Ultrasound Obstet Gynecol 2009; **33**: 382–386
Published online 10 March 2009 in Wiley InterScience (www.interscience.wiley.com). **DOI:** 10.1002/uog.6331

Maternal serum placental growth factor at 11–13 weeks in chromosomally abnormal pregnancies

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KEYWORDS: aneuploidy; first-trimester screening; placental growth factor; trisomy 21

ABSTRACT

Objectives *To investigate the potential value of maternal serum placental growth factor (PlGF) in first-trimester screening for trisomy 21 and other major chromosomal abnormalities.*

Methods *The maternal serum concentration of PlGF at 11 + 0 to 13 + 6 weeks was measured in 609 euploid and 175 chromosomally abnormal pregnancies, including 90 with trisomy 21, 28 with trisomy 18, 19 with trisomy 13, 28 with Turner syndrome and 10 with triploidy. The levels of PlGF were compared in cases and controls, and were assessed for association with free β-human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein-A (PAPP-A).*

Results *Logistic regression analysis demonstrated in the euploid group that significant independent contributions for log PlGF were provided by fetal crown–rump length, maternal weight, cigarette smoking and ethnic origin; after correction for these variables the median multiple of the median (MoM) PlGF was 0.991. Significantly lower values were observed in pregnancies with trisomy 21 (0.707 MoM), trisomy 18 (0.483 MoM), trisomy 13 (0.404 MoM), triploidy (0.531 MoM) and Turner syndrome (0.534 MoM). Significant contributions in the prediction of trisomy 21 were provided by maternal age, serum PlGF, PAPP-A and free β-hCG, and the detection rates of screening with the combination of these variables were 70% and 80% at respective false-positive rates of 3% and 5%.*

Conclusions *Maternal serum PlGF concentration at 11–13 weeks of gestation is potentially useful in first-trimester screening for trisomy 21 and other major chromosomal abnormalities. Copyright © 2009 ISUOG. Published by John Wiley & Sons, Ltd.*

INTRODUCTION

Placental growth factor (PlGF), a member of the vascular endothelial growth factor family, is a glycosylated dimeric glycoprotein that is expressed only in the placenta. It is an important local mediator of angiogenesis and it may also play a role in regulating trophoblastic invasion of the maternal spiral arteries[1–3]. Four studies investigating the maternal serum concentrations of PlGF in pregnancies with fetal trisomy 21 during the first or second trimester have reported that the levels are decreased, increased or the same in trisomy 21 compared with controls (Table 1)[4–7]. One study reported that serum PlGF is reduced in trisomy 18 pregnancies[4].

The aims of this study were, first, to investigate further the maternal serum concentration of PlGF at 11–13 weeks of gestation in chromosomally abnormal fetuses and, second, to examine whether measurement of PlGF can improve the performance of first-trimester biochemical screening for trisomy 21 provided by maternal serum free β-human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein-A (PAPP-A)[8].

METHODS

Study population

This was a case–control study. In our center we performed screening for chromosomal abnormalities by a combination of maternal age, fetal nuchal translucency (NT) thickness and maternal serum free β-hCG and PAPP-A at 11 + 0 to 13 + 6 weeks of gestation[9,10]. Written informed consent was obtained from the women agreeing to participate in a research study to identify potential biomarkers of pregnancy complications, which was approved by the King's College Hospital Ethics Committee. They agreed to aliquots of their serum used

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Accepted: 22 December 2008

Table 1 Studies reporting on maternal serum levels of placental growth factor in euploid and trisomy 21 pregnancies

		Trisomy 21		*Euploid controls*		
Reference	*Gestation (weeks)*	n	*Median MoM*	n	*Median MoM*	P
Spencer *et al.* 2001[4]	10–13	45	1.26	493	1.0	< 0.0001
Debieve *et al.* 2001[5]	15–20	24	0.69	102	0.89	< 0.001
Su *et al.* 2002[6]	14–21	36	1.45	320	1.0	< 0.001
Lambert-Messerlian and Canick 2004[7]	15–20	39	1.01	195	1.0	NS

MoM, multiples of the median; NS, not significant.

for measurement of free β-hCG and PAPP-A being stored at −80°C for future studies.

Transabdominal ultrasound examination was performed to diagnose any major fetal defects, and for measurement of fetal NT and crown–rump length (CRL). Automated machines that provide reproducible results within 30 min were used to measure PAPP-A and free β-hCG (Delfia Xpress system, PerkinElmer Life and Analytical Sciences, Waltham, MA, USA). Maternal demographic characteristics, ultrasonographic measurements and biochemical results were recorded in a computer database. Karyotype results and details of pregnancy outcomes were added to the database as soon as they became available.

The case–control study population comprised 175 cases with fetal chromosomal abnormalities and 609 controls with no pregnancy complications resulting in the live birth of phenotypically normal neonates. The cases and controls were matched for length of storage of blood samples; none of the samples was previously thawed and refrozen.

Sample analysis

Duplicate serum samples of 100 μL were used to measure PlGF concentration by a quantitative enzyme-linked immunoassay (ELISA) technique using Quantikine® human PlGF immunoassay (R&D systems Europe Ltd., Abingdon, UK). The assays were performed on an automated ELISA processor (Dade-Behring BEP 2000, Liederbach, Germany). Absorbance readings were taken on a VICTOR3™ plate reader (PerkinElmer Life and Analytical Sciences, Turku, Finland) and PlGF concentrations were determined using MultiCalc software (PerkinElmer Life and Analytical Sciences). The lower limit of detection of the assay was 7 pg/mL and lower levels were observed in only two of our 784 samples. The between-batch imprecision was 8.3% at a PlGF concentration of 48 pg/mL, 5.6% at 342 pg/mL and 5.1% at 722 pg/mL. Duplicate samples whose coefficient of variation exceeded 15% were reanalyzed.

Statistical analysis

In each case and control the measured free β-hCG, PAPP-A and PlGF were converted into multiples of the median (MoM) after adjustment for gestation, maternal age, ethnicity, weight, parity and method of conception as described previously[8,11]. A box and whisker plot of PlGF MoM of cases and controls was created. Mann–Whitney test was used to determine the significance of differences in the median MoM between each chromosomally abnormal group and controls. Regression analysis was used to determine the significance of the association between PlGF MoM and free β-hCG MoM and PAPP-A MoM. Similarly, the measured NT was expressed as a difference with respect to the expected normal mean for gestation (delta value) and regression analysis was then used to determine the significance of association between PlGF MoM and delta NT.

Logistic regression analysis was used to determine whether significant contributions to the detection of trisomy 21 were provided by maternal age, free β-hCG, PAPP-A and PlGF. The performance of screening was determined by means of receiver–operating characteristics (ROC) curves. The statistical software package SPSS version 15.0 (SPSS Inc., Chicago, IL, USA) was used for all data analyses.

RESULTS

There were 90 singleton pregnancies with trisomy 21, 28 with trisomy 18, 19 with trisomy 13, 28 with Turner syndrome and 10 with triploidy. All 10 cases of triploidy had the phenotype of digynic triploidy characterized by a thin but normal looking placenta with severe asymmetrical fetal growth restriction. The characteristics of cases and controls are compared in Table 2.

Euploid

In the euploid group, the mean ± SD log PlGF MoM was -0.004 ± 0.171. There was a significant association between log PlGF MoM and log PAPP-A MoM ($r = 0.264$, $P < 0.0001$) (Figure 1) and log free β-hCG MoM ($r = 0.183$, $P < 0.0001$), but not with delta NT ($r = 0.078$, $P = 0.054$).

Trisomy 21

In pregnancies with trisomy 21 the median free β-hCG and fetal NT were significantly higher, and PAPP-A and PlGF were significantly lower, than those in the euploid group (Figure 2 and Table 3). In trisomy 21 pregnancies, the mean log PlGF MoM ± SD was -0.150 ± 0.181.

Table 2 Characteristics in cases and euploid controls

Characteristic	*Control* (n = 609)	*Trisomy 21* (n = 90)	*Trisomy 18* (n = 28)	*Trisomy 13* (n = 19)	*Turner syndrome* (n = 28)	*Triploidy* (n = 10)
Age (years)	32.7 (16.1–45.2)	37.9 (19.1–46.5)‡	37.9 (25.3–42.6)‡	34.8 (29.6–44.6)†	29.9 (18.1–37.9)*	31.9 (20.8–37.6)
Maternal weight (kg)	65.0 (42–143)	66.5 (42–109)	71.4 (52–90)	72.0 (52–85)	66.9 (39–114)	65.7 (50–89)
Fetal crown–rump length (mm)	64.0 (45–84)	65 (47–84)	57.7 (47–71)‡	60.1 (51–73)*	64.6 (50–79)	58.4 (45–74)*
Racial origin						
White	441 (72.4)	81 (90.0)‡	19 (67.9)	15 (78.9)	26 (92.9)*	8 (80.0)
Black	99 (16.3)	4 (4.4)†	4 (14.3)	2 (10.5)	2 (7.1)	2 (20.0)
Indian or Pakistani	34 (5.6)	3 (3.3)	4 (14.3)	1 (5.3)	0 (0)	0 (0)
Chinese or Japanese	13 (2.1)	1 (1.1)	0 (0)	0 (0)	0 (0)	0 (0)
Mixed	22 (3.6)	1 (1.1)	1 (3.6)	1 (5.3)	0 (0)	0 (0)
Nulliparous	277 (45.5)	28 (31.1)*	12 (42.9)	4 (21.1)*	13 (46.4)	7 (70.0)
Cigarette smoker	31 (5.1)	6 (6.7)	1 (3.6)	1 (5.3)	2 (7.1)	1 (10.0)
Conception						
Spontaneous	594 (97.5)	64 (71.1)‡	12 (42.9)‡	15 (78.9)†	18 (64.3)‡	8 (80.0)*
Ovulation drugs	10 (1.6)	25 (27.8)‡	16 (57.1)‡	4 (21.1)†	10 (35.7)‡	2 (20.0)*
In-vitro fertilization	5 (0.8)	1 (1.1)	0 (0)	0 (0)	0 (0)	0 (0)

Values are median (range) or *n* (%). *$P < 0.05$, †$P < 0.01$, ‡$P < 0.0001$ vs. euploid group (chi-square test for categorical variables and ANOVA for continuous variables).

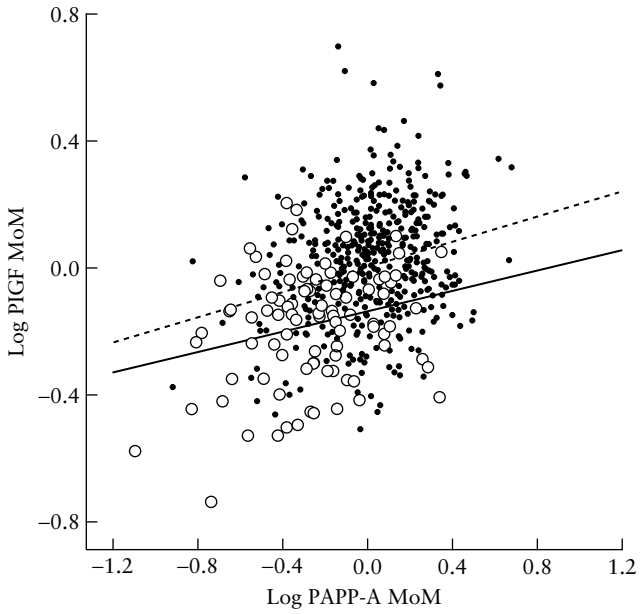

Figure 1 Relationship between log placental growth factor (PlGF) multiples of the median (MoM) and log pregnancy-associated plasma protein-A (PAPP-A) MoM in euploid (•, regression line – – –) and trisomy 21 (○, regression line ———) pregnancies.

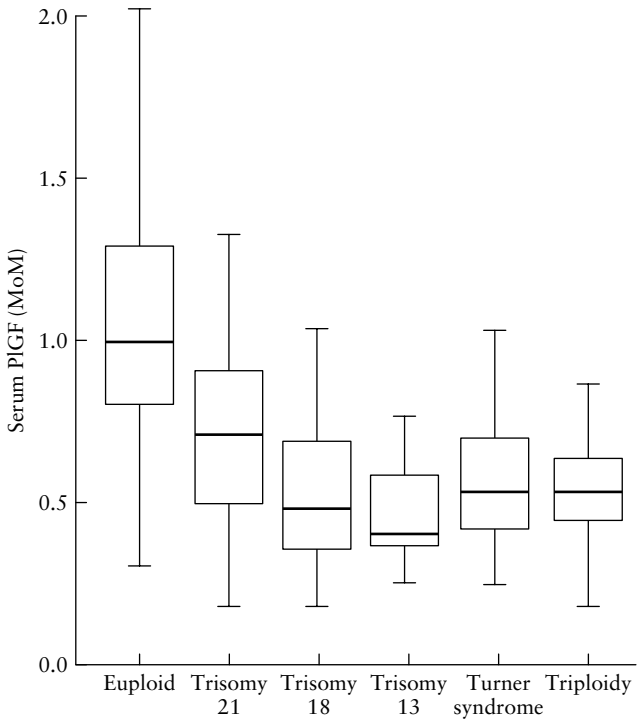

Figure 2 Box-and-whisker plot of placental growth factor (PlGF) multiples of the median (MoM) in euploid, trisomy 21, trisomy 18, trisomy 13, Turner syndrome and triploidy cases. Median, interquartile range and range are shown.

There was a significant association between log PlGF MoM and log PAPP-A MoM ($r = 0.246$, $P = 0.020$) (Figure 1) but not with log free β-hCG MoM ($r = 0.048$, $P = 0.652$) or delta NT ($r = 0.203$, $P = 0.055$). There

Table 3 Maternal serum placental growth factor (PlGF), free β-human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein-A (PAPP-A) multiples of the median (MoM), and delta nuchal translucency (NT) in euploid and chromosomally abnormal pregnancies

Karyotype	*PlGF MoM*	*Free β-hCG MoM*	*PAPP-A MoM*	*Delta NT (mm)*
Euploid	0.991 (0.799–1.286)	0.980 (0.686–1.467)	1.070 (0.735–1.455)	0.1 (−0.1 to 0.3)
Trisomy 21	0.707 (0.493–0.904)*	2.530 (1.550–3.725)*	0.550 (0.376–0.805)*	2.2 (1.2–3.8)*
Trisomy 18	0.483 (0.352–0.701)*	0.187 (0.142–0.300)*	0.173 (0.142–0.246)*	4.1 (1.0–6.0)*
Trisomy 13	0.404 (0.369–0.596)*	0.388 (0.273–0.482)*	0.252 (0.203–0.321)*	2.9 (0.3–4.7)*
Turner syndrome	0.534 (0.410–0.717)*	0.965 (0.593–1.755)	0.531 (0.409–0.820)*	8.1 (6.7–10.8)*
Triploidy	0.531 (0.437–0.668)*	0.130 (0.036–0.336)*	0.060 (0.041–0.080)*	0.1 (−0.0 to 0.7)

Values are median (interquartile range). *$P < 0.0001$ vs. euploid group (Mann–Whitney test).

Table 4 Logistic regression analysis for the prediction of trisomy 21 by a combination of maternal age, pregnancy-associated plasma protein-A (PAPP-A), free β-human chorionic gonadotropin (β-hCG) and placental growth factor (PlGF)

Independent variable	*Odds ratio (95% CI)*	P
Maternal age	1.190 (1.116–1.269)	< 0.0001
Log PAPP-A MoM	0.027 (0.006–0.115)	< 0.0001
Log β-hCG MoM	671.150 (150.215–2998.655)	< 0.0001
Log PlGF MoM	0.001 (0.000–0.013)	< 0.0001

MoM, multiples of the median.

was no significant association between log PlGF MoM and fetal CRL ($r = 0.004$, $P = 0.973$).

Logistic regression analysis demonstrated that significant contributions to the detection of trisomy 21 were provided by maternal age, free β-hCG, PAPP-A and PlGF ($R^2 = 0.662$; $P < 0.0001$) (Table 4). The areas under the ROC curves and detection rates of trisomy 21 for different false-positive rates in screening by maternal age, serum PAPP-A, serum free β-hCG, serum PlGF and by their combinations are given in Table 5.

Other aneuploidies

The median levels of PlGF in trisomy 18, trisomy 13, Turner syndrome and triploidy were significantly lower than in the euploid group (Figure 2 and Table 3). The mean ± SD log PlGF MoM was -0.293 ± 0.190. There was no significant association either in each individual chromosomal abnormality or in the combined group between log PlGF MoM and log PAPP-A MoM ($r = 0.171$, $P = 0.119$), log free β-hCG MoM ($r = 0.093$, $P = 0.396$) or delta NT ($r = 0.402$, $P = 0.701$).

DISCUSSION

The findings of this study demonstrate that the maternal serum concentration of PlGF is decreased at 11–13 weeks of gestation in trisomy 21 as well as other major chromosomal abnormalities. Measurement of PlGF can improve the performance of first-trimester biochemical screening for trisomy 21 provided by maternal serum free β-hCG and PAPP-A.

In euploid pregnancies serum PlGF increases with fetal CRL and therefore gestational age, decreases with maternal weight, and is higher in Black than in White women and in cigarette smokers than in non-smokers[11]. Consequently, as in the case of PAPP-A[8], the measured concentration of PlGF was adjusted for these variables before comparing results with pathological pregnancies. The results for trisomy 21 contradict those of previous smaller studies that did not adjust the measured values for maternal variables and reported that the levels in affected pregnancies were either increased or not significantly different from normal control values[4,6,7]. Other possible explanations for the discrepant results are differences

Table 5 Performance of maternal age, free β-human chorionic gonadotropin (β-hCG), pregnancy-associated plasma protein-A (PAPP-A) and placental growth factor (PlGF) multiples of the median in the detection of trisomy 21

Screening test	*Mean (95% CI) area under ROC curve*	*Detection rate (%) for fixed false-positive rate of:* 3%	5%
Maternal age	0.759 (0.703–0.815)	20.0	30.0
PlGF	0.775 (0.725–0.824)	22.2	27.8
Maternal age and PlGF	0.843 (0.796–0.889)	32.2	43.3
Free β-hCG and PAPP-A	0.912 (0.876–0.949)	60.0	67.8
Maternal age, free β-hCG and PAPP-A	0.926 (0.892–0.960)	71.1	76.7
Free β-hCG, PAPP-A and PlGF	0.935 (0.905–0.964)	66.7	72.2
Maternal age, free β-hCG, PAPP-A and PlGF	0.946 (0.918–0.973)	70.0	80.0

ROC, receiver–operating characteristics.

in assay methods and gestational range of the study populations.

In both the euploid and trisomy 21 pregnancies there was a significant association between serum levels of PlGF and PAPP-A, which presumably reflects the postulated roles of these peptides in placental development and/or their common origin from trophoblasts. However, in the trisomy 21 pregnancies there was no significant change in serum PlGF with fetal CRL, indicating that the deviation between trisomic and euploid pregnancies is the same at 11 and 13 weeks. In contrast, the deviation in serum PAPP-A between trisomic and euploid pregnancies is substantially greater at 11 weeks than at 13 weeks[8].

In first-trimester biochemical screening for trisomy 21 there were significant independent contributions from maternal age and serum PlGF, PAPP-A and free β-hCG. It was estimated that screening by a combination of maternal age and these three biochemical markers would identify about 70% and 80% of affected pregnancies at respective false-positive rates of 3% and 5%. These results require validation by prospective studies that will also investigate the potential improvement provided by serum PlGF to the established method of combined screening by fetal NT and serum PAPP-A and free β-hCG. The serum level of PlGF in trisomy 18, trisomy 13, Turner syndrome and triploidy is lower than in pregnancies with euploid fetuses and lower than in those with trisomy 21. It is therefore anticipated that a beneficial consequence of incorporating PlGF in first-trimester combined screening for trisomy 21 would be the detection of a high proportion of the other major aneuploidies.

ACKNOWLEDGMENTS

This study was supported by a grant from The Fetal Medicine Foundation (UK charity number 1037116). The assays were performed by Fiona Tulloch and Keith Burling, Department of Clinical Biochemistry, Addenbrookes NHS Trust, Cambridge, UK, and were sponsored by PerkinElmer Life and Analytical Sciences, Wallac Oy, Turku, Finland.

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