Fetal Growth and Placental Growth Factor Umbilical Cord Blood Levels

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Keywords

Placental growth factor · Fetal growth · Birth weight · Fetal growth pattern · Fetal growth restriction

Abstract

Objective: We assessed whether umbilical cord blood placental growth factor (PIGF) levels at delivery are associated with fetal growth. **Methods:** From a prospective population-based cohort study we included 3,461 live singleton births. Fetal growth was assessed by birth weight, fetal growth pattern, and fetal growth restriction (FGR; decrease in growth between the second trimester and birth of ≥ 40 percentiles). In all analyses the highest PIGF multiple of the median (MoM) quintile was used as the reference category. **Results:** Umbilical cord PIGF was neither correlated with maternal second-trimester PIGF ($p = 0.08$) nor placental weight ($p = 0.18$), suggesting that PIGF from umbilical cord blood was of fetal origin. Lower PIGF MoM quintiles were associated with a lower birth weight (lowest quintile -0.60 standard deviation [95% confidence interval -0.71 to -0.48 , p for trend < 0.001]) and a different fetal growth pattern ($p < 0.001$). Finally, lower PIGF MoM quintiles were associated with FGR (lowest quintile odds ratio 2.00 [95% confidence interval 1.25 to 3.21, p for trend < 0.001]). **Conclusion:** Lower umbilical cord PIGF levels are associated with lower birth weight, deviating fetal

growth patterns, and a higher odds of FGR. Hence, cord blood PIGF might be a promising biomarker to determine deviations in fetal growth and FGR retrospectively, enabling follow-up of these neonates.

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Introduction

Placental growth factor (PIGF) is a proangiogenic factor produced by the placenta during pregnancy [1]. Extraplacental tissues associated with a high grade of vascularization, such as the heart and lungs, also produce PIGF [2]. Hence, PIGF is also detectable in nonpregnant women, men, and even in cord blood. To date, the majority of studies have focused predominantly on PIGF in the maternal rather than the fetal circulation [3]. Low levels of maternal PIGF are well known to be associated with placental development and function and subsequently with placenta-mediated pregnancy processes and complications, such as preeclampsia and fetal growth restriction (FGR), demonstrated by a lower birth weight and an increased risk for a neonate to be born small for gestational age (SGA) [4–8]. Interestingly, fetal PIGF levels have been associated with fetal growth. Within our cohort lower levels of umbilical cord PIGF were associated with neonates born SGA [9].

However, SGA fetuses constitute a heterogeneous group consisting of fetuses which are constitutionally small and fetuses with FGR resulting in a low birth weight [10–12]. Moreover, neonates could have experienced FGR despite their normal birth weight. Neonates who have experienced FGR may constitute a vulnerable group with an increased risk of accelerated growth and altered cardiovascular outcomes in childhood, irrespective of their birth weight [13, 14]. Identification of FGR neonates is therefore vital.

In this study we aim to assess the association between umbilical cord blood PlGF and fetal growth measured by birth weight, fetal growth pattern, and FGR.

Subjects and Methods

Study Design and Ethical Approval

This study was embedded in the Generation R Study, a population-based prospective cohort study from early pregnancy onwards [15]. The study was approved by the Medical Ethics Committee of the Erasmus Medical Center Rotterdam, The Netherlands. Written informed consent was obtained from all participants. For the present study we included women with a live-born singleton with data available concerning fetal growth and cord blood PlGF levels (Fig. 1).

Pregnancy Dating

Dating by early ultrasonography is vital to ensure accurate pregnancy dating, especially when assessing fetal growth and FGR. Pregnancy dating was performed in early pregnancy using the first ultrasound measurement of either crown-rump length (gestational age until 12 weeks and 5 days of gestation and crown-rump length measurement <65 mm) or biparietal diameter (gestational age from 12 weeks and 5 days of gestation onwards and biparietal diameter >23 mm) [16].

Umbilical Cord Blood PlGF

Umbilical venous cord blood was sampled immediately after birth and transported to the regional laboratory for processing and storage at -80°C . Measurements were performed between 2008 and 2010, and all measurement factors were shown to be stable during long-term storage [17]. Serum levels of PlGF were analyzed using an immunoelectron chemiluminescence assay on the Architect System (Abbott Diagnostics BV). The between-run coefficients of variation for PlGF were 4.7% at 24 pg/mL and 3.8% at 113 pg/mL [8]. Umbilical cord blood PlGF is highly dependent of gestational age at birth. Therefore, multiples of the median (MoM) were created for each gestational week separately.

To investigate whether umbilical cord PlGF had a maternal origin, Pearson correlation tests were performed on umbilical cord PlGF levels with first- and second-trimester maternal PlGF levels and placental weight. The Pearson correlation coefficient for umbilical cord PlGF and placental weight was 0.025 ($p = 0.18$). For maternal PlGF the Pearson correlation coefficients were -0.01 for first-trimester PlGF ($p = 0.69$) and 0.03 for second-trimester PlGF ($p = 0.08$), suggesting that fetal PlGF levels instead of maternal PlGF levels were measured in umbilical blood.

Fig. 1. Flowchart of the selection process. EFW, estimated fetal weight; FGR, fetal growth restriction; PlGF, placental growth factor; SGA, small for gestational age.

Fetal Growth

To assess estimated fetal weight (EFW), ultrasound examinations were performed in the second (median 20.5 weeks of gestation, 90% range 18.9–22.8) and third trimester (median 30.4 weeks of gestation, 90% range 28.8–32.4) of pregnancy. Fetal biometry (head circumference [HC], abdominal circumference [AC], and femur length [FL]) was measured transabdominally. EFW was calculated using the formula of Hadlock with the parameters AC, HC, and FL (in cm): $\text{EFW} = 10^{**} [1.326 - (0.00326 \times \text{AC} \times \text{FL}) + (0.0107 \times \text{HC}) + (0.0438 \times \text{AC}) + (0.158 \times \text{FL})]$ [18]. Ultrasound examinations were performed using Aloka® model SSD-1700 (Tokyo, Japan) or the ATL-Philips® Model HDI 5000 (Seattle, WA, USA). Gestational age at birth and birth weight were obtained from midwives and hospital registries. SGA was defined as a gestational and fetal sex-adjusted birth weight below the fifth percentile based on charts derived from our cohort [16]. Five definitions were used to define FGR: a decrease in growth of at least 30, 35, 40, 45, or 50 percentiles, respectively between the second trimester and birth. Choosing cutoffs to define FGR may lead to misclassification. For a fetus to be able to deviate from its growth curve with 40 percentiles, the initial EFW in the second trimester of pregnancy should by definition have been above the 40th percentile. Hereby fetuses with an EFW below the 40th percentile cannot be classified as FGR and are thus classified as not growth restricted. Therefore, in an additional analysis, we classified all fetuses with

an EFW in the 2nd trimester below the 40th percentile and with a maximum decrease in growth (birth weight below the 1st percentile) as FGR, without any differences in results.

Covariates

We obtained information on maternal age, ethnicity, educational level, parity, smoking during pregnancy, and folic acid intake by questionnaire at enrolment. At intake height (cm) and weight (kg) were measured without shoes and heavy clothing and the body mass index (BMI) was calculated. Preeclampsia was defined as the development of a systolic blood pressure ≥ 140 mm Hg and/or a diastolic blood pressure ≥ 90 mm Hg after 20 weeks of gestation plus the presence of proteinuria (≥ 0.3 g in a 24-h urine specimen or $2+$ or greater [1 g/L] on a voided specimen, or $1+$ or greater [0.3 g/L] on a catheterized specimen) in previously normotensive women according to the former International Society for the Study of Hypertension in Pregnancy criteria [19].

Statistical Analyses

To explore nonlinearity, PlGF MoM was categorized into quintiles: first quintile (PlGF MoM >1.27), second quintile (PlGF MoM <1.27 and >1.08), third quintile (PlGF MoM <1.08 and >0.93), fourth quintile (PlGF MoM <0.93 and >0.78), and fifth quintile (PlGF MoM <0.78).

To test the differences in baseline characteristics between the five PlGF MoM quintiles, ANOVA, Mann-Whitney U, and χ^2 tests were performed. Then linear regression analyses were performed to relate cord blood PlGF MoMs to birth weight. To explore growth trajectories between different PlGF MoM categories, unbalanced repeated-measurements regression models with an unstructured covariance structure were performed. The mixed-model procedure was used with (estimated) weight expressed in standard deviation as a repeated outcome measure. These regression models take the correlation between repeated measurements of the same subject into account. Moreover, they have an optimal use of available measurements by allowing for incomplete outcome data. Then logistic regression models were used to analyze the associations of umbilical cord blood PlGF MoMs with FGR.

Basic models were adjusted for fetal sex and ethnicity. Confounder models were additionally adjusted for maternal age, educational level, parity, smoking during pregnancy, and folic acid intake. We selected these confounders on the basis of their associations with both the exposure and the outcomes of interest or a change in effect estimate of $>10\%$. We considered the confounder model to be the main model. Since angiogenic levels are disturbed in case of maternal preeclampsia, a third model was used in which we additionally adjusted for the presence of preeclampsia (preeclampsia model).

Effect modification on the multiplicative scale was tested to exclude the possibility that the effect of low cord blood PlGF MoM on fetal growth depends on fetal sex, smoking, parity, maternal BMI at intake, and SGA. If $p < 0.10$ was fulfilled, regression analyses were performed in strata of that specific variable.

For all analyses, the percentages of missing values of covariates were $<20\%$. We imputed missing data of the covariates by using multiple imputations [20]. Ten datasets were created and analyzed together. Statistical analyses were performed using the Statistical Package of Social Sciences version 21.0 for Windows (SPSS Inc., Chicago, IL, USA) or the Statistical Analysis System version 9.3 (SAS Institute Inc., Gary, NC, USA).

Fig. 2. Associations between placental growth factor and fetal growth pattern. Results are based on repeated-measurements regression models and reflect the differences in gestational age-adjusted standard deviation scores at 20, 30, 35, and 40 weeks of gestation. The highest quintile of placental growth factor is the reference category and presented as the zero line in the graph. The model was adjusted for fetal sex, ethnicity, maternal age, educational level, parity, smoking during pregnancy, and folic acid intake. SDS, standard deviation score.

Results

The baseline characteristics of the total study population are presented in Table 1. Stratification between the different PlGF MoM quintiles showed that mothers of neonates with lower PlGF MoMs were more often nulliparous ($p < 0.001$) and more often had preeclampsia ($p = 0.007$). The neonates themselves had a lower birth weight ($p < 0.001$).

The results regarding birth weight (standard deviation score) are depicted in Table 2. All quintiles, with the exception of the second quintile, were associated with lower birth weights, with a p for trend <0.001 in all models compared to the first quintile.

The results from repeated measurements showed a different growth pattern in the lowest PlGF MoM quintile compared to the highest quintile (Fig. 2, $p < 0.001$). This resulted in a lower EFW and birth weight. All other quintiles had lower EFWs and lower birth weights, however with a similar growth pattern compared with the highest PlGF MoM quintile.

The results regarding the clinical outcome FGR are depicted in Table 3. Again similar results towards lower PlGF MoMs versus decreased fetal growth were observed,

Table 1. Baseline characteristics of the total study population

	Study population (<i>n</i> = 3,461)	Highest quintile (<i>n</i> = 696)	Second quintile (<i>n</i> = 695)	Third quintile (<i>n</i> = 697)	Fourth quintile (<i>n</i> = 678)	Lowest quintile (<i>n</i> = 695)	<i>p</i> value
Maternal age	29.6 ± 5.2	29.6 ± 5.1	29.5 ± 5.3	29.6 ± 5.1	29.5 ± 5.2	29.7 ± 5.4	0.96
Anthropometrics							
Height, cm	167.7 ± 7.4	168.0 ± 7.6	167.9 ± 7.1	167.8 ± 7.1	167.6 ± 7.7	167.0 ± 7.3	0.1
Weight, kg	69.4 ± 12.9	69.4 ± 12.8	69.8 ± 13.0	68.9 ± 13.0	69.7 ± 13.1	69.0 ± 12.7	0.6
BMI	23.8 (19.3–33.2)	23.8 (19.3–33.2)	23.9 (19.5–33.3)	23.4 (19.0–33.1)	23.8 (19.5–33.4)	24.0 (19.4–33.4)	0.31
Ethnicity							0.94
Western	2,047 (61.6%)	413 (61.9%)	414 (62.3%)	418 (62.3%)	398 (61.1%)	403 (60.3%)	
Non-Western	1,276 (38.4%)	254 (39.1%)	251 (37.7%)	253 (37.7%)	253 (39.9%)	265 (39.7%)	
Educational level							0.08
Low	391 (11.3%)	77 (11.1%)	88 (12.7%)	79 (11.3%)	83 (12.2%)	65 (9.4%)	
Middle	1,589 (46.1%)	326 (46.8%)	306 (44.0%)	309 (44.3%)	311 (45.9%)	346 (49.8%)	
High	1,468 (42.6%)	293 (42.1%)	301 (43.3%)	309 (44.3%)	284 (41.9%)	284 (40.9%)	
Smoking habits							0.46
No	2,470 (71.6%)	499 (71.7%)	506 (72.8%)	486 (69.7%)	484 (71.4%)	500 (71.9%)	
Yes, stopped	291 (8.4%)	50 (7.2%)	58 (8.3%)	64 (9.2%)	68 (10.0%)	55 (7.9%)	
Yes, continued	688 (19.9%)	147 (21.1%)	131 (18.8%)	147 (21.1%)	126 (18.6%)	140 (20.1%)	
Folic acid use							0.18
No	990 (28.7%)	191 (27.4%)	201 (28.9%)	194 (27.8%)	205 (30.2%)	192 (27.6%)	
Yes, before 10 weeks	1,099 (31.8%)	218 (31.3%)	229 (32.9%)	219 (31.4%)	221 (32.6%)	229 (32.9%)	
Yes, preconception start	1,360 (39.4%)	287 (41.2%)	265 (38.1%)	284 (40.7%)	252 (37.2%)	274 (39.4%)	
Nulliparous	1,942 (56.3%)	320 (46.0%)	318 (45.8%)	399 (57.2%)	421 (62.1%)	490 (70.5%)	<0.001
Preeclampsia	42 (1.3%)	5 (0.7%)	5 (0.7%)	5 (0.7%)	10 (1.5%)	17 (2.4%)	0.007
GA at sonography, weeks							
Second trimester	20.5 (18.9–22.8)	20.5 (19.0–22.9)	20.5 (18.8–22.7)	20.5 (18.9–22.8)	20.5 (18.8–22.8)	20.5 (18.9–22.9)	0.65
Third trimester	30.4 (28.8–32.3)	30.4 (28.8–32.3)	30.4 (28.8–32.3)	30.3 (28.9–32.3)	30.3 (28.7–32.2)	30.4 (28.6–32.6)	0.72
GA at birth, weeks	40.1 (37.4–42.0)	40.1 (37.1–42.1)	40.1 (37.6–42.0)	40.0 (37.4–42.0)	40.1 (37.4–42.0)	40.3 (37.3–42.2)	0.28
EFW 2nd trimester, g	382 ± 94	384 ± 91	381 ± 95	385 ± 98	380 ± 95	383 ± 93	0.87
EFW 3rd trimester, g	1,614 ± 255	1,619 ± 241	1,627 ± 274	1,611 ± 38	1,603 ± 253	1,612 ± 265	0.52
Birth weight, g	3,458 ± 500	3,556 ± 535	3,551 ± 463	3,451 ± 471	3,431 ± 475	3,298 ± 509	<0.001
PIGF MoM	1.00 (0.58–1.75)	1.47 (1.29–5.09)	1.16 (1.08–1.26)	1.00 (0.93–1.07)	0.86 (0.79–0.92)	0.64 (0.41–0.77)	<0.001

Data are presented as *n* (%), mean ± standard deviation, or median (90% range). Differences in baseline characteristics were tested using analysis of variance, Mann-Whitney U test, and χ^2 tests. BMI, body mass index; EFW, estimated fetal weight; GA, gestational age; MoM, multiple of the median; PIGF, placental growth factor.

Table 2. Associations between cord blood PIGF and birth weight

	Basic model		Confounder model		Preeclampsia model	
	β (95% CI)	<i>p</i> value	β (95% CI)	<i>p</i> value	β (95% CI)	<i>p</i> value
<i>Birth weight (SDS)</i>						
PIGF MoM 1st quintile	reference		reference		reference	
PIGF MoM 2nd quintile	−0.02 (−0.12 to 0.09)	0.74	−0.02 (−0.12 to 0.08)	0.70	−0.02 (−0.12 to 0.08)	0.72
PIGF MoM 3rd quintile	−0.22 (−0.33 to −0.12)	<0.001	−0.19 (−0.29 to −0.08)	<0.001	−0.19 (−0.29 to −0.08)	<0.001
PIGF MoM 4th quintile	−0.29 (−0.39 to −0.18)	<0.001	−0.24 (−0.34 to −0.13)	<0.001	−0.23 (−0.33 to −0.13)	<0.001
PIGF MoM 5th quintile	−0.60 (−0.71 to −0.48)	<0.001	−0.50 (−0.61 to −0.38)	<0.001	−0.48 (−0.60 to −0.37)	<0.001
	<i>p</i> for trend	<0.001	<i>p</i> for trend	<0.001	<i>p</i> for trend	<0.001

Values are regression coefficient (95% CI) and are based on linear regression models. Basic model: adjusted for fetal sex and ethnicity. Confounder model: basic model additionally adjusted for maternal age, educational level, parity, smoking, and folic acid intake. Preeclamptic model: confounder model additionally adjusted for maternal preeclampsia. CI, confidence interval; MoM, multiple of the median; PIGF, placental growth factor; SDS, standard deviation score.

Table 3. Associations between cord blood placental growth factor and FGR

	Basic model		Confounder model		Preeclampsia	
	β (95% CI)	<i>p</i> value	β (95% CI)	<i>p</i> value	β (95% CI)	<i>p</i> value
<i>FGR 30</i>						
PIGF MoM 1st quintile	reference		reference		reference	
PIGF MoM 2nd quintile	1.05 (0.70 to 1.56)	0.82	1.03 (0.69 to 1.54)	0.90	1.03 (0.69 to 1.54)	0.90
PIGF MoM 3rd quintile	1.31 (0.89 to 1.94)	0.17	1.20 (0.81 to 1.79)	0.37	1.20 (0.80 to 1.78)	0.38
PIGF MoM 4th quintile	1.73 (1.19 to 2.52)	0.004	1.57 (1.07 to 2.31)	0.02	1.56 (1.06 to 2.29)	0.02
PIGF MoM 5th quintile	2.38 (1.67 to 3.39)	<0.001	2.01 (1.38 to 2.93)	<0.001	1.99 (1.36 to 2.90)	<0.001
	<i>p</i> for trend	<0.001	<i>p</i> for trend	<0.001	<i>p</i> for trend	<0.001
<i>FGR 35</i>						
PIGF MoM 1st quintile	reference		reference		reference	
PIGF MoM 2nd quintile	1.11 (0.71 to 1.73)	0.64	1.09 (0.69 to 1.71)	0.72	1.09 (0.69 to 1.70)	0.72
PIGF MoM 3rd quintile	1.50 (0.98 to 2.30)	0.07	1.39 (0.90 to 2.15)	0.14	1.39 (0.90 to 2.15)	0.14
PIGF MoM 4th quintile	1.84 (1.21 to 2.78)	0.004	1.69 (1.10 to 2.59)	0.016	1.68 (1.09 to 2.58)	0.018
PIGF MoM 5th quintile	2.35 (1.58 to 3.50)	<0.001	1.92 (1.26 to 2.93)	0.002	1.88 (1.24 to 2.87)	0.003
	<i>p</i> for trend	<0.001	<i>p</i> for trend	<0.001	<i>p</i> for trend	<0.001
<i>FGR 40</i>						
PIGF MoM 1st quintile	reference		reference		reference	
PIGF MoM 2nd quintile	1.04 (0.62 to 1.73)	0.89	1.02 (0.61 to 1.71)	0.95	1.02 (0.61 to 1.70)	0.96
PIGF MoM 3rd quintile	1.62 (1.01 to 2.62)	0.047	1.56 (0.96 to 2.53)	0.07	1.55 (0.95 to 2.52)	0.08
PIGF MoM 4th quintile	2.05 (1.29 to 3.26)	0.002	1.89 (1.18 to 3.04)	0.009	1.87 (1.16 to 3.01)	0.01
PIGF MoM 5th quintile	2.30 (1.47 to 3.61)	<0.001	2.00 (1.25 to 3.21)	0.004	1.97 (1.23 to 3.17)	0.005
	<i>p</i> for trend	<0.001	<i>p</i> for trend	<0.001	<i>p</i> for trend	<0.001
<i>FGR 45</i>						
PIGF MoM 1st quintile	reference		reference		reference	
PIGF MoM 2nd quintile	1.34 (0.73 to 2.47)	0.35	1.29 (0.70 to 2.39)	0.41	1.29 (0.70 to 2.38)	0.42
PIGF MoM 3rd quintile	1.79 (0.99 to 3.22)	0.053	1.70 (0.93 to 3.09)	0.08	1.70 (0.93 to 3.09)	0.08
PIGF MoM 4th quintile	2.81 (1.62 to 4.89)	<0.001	2.68 (1.52 to 4.71)	0.001	2.64 (1.50 to 4.65)	0.001
PIGF MoM 5th quintile	3.27 (1.91 to 5.60)	<0.001	2.92 (1.66 to 5.12)	<0.001	2.88 (1.64 to 5.07)	<0.001
	<i>p</i> for trend	<0.001	<i>p</i> for trend	<0.001	<i>p</i> for trend	<0.0001
<i>FGR 50</i>						
PIGF MoM 1st quintile	reference		reference		reference	
PIGF MoM 2nd quintile	1.39 (0.69 to 2.80)	0.36	1.32 (0.65 to 2.69)	0.44	1.32 (0.65 to 2.68)	0.44
PIGF MoM 3rd quintile	1.50 (0.74 to 3.05)	0.26	1.41 (0.69 to 2.89)	0.34	1.42 (0.69 to 2.90)	0.34
PIGF MoM 4th quintile	2.94 (1.56 to 5.55)	0.001	2.84 (1.49 to 5.43)	0.002	2.80 (1.46 to 5.36)	0.002
PIGF MoM 5th quintile	3.76 (2.04 to 6.93)	<0.001	3.45 (1.83 to 6.53)	<0.001	3.43 (1.81 to 5.50)	<0.001
	<i>p</i> for trend	<0.001	<i>p</i> for trend	<0.001	<i>p</i> for trend	<0.001

Values are regression coefficient (95% CI) and are based on linear regression models. Basic model: adjusted for fetal sex and ethnicity. Confounder model: basic model additionally adjusted for maternal age, educational level, parity, smoking, and folic acid intake. Preeclampsia model: confounder model additionally adjusted for maternal preeclampsia. CI, confidence interval; FGR, fetal growth restriction; MoM, multiple of the median; PIGF, placental growth factor.

with odds ratios up to 3.45 (95% confidence interval 1.83 to 6.53) for the fifth quintile for FGR with a decrease in growth of >50 percentiles.

Interaction analyses showed no effect modification of fetal sex, smoking, parity, maternal BMI at intake, and SGA on all outcomes.

Discussion

Principal Findings

This study showed that umbilical cord blood PIGF is associated with fetal growth, with consistent results for birth weight, growth pattern, and the outcome FGR. The

lower PlGF levels, the stronger the observed effect estimates.

Strengths and Limitations

The main strength of this study was the extensive prospective data collection on fetal growth and environmental influences. This enabled us to adjust for multiple confounding factors and to investigate the effects of fetal growth in a large sample of 3,461 participants.

The response rate at baseline for participation in the Generation R Study was 61%. Selective participations in this study did occur since pregnant women who participated were generally higher educated, more healthy, and more frequently of European origin than those who did not participate. Data on umbilical cord blood PlGF were available in 36.4% of our total live singleton births. The nonresponse at baseline would lead to biased effect estimates if the associations were different between those included and those not included in the analyses. However, given the prospective nature of the study, this seems unlikely. Nevertheless it might have led to the selection of a more healthy population, which affects the generalizability of our results.

For the purpose of this study we included only live singleton births. Therefore, intrauterine fetal deaths with or without FGR were excluded. As a consequence, the included pregnancies constitute a relatively healthy and homogenous study population.

In this study, we focused on a true deviation of fetal growth rather than on other parameters such as perinatal outcomes, Doppler parameters, or placental function. SGA was defined as a gestational and fetal sex-adjusted birth weight below the fifth percentile based on charts derived from our cohort. This cutoff was chosen since the fifth percentile of our cohort is more comparable with the cutoff of the tenth percentile used in general clinical practice, compared with the tenth percentile of our cohort.

Interpretation

One of the key findings of this study is that not only the lowest quintile of cord blood PlGF was associated with fetal growth, but that also associations for higher quintiles were found and that clear trends were visible. Although this is an observational study and causality can therefore not be proven, one of the most important criteria for causation according to the Bradford Hill criteria is a biological gradient [21]. In our study it is clear that a clear trend was visible in the association between neonatal PlGF levels and fetal growth parameters. Moreover,

the effect sizes are substantial and consistent despite adjustment for multiple confounding factors.

Fetal cord blood may contain PlGF of fetal and maternal or placental origin since PlGF crosses the placenta. However, very low correlations were found between umbilical cord blood PlGF levels on the one hand and maternal PlGF levels and placental weight on the other. Furthermore, there were hardly any differences in effect estimates of the confounder models and the preeclampsia models. This implies that the vast majority of PlGF measured in umbilical cord blood is of fetal origin. Unfortunately no maternal PlGF levels at the end of pregnancy were available within our cohort. During pregnancy, PlGF reaches its peak around 31 weeks of gestation, after which it declines [7, 22]. Around the time of delivery, levels of maternal PlGF are comparable with levels around 19 weeks of gestation. Correlation coefficients might therefore not be drastically different even if term maternal PlGF at term had been available. This is confirmed by one study in which there was a 10-fold difference for PlGF in the maternal and fetal circulation during delivery and without a significant correlation between the two [23]. It is suggested that the umbilical artery PlGF would be a better representation of the fetal circulation compared to the umbilical vein PlGF which we used in our study. Some studies investigated PlGF in both the umbilical vein as well as arteries in SGA neonates compared with appropriate for gestational age neonates and considered the umbilical vein as a representation of the placental compartment [24]. They observed differences in PlGF levels between SGA and appropriate for gestational age neonates in the umbilical vein, but not in the umbilical artery, and concluded that fetal PlGF was not altered in SGA. However, if the umbilical vein indeed is a representation of the placental compartment, a significant correlation would be expected between umbilical vein PlGF and maternal PlGF or measures of placental function such as placental weight. In our study these associations were not present, from which we conclude that also the umbilical vein is representative for the fetal circulation.

In the current literature, the clinical outcome FGR is often defined as SGA [25]. However, FGR is not necessarily synonymous with SGA. FGR fetuses may experience a failure to reach their biological growth potential because of a pathological slowdown in the fetal growth pace. Recently, the Delphi study also established a definition for FGR by expert consensus, acknowledging the significant difference between FGR and SGA [25]. The definitions of both early and late FGR were based on

measurements taken at one single point in time. Although crossing centiles for AC or EFW were allowed in the definition for late FGR, it must be combined with another criterion. We, on the other hand, primarily and solely focused on deviating growth patterns. Since there is no international consensus on how much a fetal growth curve needs to deviate before it can be designated as FGR, we used several cutoffs of a decrease in growth between the second trimester of pregnancy and birth. In previous studies, FGR fetuses without SGA were not the subject of research. Nevertheless, this might be a vulnerable group with increased risks of adverse health in later life. The current study shows an association between fetal PlGF and the clinical outcome FGR. These associations were not only found for the severe growth restricted fetuses, i.e., a decrease of ≥ 50 percentiles, but also for the mildly growth-restricted fetuses, i.e., a decrease of ≥ 30 percentiles. Future research has to focus on the potential of fetal PlGF to identify those neonates at risk for adverse childhood outcomes.

Since little research has been performed on neonatal PlGF, not much is known about the exact origin of neonatal PlGF. PlGF is not only expressed by the placenta, but also by tumor cells, endothelial cells, and inflammatory cells [26]. Especially endothelial cells could be a potential source of PlGF within the neonate. PlGF exerts a strong effect upon blood vessel growth and maturation and has direct proangiogenic effects on endothelial cells [27]. Within cardiovascular disease, endothelial function is crucial and often precedes clinical manifestations [28, 29]. This is of importance when taking the Development and Origins of Health and Disease theory into consideration. According to this theory, the unborn fetus can modify its own development such that it will be prepared for survival in an environment in which recourses are likely to be short. Although these adaptations may be beneficial for short-term survival, they may have adverse consequences at delivery or in later life [30]. Since FGR fetuses have an increased risk of developing cardiac diseases in later life, low cord blood levels of PlGF could well be one of the first signs of this increased risk. Nevertheless, we should consider the possibility that small fetuses produce less PlGF. In that case the underlying mechanisms of the fetuses being small cause the potential adverse outcomes in later life associated with FGR rather than PlGF itself. However, this seems unlikely considering the incidence of preeclampsia as shown in Table 1, which differs considerably across the different PlGF quintiles.

Conclusion

This study demonstrates that umbilical cord blood PlGF is associated with birth weight, fetal growth pattern, and FGR. Fetuses born with FGR are often born with a normal birth weight, but nevertheless constitute a vulnerable group with an increased risk for cardiovascular diseases in later life. Identification of this vulnerable group enables follow-up in the postnatal period, preventing possible adverse events in later life. Future research is needed to investigate whether fetal PlGF can be used to identify those fetuses at risk for adverse childhood outcomes.

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Author Contributions

Z.A. Broere-Brown analyzed the data and wrote the article. S. Schalekamp-Timmermans contributed to the design of the article and assisted with the analyses and writing of the article. V.W.V. Jaddoe contributed to the design of the study, the interpretation of the data, and revisions and gave input at all stages of the study. E.A.P. Steegers contributed to the design of the study, the writing of the article, interpretation of the data, and revisions and gave input at all stages of the study. All authors have approved the final version of the manuscript.

References

- Maglione D, Guerriero V, Viglietto G, Delli-Bovi P, Persico MG: Isolation of a human placenta cDNA coding for a protein related to the vascular permeability factor. *Proc Natl Acad Sci USA* 1991;88:9267–9271.
- Andraweera PH, Dekker GA, Roberts CT: The vascular endothelial growth factor family in adverse pregnancy outcomes. *Hum Reprod Update* 2012;18:436–457.
- Triunfo S, Lobmaier S, Parra-Saavedra M, Crovetto F, Peguero A, Nadal A, Gratacos E, Figueras F: Angiogenic factors at diagnosis of late-onset small-for-gestational age and histological placental underperfusion. *Placenta* 2014;35:398–403.
- Levine RJ, Maynard SE, Qian C, Lim KH, England LJ, Yu KF, Schisterman EF, Thadhani R, Sachs BP, Epstein FH, Sibai BM, Sukhatme VP, Karumanchi SA: Circulating angiogenic factors and the risk of preeclampsia. *N Engl J Med* 2004;350:672–683.
- Taylor RN, Grimwood J, Taylor RS, McMaster MT, Fisher SJ, North RA: Longitudinal serum concentrations of placental growth factor: evidence for abnormal placental angiogenesis in pathologic pregnancies. *Am J Obstet Gynecol* 2003;188:177–182.
- Tidwell SC, Ho HN, Chiu WH, Torry RJ, Torry DS: Low maternal serum levels of placenta growth factor as an antecedent of clinical preeclampsia. *Am J Obstet Gynecol* 2001;184:1267–1272.
- Sundrani D, Khot V, Pisal H, Mehendale S, Wagh G, Joshi A, Joshi S: Gestation dependent changes in angiogenic factors and their associations with fetal growth measures in normotensive pregnancy. *PLoS One* 2013;8:e54153.
- Coolman M, Timmermans S, de Groot CJ, Russcher H, Lindemans J, Hofman A, Geurts-Moespot AJ, Sweep FC, Jaddoe VV, Steegers EA: Angiogenic and fibrinolytic factors in blood during the first half of pregnancy and adverse pregnancy outcomes. *Obstet Gynecol* 2012;119:1190–1200.
- Bergen NE, Bouwland-Both MI, Steegers-Theunissen RP, Hofman A, Russcher H, Lindemans J, Jaddoe VV, Steegers EA: Early pregnancy maternal and fetal angiogenic factors and fetal and childhood growth: the Generation R Study. *Hum Reprod* 2015;30:1302–1313.
- Alberry M, Soothill P: Management of fetal growth restriction. *Arch Dis Child Fetal Neonatal Ed* 2007;92:F62–F67.
- Illanes S, Soothill P: Management of fetal growth restriction. *Semin Fetal Neonatal Med* 2004;9:395–401.
- Figueras F, Gratacos E: Stage-based approach to the management of fetal growth restriction. *Prenat Diagn* 2014;34:655–659.
- Crispi F, Bijns B, Figueras F, Bartrons J, Eixarch E, Le Noble F, Ahmed A, Gratacos E: Fetal growth restriction results in remodeled and less efficient hearts in children. *Circulation* 2010;121:2427–2436.
- Figueras F, Oros D, Cruz-Martinez R, Padilla N, Hernandez-Andrade E, Botet F, Costas-Moragas C, Gratacos E: Neurobehavior in term, small-for-gestational age infants with normal placental function. *Pediatrics* 2009;124:e934–e941.
- Jaddoe VW, van Duijn CM, Franco OH, van der Heijden AJ, van Iizendoorn MH, de Jongste JC, van der Lugt A, Mackenbach JP, Moll HA, Raat H, Rivadeneira F, Steegers EA, Tie-meier H, Uitterlinden AG, Verhulst FC, Hofman A: The Generation R Study: design and cohort update 2012. *Eur J Epidemiol* 2012;27:739–756.
- Verburg BO, Steegers EA, De Ridder M, Snijders RJ, Smith E, Hofman A, Moll HA, Jaddoe VW, Witteman JC: New charts for ultrasound dating of pregnancy and assessment of fetal growth: longitudinal data from a population-based cohort study. *Ultrasound Obstet Gynecol* 2008;31:388–396.
- Law LW, Sahota DS, Chan LW, Chen M, Lau TK, Leung TY: Effect of long-term storage on placental growth factor and fms-like tyrosine kinase 1 measurements in samples from pregnant women. *J Matern Fetal Neonatal Med* 2010;23:1475–1480.
- Hadlock FP, Harrist RB, Sharman RS, Deter RL, Park SK: Estimation of fetal weight with the use of head, body, and femur measurements – a prospective study. *Am J Obstet Gynecol* 1985;151:333–337.
- Report of the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy. *Am J Obstet Gynecol* 2000;183:S1–S22.
- Sterne JA, White IR, Carlin JB, Spratt M, Royston P, Kenward MG, Wood AM, Carpenter JR: Multiple imputation for missing data in epidemiological and clinical research: potential and pitfalls. *BMJ* 2009;338:b2393.
- Hill AB: The environment and disease: association or causation? *Proc R Soc Med* 1965;58:295–300.
- Tsiakkas A, Duvdevani N, Wright A, Wright D, Nicolaides KH: Serum placental growth factor in the three trimesters of pregnancy: effects of maternal characteristics and medical history. *Ultrasound Obstet Gynecol* 2015;45:591–598.
- Algeri P, Ornaghi S, Bernasconi DP, Cappellini F, Signorini S, Brambilla P, Urban G, Vergani P: Feto-maternal correlation of PTX3, sFlt-1 and PlGF in physiological and pre-eclamptic pregnancies. *Hypertens Pregnancy* 2014;33:360–370.
- Wallner W, Sengenberger R, Strick R, Strissel PL, Meurer B, Beckmann MW, Schlembach D: Angiogenic growth factors in maternal and fetal serum in pregnancies complicated by intrauterine growth restriction. *Clin Sci (Lond)* 2007;112:51–57.
- Gordijn SJ, Beune IM, Thilaganathan B, Pappageorgiou A, Baschat AA, Baker PN, Silver RM, Wynia K, Ganzevoort W: Consensus definition of fetal growth restriction: a Delphi procedure. *Ultrasound Obstet Gynecol* 2016;48:333–339.
- Cao Y: Positive and negative modulation of angiogenesis by VEGFR1 ligands. *Sci Signal* 2009;2:re1.
- Carmeliet P, Moons L, Lutun A, Vincenti V, Compernelle V, De Mol M, Wu Y, Bono F, Devy L, Beck H, Scholz D, Acker T, DiPalma T, Dewerchin M, Noel A, Stalmans I, Barra A, Blacher S, VandenDriessche T, Ponten A, Eriksson U, Plate KH, Foidart JM, Schaper W, Charnock-Jones DS, Hicklin DJ, Herbert JM, Collen D, Persico MG: Synergism between vascular endothelial growth factor and placental growth factor contributes to angiogenesis and plasma extravasation in pathological conditions. *Nat Med* 2001;7:575–583.
- Hadi HA, Carr CS, Al Suwaidi J: Endothelial dysfunction: cardiovascular risk factors, therapy, and outcome. *Vasc Health Risk Manag* 2005;1:183–198.
- Gidullin NS, Valeeva KF, Gassanov N, Zagidullin SZ: Value of endothelial dysfunction in cardiovascular diseases and methods of its correction with drugs (in Russian). *Kardiologiia* 2010;50:54–60.
- Barker DJ, Osmond C: Infant mortality, childhood nutrition, and ischaemic heart disease in England and Wales. *Lancet* 1986;1:1077–1081.