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Placental growth hormone and growth hormone binding protein are first trimester maternal serum markers of Down syndrome†

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Background Placental growth hormone (PGH) is synthesised by the placenta, and its function is modulated by growth hormone binding protein (GHBP). The potential of PGH and GHBP as maternal serum screening markers for Down syndrome (DS) was examined.

Materials and methods Maternal serum concentrations of PGH and GHBP were determined by ELISA in 74 DS and 261 control pregnancies in gestational week 8^{+0} to 13^{+4}. $\log_{10}$ MoM distributions of the markers were established. The performance of DS screening was estimated by Monte Carlo simulation.

Results PGH $\log_{10}$ MoM (SD) was decreased ($p < 0.001$) to -0.201 (0.373) and GHBP $\log_{10}$ MoM to -0.116 (0.265) ($p = 0.04$), in DS pregnancies ($n = 34$) in week 8^{+0} to 10^{+0}. In week 10^{+1} to 13^{+4}, neither PGH ($p = 0.16$) nor GHBP ($p = 0.13$) was reduced in DS pregnancies. The detection rate (DR) for PGH in screening for DS in week 8^{+0} to 10^{+0} was 39% for a false positive rate (FPR) of 5%; increasing to 72% in combination with PAPP-A + hCGβ. PGH + GHBP in combination with PAPP-A + hCGβ + nuchal translucency (NT) (CUB test) had a DR of 91% compared with 80% for the CUB test.

Conclusion PGH and GHBP are early first trimester maternal serum markers for DS [Correction made here after initial online publication]. Copyright © 2009 John Wiley & Sons, Ltd.

KEY WORDS: PGH; GHBP; prenatal screening; Down syndrome; aneuploidy; placenta

INTRODUCTION

First trimester screening for Down syndrome (DS) is routinely performed in gestational week 10–14 using a combination of the serum markers, pregnancy associated plasma protein-A (PAPP-A) and the free β-form of human chorionic gonadotrophin (hCGβ) along with a measurement of the nuchal translucency thickness (Nicolaides *et al*., 2005; Wojdemann *et al*., 2005; Spencer, 2007; Ekelund *et al*., 2008). Despite the major advances in prenatal screening for DS during the last decade where screening performance increased dramatically and the time of screening was changed from second to first trimester of pregnancy (Benn, 2002a, 2002b), we are still faced with the problem of a fairly high proportion, in the order of 2–4%, of false positives and a false negative rate of approx. 5–10% (Spencer *et al*., 2003; Nicolaides *et al*., 2005). As the false positive cases often result in invasive diagnostic procedures associated with a 1% risk of foetal death (Tabor *et al*., 1986; Smidt-Jensen *et al*., 1992), advances in screening efficiency are still of high priority.

One way of improving screening performance is to include additional markers. Several have been suggested; including the ultrasound (US) markers: nasal bone (Kagan *et al*., 2009) and tricuspid regurgitation (Falcon *et al*., 2006). Additional biochemical markers: SP1 (Qin *et al*., 1997), ProMBP (Christiansen *et al*., 1999), Placental Growth Factor (PLGF) (Spencer *et al*., 2001), Inhibin A (Christiansen and Norgaard-Pedersen, 2005), hPL (Christiansen *et al*., 2007a) and ADAM12 (Laigaard *et al*., 2003, 2006; Poon *et al*., 2009) have also been tested.

Several of the first trimester maternal serum markers are involved in the insulin-like growth factor axis (IGF-axis); PAPP-A is an IGFBP4-protease (Conover *et al*., 2001), ProMBP is a physiological inhibitor of PAPP-A (Overgaard *et al*., 2000) and ADAM 12 is an IGFBP3- and IGFBP5-protease (Loechel *et al*., 2000). In general, the IGF-axis-related markers in DS pregnancies are decreased in first trimester maternal serum (Christiansen *et al*., 2004; Spencer, 2007) and increased in second trimester (Christiansen *et al*., 1999; Rode *et al*., 2003; Christiansen *et al*., 2004, 2007b). The general reduction in the level of IGF-axis associated molecules suggests that other members might have perturbed concentrations in DS pregnancies and potentially be useful as DS markers.

During pregnancy, the placenta synthesises a growth hormone (GH) analogue, placental growth hormone (PGH) (Liebhaber *et al*., 1989). Gradually, during pregnancy, PGH replaces the pulsatile GH secretion (Frankenne *et al*., 1988; Eriksson *et al*., 1989) and takes

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over the somatotrophic and lipolytic effects of GH (Fuglsang and Ovesen, 2006). PGH is synthesised by both the syncytiotrophoblast (Scippo *et al*., 1993) and the extravillous trophoblast (Lacroix *et al*., 2005) and is detectable in maternal serum from early first trimester and the concentration increases towards term (Alsat *et al*., 1998; Chellakooty *et al*., 2002, 2004). In DS pregnancies, the *in vitro* PGH expression of the syncytiotrophoblast is decreased (Frendo *et al*., 2000), but several studies in second trimester have shown that the maternal serum concentration of PGH is increased in DS pregnancies (Moghadam *et al*., 1998; Baviera *et al*., 2004; Papadopoulou *et al*., 2008) and the amniotic fluid concentration of PGH is likewise increased in DS pregnancies (Sifakis *et al*., 2009). The maternal serum concentration of PGH has been found elevated in pre-eclampsia (Mittal *et al*., 2007), probably a consequence of the role of PGH in trophoblast invasion (Lacroix *et al*., 2005) and/or the relation to placental arterial resistance (Wu *et al*., 2003).

Growth hormone binding protein (GHBP) is the proteolytically shedded form of the growth hormone receptor (GHR) (Leung *et al*., 1987; Zhang *et al*., 2000) and is synthesised by the liver, adipose tissue and the placenta (Frankenne *et al*., 1992). In maternal serum, GHBP is found in decreasing concentration from the end of the first trimester to term (Barnard *et al*., 1997) and it is believed to bind PGH and modulate its physiological function (McIntyre *et al*., 2000). GHBP has been found slightly elevated in second trimester maternal serum samples from DS pregnancies and decreased in trisomy 18 pregnancies (Wallace *et al*., 2001).

The present study examines the first trimester maternal serum concentrations of PGH and GHBP in DS pregnancies and assesses whether these analytes may be used in maternal serum screening for DS.

MATERIALS AND METHODS

Serum samples

Serum samples from 74 women, median age 37.5 years, with a DS foetus sampled at median gestational age 73 days (range: 56–95 days) and 261 age-matched control pregnant women, median age 36.4 years, sampled at median gestational age 70 days (range: 56–95 days) were retrieved from samples received as part of routine first trimester screening (PAPP-A and hCGβ) performed at Statens Serum Institut (SSI), Copenhagen. Controls were collected at random but matched for length of storage ($\pm$3 months) and maternal age. Initially, four controls per DS sample were selected but shortage of serum for analysis resulted in the number of controls only reaching 261. Samples had been stored at $-20\,^{\circ}$C in a biobank as part of the quality control function at the Pregnancy Screening Registry at SSI. All DS cases were identified by cross referencing with the Danish Cytogenetic Central Registry so that both samples identified by screening and not identified were included. All cases were singleton pregnancies. Information about gestational age based on crown rump length (CRL) was obtained from referral sheets. When a US based gestational age was not available, a gestational age based on last menstrual period was used.

Biochemical measurements

The PGH concentrations were determined using a commercially available double monoclonal ELISA (DSL-10-19 200, Diagnostic Systems Laboratory Inc, Webster, TX, USA). All quantifications were performed as single determinations following the manufacturer's instructions. The assay exhibits no cross-reactivity with either pituitary-derived growth hormone (hGH) or hPL. The detection limit is 8.0 pg/mL and the interassay coefficient of variation of controls was $<$15%. The GHBP concentrations were determined by the commercially available emzyme-amplified ELISA (DSL-10-48 100, Diagnostic Systems Laboratory Inc, Webster, TX, USA). All quantifications were performed as single determinations according to the manufacturer's instructions. The assay exhibits no cross-reactivity with either GH or PGH. The assay sensitivity is 1.69 pmol/L and the interassay coefficient of variation was $<$15%. Biochemical measurements were performed in batch. The concentrations of PAPP-A and hCGβ were determined in maternal sera as part of the routine screening on the analytical platforms AutoDelfia (PerkinElmer, Turku, Finland) or Kryptor (Brahms, Henningsdorf, Germany) using kits and procedures provided by the manufacturers.

Data analysis

Means were compared using Mann–Whitney U-test or ANOVA with F-test. LOESS regression was used to study the gestational age dependence of analytes. Log-regression of analytes on gestational age in days was used to calculate median formulae as described in detail in the Section on Results. Correlations were performed by the method of Pearson. Compatibility with the normal distribution was assessed by Shapiro–Wilk's test and normal probability plots.

Monte Carlo simulation of screening performance

The population performance of various marker combinations in DS screening was examined using Monte Carlo simulation as described elsewhere (Larsen *et al*., 1998). Using the observed parameters for GHBP and PGH and published parameters for PAPP-A and hCGβ in the relevant gestational age window (Spencer *et al*., 2002), a series of random $\log_{10}$ MoM values were selected from the distributions in unaffected and affected pregnancies. The $\log_{10}$ MoM values (standard deviation) for NT were 0.305 (0.235) and 0 (0.120) in DS pregnancies and controls, respectively, and no correlation with biochemical markers was assumed (Christiansen and Olesen, 2002). These values—in combination with the

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empirically established correlations between the markers—were then used to calculate likelihood ratios for the combinations. The estimation of population performance was performed for the gestational age windows 8^{+0} to 10^{+0} and 11^{+0} to 13^{+4} weeks separately. MOM values of −0.6 and 0.6 were used as lower and upper truncation limits for all parameters The likelihood ratios were then used together with the age-related risk of having a DS child at birth (Cuckle *et al*., 1987) to calculate the expected detection rate of affected pregnancies at various false positive rates (FPR), in a population with a standardised maternal age distribution (van der Veen *et al*., 1997).

RESULTS

Placental growth hormone

The distribution of maternal serum PGH concentrations in DS and control pregnancies is shown in Figure 1. The PGH concentration in controls increases until gestational age 74 days from which time it remains constant. In controls, the empirical median PGH concentration in each completed week was 531 pg/mL (8th week), 935 pg/mL (9th week), 962 pg/mL (10th week), 1103 pg/mL (11th week), 1204 pg/mL (12th week) and 1169 pg/mL (13th week). In DS pregnancies, the concentration increases through the examined period from a decreased level, compared with controls, prior to gestational age 85 to become increased after day 85. In order to obtain gestational age, independent distributions of PGH two median formulae were established in control pregnancies by log-linear regression of PGH on gestational age in days, separately for the gestational age windows <75 days and ≥75 days. The median formula for the ≥75 days window was $\log_{10}$ PGH = 0.023 × days + 1336. ($p < 0.001$, $n = 174$) and for the ≥75 days window, no significant relation between $\log_{10}$ PGH and gestational age ($p = 0.74$, $n = 87$) was found with the regression line: $\log_{10}$ PGH = 0.001 × days + 2894. For the upper interval, the constant 2894 was used as $\log_{10}$ PGH median. The median formulae were used to convert all PGH concentrations into $\log_{10}$ MoM values. The distribution of $\log_{10}$ MoM PGH values in DS pregnancies as a function of gestational age is shown in Figure 2. Log_{10} MoM PGH in DS pregnancies increased significantly with gestational age, $r = 0.425$, $p < 0.001$. The $\log_{10}$ MoM PGH distributions in controls and DS pregnancies in the gestational age window 8^{+0} to 10^{+0} weeks, where the $\log_{10}$ MoM PGH are significantly decreased compared with controls, are given in Table 1. In the gestational age window 10^{+1} to 13^{+4} weeks, $\log_{10}$ MoM PGH was not significantly different when comparing DS and control pregnancies ($p = 0.16$). The significant deviation from log-normality identified by Shapiro–Wilk's test in Table 1 is due to the presence of a single outlier in DS pregnancies (normal probability plot not shown).

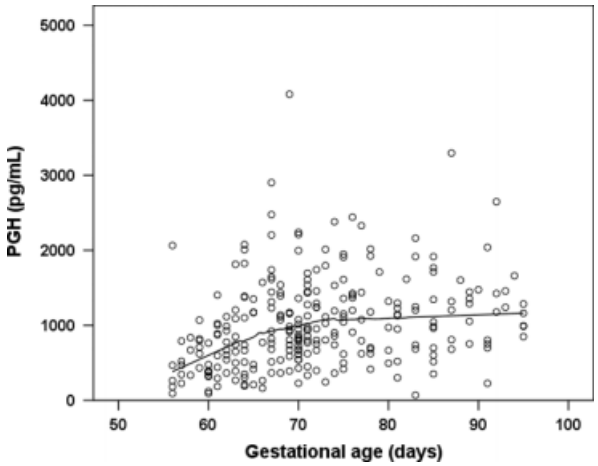

Figure 1—Scatterplot of PGH concentrations in control pregnancies. The line is the LOESS regression line

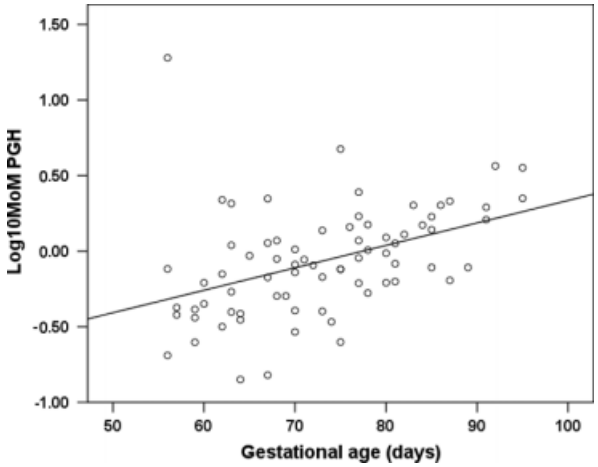

Figure 2—Scatterplot of $\log_{10}$ MoM PGH in DS pregnancies as a function of gestational age. The line is the linear fit, $r = 0.425$, $p < 0.001$

Table 1—The distribution of $\log_{10}$ MoM PGH and GHBP values in controls and DS pregnancies in early first trimester (week 8^{+0} to 10^{+0})

Gestational age	N	Mean $\log_{10}$ MoM	SD	Shapiro–Wilk's W′	*p*
PGH					
Controls	135	0.030*	0.285	0.98	0.096
DS pregnancies	34	−0.206	0.395	0.89	0.001
GHBP					
Controls	135	−0.029**	0.206	0.99	0.165
DS pregnancies	34	−0.116	0.266	0.96	0.183

Controls versus DS pregnancies: * $p < 0.001$ and ** $p = 0.04$, ANOVA.

Growth hormone binding protein

The distributions of maternal serum GHBP concentrations in DS and control pregnancies are shown in

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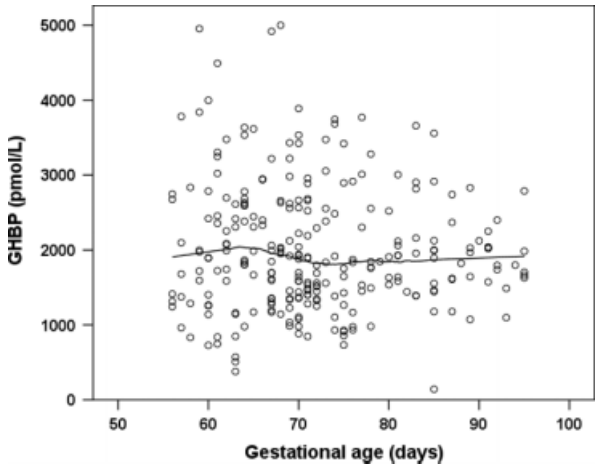

Figure 3—Scatterplot of GHBP concentrations in control pregnancies. The line is the LOESS regression line

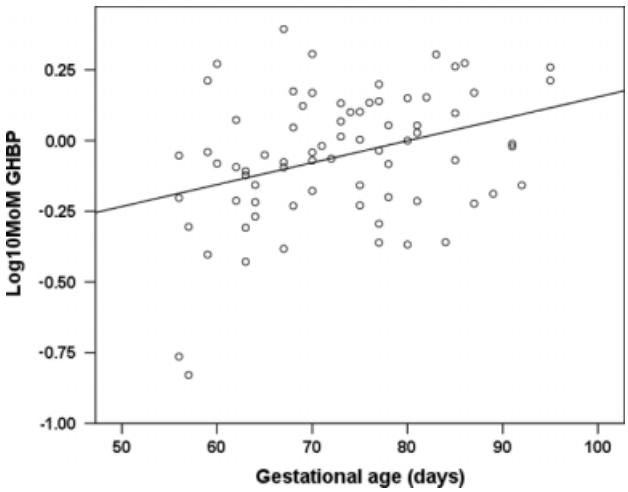

Figure 4—Scatterplot of $\log_{10}$ MoM GHBP in DS pregnancies as a function of gestational age. The line is the linear fit, $r = 0.0.345$, $p = 0.003$

Figure 3. The concentration of GHBP is constant, at 2004 pmol/L, through the period of gestation examined in control pregnancies, whereas it increases through the period in DS pregnancies from a very low level prior to gestational age 70 days to an increased level after gestational age 77 days. In controls, the empirical median GHBP concentration in each completed week was 1982 pmol/L (8th week), 1982 pmol/L (9th week), 1645 pmol/L (10th week), 1917 pmol/L (11th week), 1893 pmol/L (12th week) and 1796 pmol/L (13th week). In order to facilitate an assessment of the screening performance of GHBP; all GHBP concentrations were converted into $\log_{10}$ MoM values by $\log_{10}$ transforming the concentrations divided by 2004 pmol/L. In both controls and DS pregnancies, the $\log_{10}$ MoM GHBP distributions were compatible with the normal distribution. The $\log_{10}$ MoM GHBP values in DS pregnancies increased significantly with gestational age, $r = 0.345$, $p = 0.003$, as shown in Figure 4. The $\log_{10}$ MoM GHBP distributions in controls and DS pregnancies in early first trimester are given in Table 1. In the 8^{+0} to 10^{+0} week window, the difference between controls and DS pregnancies is significant (Table 1), so this window is chosen as the screening window applicable for GHBP. In week 10^{+1} to 13^{+4}, the $\log_{10}$ MoM GHBP values did not differ significantly between DS and control pregnancies ($p = 0.13$). The correlation between $\log_{10}$ MoM GHBP and $\log_{10}$ MoM PGH was not significant in either controls or DS pregnancies.

PGH and GHBP screening

The performance of PGH screening, alone with age, or in combination with PAPP-A or hCGβ is given in Table 2. The gestational age window used was from 8^{+0} to 10^{+0} weeks. There was no correlation between PGH and maternal age in either controls or DS pregnancies. The correlations between PGH and hCGβ in both controls and DS pregnancies in the examined gestational age were not significant. However, a highly significant correlation was found between $\log_{10}$ MoM PGH and $\log_{10}$ MoM PAPP-A in both controls, $r = 0.457$, $p < 0.001$, and DS pregnancies, $r = 0.596$, $p < 0.001$. The performance of GHBP screening in gestational age window 8^{+0} to 10^{+0} weeks is given in Table 3 together with the screening performance of combinations of GHBP with PAPP-A, hCGβ, PGH and NT. The gestational age window used was from 8^{+0} to 10^{+0} weeks. The correlations between GHBP, PAPP-A and hCGβ in both controls and DS pregnancies in the 8^{+0} to 10^{+0} week gestational age window were not significant.

Table 2—The estimated performance of age + PGH and combinations of PGH, PAPP-A, hCGβ and NT in DS screening in week 8^{+0} to 10^{+0}

	Risk cut-off						Fixed FPR		
	1 : 100		1 : 250		1 : 400		1%	3%	5%
Markers	FPR(%)	DR(%)	FPR(%)	DR(%)	FPR(%)	DR(%)	DR(%)	DR(%)	DR(%)
PGH	1.2	21	5.9	41	12.8	57	19	30	39
PGH + PAPP-A	2.0	41	7.0	65	11.8	76	29	48	57
PGH + hCGβ	1.7	34	7.3	63	12.7	76	27	45	55
PAPP-A + hCGβ	2.1	53	5.8	74	9.0	82	40	61	71
PGH + PAPP-A + hCGβ	2.1	55	5.9	75	9.1	82	42	61	72
PGH + PAPP-A + hCGβ + NT	1.0	73	2.5	82	4.0	85	73	83	86

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Table 3—The estimated performance of age + GHBP and combinations of PGH, PAPP-A and hCGβ in week 8^{+0} to 10^{+0} and NT in week 11^{+6} to 13^{+6}

	Risk cut-off						Fixed FPR		
	1 : 100		1 : 250		1 : 400		1%	3%	5%
Markers	FPR(%)	DR(%)	FPR(%)	DR(%)	FPR(%)	DR(%)	DR(%)	DR(%)	DR(%)
GHBP	0.6	8	5.2	27	17.5	55	11	20	27
GHBP + PAPP-A	2.1	45	6.9	66	11.3	76	30	50	60
GHBP + hCGβ	2.0	31	8.6	61	14.7	75	20	37	48
GHBP + PGH	1.4	26	6.4	47	12.9	63	20	35	43
PAPP-A + hCGβ	2.1	53	5.8	74	9.0	82	40	61	71
PAPP-A + hCGβ + NT	2.3	65	5.5	82	7.9	88	50	71	80
GHBP + PAPP-A + hCGβ	2.1	56	5.8	75	8.7	83	44	63	73
GHBP + PGH + PAPP-A + hCGβ	1.9	57	5.2	75	8.1	83	45	65	75
GHBP + PGH + PAPP-A + hCGβ + NT	0.9	77	2.3	85	3.6	88	78	87	91

DISCUSSION

The major finding in this study is the first demonstration that both PGH and GHBP maternal serum concentrations are reduced in early first trimester of pregnancy in DS pregnancies. This is somewhat surprising, as other studies in second trimester pregnancies have demonstrated elevated levels of PGH in both maternal serum (Moghadam *et al*., 1998; Baviera *et al*., 2004; Papadopoulou *et al*., 2008) and amniotic fluid (Sifakis *et al*., 2009) and marginally elevated GHBP in maternal serum (Wallace *et al*., 2001). It is well established that some first trimester markers, for example, PAPP-A, ProMBP, SP1 and ADAM12 are decreased in first trimester and increased in second trimester (Qin *et al*., 1997; Christiansen *et al*., 2004, 2007b). Thus, PGH and GHBP behave in a way similar to that of these other markers. This similarity makes it unlikely that the differential distribution of PGH and GHBP values in early (8^{+0} to 10^{+0} weeks) and late (10^{+1} to 13^{+4} weeks) first trimester is a chance finding. However, as the age limits for the informative gestational age window are defined in the same population as the one used for assessment of screening performance, the performance may be overestimated. This is particularly the case for GHBP, where the difference between DS and control pregnancies is small.

Why some serum markers are decreased in DS pregnancies in first trimester is not known, but our results confirm the hypothesis that DS pregnancies are characterised by a profound reduction in the maternal serum concentration of IGF-axis analytes in first trimester of pregnancy.

The transcription of the genes coding for PGH, PAPP-A and hCG are all regulated by the PPARγ receptor which is essential for placental development and trophoblast invasion (Fournier *et al*., 2008). The strong positive correlation between PGH and PAPP-A may reflect such a combined regulation or be a result of an autocrine relation between these two proteins which are both synthesised by the trophoblast.

The performance estimates of screening involving PGH and GHBP (Table 2 and 3) show that adding PGH to the conventional double test (PAPP-A and hCGβ) does not increase screening efficiency appreciably. This is probably because of the highly significant correlation between $\log_{10}$ MoM PGH and $\log_{10}$ MoM PAPP-A. The improvement of the screening efficiency of the double test by adding GHBP is slightly better (Table 3), but still not sufficient to warrant an inclusion of GHBP in the routine prenatal screening programme. However, when adding PGH and GHBP to the double test, an increase of 4–5% in DR is seen for a fixed FPR (Table 3). Furthermore, in combined screening, i.e. the double test supplemented with an NT-screening in late first trimester, the addition of PGH and GHBP improves screening efficiency considerably (Table 3).

In conclusion, both PGH and GHBP are interesting new early first trimester maternal serum markers for foetal DS, but, because of the highly significant correlation between PGH and PAPP-A, their use requires the use of US screening. They are probably not going to be of value in a contingent screening model (Christiansen and Olesen, 2002). However, the final assessment of the clinical significance of these markers requires prospective clinical studies.

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REFERENCES

Alsat E, Guibourdenche J, Couturier A, Evain-Brion D. 1998. Physiological role of human placental growth hormone. *Mol Cell Endocrinol* **140**: 121–127.

Barnard R, Chan FY, McIntyre HD. 1997. Growth hormone-binding protein in normal and pathologic gestation: correlations with maternal diabetes and fetal growth. *J Clin Endocrinol Metab* **82**: 1879–1884.

Baviera G, Carbone C, Corrado F, Mastrantonio P. 2004. Placental growth hormone in Down's syndrome screening. *J Matern Fetal Neonatal Med* **16**: 241–243.

Benn PA. 2002a. Advances in prenatal screening for Down syndrome: I. General principles and second trimester testing. *Clin Chim Acta* **323**: 1–16.

Benn PA. 2002b. Advances in prenatal screening for Down syndrome: II first trimester testing, integrated testing, and future directions. *Clin Chim Acta* **324**: 1–11.

Chellakooty M, Skibsted L, Skouby SO, *et al*. 2002. Longitudinal study of serum placental GH in 455 normal pregnancies: correlation to gestational age, fetal gender, and weight. *J Clin Endocrinol Metab* **87**: 2734–2739.

Chellakooty M, Vangsgaard K, Larsen T, *et al*. 2004. A longitudinal study of intrauterine growth and the placental growth hormone (GH)-insulin-like growth factor I axis in maternal circulation: association between placental GH and fetal growth. *J Clin Endocrinol Metab* **89**: 384–391.

Christiansen M, Larsen SO, Oxvig C, *et al*. 2004. Screening for Down's syndrome in early and late first and second trimester using six maternal serum markers. *Clin Genet* **65**: 11–16.

Christiansen M, Norgaard-Pedersen B. 2005. Inhibin A is a maternal serum marker for Down's syndrome early in the first trimester. *Clin Genet* **68**: 35–39.

Christiansen M, Olesen LS. 2002. An increase in cost-effectiveness of first trimester maternal screening programmes for fetal chromosome anomalies is obtained by contingent testing. *Prenat Diagn* **22**: 482–486.

Christiansen M, Oxvig C, Wagner JM, *et al*. 1999. The proform of eosinophil major basic protein: a new maternal serum marker for Down syndrome. *Prenat Diagn* **19**: 905–910.

Christiansen M, Sorensen TL, Norgaard-Pedersen B. 2007a. Human placental lactogen is a first-trimester maternal serum marker of Down syndrome. *Prenat Diagn* **27**: 1–5.

Christiansen M, Spencer K, Laigaard J, Cowans NJ, Larsen SO, Wewer UM. 2007b. ADAM 12 as a second-trimester maternal serum marker in screening for Down syndrome. *Prenat Diagn* **27**: 611–615.

Conover CA, Faessen GF, Ilg KE, *et al*. 2001. Pregnancy-associated plasma protein-a is the insulin-like growth factor binding protein-4 protease secreted by human ovarian granulosa cells and is a marker of dominant follicle selection and the corpus luteum. *Endocrinology* **142**: 2155–2158.

Cuckle HS, Wald NJ, Thompson SG. 1987. Estimating a woman's risk of having a pregnancy associated with Down's syndrome using her age and serum alpha-fetoprotein level. *Br J Obstet Gynaecol* **94**: 387–402.

Ekelund CK, Jorgensen FS, Petersen OB, Sundberg K, Tabor A. 2008. Impact of a new national screening policy for Down's syndrome in Denmark: population based cohort study. *Br Med J* **337**: a2547.

Eriksson L, Frankenne F, Eden S, Hennen G, Von SB. 1989. Growth hormone 24-h serum profiles during pregnancy–lack of pulsatility for the secretion of the placental variant. *Br J Obstet Gynaecol* **96**: 949–953.

Falcon O, Auer M, Gerovassili A, Spencer K, Nicolaides KH. 2006. Screening for trisomy 21 by fetal tricuspid regurgitation, nuchal translucency and maternal serum free beta-hCG and PAPP-A at $11+0$ to $13+6$ weeks. *Ultrasound Obstet Gynecol* **27**: 151–155.

Fournier T, Therond P, Handschuh K, Tsatsaris V, Evain-Brion D. 2008. PPARgamma and early human placental development. *Curr Med Chem* **15**: 3011–3024.

Frankenne F, Alsat E, Scippo ML, Igout A, Hennen G, Evain-Brion D. 1992. Evidence for the expression of growth hormone receptors in human placenta. *Biochem Biophys Res Commun* **182**: 481–486.

Frankenne F, Closset J, Gomez F, Scippo ML, Smal J, Hennen G. 1988. The physiology of growth hormones (GHs) in pregnant women and partial characterization of the placental GH variant. *J Clin Endocrinol Metab* **66**: 1171–1180.

Frendo JL, Vidaud M, Guibourdenche J, *et al*. 2000. Defect of villous cytotrophoblast differentiation into syncytiotrophoblast in Down's syndrome. *J Clin Endocrinol Metab* **85**: 3700–3707.

Fuglsang J, Ovesen P. 2006. Aspects of placental growth hormone physiology. *Growth Horm IGF Res* **16**: 67–85.

Kagan KO, Cicero S, Staboulidou I, Wright D, Nicolaides KH. 2009. Fetal nasal bone in screening for trisomies 21, 18 and 13 and Turner syndrome at 11–13 weeks of gestation. *Ultrasound Obstet Gynecol* **33**: 259–264.

Lacroix MC, Guibourdenche J, Fournier T, *et al*. 2005. Stimulation of human trophoblast invasion by placental growth hormone. *Endocrinology* **146**: 2434–2444.

Laigaard J, Sorensen T, Frohlich C, *et al*. 2003. ADAM12: a novel first-trimester maternal serum marker for Down syndrome. *Prenat Diagn* **23**: 1086–1091.

Laigaard J, Spencer K, Christiansen M, *et al*. 2006. ADAM 12 as a first-trimester maternal serum marker in screening for Down syndrome. *Prenat Diagn* **26**: 973–979.

Larsen SO, Christiansen M, Norgaard-Pedersen B. 1998. Calculation of roc curves in multidimensional likelihood ratio based screening with Down's syndrome as a special case. *J Med Screen* **5**: 57–62.

Leung DW, Spencer SA, Cachianes G, *et al*. 1987. Growth hormone receptor and serum binding protein: purification, cloning and expression. *Nature* **330**: 537–543.

Liebhaber SA, Urbanek M, Ray J, Tuan RS, Cooke NE. 1989. Characterization and histologic localization of human growth hormone-variant gene expression in the placenta. *J Clin Invest* **83**: 1985–1991.

Loechel F, Fox JW, Murphy G, Albrechtsen R, Wewer UM. 2000. ADAM 12-S cleaves IGFBP-3 and IGFBP-5 and is inhibited by TIMP-3. *Biochem Biophys Res Commun* **278**: 511–515.

McIntyre HD, Serek R, Crane DI, *et al*. 2000. Placental growth hormone (GH), GH-binding protein, and insulin-like growth factor axis in normal, growth-retarded, and diabetic pregnancies: correlations with fetal growth. *J Clin Endocrinol Metab* **85**: 1143–1150.

Mittal P, Espinoza J, Hassan S, *et al*. 2007. Placental growth hormone is increased in the maternal and fetal serum of patients with preeclampsia. *J Matern Fetal Neonatal Med* **20**: 651–659.

Moghadam S, Engel W, Bougoussa M, Hennen G, Igout A, Sancken U. 1998. Maternal serum placental growth hormone and insulinlike growth factor binding proteins 1 and 3 in pregnancies affected by fetal aneuploidy and other abnormalities: implications for prenatal diagnosis of trisomy 21. *Fetal Diagn Ther* **13**: 291–297.

Nicolaides KH, Spencer K, Avgidou K, Faiola S, Falcon O. 2005. 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* **25**: 221–226.

Overgaard MT, Haaning J, Boldt HB, *et al*. 2000. Expression of recombinant human pregnancy-associated plasma protein-A and identification of the proform of eosinophil major basic protein as its physiological inhibitor. *J Biol Chem* **275**: 31128–31133.

Papadopoulou E, Sifakis S, Giahnakis E, *et al*. 2008. Human placental growth hormone is increased in maternal serum in pregnancies affected by Down syndrome. *Fetal Diagn Ther* **23**: 211–216.

Poon LC, Chelemen T, Minshawa R, Frisova V, Nicolaides KH. 2009. Maternal serum ADAM12 (A disintegrin and metalloprotease) in chromosomally abnormal pregnancies at 11–13 weeks. *Am J Obstet Gynecol* **200**: e1–e6.

Qin QP, Christiansen M, Nguyen TH, Sorensen S, Larsen SO, Norgaard-Pedersen B. 1997. Schwangerschaftsprotein 1 (SP1) as a maternal serum marker for Down syndrome in the first and second trimesters. *Prenat Diagn* **17**: 101–108.

Rode L, Wojdemann KR, Shalmi AC, *et al*. 2003. Combined first- and second-trimester screening for Down syndrome: an evaluation of proMBP as a marker. *Prenat Diagn* **23**: 593–598.

Scippo ML, Frankenne F, Hooghe-Peters EL, Igout A, Velkeniers B, Hennen G. 1993. Syncytiotrophoblastic localization of the human growth hormone variant mRNA in the placenta. *Mol Cell Endocrinol* **92**: R7–13.

Sifakis S, Papadopoulou E, Konstantinidou A, *et al*. 2009. Increased levels of human placental growth hormone in the amniotic fluid of pregnancies affected by Down syndrome. *Growth Horm IGF Res* **19**: 121–125.

Smidt-Jensen S, Permin M, Philip J, *et al*. 1992. Randomised comparison of amniocentesis and transabdominal and transcervical chorionic villus sampling. *Lancet* **340**: 1237–1244.

Spencer K. 2007. Aneuploidy screening in the first trimester. *Am J Med Genet C Semin Med Genet* **145C**: 18–32.

Spencer K, Crossley JA, Aitken DA, Nix AB, Dunstan FD, Williams K. 2002. Temporal changes in maternal serum biochemical markers of trisomy 21 across the first and second trimester of pregnancy. *Ann Clin Biochem* **39**: 567–576.

Spencer K, Liao AW, Ong CY, Geerts L, Nicolaides KH. 2001. First trimester maternal serum placenta growth factor (PLGF) concentration in pregnancies with fetal trisomy 21 or trisomy 18. *Prenat Diagn* **21**: 718–722.

Spencer K, Spencer CE, Power M, Dawson C, Nicolaides KH. 2003. 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. *Br J Obstet Gynecol* **110**: 281–286.

Tabor A, Philip J, Madsen M, Bang J, Obel EB, Norgaard-Pedersen B. 1986. Randomised controlled trial of genetic amniocentesis in 4606 low-risk women. *Lancet* **1**: 1287–1293.

van der Veen WJ, Beekhuis JR, Cornel MC, Mantingh A, de Walle HE, de Wolf BT. 1997. A demographic approach to the assessment of Down syndrome screening performance. *Prenat Diagn* **17**: 717–724.

Wallace EM, D'Antona D, Veveris-Lowe T, Crossley JA, Barnard R. 2001. Growth hormone binding protein in normal and aneuploid pregnancy: a paradoxical decrease in trisomy 18. *Br J Obstet Gynecol* **108**: 701–703.

Wojdemann KR, Shalmi AC, Christiansen M, *et al*. 2005. Improved first-trimester Down syndrome screening performance by lowering the false-positive rate: a prospective study of 9941 low-risk women. *Ultrasound Obstet Gynecol* **25**: 227–233.

Wu Z, Bidlingmaier M, Friess SC, *et al*. 2003. A new nonisotopic, highly sensitive assay for the measurement of human placental growth hormone: development and clinical implications. *J Clin Endocrinol Metab* **88**: 804–811.

Zhang Y, Jiang J, Black RA, Baumann G, Frank SJ. 2000. Tumor necrosis factor-alpha converting enzyme (TACE) is a growth hormone binding protein (GHBP) sheddase: the metalloprotease TACE/ADAM-17 is critical for (PMA-induced) GH receptor proteolysis and GHBP generation. *Endocrinology* **141**: 4342–4348.

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