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Maternal serum levels of total activin-A in first-trimester trisomy 21 pregnancies

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Maternal serum total activin-A concentration was measured in 45 pregnancies affected by trisomy 21 and 493 control unaffected pregnancies at 10–14 weeks of gestation. In the trisomy 21 pregnancies total activin-A concentration was significantly higher (1.36 MoM of the unaffected pregnancies) and in 16% of cases the level was above the 95th centile of normal. The $\log_{10}$ SD for the control group and the trisomy 21 group were 0.17 and 0.22, respectively. The median pregnancy associated plasma protein-A (PAPP-A) in this trisomy 21 series was 0.49 and for free β-hCG was 2.05. In the trisomy group there were significant positive associations between total activin-A and PAPP-A (0.6071) and free β-hCG (0.4255). The low median difference and the high overlap in values between trisomic and unaffected pregnancies make total activin-A of little practical use in first-trimester screening for trisomy 21.

KEY WORDS: prenatal screening; Down syndrome; inhibin; free β-hCG; PAPP-A

INTRODUCTION

In both the first and second trimester of pregnancy, levels of maternal serum free β-hCG are elevated in pregnancies affected by fetal trisomy 21 (Spencer, 1991; Spencer *et al*., 1993). In conjunction with alpha-fetoprotein (AFP) in the second trimester (Spencer, 1999), and PAPP-A and fetal nuchal translucency in the first trimester (Spencer *et al*., 1999), successful screening programmes for these and other chromosomal anomalies have been developed. The mechanism behind this elevation of free β-hCG is unclear, but it is know that amniotic fluid levels of free β-hCG are increased (Spencer *et al*., 1997) and that placental tissue levels (Newby *et al*., 1997) are also increased in pregnancies affected by trisomy 21. Furthermore, at least one study (Eldar-Geva *et al*., 1995) has shown placental tissue hCG mRNA levels to be elevated in cases with trisomy 21, leading to speculation of altered synthesis rates by a transcriptional factor located on chromosome 21 which may upregulate the β-subunit transcription (Goshen *et al*., 1999; Knofler, 1999).

Control of hCG production in the placenta may well involve activin, which is known to stimulate placental hCG synthesis *in vitro* through increased placental GnRH release (Petraglia *et al*., 1989) and through interaction with inhibin (Steele *et al*., 1993). Activins are a group of homodimeric proteins consisting of two β-subunits, belonging to the family of transforming growth factor β-proteins, which are involved in cellular differentiation, proliferation and morphogenesis (Ying, 1988; Massague, 1990). In pregnancy activin-A is produced by the placenta and maternal serum levels increase with gestation (Qu and Thomas, 1995), whilst activin-B is not present in maternal serum but is present in amniotic fluid and cord serum. The development of reliable immunoassays for activins has been complicated by the fact that circulating activins may be bound to binding proteins such as follistatin, but recently new assays have emerged which overcome the binding protein interferences. (Knight *et al*., 1996) and enable total activin-A to be measured.

In view of the possible central role of activin-A in the control of hCG regulation we chose to investigate if levels of maternal serum activin-A were increased in pregnancies affected by trisomy 21 in the first trimester.

METHODS

In our two centres maternal serum samples from pregnancies in the first trimester (10–14 weeks of gestation) have been collected as part of screening for trisomy 21 and other chromosomal anomalies incorporating nuchal translucency scanning (Spencer *et al*., 1999, 2000). All serum was aliquoted and stored at -20°C. From the stored database of normal pregnancies and those affected by trisomy 21, 45 cases of trisomy 21 were selected together with 493 control unaffected pregnancies. Data summarising the clinical details of these pregnancies were extracted from the fetal database.

Maternal serum total activin-A was measured in all samples in duplicate using a commercial quantitative enzyme immunoassay technique (Serotec, Kidlington, Oxford, UK). The inter-assay precision was 8% and the intra-assay precision was 10% at 0.1 ng/ml and 3% and 3.5% at 4 ng/ml.

Maternal serum PAPP-A and free β-hCG were also measured, as part of the screening programme, using the CIS Kryptor system as described previously (Spencer *et al*., 1999).

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Statistical analysis

Median activin-A concentrations were established across the gestational window of 10–14 weeks using gestational age determined by crown–rump length. Since activin-A concentrations did not change significantly with gestation ($r = -0.02$) (Table 1) all measurements of activin-A were expressed as multiples of the median (MoM) for all gestations (median value 2.83 ng/ml).

Statistical analyses of the various markers were carried out using Analyse-It (Smart Software, Leeds, UK) a statistical software add-in for Microsoft Excel 7. The Kolmogorov-Smirnov test was used to confirm a Gaussian distribution of raw or log-transformed data. Significance of marker levels in the affected group were analysed using *t*-tests of unequal variance on Gaussian-confirmed marker distributions.

RESULTS

Table 2 outlines the characteristics of the trisomy 21 and the control group. Activin-A MoMs were not correlated with maternal age in either the trisomy 21 group ($r = -0.1049$) or in the unaffected group ($r = -0.0707$). Activin-A followed a Gaussian distribution after log transformation in both the unaffected and trisomy 21 group as shown in Figure 1. The individual marker levels in cases of trisomy 21 and in the unaffected group are summarised in Table 3. The median MoM of activin-A levels was significantly higher in the trisomy 21 group (1.355 MoM) compared with the control group (1.001 MoM) ($p < 0.001$). The $\log_{10}$ mean MoM in controls was 0.007 compared with 0.144 in the trisomy 21 group, with $\log_{10}$ SDs of 0.1657 and 0.2157, respectively. In 16% of the trisomy 21 pregnancies activin-A concentration was above the 95th centile of normal.

Table 1—Variation of total activin-A concentration across the first trimester

Gestation (week)	*n*	Median activin-A (ng/ml)
10	43	2.66
11	159	2.96
12	219	2.79
13	93	2.68

Table 2—Characteristics of the control and trisomy 21 study populations

	Controls ($n = 493$)	Trisomy 21 ($n = 45$)
Maternal age (years)	28.1 (SD 5.2)	36.62 (SD 5.28)
Maternal weight (kg)	68.0 (SD 13.6)	67.5 (SD 10.6)
Caucasian (%)	430 (87.2%)	41 (91%)
Cigarette smokers (%)	78 (15.8%)	5 (11.1%)
Primigravidae (%)	240 (48.7%)	13 (28.9%)
Gestational age (weeks)	12 (SD 0.8)	12 (SD 0.6)

In the trisomy group there were significant positive associations between activin-A and both PAPP-A ($r = 0.6071$, $p < 0.0001$) and free β-hCG ($r = 0.4255$, $p < 0.0001$). Free β-hCG and PAPP-A median MoMs in the trisomy 21 group were largely similar to those we have observed in larger series (Spencer *et al.*, 1999).

DISCUSSION

This study has demonstrated that at 10–14 weeks of gestation maternal serum activin-A in trisomy 21 pregnancies is increased but the degree of increase is small and the overlap in levels with normal pregnancies is large (see Figure 1). In addition there is an association between activin-A and free β-hCG levels and between activin-A and PAPP-A. Consequently, activin-A is of little practical use in first-trimester screening for trisomy 21.

Previous studies investigating activin in trisomy 21 are confined to the second trimester of pregnancy. Lambert-Messerlian et al. (1996a) used an assay measuring only free (non-follistatin-bound) activin and reported that in 20 trisomy 21 pregnancies the median (1.16 MoM) was not significantly different from 100 normal controls. Similarly, in a study of total activin-A, measured with the same assay as in the present study, the median value in ten trisomy 21 pregnancies (1.25 MoM) was not significantly different from 50 normal controls (Lambert-Messerlian *et al.*, 1996b). The same group then used a different assay for total activin-A and reported that the median MoM in 20 trisomy 21 pregnancies was 0.82 (Lambert-Messerlian *et al.*, 1998). Cuckle *et al.* (1999) reported that the median total activin-A in 30 cases of trisomy 21 (1.19 MoM) was significantly higher than in 199 unaffected pregnancies. However, the conclusion again was that the extent of the overlap between trisomy 21 and unaffected pregnancies was too great for it to be of practical use in screening.

Our results at 10–14 weeks of gestation are largely in agreement with those in the second trimester. Particularly our measured standard deviation of the log transformed MoM values of 0.166 (controls) and 0.216 (trisomy 21) are very close to the 0.15 and 0.20 estimated by Cuckle *et al.* (1999), producing a similar number of cases above the 95th centile (16% vs 20% for Cuckle *et al.*, 1999).

In trisomy 21 pregnancies the increase in maternal serum hCG is mirrored by increased levels in amniotic fluid (Spencer *et al.*, 1997). In contrast, the increased maternal serum activin-A in trisomy 21 pregnancies is associated with reduced levels in amniotic fluid (Wallace *et al.*, 1999). A similar difference in the distribution between maternal serum and amniotic fluid has also been reported for inhibin-A (Wallace *et al.*, 1997). It has been suggested that the major source of activin in the maternal circulation is the placenta (Qu and Thomas, 1995) whilst the amniotic and chorionic membranes are the most likely source in the amniotic fluid. The observed discordance in activin

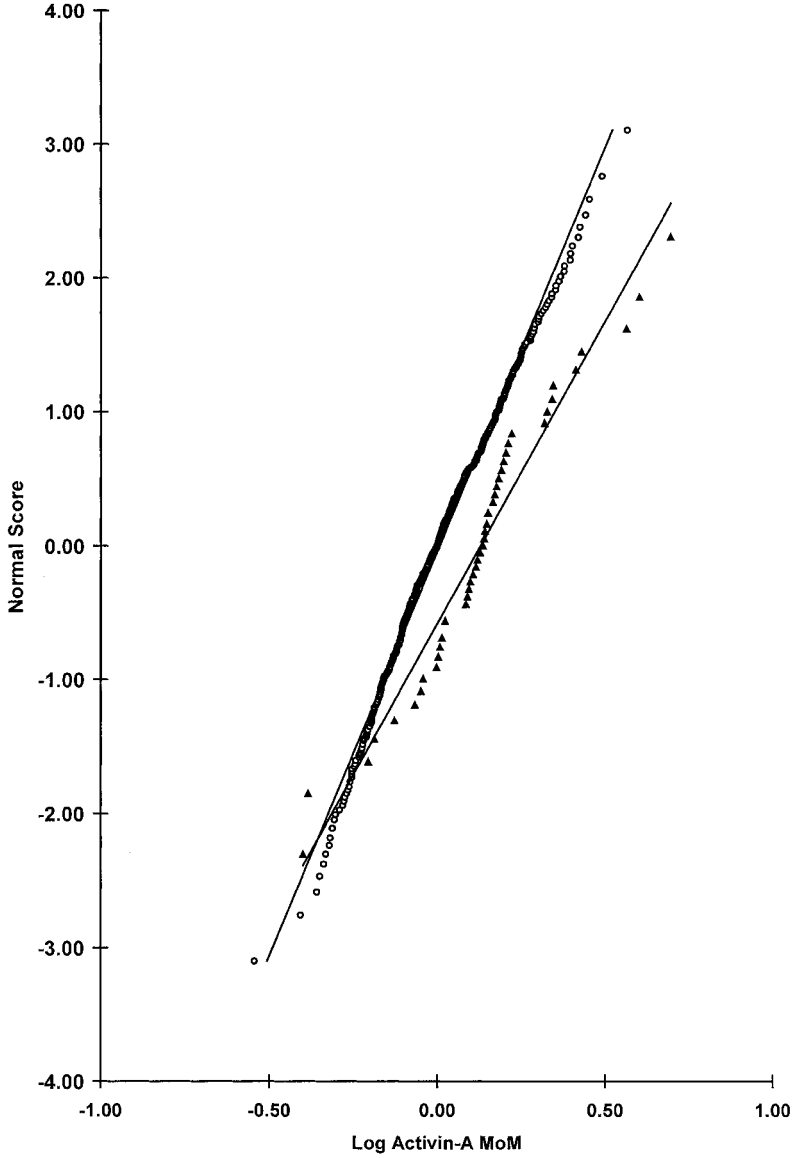

Figure 1—Probability plot of $\log_{10}$ total activin-A MoM in unaffected (○) and trisomy 21 (▲) affected pregnancies. The solid line represents the line of best fit

Table 3—Summary of marker values in the trisomy 21 and unaffected groups

	Trisomy 21			Unaffected		
	Activin-A	PAPP-A	Free β-hCG	Activin-A	PAPP-A	Free β-hCG
Median MoM	1.36	0.49	2.05	1.00	1.00	1.00
Log_{10}SD MoM	0.1657	0.2851	0.2900	0.2157	0.2315	0.2617

Correlation

	Trisomy 21	Unaffected
Activin-A vs PAPP-A	0.6071	0.2093
Activin-A vs free β-hCG	0.4255	0.1204
Free β-hCG vs PAPP-A	0.0663	0.2074

concentration in the two compartments may reflect differences in regulation of synthesis in various tissues.

Although activin-A is partially correlated with free β-hCG it seems that in the aetiology of trisomy 21, disturbance in activin-A synthesis is unlikely to be an explanation for the increase in free β-hCG in either the first or second trimester.

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