregnancies with fetal trisomy 21, the maternal serum concentration of free $\beta$-hCG at 11–13 weeks' gestation is on average twice as high as in euploid pregnancies, whereas in trisomy 18 the levels are one fifth of normal (Macri *et al*., 1990; Spencer *et al*., 1999; Tul *et al*., 1999; Kagan *et al*., 2008a). It is therefore anticipated that in aneuploid pregnancies the maternal serum concentration of TSH would be altered. However, a case control study of 23 pregnancies with fetal trisomy 21 and 115 with euploid fetuses at 9–11 weeks reported that although in the unaffected pregnancies there was a correlation between serum hCG and TSH ($r = 0.21$, $p = 0.02$), there was no significant difference between the trisomic and euploid pregnancies in either hCG or TSH (Weinans *et al*., 2001). This may be due to the early gestational age at measurement before the peak of serum hCG. In this study, no corrections were made for maternal characteristics and gestational age that are known to affect the measured concentrations of hCG and TSH. We have previously reported that normally serum TSH increases with gestational age and body mass index (BMI), and is lower in women of African racial origin than in Caucasian women (Ashoor *et al*., 2010). Additionally, the levels are lower in women with positive than negative anti-thyroperoxidase (anti-TPO) and anti-thyroglobulin (anti-Tg) antibodies (Ashoor *et al*., 2010). Maternal serum free $\beta$-hCG decreases with gestational age and maternal weight, it is decreased in cigarette smokers and in parous women and it is increased in women of African racial origin and in those conceiving after ovulation-induction drugs (Kagan *et al*., 2008b).

The aim of this study is to examine further the association between maternal serum levels of TSH and hCG in trisomy 21, trisomy 18 and euploid pregnancies at 11–13 weeks, assess any differences in free thyroxine (FT4) and free triiodothyronine (FT3) between the three groups and investigate the potential value of TSH in first-trimester screening for aneuploidies.

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## METHODS

This was a prospective screening study for adverse obstetric outcomes in women attending their routine first hospital visit in pregnancy. In this visit, which is held at $11^{+0}$–$13^{+6}$ weeks of gestation, we record maternal characteristics, including age, racial origin (White, Black, South Asian, East Asian and mixed), cigarette smoking during pregnancy (yes or no), method of conception (spontaneous or assisted), medical history of chronic hypertension, parity (parous or nulliparous if no delivery beyond 23 weeks), weight, height and BMI. We then perform an ultrasound scan to confirm gestational age from the measurement of the fetal crown-rump length (CRL), to diagnose any major fetal abnormalities and to measure fetal nuchal translucency (NT) thickness. We also measure maternal serum free $\beta$-hCG and pregnancy-associated plasma protein-A (PAPP-A) (DELFIA XPRESS analyzer, PerkinElmer, Waltham, MA, USA) as part of the screening for chromosomal abnormalities by a combination of fetal NT and serum biochemistry (Snijders *et al*., 1998; Kagan *et al*., 2008b). Additionally, blood is collected for research and the separated plasma and serum are stored at −80 °C for subsequent biochemical analysis. Written informed consent was obtained from the women agreeing to participate in the study, which was approved by the King's College Hospital Ethics Committee.

In this study, we retrospectively examined maternal thyroid function at 11–13 weeks in 30 pregnancies with fetal trisomy 21, 25 with fetal trisomy 18 and 2 with paternally derived triploidy. The diagnosis of aneuploidy was made by chorionic villus sampling after first-trimester screening between March 2006 and December 2006. The maternal serum concentrations of FT3, FT4, TSH, anti-TPO and anti-Tg were measured by immunoassay using direct, chemiluminometric technology (Siemens Advia Centaur assays, Siemens Healthcare Diagnostics Ltd, Surrey, UK), as previously described (Ashoor *et al*., 2010). In five of the trisomy 21 cases the maternal serum concentration of anti-TPO and/or anti-Tg was more than 60 U/mL, and these cases were excluded from further analysis because their values were above the manufacturer's reference limit.

The values of FT3, FT4 and TSH in the 25 cases of trisomy 21, 25 cases of trisomy 18 and 2 with triploidy were compared with the results of our previous study of 3592 antithyroid antibody-negative singleton pregnancies with no history of thyroid disease, which resulted in live birth after 34 weeks of phenotypically normal neonates with birth weight above the fifth centile (Ashoor *et al*., 2010).

### Statistical analysis

The characteristics of the trisomy 21, trisomy 18 and unaffected groups were compared using the one-way analysis of variance (ANOVA) test, followed by Bonferroni *post-hoc* test if variances were equal or Tamhane *post-hoc* test if variances were unequal, for continuous variables, and Fisher's exact test or Chi-square test followed by Bonferroni *post-hoc* test for categorical variables. The measured concentrations of FT3, FT4 and TSH were converted to MoMs corrected for gestational age, maternal age, racial origin and BMI (Ashoor *et al*., 2010). Similarly, the measured concentration of maternal serum free $\beta$-hCG was MoMs corrected for fetal CRL, maternal weight, smoking status, racial origin, parity and method of conception (Kagan *et al*., 2008a,b). Comparison of TSH MoM, FT3 MoM and FT4 MoM between aneuploid and unaffected groups was by one-way ANOVA test, followed by the Bonferroni *post-hoc* test for equal variances or the Tamhane *post-hoc* test for unequal variances. The Pearson correlation was used to determine the significance of the inter-relations between serum square root (SQR) TSH MoM, $\log_{10}$ FT3 MoM, $\log_{10}$ FT4 MoM, $\log_{10}$ PAPP-A MoM and $\log_{10}$ free $\beta$-hCG MoM.

The added value of including thyroid function markers in aneuploidy screening was estimated by standard modeling techniques (Royston and Thompson, 1992). The model parameters—means, standard deviations and correlation coefficients and maternal age distribution—were derived directly from the study data. Trisomy 21 detection rates were estimated for fixed 3 and 5% false-positive rates; trisomy 18 rates for 0.5 and 1%.

The statistical software package statistics 18.0 (SPSS Inc., Chicago, IL, USA) was used for the data analyses.

## RESULTS

The maternal characteristics and results of first-trimester combined screening for aneuploidies in trisomy 21, trisomy 18 and unaffected pregnancies are compared in Table 1. In both the trisomy 21 and trisomy 18 groups, compared with the unaffected group, maternal age and fetal NT thickness were higher and serum PAPP-A was lower. Serum free $\beta$-hCG in trisomy 21 was increased, and in trisomy 18 it was decreased.

Serum TSH in the trisomy 21 pregnancies was lower, and in trisomy 18 it was higher than in unaffected pregnancies (Table 2). There were no significant differences between the groups in serum FT4 but in trisomy 18 pregnancies FT3 was significantly reduced. In the unaffected pregnancies, but not in those with trisomy 21 or trisomy 18, there were significant intercorrelations between TSH, free $\beta$-hCG, FT3 and FT4 (Table 3).

None of the thyroid function markers increased the model-predicted detection rates for trisomy 21 compared with the existing first-trimester screening protocol by more than 0.8%. The increase in detection for trisomy 18 was at most 0.2%. The Mahalanobis distance for the decrease in TSH MoM values in trisomy 21 was 0.46 and for the increase in trisomy 18 was 0.37.

In the two cases of paternally derived fetal triploidy, the gestational age was 13 weeks and the fetal CRL was 63.5 and 73.7 mm, respectively. The maternal serum free $\beta$-hCG was 7.51 and 6.77 MoM, the levels of TSH were undetectable and both FT3 (1.63 and 3.98 MoM) and FT4 (1.71 and 3.41 MoM) were increased.



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Table 1—Maternal characteristics and results of first-trimester combined screening for aneuploidies in trisomy 21, trisomy 18 and unaffected pregnancies

| Variables | Unaffected ($n = 3592$) | Trisomy 21 ($n = 25$) | Trisomy 18 ($n = 25$) |
|---|---|---|---|
| Maternal age in years (median, IQR) | 32.2 (27.9–36.0) | 38.7 (35.2–41.8)* | 37.9 (33.6–40.4)* |
| BMI in kg/m$^2$, median (IQR) | 24.7 (22.2–27.9) | 24.8 (22.5–28.5) | 25.1 (21.6–29.2) |
| Racial origin | | | |
| Caucasian, *n* (%) | 2543 (70.8) | 20 (80.0) | 18 (72.0) |
| African, *n* (%) | 708 (19.7) | 4 (16.0) | 4 (16.0) |
| South Asian, *n* (%) | 148 (4.1) | 0 | 2 (8.0) |
| East Asian, *n* (%) | 57 (1.6) | 1 (4.0) | 0 |
| Mixed, *n* (%) | 136 (3.8) | 0 | 1 (4.0) |
| Parity | | | |
| Nulliparous, *n* (%) | 1684 (46.9) | 9 (36.0) | 9 (36.0) |
| Parous | 1908 (53.1) | 16 (64.0) | 16 (64.0) |
| Cigarette smoker, *n* (%) | 322 (9.0) | 3 (12.0) | 2 (8.0) |
| Conception by ovulation drugs, *n* (%) | 101 (2.8) | 4 (16.0)* | 6 (24.0)* |
| Fetal CRL in mm (median, IQR) | 63.5 (59.0–68.7) | 64.2 (58.0–70.1) | 57.3 (51.0–61.8)* |
| Fetal NT thickness in mm (median, IQR) | 1.8 (1.5–2.0) | 3.7 (2.8–5.0)* | 5.4 (2.0–6.4)* |
| Delta NT thickness in mm (median, IQR) | 0.09 (−0.11 to 0.29) | 2.13 (1.30 to 3.23)* | 3.88 (0.39 to 5.04)* |
| Serum free $\beta$-hCG MoM (median, IQR) | 0.96 (0.66–1.50) | 1.87 (1.46–3.35)* | 0.23 (0.17–0.34)* |
| Serum PAPP-A MoM (median, IQR) | 1.00 (0.69–1.42) | 0.54 (0.33–0.69)* | 0.26 (0.16–0.38)* |

Comparison between each aneuploid group and the unaffected pregnancies was by Chi-square or Fisher exact test for categorical variables with *post-hoc* Bonferroni correction and analysis of variance (ANOVA) test for categorical both with *post-hoc* Bonferroni test for maternal age, BMI and CRL, and *post-hoc* Tamhane test for fetal NT, delta NT and serum free $\beta$-hCG MoM and PAPP-A.
BMI, body mass index; CRL, crown-rump length; hCG, human chorionic gonadotrophin; IQR, interquartile range; MoM, multiples of the normal median; NT, nuchal translucency; PAPP-A, pregnancy-associated plasma protein-A.
* $p < 0.001$.

Table 2—Maternal thyroid function in trisomy 21, trisomy 18 and unaffected pregnancies

| | Unaffected ($n = 3592$) | Trisomy 21 ($n = 25$) | Trisomy 18 ($n = 25$) |
|---|---|---|---|
| Thyroid stimulating hormone | | | |
| MoM (median, IQR) | 1.01 (0.61–1.51) | 0.76 (0.46–1.09)* | 1.25 (0.88–1.98)* |
| mIU/L (median, IQR) | 1.10 (0.67–1.67) | 0.85 (0.49–1.22)* | 1.46 (1.03–2.11)* |
| Free thyroxine | | | |
| MoM (median, IQR) | 0.99 (0.91–1.09) | 1.04 (0.91–1.23) | 0.97 (0.83–1.03) |
| Pmol/L (median, IQR) | 14.9 (13.6–16.3) | 15.4 (13.5–18.2) | 14.4 (12.2–15.4) |
| Free triiodothyronine | | | |
| MoM (median, IQR) | 0.99 (0.93–1.06) | 1.02 (0.97–1.18) | 0.95 (0.91–1.00)* |
| Pmol/L (median, IQR) | 4.6 (4.4–5.0) | 4.7 (4.5–5.5) | 4.4 (4.2–4.8)* |

Comparison between each aneuploid group and the unaffected pregnancies was by analysis of variance (ANOVA) test for categorical both with *post-hoc* Bonferroni test for free thyroxine and *post-hoc* Tamhane test for thyroid stimulating hormone and free triiodothyronine.
IQR, interquartile range; MoM, multiples of the normal median.
* $p < 0.05$.

## DISCUSSION

This study has demonstrated that in euploid pregnancies, there is a weak inverse association between free $\beta$-hCG MoM and TSH MoM. In trisomy 21 pregnancies, free $\beta$-hCG is increased and TSH is decreased, and in trisomy 18 pregnancies free $\beta$-hCG is decreased and TSH is increased.

In first-trimester screening for aneuploidy, the observed decrease in TSH MoM values in trisomy 21 and the increase in trisomy 18 pregnancies had only modest discriminatory value, about half that of alpha-fetoprotein which is the weakest current marker. Moreover, the negative correlation between free $\beta$-hCG and TSH means that the use of both markers in screening will effectively decrease the Mahalanobis distances. This is reflected in the lack of any model-predicted additional detection if TSH was added to current first-trimester screening protocols.

The finding of an inverse association between free $\beta$-hCG MoM and TSH MoM is compatible with the known thyrotropic properties of hCG (Braunstein and Hershman, 1976; Pekonen *et al*., 1988; Glinoer *et al*., 1990; Ballabio *et al*., 1991). In pregnancy, there is a mirror image between TSH and hCG levels with high hCG and low TSH at 8–14 weeks and subsequent increase in TSH during the second and third trimesters coinciding with the decline in hCG (Glinoer *et al*., 1990). Pregnancy is associated with an approximate 50% increase in demand for thyroid hormones which is apparent within the first 16 weeks of gestation and is mainly attributed to the estrogen-driven doubling in thyroxine-binding globulin concentrations (Glinoer *et al*., 1990; Alexander *et al*., 2004). Consequently, the increased demands for maternal thyroid hormones



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Table 3—Pearson correlation between square root (SQR) TSH MoM, log FT4 MoM and log FT3 MoM and log free $\beta$-hCG MoM in trisomy 21, trisomy 18 and unaffected pregnancies

| | Unaffected ($n = 3592$) | | Trisomy 21 ($n = 25$) | | Trisomy 18 ($n = 25$) | |
|---|---|---|---|---|---|---|
| Correlations | $r$ | $p$ | $r$ | $p$ | $r$ | $p$ |
| SQR TSH MoM with log FT4 MoM | −0.245 | <0.0001 | 0.106 | 0.613 | −0.111 | 0.596 |
| SQR TSH MoM with log FT3 MoM | −0.182 | <0.0001 | −0.307 | 0.135 | 0.238 | 0.251 |
| Log FT4 MoM with log FT3 MoM | 0.476 | <0.0001 | 0.376 | 0.064 | 0.359 | 0.078 |
| Log free $\beta$-hCG MoM with SQR TSH MoM | −0.214 | <0.0001 | −0.157 | 0.452 | −0.176 | 0.401 |
| Log free $\beta$-hCG MoM with Log FT4 MoM | 0.124 | <0.0001 | 0.252 | 0.223 | −0.015 | 0.944 |
| Log free $\beta$-hCG MoM with Log FT3 MoM | 0.104 | <0.0001 | 0.205 | 0.325 | −0.093 | 0.658 |

FT3, free triiodothyronine; FT4, free thyroxine; hCG, human chorionic gonadotrophin; MoM, multiples of the normal median; TSH, thyroid stimulating hormone.

of early pregnancy are under the influence of the placenta rather than the maternal pituitary gland. It could even be postulated that the otherwise unknown role of hCG resides in its thyrotropic properties at this critical stage of development when the fetal requirements for thyroid hormones are entirely dependent on the mother. Although the fetal thyroid gland begins to produce thyroid hormones from as early as 11 weeks of gestation (Shepard, 1967), functional maturation of the fetal pituitary–thyroid axis occurs only during the second half of pregnancy (Thorpe-Beeston *et al*., 1991a,b).

In this study we measured the screening marker free $\beta$-hCG, which is not a hormonally active molecule but its levels reflect those of the hormonally active intact hCG molecule. This interrelation between free $\beta$-hCG and TSH maintains normal FT3 and FT4 levels and this is reflected in trisomy 21 pregnancies where despite doubling in free $\beta$-hCG levels, FT3 and FT4 were not significantly different from euploid pregnancies. However, in cases of very high levels in free $\beta$-hCG, as observed in our two cases of androgenic triploidy, serum FT3 and FT4 were increased with complete suppression of TSH production. The opposite was true in the case of trisomy 18 where very low levels of free $\beta$-hCG were accompanied by an increase in TSH, but the level of FT3 was reduced. Although the level of FT4 was also lower than in euploid pregnancies, the difference was not significant. These data provide further support to the hypothesis that hCG, rather than TSH, may be the primary thyrotropic factor in early pregnancy. Although serum TSH is altered in pregnancies with fetal trisomies 21 and 18, this measurement does not improve the performance of screening for these aneuploidies provided by NT, free $\beta$-hCG and PAPP-A.

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