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First trimester maternal serum placental growth factor in trisomy 21 pregnancies

N. J. Cowans, A. Stamatopoulou and K. Spencer*

Prenatal Research Unit, Clinical Biochemistry Department, King George Hospital, Goodmayes, UK

Objective To examine placental growth factor (PlGF) levels in first trimester maternal serum in trisomy 21 pregnancies and to investigate the potential value of PlGF in a first trimester screening test.

Methods First trimester maternal serum from 70 trisomy 21 cases and 375 euploid controls were retrospectively analyzed for PlGF using a DELFIA® Xpress immunoassay platform. Results were expressed as multiples of medians (MoM) for comparison.

Results PlGF levels were significantly decreased in pregnancies with trisomy 21, 0.76 MoM versus 0.98 MoM in controls. Inclusion of PlGF into the first trimester combined test [maternal age, pregnancy associated plasma protein-A (PAPP-A), free-β human chorionic gonadotrophin (β-hCG) and nuchal translucency] would increase the detection rate by 0.5% at a 5% false positive rate.

Conclusion PlGF at 11 weeks to 13 weeks 6 days has the potential to be included as a marker for the detection of pregnancies with trisomy 21. Copyright © 2010 John Wiley & Sons, Ltd.

KEY WORDS: PlGF; screening; trisomy 21; first trimester; aneuploidy; PAPP-A; free β-hCG; nuchal translucency

INTRODUCTION

Placental growth factor (PlGF) is a dimeric glycoprotein that belongs to the angiogenic vascular growth factor family. It is predominantly expressed in trophoblasts and it plays an important role in placental development during pregnancy. Dysfunctions in the production and expression of the molecule lead to impaired trophoblast invasion and insufficient vascular remodeling of the spiral arteries (Maglione *et al*., 1991; Shore *et al*., 1997; Vuorela *et al*., 1997).

Maternal serum PlGF has been reported to be decreased in the first trimester in pregnancies which later develop preeclampsia (PE) or deliver small for gestational age (SGA) fetuses (Tidwell *et al*., 2001; Akolekar *et al*., 2008; Poon *et al*., 2008; Cowans *et al*., 2010). However, there are conflicting reports in the literature surrounding the use of maternal serum PlGF in the first trimester of aneuploidy pregnancies, summarized in Table 1 (Debieve *et al*., 2001; Spencer *et al*., 2001; Su *et al*., 2002; Lambert-Messerlian and Canick, 2004; Zaragoza *et al*., 2009). In particular, first trimester maternal serum PlGF has been reported both to increase (Spencer *et al*., 2001) and decrease (Zaragoza *et al*., 2009) in trisomy 21 pregnancies.

This study aims to investigate the maternal serum PlGF concentrations at 11 weeks to 13 weeks 6 days of gestation in trisomy 21 pregnancies compared to euploid controls. The current most widely used method for fetal chromosomal anomaly detection is the combined test which calculates risk of aneuploidy using maternal age and measurements of first trimester maternal serum pregnancy associated plasma protein-A (PAPP-A), and free-β human chorionic gonadotrophin (β-hCG) and ultrasound measurement of the nuchal translucency (NT) (Spencer *et al*., 2003c). This study also aims to determine the effect that inclusion of PlGF in a screening test might have on detection rates.

MATERIALS AND METHODS

Study population and screening

This was a retrospective case–control study in pregnancies which were screened between 11 weeks and 13 weeks 6 days of gestation at our center from January 2007 to October 2008.

Serum PAPP-A and free β-hCG concentrations were determined at the time of screening (Kryptor analyser, Brahms AG, Germany). Women had given written informed consent for surplus blood to be used for research, approved by our Institution Review Board. Following screening, serum samples were stored at $-20\,^{\circ}C$ awaiting use in research studies. Fetal NT and crown-rump length (CRL) measurements were taken by ultrasonographers with the Fetal Medicine Foundation Certificate of competence in the first trimester scan. The pregnancy was dated for screening purposes by use of the CRL. Maternal demographic data were obtained at the time of screening and recorded in a computer database. Karyotype results and pregnancy outcome were added to the database after being received from the cytogenetics laboratories and maternity units in which the deliveries happened.

Before measurement of PlGF, the samples used in this study had been thawed and re-frozen once for

*Correspondence to: K. Spencer, Clinical Biochemistry Department, King George Hospital, Barley Lane, Goodmayes IG3 8YB, UK. E-mail: kevinspencer1@aol.com

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Table 1—Summary of published reports of maternal serum PlGF in aneuploid pregnancies

Study	Gestational age range (weeks)	Euploid		Aneuploid		
		n	Median MoM	*n*	Median MoM	*p*
Trisomy 21						
Spencer *et al.* (2001)	10–13	493	1.00	45	1.26	<0.0001
Debieve *et al.* (2001)	15–20	102	0.89	24	0.69	<0.001
Su *et al.* (2002)	14–21	320	1.00	36	1.45	<0.001
Lambert-Messerlian and Canick (2004)	15–20	195	1.00	39	1.01	NS
Zaragoza *et al.* (2009)	11–14	609	0.99	90	0.71	<0.0001
Trisomy 18						
Spencer *et al.* (2001)	10–13	493	1.00	45	0.16	<0.0001
Zaragoza *et al.* (2009)	11–14	609	0.99	28	0.48	<0.0001
Trisomy 13						
Zaragoza *et al.* (2009)	11–14	609	0.99	19	0.40	<0.0001

p, from hypothesis test; NS, not significant.

use in previous study. Only patients of racial origin 'Caucassian' were selected due to the inclusion criteria of this previous study. The study population consisted of 70 cases of trisomy 21 and 375 euploid controls (5 per case), which were matched to the cases based on sample storage time (±1 day) and gestational age (±1 day). An additional four cases of trisomy 21 were excluded from the study due to incomplete patient records.

PlGF analysis

PlGF was analyzed using a new solid phase, two-site fluoroimmunometic research assay (4083-0010) on the 6000 DELFIA® Xpress random access platform (PerkinElmer, Turku, Finland). In the automated process, 40 μL of sample, calibrator or internal control are added along with 35 μL buffer and 5 μL tracer (europium-labeled anti-PlGF mouse monoclonal antibody) to a single cup bound with anti-PlGF rabbit polyclonal capture antibodies. Following a 20-min incubation at 35 °C in which protein–antibody complexes form, the cup is washed, 100 μL DELFIA® Inducer is added and the PlGF concentration is reported after the fluorescence has been measured.

Statistics

PAPP-A and free β-hCG concentrations were converted to multiples of the median (MoM) following adjustment for gestational age, maternal weight, smoking and ethnicity as previously described (Spencer *et al.*, 2003a, 2004, 2005). NT was incorporated into the risk algorithm by converting measured NT results to delta NT at the same CRL using the methodology described earlier (Spencer *et al.*, 2003b). PlGF MoMs were calculated after multiple regression was used to determine which gestational and maternal variables had an influencing effect on PlGF levels. Mann–Whitney testing was used to determine the significance of differences in medians between the euploid and trisomy 21 cases. Pearson Product Moment Correlation analysis was carried out to determine whether Log_{10} PlGF MoMs correlated with Log_{10} PAPP-A MoMs and Log_{10} free β-hCG MoMs.

Data were analyzed using R (GNU Project, www.r-project.org). Detection rates at fixed false positive rates were obtained using Monte Carlo methods to sample from the log biomarker MoMs and ΔNT in trisomy 21 and unaffected pregnancies using an R function (Wright, D. E., School of Mathematics and Statistics, University of Plymouth, UK, Personal Communication). The risk cut-off above which pregnancies were screened positive was determined to fix the false positive rate. Samples of 500 000 observations were drawn for the trisomy 21, and unaffected pregnancies and likelihood ratios were computed for each observation and then used to produce detection rates for each maternal age. Overall population detection rates were obtained by combining the maternal age specific rates according to the maternal age distribution for trisomy 21. Median, standard deviation and correlation parameters for PAPP-A, free β-hCG and ΔNT were obtained from published work (Spencer *et al.*, 2003b; Kagan *et al.*, 2008) and for PlGF, they were obtained from the current study. The maternal age related risk of trisomy 21 was obtained from the model of Wright and Bray (Wright and Bray, 2000), with the UK 2002 maternal age distribution (Office of National Statistics, 2002).

RESULTS

PlGF analysis was carried out over a 2-week period. The kit was calibrated at the beginning of the study, and three-level internal maternal health controls (Sero, AS, Billingstad, Norway) were run before the samples each day. The inter-day variance of the internal controls was 4.5%, 2.8% and 3.1% at 34.1 pg/mL, 103.9 pg/mL and 139.3 pg/mL, respectively. The lower limit of detection of the assay was 7 pg/mL, and only 1 out of 445 of our samples measured below this level.

Maternal demographics of the two study populations are summarized in Table 2.

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Table 2—Screening and maternal demographics in cases and controls [Medians (and inter quartile range, IQR) or % shown]

	Control	Trisomy 21
n	375	70
Gestational age (days)	88 (85–91)	89 (86–91)
Maternal age (years)	32.4 (29.0–35.9)	37.0 (32.9–40.5)*
Maternal weight (kg)	66.0 (60.0–76.0)	68.8 (57.4–76.4)
Smokers (%)	14.9	14.3
IVF (%)	2.1	1.4

* $p < 0.0001$ case versus control: Mann–Whitney test for continuous variables, Pearsons chi-squared test for categorical variables.

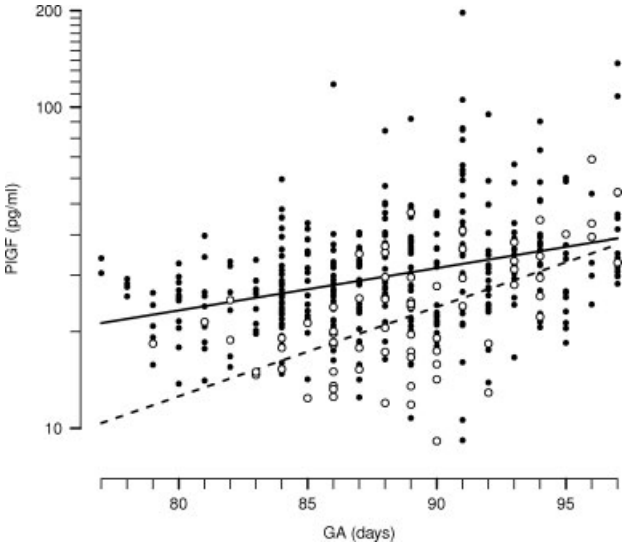

Figure 1—Distribution of PlGF as a function of gestational age. ● (—) euploid controls, ○ (------) trisomy 21 cases

The median concentration of PlGF was 28.4 pg/mL [inter quartile range (IQR) 22.8–36.6 pg/mL] in the euploid controls and 21.4 pg/mL (IQR 16.8–29.6 pg/mL) in the trisomy 21 cases ($p < 0.0001$). The distributions of PlGF as a function of gestational age with least squared regression lines in the euploid and trisomy 21 groups are shown in Figure 1.

Multiple regression determined that gestational age ($p < 0.0001$), maternal weight ($p < 0.001$) and smoking status ($p < 0.0001$) had confounding effects on euploid control $\log_{10}$ PlGF concentrations ($r^2 = 0.2591$). The following equation, created from the coefficients from this analysis, was used to predict expected $\log_{10}$ PlGF concentrations for each patient, which was in turn used to calculate patient PlGF MoMs:

$$\text{Expected } \log_{10} \text{ PlGF} = 1.309 + 0.0122\times [\text{gestational age (days)} - 77] - 0.0019\times [\text{maternal weight (kg)} - 69] + 0.1859 \text{ (if smoker)}$$

The median PlGF, PAPP-A and β-hCG MoMs and ΔNT (mm) were significantly different in the trisomy 21 cases compared to the euploid controls, as shown in Table 3. The correlation coefficients between $\log_{10}$ PlGF MoM and other markers are shown in Table 4. Detection rates for trisomy 21 using maternal age plus maternal serum markers PAPP-A, β-hCG and PlGF and ΔNT were estimated using modeling software which uses UK 2002 maternal age data (mean maternal age 29.28 years, standard deviation 6.0 years), and are displayed in Table 5. In addition, to compare with Zaragoza *et al.* (2009), an artificial maternal age distribution with normal distribution, mean age of 32.7 years and standard deviation of 6.0 years were created and the modeling software was rerun (Table 5).

Table 3—Median (IQR) maternal serum marker MoMs and ΔNT in control and trisomy 21 pregnancies

	Control	Trisomy 21
PlGF MoM	0.98 (0.80–1.28)	0.76 (0.58–0.96)*
PAPP-A MoM	1.06 (0.76–1.46)	0.50 (0.31–0.66)*
Free-β hCG MoM	1.13 (0.76–1.68)	2.08 (1.41–2.76)*
ΔNT (mm)	−0.01 (−0.22–0.20)	1.72 (0.86–3.30)*

* $p < 0.0001$ case versus control: Mann–Whitney test.

Table 4—Pearson's product moment correlation

	Euploid		Trisomy 21	
	r	*p*	*r*	*p*
Log_{10} PlGF and Log_{10} PAPP-A	0.28	<0.0001	0.33	0.005
Log_{10} PlGF and Log_{10} free β-hCG	0.22	<0.0001	−0.02	0.89
Log_{10} PlGF and ΔNT (mm)	0.03	0.52	−0.13	0.27

DISCUSSION

This study found that trisomy 21 pregnancies have significantly decreased levels of maternal serum PlGF in the first trimester compared with euploid controls. Further, it has been demonstrated that the addition of PlGF to a screening test of maternal age, PAPP-A and free β-hCG would increase the detection rate by approximately 2.1% at a 5% false positive rate; however, the increase is much smaller, 0.5%, in a screening test which also included ΔNT.

As expected, maternal age was significantly higher in the trisomy 21 group compared to the euploid group; however, no other screening and maternal demographics were significantly different between the two groups. In euploid pregnancies, PlGF increased with gestational age, decreased with increasing maternal weight and was higher in smokers than non-smokers, as described earlier (Ong *et al.*, 2001; Tidwell *et al.*, 2001; Akolekar *et al.*, 2008; Poon *et al.*, 2008; Cowans *et al.*, 2010; Zaragoza *et al.*, 2009).

The main finding of this study is in contrary to a previous study (Spencer *et al.*, 2001), which reported an

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Table 5—Estimated detection rates of trisomy 21 in various screening set-ups, excluding and including PlGF as a first trimester screening marker

Screening markers	Detection rates (%)	
	FPR = 3	FPR = 5
UK 2002 maternal age distribution (mean: 29.28 years, standard deviation: 6.0 years)		
Maternal age, PAPP-A and β-hCG	62.7	69.9
Maternal age, PAPP-A, β-hCG and PlGF	64.9	72.0
Maternal age, PAPP-A, β-hCG and ΔNT	91.2	93.1
Maternal age, PAPP-A, β-hCG, ΔNT and PlGF	91.7	93.6
Artificial maternal age distribution (mean: 32.7 years, standard deviation: 6.0 years)		
Maternal age, PAPP-A and β-hCG	69.2	76.1
Maternal age, PAPP-A, β-hCG and PlGF	71.0	77.6
Maternal age, PAPP-A, β-hCG and ΔNT	92.9	94.5
Maternal age, PAPP-A, β-hCG, ΔNT and PlGF	93.3	94.9

FPR, false positive rate.

increase in PlGF in maternal serum in trisomy 21 pregnancies at 11–14 weeks. One possible explanation for the discrepancy with the original article is that it did not correct for maternal factors, although the current article reports a significant decrease even in uncorrected raw PlGF levels in trisomy 21 cases compared to euploids. The assay used might have some effect on PlGF levels: at the time of submission, this is the first study to look at PlGF levels in aneuploid pregnancies to use the DELFIA® Xpress (PerkinElmer) PlGF immunoassay, whereas previous articles used a Quantikine® assay (R&D Systems Europe Ltd, Abingdon, UK). PlGF circulates largely bound to the soluble form of Flt1 and it is possible that the two assays may not recognise free and bound PIGF in the same way. Furthermore there are different spliced forms of PIGF and these may be recognised differently by the two methods. Thus, these two factors may explain some of the differences between the two assay systems.

The current study is, however, in agreement with a more recent study (Zaragoza *et al*., 2009), which reported a similar decrease in trisomy 21 cases using the Quantikine® assay for the same gestational age range. Zaragoza *et al*. found a slightly higher net increase in the detection rate of trisomy 21 when including PlGF in a test of maternal age, PAPP-A and free β-hCG: 3.3% increase at a fixed false positive rate of 5%. However, this group uses an age biased study population with a median maternal age over 3 years above the 2002 mean, and therefore report much higher detection rates over all: 80% detection rate at 5% false positive rate for a screening test of maternal age, PAPP-A, free β-hCG and PlGF. When we used an age distribution similar to that of Zaragoza *et al*. we modeled detection rates of 77.6% at a 5% false positive rate using maternal age and the same markers (Table 5), indicating that the higher detection rate they reported may come predominantly from increased maternal age in their population.

Controversy also exists in the literature between studies looking at maternal serum PlGF in trisomy 21 cases in the second trimester, with reports of no change (Lambert-Messerlian and Canick, 2004), an increase (Su *et al*., 2002) and a decrease (Debieve *et al*., 2001) in trisomy 21 cases. The current study found that PlGF levels increased across the first trimester gestation age window at a greater rate in trisomy 21 cases than controls (Figure 1), indicating that PlGF may be a stronger discriminatory marker of trisomy 21 in the early first trimester (prior to 10 weeks) than nearer to the second trimester, as is found with PAPP-A. This is in contrary to the article by Zaragoza *et al*. who report that PlGF levels do not change with CRL in trisomy 21 cases, and only increases with CRL in controls (Zaragoza *et al*., 2009).

Although not investigated in this study, there is agreement in the two studies so far, that first trimester maternal serum levels of PlGF are decreased in cases of trisomies 13 and 18 (Spencer *et al*., 2001; Zaragoza *et al*., 2009).

In conclusion, this is the second recent retrospective study to show that first trimester maternal serum PlGF levels in trisomy 21 pregnancies are decreased, and that PlGF may add to detection rates when used in a screening test with maternal age, PAPP-A, free β-hCG and to less of an extent, NT. Further research, including prospective non-interventional studies, is required to confirm these findings and to extend them to pregnancies prior to 10 weeks. Whether the marginal increase in detection is realistically achievable also requires further study before this marker can be considered as an additional practical screening marker. In addition to any decrease in PlGF in trisomy 21 and other aneuploidies, the interest in PlGF as a marker for PE makes it an interesting potential contender for a first trimester screening marker of this condition; however, views are rather polarized as to whether screening for a condition for which there is no clear prophylaxis is a valid and ethical procedure.

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