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Maternal Serum Placental Growth Factor (PlGF) Isoforms 1 and 2 at 11–13 Weeks' Gestation in Normal and Pathological Pregnancies

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Key Words
Aneuploidy · First trimester · Preeclampsia · Screening · Placental growth factor · Isoform · Small for gestational age

Abstract
Objective: To compare the maternal serum concentration of placental growth factor-1 (PlGF-1) and PlGF-2 at 11–13 weeks' gestation in normal pregnancies and in those complicated by preeclampsia (PE), delivery of small for gestational age (SGA) neonates and fetal trisomies 21, 18 and 13. ***Methods:*** Serum PlGF-1 and PlGF-2 were measured in 270 pathological pregnancies (PE, n = 80; SGA, n = 80; trisomy 21, n = 44; trisomy 18, n = 38; trisomy 13, n = 28) and 590 normal controls. The values were expressed as multiple of the median (MoM) after adjustment for maternal characteristics and corrected for adverse pregnancy outcomes and the median MoM values in each pathological pregnancy were compared to the normal group. ***Results:*** There were significant contributions to PlGF-1 and PlGF-2 from gestational age, smoking and racial origin. In addition, there were significant contributions to PlGF-1 from parity and method of conception. The median MoM of PlGF-1 and PlGF-2 was significantly decreased in PE (0.783 and 0.916 MoM), SGA (0.891 and 0.851 MoM), trisomy 21 (0.609 and 0.749 MoM), trisomy 18 (0.529 and 0.730 MoM) and trisomy 13 (0.373 and 0.699 MoM). ***Conclusions:*** In pathological pregnancies, except SGA, the decrease in serum PlGF-1 at 11–13 weeks' gestation is more marked than the decrease in PlGF-2.

Introduction

Placenta growth factor (PlGF), an angiogenic glycoprotein that is homologous to vascular endothelial growth factor, exists in at least four isoforms due to alternative mRNA splicing of the PlGF primary transcript [1]. The main difference between the four isoforms is that PlGF-1 and -3 are non-heparin binding and can potentially affect targets in a paracrine manner, whereas PlGF-2 and -4 have additional heparin-binding domains and most likely work in an autocrine way. The major isoforms are thought to be PlGF-1 and PlGF-2.

In pregnancy, PlGF is implicated in placental development and several studies have reported that maternal serum PlGF concentration at 11–13 weeks' gestation is reduced in pregnancies that subsequently develop preeclampsia (PE), in those that deliver small for gestational age (SGA) neonates and in those with fetal trisomies 21,

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18 and 13 [2–10]. Although in these studies it was assumed that PlGF-1 was measured, in the various commercially available assays there is considerable cross-reactivity with other PlGF isoforms. For example, the method instructions of the PlGF-1 assays report cross-reactivity to PlGF-2 of 13–50% (Brahms Kryptor; Thermo Fisher Scientific, Hennigsdorf, Germany; Delfia Xpress, PerkinElmer Inc., Waltham, Mass., USA; Roche Cobas and Elecsys Systems; Roche Diagnostics, Mannheim, Germany; R&D Systems Inc., Minneapolis, Minn., USA). There are no studies reporting on maternal serum concentration of PlGF-2 in normal or pathological pregnancies.

The objective of this study is to compare the maternal serum concentration of PlGF-1 and PlGF-2 at 11–13 weeks' gestation in normal pregnancies and in those complicated by PE, delivery of SGA neonates and fetal trisomies 21, 18 and 13.

Methods

Study Population

This was a case-control study drawn from a prospective observational study for adverse pregnancy outcomes in women attending for their routine first-trimester hospital visit in pregnancy at King's College Hospital London. During this visit, an ultrasound scan was carried out to, firstly, confirm gestational age from the measurement of the fetal crown-rump length (CRL) [11], secondly, to diagnose any major fetal abnormalities, and thirdly, to measure fetal nuchal translucency (NT) thickness as part of combined screening for aneuploidies [12]. Automated machines that provide reproducible results within 30 min were used to measure pregnancy-associated plasma protein-A (PAPP-A) and free β-human chorionic gonadotropin (β-hCG) (Delfia Xpress System; PerkinElmer Life and Analytical Sciences) as part of combined screening for aneuploidies. Samples of serum were stored at –80°C for subsequent biochemical analysis. Written informed consent was obtained from the women agreeing to participate in the study, which was approved by the NHS Research Ethics Committee.

The study population comprised 80 pregnancies that subsequently developed PE, including 30 with early PE (requiring delivery before 34 weeks) and 50 with late PE (requiring delivery at or after 34 weeks), 80 cases who delivered SGA neonates, 44 cases of trisomy 21, 38 cases of trisomy 18, and 28 cases of trisomy 13. The controls comprised 590 cases matched with the cases for storage time; they did not develop any pregnancy complication and resulted in the live birth of phenotypically normal neonates.

Maternal History

Patients were asked to complete a questionnaire on maternal age, racial origin (Caucasian, Afro-Caribbean, South Asian, East Asian and Mixed), cigarette smoking during pregnancy (yes or no), method of conception (spontaneous, use of ovulation drugs and in vitro fertilization) and parity (parous or nulliparous if no delivery beyond 23 weeks). Maternal weight and height were measured and recorded.

Outcome Measures

Maternal demographic characteristics, ultrasonographic measurements and biochemical results were recorded in a computer database. Data on pregnancy outcomes were collected from the hospital maternity records or the general medical practitioners of the women. The obstetric records of all women with preexisting or pregnancy-associated hypertension were examined to determine if the condition was chronic hypertension, PE or non-proteinuric gestational hypertension. The definition of PE was that of the International Society for the Study of Hypertension in Pregnancy [13]. The definitions of SGA were birth weight below the 5th percentile of a reference range derived from our population [14].

Sample Analysis

Single measurements were performed for PlGF and specific isoform PlGF-2 on the fully automated Kryptor compact Plus system (Brahms PlGF Kryptor and Brahms PlGF-2 Kryptor; Thermo Fisher Scientific). Both assays were homogeneous sandwich immunoassays based on the Trace technology [15]. The total duration of the assays was 29 min and the sample volume was 70 µl. The PlGF assay was calibrated with recombinant human PlGF and standardized against the Quantikine PlGF ELISA (R&D Systems Europe Ltd, Abingdon, UK). According to the manufacturer's instructions for use, the Brahms PlGF Kryptor assay covered a measuring range of 3.6–7,000 pg/ml. The limit of detection was 3.6 pg/ml and the limit of quantitation (functional sensitivity) was <6.9 pg/ml. The intra- and inter-assay variations at a PlGF concentration of 35 pg/ml were 4.6 and 7.3%, respectively, at a concentration of 103 pg/ml they were 2.1 and 3.1%, and at a concentration of 430 pg/ml they were 0.9 and 2.3%. The cross-reactivity of the PlGF assay, which was determined in accordance with CLSI EP7-A2 (Clinical Laboratory Standards Institute guideline), was 13% for PlGF-2 and 4% for PlGF-3 and in this study this assay is referred to as PlGF-1.

The specific PlGF-2 immunoassay was set up using a polyclonal anti-human PlGF antibody (R&D Systems Europe Ltd) and a monoclonal anti-human PlGF-2 antibody generated by immunization of female BALB/c mice with human PlGF-2 recombinant protein (R&D Systems Europe Ltd). This assay was calibrated with recombinant human PlGF-2. Brahms PlGF-2 Kryptor had an assay range of 15–10,000 pg/ml. The variation of PlGF-2 assay was determined for the control samples in 26 runs with two replicates. The calibration curve of the first run was used as a reference curve during the 7-day period. The limit of detection was 15 pg/ml and the limit of quantitation (functional sensitivity) was 42 pg/ml. The intra- and inter-assay variations at a PlGF-2 concentration of 100 pg/ml were 8.8 and 9.4%, respectively, at a concentration of 505 pg/ml they were 2.3 and 2.9%, and at a concentration of 1,018 pg/ml they were 1.3 and 2.4%.

Statistical Analysis

Comparisons of pregnancy characteristics between outcome groups were by the student's t-test or Mann-Whitney U test or χ^2 test or Fisher's exact test for categorical variables, with post hoc Bonferroni correction (adjusted significance level $p < 0.01$).

The measured concentrations of PlGF-1 and PlGF-2 were $\log_{10}$ transformed to make the distribution gaussian. Backward stepwise multiple regression analysis was used to determine which of the factors amongst the maternal characteristics and gestation were significant predictors of the $\log_{10}$ PlGF-1 and $\log_{10}$ PlGF-2, adjusting for the adverse pregnancy outcomes (PE, SGA and aneuploid).

Table 1. Characteristics in the study population

Characteristic	Normal (n = 590)	Preeclampsia (n = 80)	SGA (n = 80)	Trisomy 21 (n = 44)	Trisomy 18 (n = 38)	Trisomy 13 (n = 28)
Maternal age, years (median)	32.3	33.7	30.8	39.2*	38.1*	34.7
(IQR)	28.4–35.6	27.9–36.5	25.4–35.3	35.7–41.3	32.2–41.1	29.5–38.6
Maternal weight, kg (median)	65.0	71.4*	62.5	68.0	66.4	63.0
(IQR)	58.6–73.2	64.2–82.5	55.4–80.2	60.5–75.5	60.5–73.4	57.4–72.0
Maternal height, cm (median)	165	162	162*	166	165	163
(IQR)	160–169	159–167	156–165	160–168	158–167	158–167
Gestation at screening, weeks (median)	12.7	12.7	12.6	13.0*	12.2*	12.3
(IQR)	12.3–13.0	12.3–13.0	12.1–12.9	12.5–13.6	11.8–12.8	12.0–13.0
Racial origin, n (%)						
Caucasian	366 (62.0)	43 (53.8)	41 (51.3)	32 (72.7)	33 (86.8)*	23 (82.1)
Afro-Caribbean	151 (25.6)	29 (36.3)	33 (41.3)*	10 (22.7)	4 (10.5)	4 (14.3)
South Asian	31 (5.3)	4 (5.0)	4 (5.0)	1 (2.3)	1 (2.6)	1 (3.6)
East Asian	26 (4.4)	3 (3.8)	0	1 (2.3)	0	0
Mixed	16 (2.7)	1 (1.3)	2 (2.5)	0	0	0
Nulliparous, n (%)	310 (52.5)	51 (63.8)	52 (65.0)	7 (15.9)*	12 (31.6)	10 (35.7)
Cigarette smoker, n (%)	27 (4.6)	5 (6.3)	18 (22.5)*	3 (6.8)	3 (7.9)	1 (3.6)
Conception, n (%)						
Spontaneous	561 (95.1)	75 (93.8)	75 (93.8)	35 (79.5)*	37 (97.4)	26 (92.9)
Ovulation drugs	11 (1.9)	0	1 (1.3)	5 (11.4)*	1 (2.6)	1 (3.6)
In vitro fertilization	18 (3.1)	5 (6.3)	4 (5.0)	4 (9.1)	0	1 (3.6)
Fetal CRL, mm (median)	62.7	62.9	61.5	68.4*	57.0*	57.7
(IQR)	57.5–67.3	58.0–67.1	56.1–65.8	60.5–75.7	52.1–64.2	54.2–67.4
ΔNT, mm (median)	0.076	0.054	0.012	2.517*	4.531*	4.196*
(IQR)	–0.108 to 0.278	–0.170 to 0.277	–0.198 to 0.228	1.064–3.842	0.257–6.239	1.623–5.673
PAPP-A MoM (median)	1.066	0.943	0.814*	0.563*	0.184*	0.218*
(IQR)	0.756–1.449	0.580–1.413	0.514–1.383	0.409–1.070	0.090–0.257	0.141–0.308
β-hCG MoM (median)	1.047	1.159	1.047	2.439*	0.241*	0.556*
(IQR)	0.744–1.590	0.733–1.741	0.592–1.488	1.597–4.773	0.117–0.358	0.373–0.960

Comparison between outcome groups by the Mann-Whitney U test with post hoc Bonferroni correction and the χ^2 test or Fisher's exact test for categorical variables; adjusted significance level: * $p < 0.01$.

Variables were not considered to be significant predictors if the p value was >0.05 or that the ratio of the regression coefficients to the standard deviation of the $\log_{10}$ multiple of the median (MoM) of PlGF-1 and PlGF-2 was <0.1. Gestational age at screening was centred by subtracting 12 weeks, maternal weight was centred by subtracting 65 kg and maternal height was centred by subtracting 165 cm.

The distributions of PlGF-1 and PlGF-2 were expressed as MoM in all cases, correcting for the significant predictors as defined in the multiple regression. The Mann-Whitney U test was used to determine the significance of differences in the median MoM values in each outcome group to that in the controls. The Wilcoxon signed-rank test was used to compare the median MoM values of PlGF-1 and PlGF-2 within each outcome group. The measured NT was expressed as a difference from the expected normal mean for gestation (Δ value) and the measured PAPP-A and free β-hCG were converted into MoM after adjustment for gestation, maternal age, racial origin, weight, parity and method of conception as previously described [16, 17].

Regression analysis was used to determine the significance of association between $\log_{10}$ MoM values of PlGF-1 and PlGF-2 in each outcome group, between $\log_{10}$ MoM values of PlGF-1 and PlGF-2 with gestation at screening, ΔNT, PAPP-A $\log_{10}$ MoM and free β-hCG $\log_{10}$ MoM in the trisomic groups and between $\log_{10}$ MoM values of PlGF-1 and PlGF-2 with gestation at delivery in the PE and SGA groups. The performance of screening was determined by receiver operating characteristic (ROC) curves.

The statistical software package SPSS 20.0 (IBM SPSS Statistics for Windows, Version 20.0; IBM Corp., Armonk, N.Y., USA) and MedCalc (MedCalc Software, Mariakerke, Belgium) were used for all data analyses.

Results

The maternal characteristics of each of the outcome groups are summarized in table 1. The median measured concentration of serum PlGF-2 (139.1 pg/ml, interquartile range (IQR) 108.8–175.2) was 3.7 times higher than that of PlGF-1 (37.5 pg/ml, IQR 26.9–52.9; $p < 0.0001$; fig. 1).

Normal Pregnancy Outcomes

Multiple regression analysis demonstrated that for $\log_{10}$ PlGF-1, significant independent contributions were

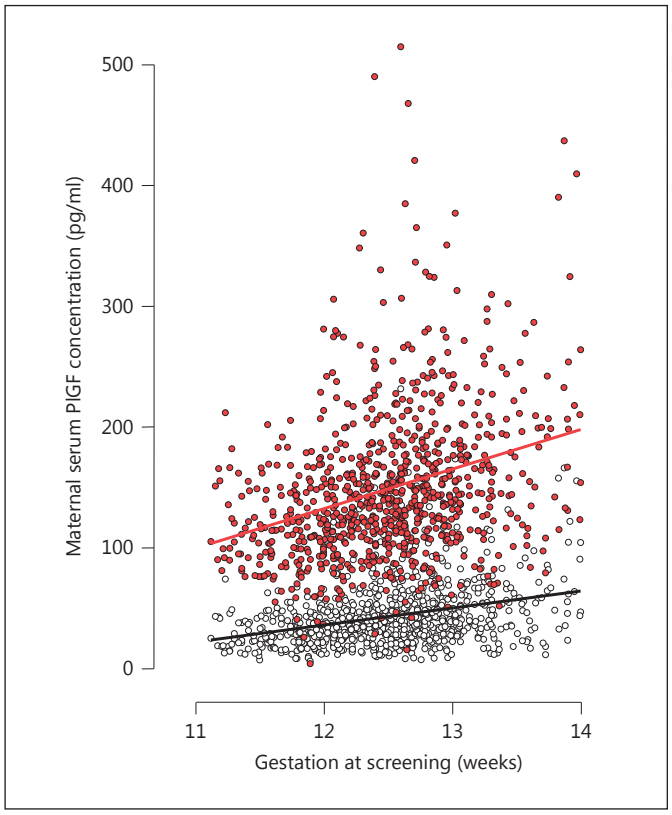

Fig. 1. Serum concentration of PlGF-1 (open circles) and PlGF-2 (filled circles) with regression lines of the relationship with gestational age.

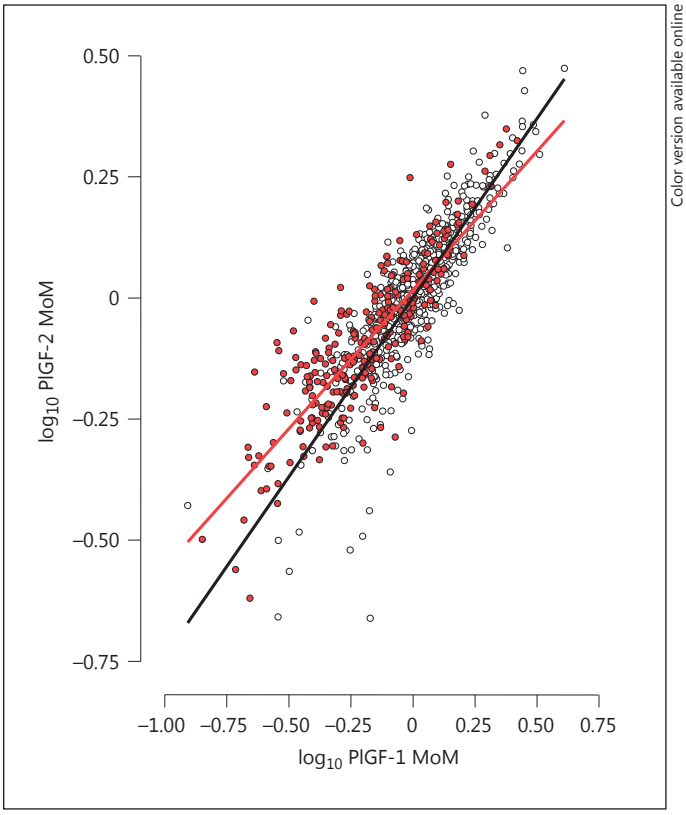

Fig. 2. Relationship between $\log_{10}$ MoM values of PlGF-1 and PlGF-2 in the normal control (open circles) and pathological pregnancies (filled circles).

provided by gestational age at screening, cigarette smoking, racial origin, parity and method of conception (R^2 = 0.396; table 2) but not maternal age (p = 0.347), weight (p = 0.878) and height (p = 0.119). Similarly, multiple regression analysis demonstrated that for $\log_{10}$ PlGF-2, significant independent contributions were provided by gestational age at screening, cigarette smoking and racial origin (R^2 = 0.251; table 2) but not maternal age (p = 0.529), weight (p = 0.831), height (p = 0.721), parity (p = 0.890) and method of conception (p = 0.444).

In each patient we used these formulae to derive the expected $\log_{10}$ values of PlGF-1 and PlGF-2 and then expressed the observed value as MoM of the expected (table 3). The median MoM of PlGF-1 and PlGF-2 were not significantly different in the controls but there was a significant direct correlation between the $\log_{10}$ MoM values of the two PlGF isoforms (r = 0.845, p < 0.0001; fig. 2).

Preeclampsia

In the PE group the median MoM of PlGF-1 was significantly lower than that of PlGF-2 and both the median MoM of PlGF-1 and PlGF-2 were significantly lower than in the controls. There was a significant association with gestational age at delivery for both PlGF-1 $\log_{10}$ MoM (r = 0.546, p < 0.0001; fig. 3) and PlGF-2 $\log_{10}$ MoM (r = 0.379, p = 0.001; fig. 3). There was a significant direct correlation between the $\log_{10}$ MoM values of the two PlGF isoforms (r = 0.870, p < 0.0001; fig. 2). The PlGF-1 MoM was below the 10th percentile in 46.7% (95% CI 30.2–63.9) of women who developed early PE requiring delivery before 34 weeks and in 10.0% (95% CI 4.3–21.4) of those with late PE delivering at or after 34 weeks. The respective values for PlGF-2 MoM were 30.0% (95% CI 16.7–47.9) and 14.0% (95% CI 7.0–26.2).

In the prediction for PE, multivariate logistic regression analysis demonstrated that there were independent significant contributions from PlGF-1 $\log_{10}$ MoM and PlGF-2 $\log_{10}$ MoM (R^2 = 0.125, p < 0.0001). The areas un-

Table 2. Fitted regression models for log_{10} placental growth factor at 11–13 weeks

Coefficient	Estimate	Standard error	LCL	UCL	p value
PlGF-1					
Intercept	1.52325	0.014011	1.49575	1.55076	<0.0001
Preeclampsia	–0.12572	0.022706	–0.17028	–0.081149	<0.0001
SGA	–0.088591	0.023376	–0.13447	–0.042709	0.0002
Trisomy 21	–0.20459	0.030240	–0.26374	–0.14504	<0.0001
Trisomy 18	–0.27830	0.032105	–0.34131	–0.21528	<0.0001
Trisomy 13	–0.41711	0.036807	–0.48935	–0.34487	<0.0001
(GA 12 weeks)	0.10715	0.011494	0.084592	0.12971	<0.0001
Smoking	0.16019	0.026775	0.10764	0.21275	<0.0001
Afro-Caribbean racial origin	0.15372	0.015121	0.12404	0.18340	<0.0001
In vitro fertilization	–0.088519	0.034558	–0.15635	–0.020689	0.011
Nulliparity	–0.026764	0.013466	–0.053194	–0.00033324	0.047
PlGF-2					
Intercept	2.075556	0.0094146	2.057078	2.094035	<0.0001
Preeclampsia	–0.046254	0.018986	–0.083518	–0.0089897	0.015
SGA	–0.045229	0.019515	–0.083533	–0.0069253	0.021
Trisomy 21	–0.13305	0.025012	–0.18214	–0.083954	<0.0001
Trisomy 18	–0.14105	0.026799	–0.19365	–0.088454	<0.0001
Trisomy 13	–0.17327	0.030826	–0.23377	–0.11277	<0.0001
(GA 12 weeks)	0.082300	0.0096100	0.063438	0.10116	<0.0001
Smoking	0.13135	0.022347	0.087483	0.17521	<0.0001
Afro-Caribbean racial origin	0.10267	0.012539	0.078058	0.12728	<0.0001

LCL = Lower confidence limit; UCL = upper confidence limit.

Table 3. Comparison of median (IQR) of MoM values of PlGF-1 and PlGF-2 of each adverse outcome group with the controls

Outcome group	PlGF-1		PlGF-2		Within outcome groups
	median MoM	between outcome groups	median MoM	between outcome groups	
Control (n = 590)	0.997 (0.784–1.278)	–	1.007 (0.848–1.240)	–	0.231
Preeclampsia (n = 80)	0.783 (0.633–0.979)	<0.0001*	0.916 (0.745–1.170)	0.007*	<0.0001**
Delivery <34 weeks (n = 30)	0.638 (0.398–0.787)	<0.0001*	0.856 (0.619–1.076)	0.002*	<0.0001**
Delivery ≥34 weeks (n = 50)	0.870 (0.730–1.171)	0.035	0.948 (0.828–1.227)	0.270	0.004**
SGA (n = 80)	0.891 (0.575–1.126)	<0.0001*	0.851 (0.704–1.118)	0.001*	0.013**
Trisomy 21 (n = 44)	0.609 (0.425–0.908)	<0.0001*	0.749 (0.612–0.973)	<0.0001*	<0.0001**
Trisomy 18 (n = 38)	0.529 (0.407–0.683)	<0.0001*	0.730 (0.588–0.890)	<0.0001*	<0.0001**
Trisomy 13 (n = 28)	0.373 (0.276–0.501)	<0.0001*	0.699 (0.531–0.798)	<0.0001*	<0.0001**

Comparison between outcome groups by the Mann-Whitney U test with post hoc Bonferroni correction; adjusted significance level: * $p < 0.01$. Comparison of PlGF-1 and PlGF-2 within outcome groups by the Wilcoxon signed-rank test; significance level: ** $p < 0.05$.

der the ROC curves (AUROC) and detection rates of PE for different false positive rates in screening by PlGF-1, PlGF-2 and their combination are given in table 4. The AUROC of PlGF-1 was significantly higher than PlGF-2 ($p < 0.001$) and the AUROC of the combination of PlGF-1 and PlGF-2 was significantly higher than PlGF-2 ($p = 0.004$), but not PlGF-1 ($p = 0.150$).

Small for Gestational Age

In the SGA group the median MoM of PlGF-2 was significantly lower than that of PlGF-1 and both the median MoM of PlGF-1 and PlGF-2 were significantly lower than in the controls. There was a significant association with gestational age at delivery for both PlGF-1 log_{10} MoM ($r = 0.448$, $p < 0.0001$; fig. 4) and PlGF-2 log_{10} MoM (r =

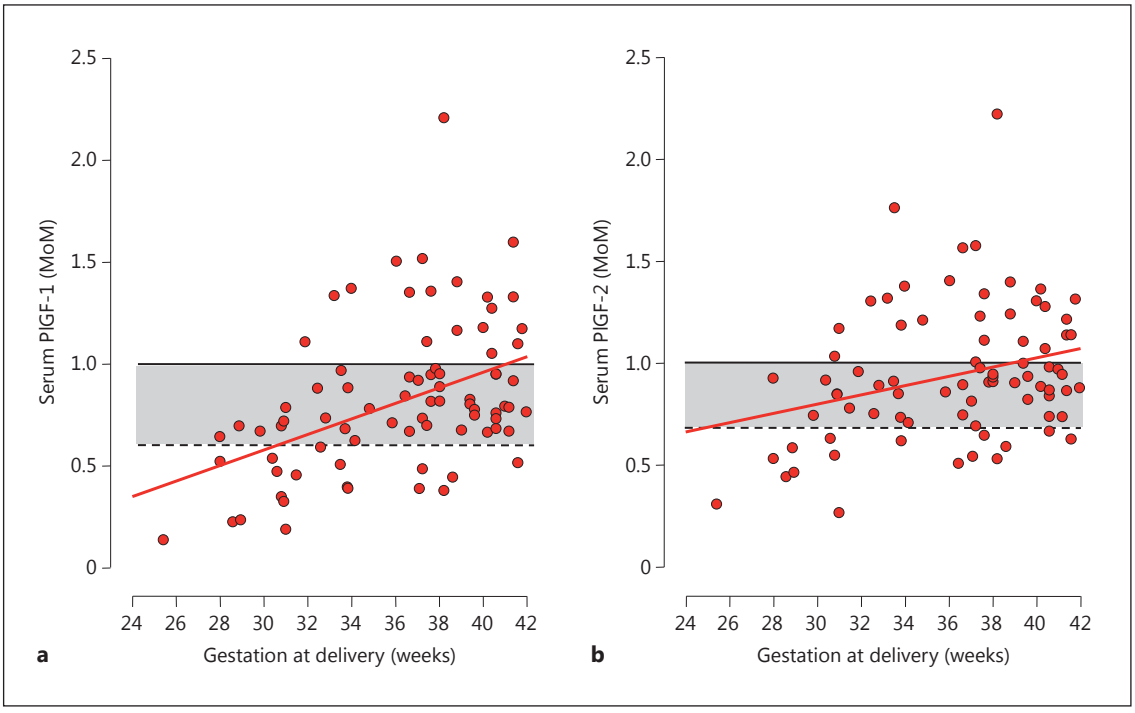

Fig. 3. Serum PlGF-1 (**a**) and PlGF-2 (**b**) MoM values in pregnancies that developed PE plotted on the reference range for gestation in normal pregnancies (median and 10th percentile, grey band).

Table 4. Performance of PlGF-1 and PlGF-2 in the detection of preeclampsia, SGA and trisomies 21, 18 and 13

Screening test	AUROC (95% CI)	Detection rates (95% CI) for fixed false positive rate	
		5%	10%
Preeclampsia			
PlGF-1	0.675 (0.638–0.710)	18.9 (10.9–29.0)	23.8 (15.0–34.6)
PlGF-2	0.593 (0.555–0.631)	11.3 (4.2–20.3)	20.0 (11.9–30.4)
PlGF-1 and PlGF-2	0.710 (0.674–0.744)	28.8 (19.2–40.0)	37.5 (26.9–49.0)
SGA			
PlGF-1	0.623 (0.585–0.660)	17.5 (9.9–27.6)	28.8 (19.2–40.0)
PlGF-2	0.610 (0.572–0.647)	6.3 (2.1–14.0)	22.5 (13.9–33.2)
Trisomy 21			
PlGF-1	0.762 (0.727–0.795)	40.9 (26.3–56.7)	50.0 (34.6–65.4)
PlGF-2	0.739 (0.703–0.772)	13.6 (5.2–27.4)	38.6 (24.4–54.5)
Trisomy 18			
PlGF-1	0.887 (0.859–0.911)	47.4 (31.0–64.2)	68.4 (51.3–82.5)
PlGF-2	0.789 (0.755–0.820)	15.8 (6.1–31.3)	39.5 (24.1–56.6)
PlGF-1 and PlGF-2	0.903 (0.877–0.925)	42.1 (26.3–59.2)	71.1 (54.1–84.6)
Trisomy 13			
PlGF-1	0.957 (0.938–0.972)	82.1 (63.1–93.9)	89.3 (71.7–97.6)
PlGF-2	0.831 (0.799–0.860)	28.6 (13.3–48.7)	46.4 (27.5–66.1)
PlGF-1 and PlGF-2	0.971 (0.954–0.983)	82.1 (63.1–93.9)	89.3 (71.7–97.6)

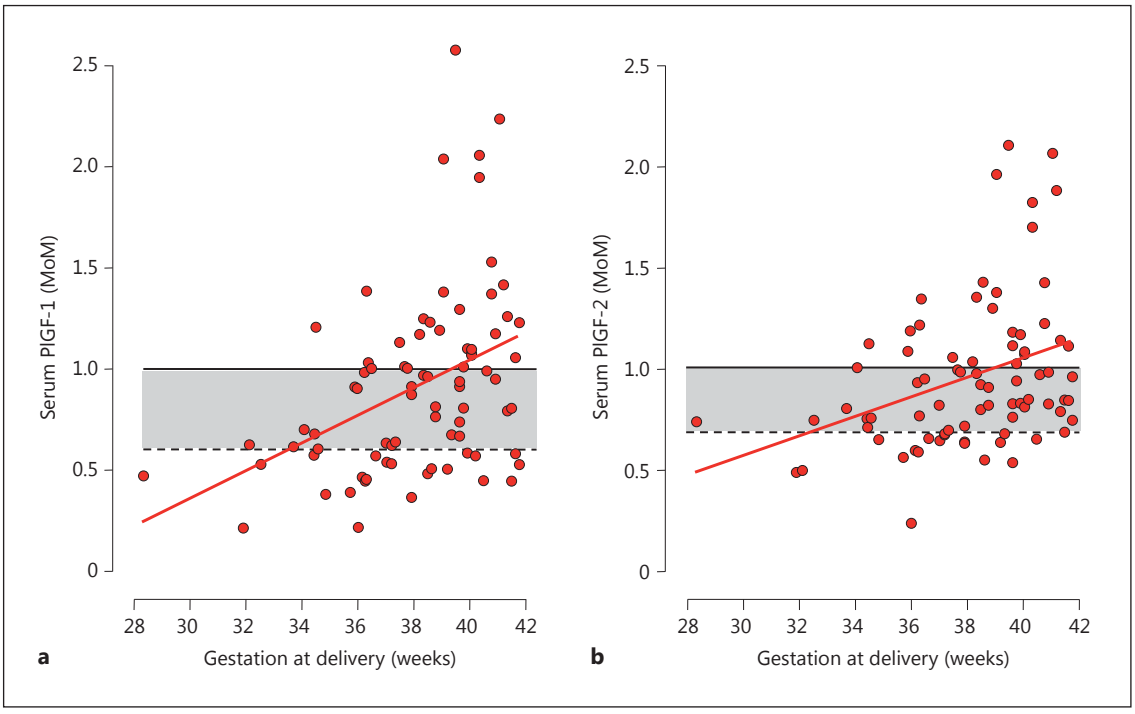

Fig. 4. Serum PlGF-1 (**a**) and PlGF-2 (**b**) MoM values in pregnancies that delivered SGA neonates plotted on the reference range for gestation in normal pregnancies (median and 10th percentile, grey band).

0.373, p = 0.001; fig. 4). There was a significant direct correlation between the $\log_{10}$ MoM values of the two PlGF isoforms (r = 0.893, p < 0.0001; fig. 2).

In the prediction for SGA, multivariate logistic regression analysis demonstrated that there was independent significant contribution from PlGF-1 $\log_{10}$ MoM but not from PlGF-2 $\log_{10}$ MoM. The AUROC and detection rates of SGA for different false positive rates in screening by PlGF-1 and PlGF-2 are given in table 4. The AUROC was not significantly different between PlGF-1 and PlGF-2 (p = 0.501).

Trisomy 21

In the trisomy 21 group the median MoM of PlGF-1 was significantly lower than that of PlGF-2 and both the median MoM of PlGF-1 and PlGF-2 were significantly lower than in the controls. There was a significant direct correlation between the $\log_{10}$ MoM values of the two PlGF isoforms (r = 0.508, p < 0.0001). There was no significant association between PlGF-1 $\log_{10}$ MoM with gestational age at screening (r = 0.056, p = 0.719; fig. 5), ΔNT (r = 0.080, p = 0.604), PAPP-A $\log_{10}$ MoM (r = 0.015, p = 0.921) or free β-hCG $\log_{10}$ MoM (r = 0.130, p = 0.400). Similarly, there was no significant association between PlGF-2 $\log_{10}$ MoM with gestational age at screening (r = 0.008, p = 0.957; fig. 5), ΔNT (r = –0.266, p = 0.081), PAPP-A $\log_{10}$ MoM (r = 0.147, p = 0.342) or free β-hCG $\log_{10}$ MoM (r = 0.228, p = 0.137).

In the prediction for trisomy 21, multivariate logistic regression analysis demonstrated that there was independent significant contribution from PlGF-1 $\log_{10}$ MoM but not from PlGF-2 $\log_{10}$ MoM. The AUROC and detection rates of trisomy 21 for different false positive rates in screening by PlGF-1 and PlGF-2 are given in table 4. The AUROC was not significantly different between PlGF-1 and PlGF-2 (p = 0.433).

Trisomy 18

In the trisomy 18 group the median MoM of PlGF-1 was significantly lower than that of PlGF-2 and both the median MoM of PlGF-1 and PlGF-2 were significantly lower than in the controls. There was a significant direct correlation between the $\log_{10}$ MoM values of the two PlGF isoforms (r = 0.807, p < 0.0001). There was no significant association between PlGF-1 $\log_{10}$ MoM with gestational age at screening (r = –0.213, p = 0.200; fig. 6),

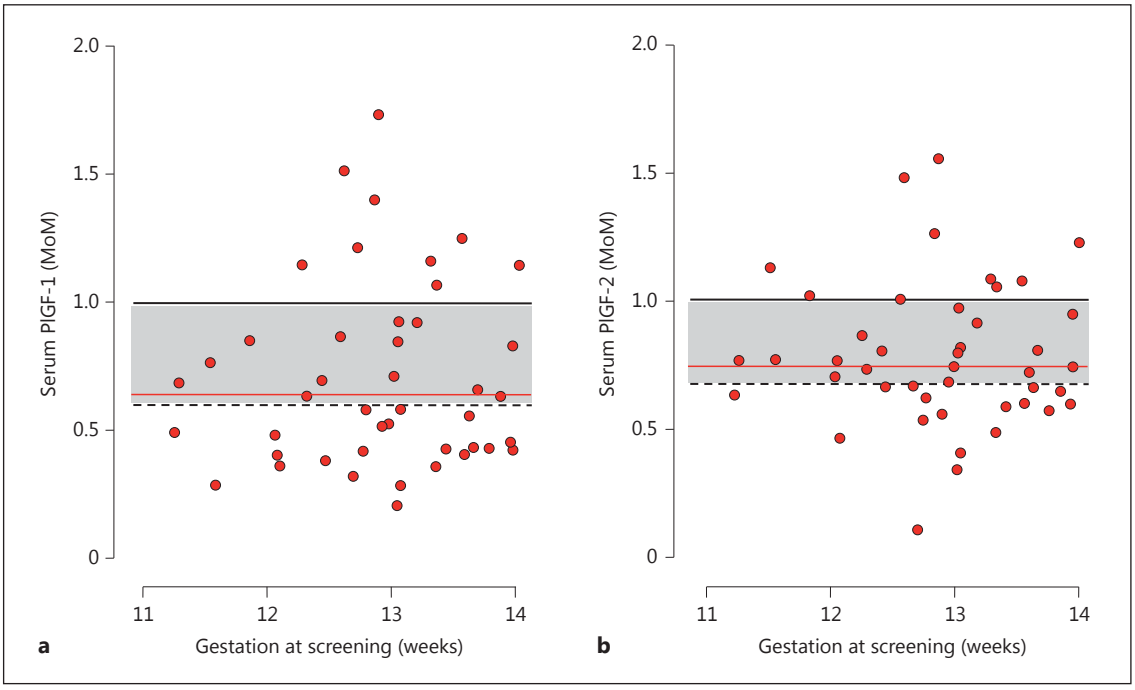

Fig. 5. Serum PlGF-1 (**a**) and PlGF-2 (**b**) MoM values in trisomy 21 pregnancies plotted on the reference range for gestation (median and 10th percentile).

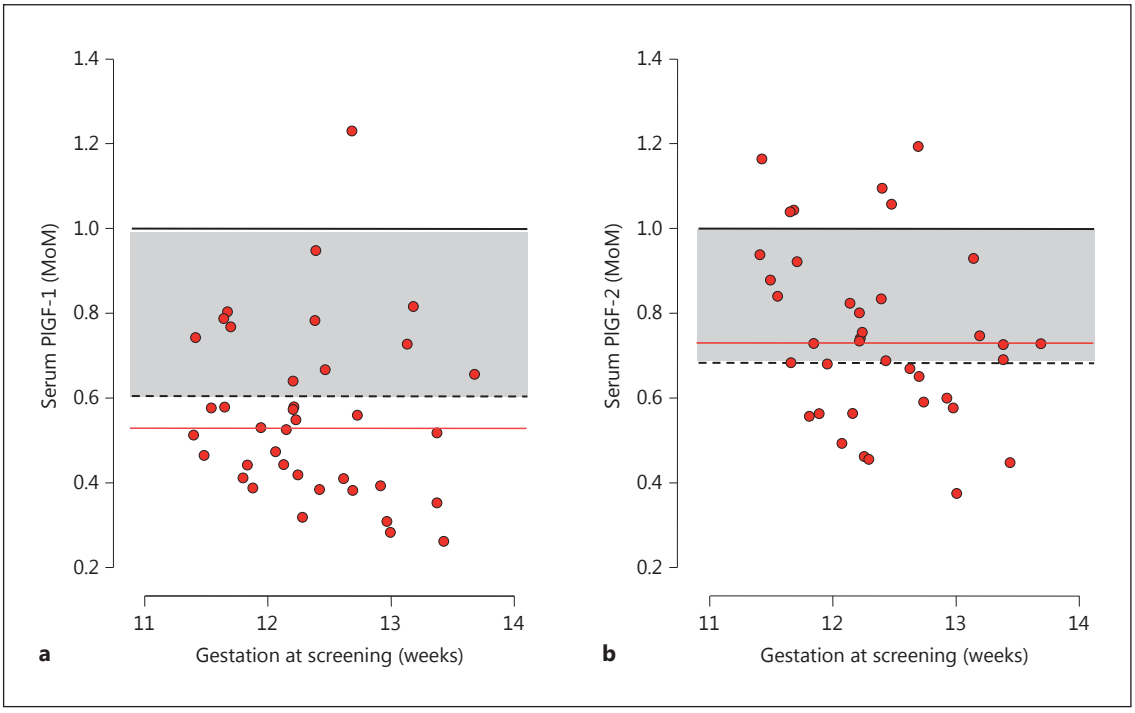

Fig. 6. Serum PlGF-1 (**a**) and PlGF-2 (**b**) MoM values in trisomy 18 pregnancies plotted on the reference range for gestation (median and 10th percentile).

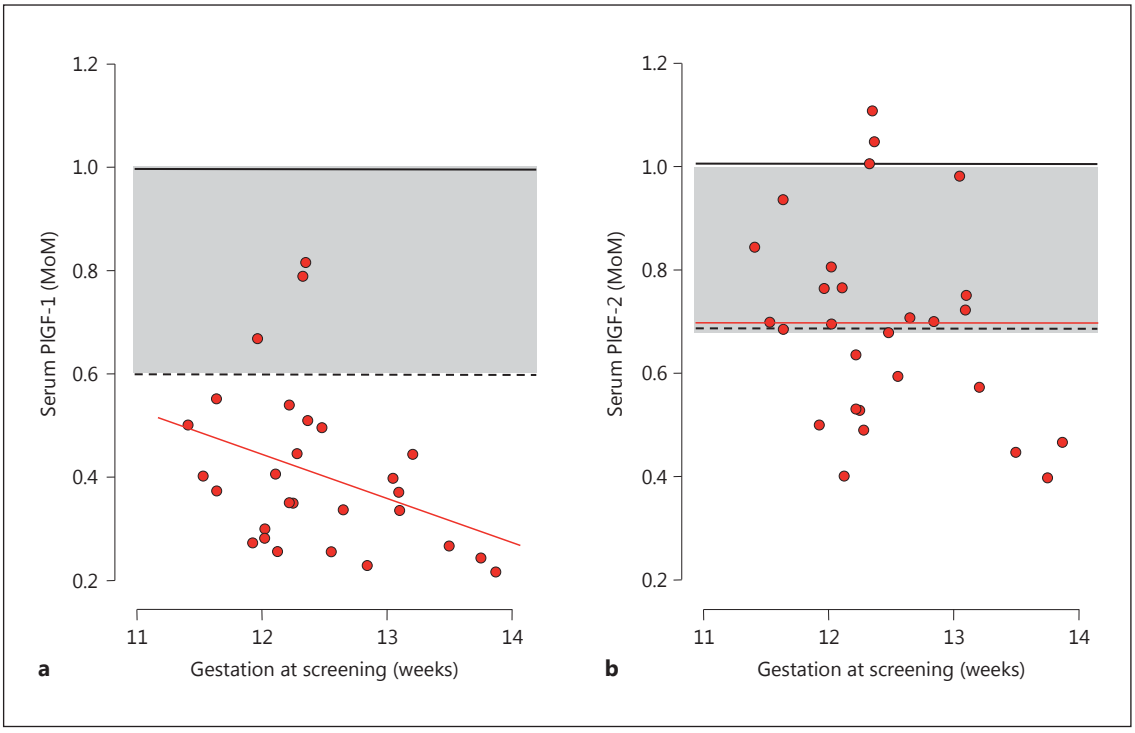

Fig. 7. Serum PlGF-1 (**a**) and PlGF-2 (**b**) MoM values in trisomy 13 pregnancies plotted on the reference range for gestation (median and 10th percentile).

ΔNT (r = 0.141, p = 0.400), PAPP-A $\log_{10}$ MoM (r = –0.172, p = 0.302) and free β-hCG $\log_{10}$ MoM (r = –0.076, p = 0.650). Similarly, there was no significant association between PlGF-2 $\log_{10}$ MoM with gestational age at screening (r = –0.317, p = 0.053; fig. 6), ΔNT (r = –0.282, p = 0.087), PAPP-A $\log_{10}$ MoM (r = 0.213, p = 0.199) and free β-hCG $\log_{10}$ MoM (r = –0.003, p = 0.985).

In the prediction for trisomy 18, multivariate logistic regression analysis demonstrated that there were independent significant contributions from PlGF-1 $\log_{10}$ MoM and PlGF-2 $\log_{10}$ MoM (R^2 = 0.336, p < 0.0001). The AUROC and detection rates of trisomy 18 for different false positive rates in screening by PlGF-1, PlGF-2 and their combination are given in table 4. The AUROC of PlGF-1 was significantly higher than PlGF-2 (p < 0.001) and the AUROC of the combination of PlGF-1 and PlGF-2 was significantly higher than PlGF-2 (p = 0.004), but not PlGF-1 (p = 0.288).

Trisomy 13

In the trisomy 13 group the median MoM of PlGF-1 was significantly lower than that of PlGF-2 and both the median MoM of PlGF-1 and PlGF-2 were significantly lower than in the controls. There was a significant direct correlation between the $\log_{10}$ MoM values of the two PlGF isoforms (r = 0.675, p < 0.0001). There was a significant association between PlGF-1 $\log_{10}$ MoM with gestational age at screening (r = –0.415, p = 0.028; fig. 7) but not with ΔNT (r = 0.372, p = 0.051), PAPP-A $\log_{10}$ MoM (r = 0.124, p = 0.529) and free β-hCG $\log_{10}$ MoM (r = –0.368, p = 0.054). There was a significant association between PlGF-2 $\log_{10}$ MoM with ΔNT (r = 0.420, p = 0.026) but not with gestational age at screening (r = 0.359, p = 0.060; fig. 7), PAPP-A $\log_{10}$ MoM (r = –0.139, p = 0.481) and free β-hCG $\log_{10}$ MoM (r = 0.033, p = 0.867).

In the prediction for trisomy 13, multivariate logistic regression analysis demonstrated that there were independent significant contributions from PlGF-1 $\log_{10}$ MoM and PlGF-2 $\log_{10}$ MoM (R^2 = 0.577, p < 0.0001). The AUROC and detection rates of trisomy 13 for different false positive rates in screening by PlGF-1, PlGF-2 and their combination are given in table 4. The AUROC of PlGF-1 was significantly higher than PlGF-2 (p = 0.001) and the AUROC of the combination of PlGF-1 and PlGF-2 was significantly higher than PlGF-2 (p = 0.003), but not PlGF-1 (p = 0.389).

Discussion

The findings of this study demonstrate that at 11–13 weeks' gestation, firstly, there are significant contributions to serum PlGF-1 from gestational age, smoking, racial origin, parity and method of conception and there are significant contributions to serum PlGF-2 from gestational age, smoking and racial origin, secondly, serum PlGF-1 and PlGF-2 are significantly decreased in pregnancies that develop PE, those that deliver SGA neonates and those with fetal trisomies 21, 18 and 13, and thirdly, there are significant correlations PlGF-1 and PlGF-2 in the normal and each pathological group.

Serum PlGF-1 and PlGF-2 concentration increases with gestational age and is higher in smokers and in women of Afro-Caribbean racial origin than in non-smokers and Caucasian women. In addition, serum PlGF-1 is lower in nulliparous women and those who conceived with in vitro fertilization than in parous women and those who conceived spontaneously or by ovulation drugs. In a large screening study involving more than 12,000 pregnancies at 11–13 weeks' gestation, we found that serum PlGF is also affected by maternal age, weight, preexisting diabetes mellitus and method of conception [10].

The finding that at 11–13 weeks serum PlGF-1 and PlGF-2 is decreased in pregnancies complicated by PE and SGA are compatible with those of previous studies that reported on levels of PlGF in these conditions [3–5]. The finding that in the case of PE and SGA the magnitude of decrease in PlGF is inversely related to gestational age at delivery is also compatible with that of previous studies and reflects the degree of impairment in placental development and function [3–5]. The decrease is serum PlGF is likely to be the consequence of impaired trophoblastic invasion of the spiral arteries and their conversion from high impedance narrow vessels to wide non-muscular channels, which is thought to be the underlying cause of PE and some cases of SGA [18, 19]. Impaired placentation is associated with placental hypoxia and in vitro studies have shown that in cultured trophoblast cells the production of PlGF mRNA is inhibited under experimental hypoxia [20]. Some evidence suggests a different function for PlGF-1 and PlGF-2. The addition of PlGF-1 to a spontaneously transformed first-trimester cytotrophoblast cell line stimulated cell proliferation while PlGF-2 had little effect [21]. In contrast, the addition of PlGF-1 had little effect on endothelial cell proliferation while this was inhibited by PlGF-2.

The median MoM in serum PlGF-1 in trisomies 21, 18 and 13 (0.61, 0.53 and 0.37 MoM) are similar to those in our prospective screening study, which included 12,154 normal pregnancies, 44 with trisomy 21 (0.62 MoM), 18 with trisomy 18 (0.57 MoM) and 3 with trisomy 13 (0.48 MoM) [10]. Our study provides further evidence that measurement of PlGF-1 is useful in improving the performance on the combined test in early screening for trisomies. In trisomies 21 and 18 the PlGF MoM did not change with gestational age but in trisomy 13 the deviation from normal increased with gestation. In screening for these aneuploidies the detection rate achieved by PlGF-1 is not improved by the addition of PlGF-2.

The high correlation between serum PlGF-1 and PlGF-2 in the normal and each pathological group presumably reflects the common origin and control mechanisms for the production of these isoforms. The finding that in PE and trisomies the deviation from normal is greater for PlGF-1 than PlGF-2 suggests that the performance of screening for these conditions by serum PlGF-1 at 11–13 weeks' gestation is likely to be inversely related to the percentage of cross-reactivity of the assay to PlGF-2. An assay with high cross-reactivity to PlGF-2 is likely to be less sensitive in predicting PE and trisomies than an assay with low cross-reactivity. Further studies are needed to determine whether the higher performance of PlGF-1 than PlGF-2 in screening for PE at 11–13 weeks persists during the second and third trimesters or whether with advancing gestation the relative performance of the two isoforms is reversed.

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