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Maternal Serum Placental Protein 13 at Eleven to Thirteen Weeks in Chromosomally Abnormal Pregnancies

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Key Words
Placental protein 13 · First-trimester screening · Trisomy 21 · Trisomy 18 · Pregnancy-associated plasma protein A · Free β-human chorionic gonadotropin

Abstract
Objective: To investigate whether the maternal serum concentration of placental protein 13 (PP13) is altered in chromosomally abnormal pregnancies and to examine the potential value of this placental protein in screening for aneuploidies at 11–13 weeks. ***Methods:*** The maternal serum concentration of PP13 at 11–13 weeks was compared in 536 euploid and 134 aneuploid pregnancies (trisomy 21: n = 49; trisomy 18: n = 28; trisomy 13: n = 19; Turner syndrome: n = 28; triploidy: n = 10). ***Results:*** Serum PP13, expressed as multiples of the median (MoM) of the euploid group, was not significantly different in trisomy 21 (1.12 MoM) pregnancies, but the levels were decreased in trisomy 18 (0.75 MoM), trisomy 13 (0.65 MoM), Turner syndrome (0.61 MoM) and triploidy (0.19 MoM). In both euploid and aneuploid pregnancies there was a significant association of serum PP13 with both serum pregnancy-associated plasma protein A (PAPP-A) and free β-human chorionic gonadotropin (β-hCG; $p < 0.0001$ for both). Logistic regression analysis demonstrated that the addition of serum PP13 did not improve the prediction of trisomy 13 and 18 provided by a combination of maternal age, nuchal translucency, and serum free β-hCG and PAPP-A. ***Conclusion:*** The measurement of maternal serum PP13 at 11–13 weeks does not improve the performance of screening for aneuploidies achieved by current algorithms.

Introduction

Placental protein 13 (PP13), which is produced by trophoblasts, is believed to play an important role in implantation and placental development [1–3]. Reduced maternal serum levels of PP13 in the first trimester have been reported in pregnancies that develop preeclampsia and fetal growth restriction [4–8].

In pregnancies with fetal aneuploidies, there is evidence from histological studies that there is abnormal placentation characterized by trophoblastic hypoplasia, stromal oedema and reduced vascularity [9–11]. This is reflected in altered maternal serum concentrations of placental proteins such as pregnancy-associated plasma protein A (PAPP-A) and free β-human chorionic gonadotropin (β-hCG), which are established markers of fetal aneuploidies in the first trimester of pregnancy [12, 13].

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The aims of this study were, firstly, to investigate whether the maternal serum concentration of PP13 at 11–13 weeks of gestation is altered in chromosomally abnormal pregnancies; secondly, to examine any possible association with maternal serum PAPP-A, free β-hCG and fetal nuchal translucency (NT) thickness, and thirdly, to examine the potential value of this placental protein in screening for fetal aneuploidies.

Methods

Study Population

This was a case-control study drawn from a large prospective study to identify potential biomarkers of pregnancy complications in women attending for their routine first hospital visit in pregnancy at King's College Hospital, London, UK. On this visit, which is held at 11+0 to 13+6 weeks of gestation, all women have an ultrasound scan to, firstly, confirm the gestational age by measurement of the fetal crown-rump length (CRL), secondly, diagnose any major fetal abnormalities and, thirdly, measure the fetal NT thickness as part of screening for chromosomal abnormalities. In addition, the maternal serum PAPP-A and free β-hCG are determined and the results combined with maternal age and fetal NT to calculate the patient-specific risk of trisomy 21 [12, 14].

Maternal demographic characteristics, ultrasonographic measurements and biochemical results were recorded in a computer database. Karyotype results and details on pregnancy outcomes were added into the database as soon as they became available. 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. They agreed that aliquots of their serum used for the measurement of free β-hCG and PAPP-A could be stored at –80°C for future studies.

The case-control study population comprised 49 cases with fetal trisomy 21, 28 cases with trisomy 18, 19 cases with trisomy 13, 28 cases with Turner syndrome, 10 cases with triploidy and 536 euploid controls. All 10 cases of triploidy had the phenotype for digynic triploidy characterized by a thin but normal-looking placenta with severe asymmetrical fetal growth restriction. Each case was matched with 4 controls who had blood collected within 6 months of each other, had a normal karyotype and delivered a phenotypically normal neonate. None of the samples in the case-control study had previously been thawed and refrozen.

Sample Analysis

DELFIA® (Dissociation-Enhanced Lanthanide Fluorescent Immunoassay) research reagents (Perkin Elmer Life and Analytical Sciences, Turku, Finland) were used to measure PP13 in maternal serum samples (25 μl/well in duplicate). The measured concentration of PP13 was directly proportional to the fluorescence measured on a time-resolved fluorometer at 615 nm. The coefficient of variation was 4.1% at a PP13 concentration of 16.6 pg/ml, 2.0% at 60.4 pg/ml and 2.7% at 136.2 pg/ml. Samples with duplicate coefficients of variation of >10% were reanalysed.

Statistical Analysis

The distribution of PP13 was made gaussian after logarithmic transformation, and normality was confirmed using the Kolmogorov-Smirnov test. Multiple regression analysis was used to determine which of the factors amongst the maternal characteristics and gestation were significant predictors of log PP13 in the euploid group. Then the distribution of log PP13, expressed as multiples of the median (MoM) of the euploid group, was determined in the different aneuploid groups. The measured PAPP-A and free β-hCG were converted into MoM after adjustment for gestation, racial origin, weight, parity, cigarette smoking status and method of conception, as previously described [15]. In each euploid and aneuploid case, the measured NT was expressed as a difference from the expected normal mean for CRL (Δ) [16]. The Mann-Whitney U test with post hoc Bonferroni correction was used to compare the median MoM of PP13, free β-hCG, PAPP-A and ΔNT between the outcome groups. Regression analysis was used to determine the significance of the association of log PP13 MoM with log free β-hCG MoM, log PAPP-A MoM and ΔNT in the different outcome groups. Logistic regression analysis was used to determine if the addition of PP13 MoM improved the screening performance for chromosomal aneuploidies that is achieved by maternal age, fetal NT, maternal serum PAPP-A and free β-hCG. The detection and false-positive rates were calculated as the respective proportions of aneuploid pregnancies (detection rate) to euploid controls (false-positive rate) with MoM values above given cutoffs. The performance of the screening was determined by receiver operating characteristic curve analysis.

The statistical software package SPSS 16.0 (SPSS Inc., Chicago, Ill., USA) and XLSTAT-Pro 2008 (Addinsoft, New York, N.Y., USA) were used for data analyses.

Results

The maternal characteristics of the cases and controls are compared in table 1.

Serum PP13 in Euploid and Aneuploid Pregnancies

In the euploid group, multiple regression analysis demonstrated that for log PP13, significant independent contributions were provided by maternal weight and smoking status but not by racial origin ($p = 0.830$), parity ($p = 0.602$), method of conception ($p = 0.982$) or fetal CRL ($p = 0.944$): log expected PP13 = 2.070 + 0.003 × maternal weight in kg + (–0.265 if smoker, 0 if non-smoker) ($R^2 = 0.149$; $p < 0.0001$).

In pregnancies with fetal trisomy 21, compared to the euploid group, the median free β-hCG and fetal NT were increased, PAPP-A was decreased but PP13 was not significantly different (table 2). The median levels of PP13 in trisomy 18, trisomy 13, Turner syndrome and triploidy were significantly lower than in the euploid group (table 2).

Table 1. Maternal characteristics in cases and euploid controls

	Euploid (n = 536)	Trisomy 21 (n = 49)	Trisomy 18 (n = 28)	Trisomy 13 (n = 19)	Turner syndrome (n = 28)	Triploidy (n = 10)
Median maternal age, years	32.4 (28.7–36.2)	38.1 (34.9–41.0)*	37.9 (34.1–40.3)*	34.8 (33.5–38.8)	29.9 (25.4–34.6)	31.9 (29.5–33.9)
Median maternal weight, kg	65.0 (59.0–73.0)	68.0 (60.0–75.7)	71.4 (61.8–79.0)	72.0 (60.0–79.5)	66.9 (57.4–70.3)	65.7 (59.5–68.8)
Median crown-rump length, mm	64.1 (59.7–69.8)	63.4 (58.7–69.3)	57.7 (51.6–61.1)*	60.1 (55.1–66.6)	64.6 (56.3–67.8)	58.4 (50.9–64.3)
Racial origin, n						
White	391 (72.9%)	43 (87.8%)	19 (67.9%)	15 (78.9%)	26 (92.9%)	8 (80.0%)
Black	83 (15.5%)	2 (4.1%)*	4 (14.3%)	2 (10.4%)	2 (7.1%)	2 (20.0%)
Indian or Pakistani	31 (5.8%)	3 (6.1%)	4 (14.3%)	1 (5.3%)	0	0
Chinese or Japanese	11 (2.1%)	1 (2.0%)	0	0	0	0
Mixed	20 (3.7%)	0	1 (3.6%)	1 (5.3%)	0	0
Nulliparous, n	263 (49.1%)	19 (38.8%)	12 (42.9%)	4 (21.1%)	13 (46.4%)	7 (70.0%)
Cigarette smoker, n	25 (4.7%)	5 (10.2%)	1 (3.6%)	1 (5.3%)	2 (7.1%)	1 (10.0%)
Conception, n						
Spontaneous	520 (97.0%)	23 (46.9%)	12 (42.9%)	15 (78.9%)	18 (64.3%)	8 (80.0%)
Ovulation drugs	10 (1.9%)	25 (51.0%)*	16 (57.1%)*	4 (21.1%)*	10 (35.7%)*	2 (20.0%)
In vitro fertilization	6 (1.1%)	1 (2.0%)	0	0	0	0

Values in parentheses denote interquartile ranges unless otherwise specified. * $p < 0.01$, comparison with euploid group (χ^2 test or Fisher's exact test for categorical variables and Mann-Whitney U test with post hoc Bonferroni correction for continuous variables).

Table 2. Medians of maternal serum PP13 MoM, free β-hCG MoM, PAPP-A MoM and ΔNT in euploid and chromosomally abnormal pregnancies

Outcome	PP13 MoM	Free β-hCG MoM	PAPP-A MoM	ΔNT, mm
Euploid	1.00 (0.76–1.30)	1.00 (0.68–1.47)	1.02 (0.71–1.37)	0.10 (–0.11–0.34)
Trisomy 21	1.12 (0.84–1.44)	2.26 (1.42–3.50)**	0.47 (0.34–0.70)**	2.21 (1.18–4.33)**
Trisomy 18	0.75 (0.54–1.02)**	0.19 (0.14–0.30)**	0.17 (0.14–0.25)**	4.17 (1.03–6.00)**
Trisomy 13	0.65 (0.50–0.80)**	0.39 (0.27–0.48)**	0.25 (0.20–0.32)**	2.92 (0.36–4.80)**
Turner syndrome	0.61 (0.48–0.93)**	0.97 (0.59–1.76)	0.53 (0.41–0.82)**	8.14 (6.75–10.82)**
Triploidy	0.19 (0.10–1.06)*	0.13 (0.04–0.34)**	0.07 (0.04–0.11)**	0.24 (0.05–0.69)

Values in parentheses denote interquartile ranges.
* $p < 0.01$, ** $p < 0.0001$, comparison with euploid group (Mann-Whitney U test with post hoc Bonferroni correction).

Association between PP13 and PAPP-A, free β-hCG and NT

In the euploid group, the mean log PP13 MoM was 0.0 with a standard deviation of 0.175. There was a significant association of log PP13 MoM with log PAPP-A MoM ($r = 0.271$; $p < 0.0001$) and log free β-hCG MoM ($r = 0.338$; $p < 0.0001$), but not with ΔNT ($p = 0.095$).

In the aneuploid pregnancies there was a significant association of log PP13 MoM with log PAPP-A MoM ($r = 0.469$; $p < 0.0001$) and log free β-hCG MoM ($r = 0.526$; $p < 0.0001$), but not with ΔNT ($p = 0.540$). Multiple regression analysis demonstrated that in predicting log PP13 MoM there were significant contributions from log free β-hCG MoM ($p < 0.0001$) and log PAPP-A MoM ($p < 0.0001$), but not of ΔNT ($p = 0.393$) or fetal karyotype ($p = 0.935$). Figures 1 and 2 illustrate the association of PP13 with PAPP-A and free β-hCG in both euploid and aneuploid pregnancies.

Screening for Chromosomal Aneuploidies

Multivariate logistic regression analysis demonstrated that in the prediction of trisomy 13 and 18 there were significant contributions from maternal age (OR = 1.31; 95% CI: 1.08–1.58; $p = 0.006$), ΔNT (OR = 6.40; 95% CI: 2.36–17.37; $p < 0.0001$), log PAPP-A MoM ($OR = 7.21 \cdot 10^{-5}$; 95% CI from $5.89 \cdot 10^{-7}$ to 0.01; $p < 0.0001$) and log free β-hCG

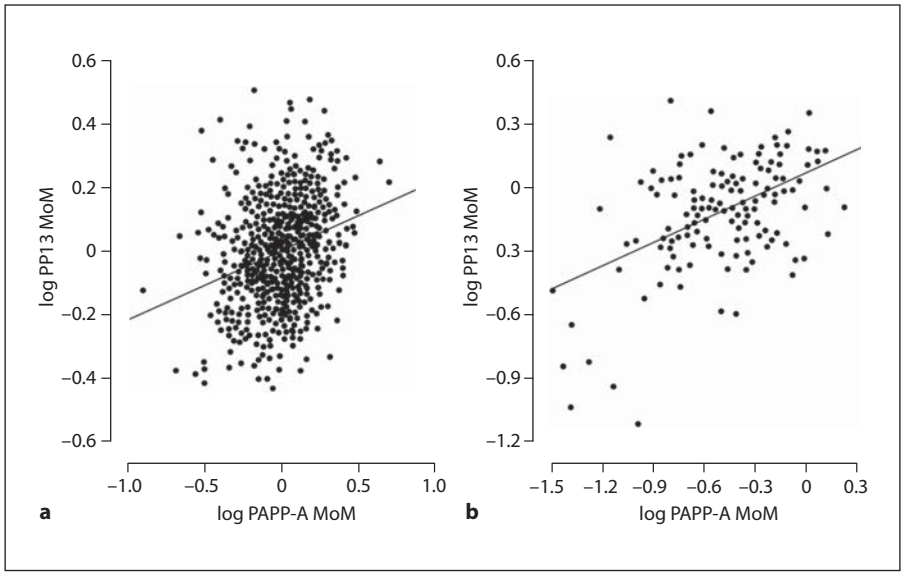

Fig. 1. Association of maternal serum PP13 MoM with PAPP-A MoM in euploid (**a**) and aneuploid (**b**) pregnancies.

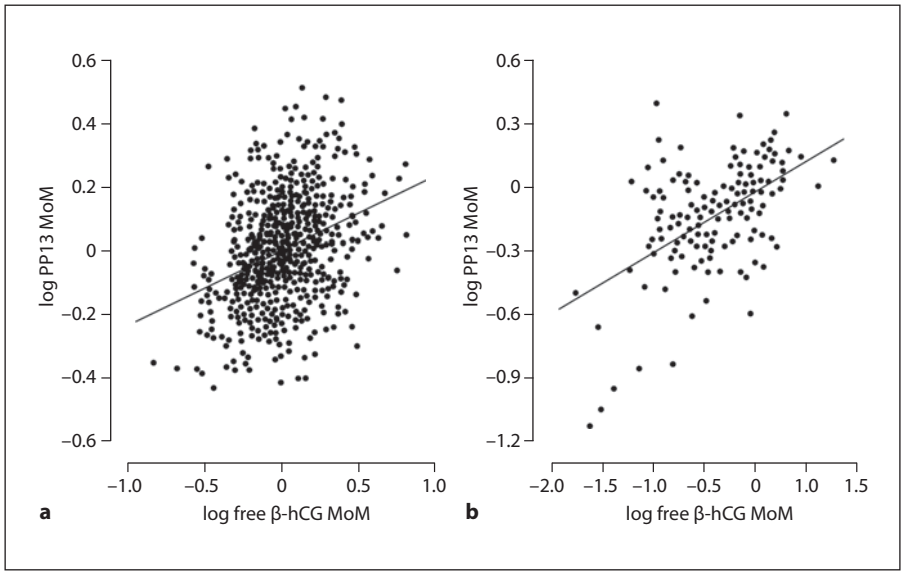

Fig. 2. Association of maternal serum PP13 MoM with free β-hCG MoM in euploid (**a**) and aneuploid (**b**) pregnancies.

Table 3. Detection rates of trisomy 13 and 18 at fixed false-positive rates and comparison of screening performance by receiver operating characteristic curve analysis in screening by maternal age, NT, PAPP-A, free β-hCG and PP13, and by their combinations

Screening test	FPR 3%	FPR 5%	AUROC
Maternal age and NT	70.2	72.3	0.939 (0.897–0.981)
PP13	19.1	25.5	0.733 (0.660–0.807)
Free β-hCG and PAPP-A	91.5	93.6	0.986 (0.974–0.997)
Maternal age and NT plus			
PP13	78.7	85.1	0.964 (0.930–0.998)
Free β-hCG and PAPP-A	95.7	97.9	0.997 (0.993–1.001)

Values in parentheses denote 95% CI. FPR = False-positive rate; AUROC = area under receiver operating characteristic curve.

MoM (OR = $1.46 \cdot 10^{-3}$; 95% CI from $3.03 \cdot 10^{-5}$ to 0.07; $p < 0.0001$), but not from log PP13 MoM ($p = 0.091$).

The estimated detection rates of trisomy 13 and 18 at fixed false-positive rates of 3 and 5%, respectively, and their respective areas under the receiver operating characteristic curves in screening by maternal age, ΔNT, serum PAPP-A and free β-hCG, and by their combinations, are shown in table 3.

Discussion

The findings of this study demonstrate a significant association between maternal serum PP13 levels and free β-hCG and PAPP-A in both euploid and aneuploid pregnancies. All three analytes are placental products, and immunohistochemical studies have localized hCG and PP13 to the syncytiotrophoblast [3, 17, 18] and PAPP-A to the villous cytotrophoblast [19]. In digynic triploidy and trisomies 18 and 13, all three analytes are decreased, whereas in trisomy 21, where free β-hCG is increased and PAPP-A is decreased, PP13 is not significantly altered. Serum PP13 is also decreased in Turner's syndrome where PAPP-A is decreased but free β-hCG is not significantly different from euploid pregnancies. The findings are similar to those of a recent study by Koster et al. [20] that reported normal levels in trisomy 21 (0.91 MoM) and low levels in trisomy 18 (0.64 MoM) and trisomy 13 (0.46 MoM).

In the euploid controls, the measured concentration of maternal serum PP13 decreased with maternal weight and was lower in smokers compared to non-smokers. Consequently, as in the case of serum PAPP-A and free β-hCG, the measured concentration of PP13 must be adjusted for these variables before comparing it with pathological pregnancies [15]. The association of maternal serum PP13 concentration with maternal weight and smoking had also been reported in previous studies [4, 20, 21]. There is evidence from a longitudinal study at 5–40 weeks of gestation in 41 unaffected pregnancies that the maternal serum concentration of PP13 increases with gestational age and then declines to undetectable levels 8–12 weeks after delivery [22]. In both our study [4] and a previous one by Cowans et al. [21], there was no significant change in serum PP13 with the fetal CRL within the narrow gestational range of 11–13 weeks.

Effective screening for trisomy 21 is provided by a combination of maternal age, fetal NT thickness and maternal serum free β-hCG and PAPP-A at 11–13 weeks of gestation [12, 14, 23]. At 11–13 weeks, the prevalence ratios of trisomies 18 and 13 to trisomy 21 are 1:3 and 1:7, respectively [24–26]. All three trisomies are associated with increased maternal age, increased fetal NT and decreased maternal serum PAPP-A, but in trisomy 21 serum free β-hCG is increased, whereas in trisomies 18 and 13 this is decreased [14, 16, 23–28]. In addition, trisomy 13, unlike trisomies 21 and 18, is associated with fetal tachycardia [29–31].

A beneficial consequence of screening for trisomy 21 is the early diagnosis of trisomies 18 and 13. At a 3% false-positive rate, the estimated detection rates of trisomies 21, 18 and 13 – using the algorithm for trisomy 21 – are 90, 74 and 77%, respectively. When specific algorithms for trisomies 18 and 13 in addition to the one for trisomy 21 are also used, about 90% of the fetuses with trisomy 21 and 95% of those with trisomies 13 and 18 can be detected at an overall false-positive rate of 3.1% [13]. Although in both trisomies 18 and 13 maternal serum PP13 is decreased, there are strong associations with serum free β-hCG and PAPP-A, and PP13 does not improve the existing method of early screening for these aneuploidies.

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