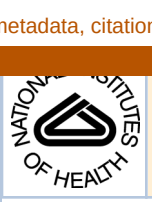

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GLYCOGEN PHOSPHORYLASE ISOENZYME BB PLASMA CONCENTRATION IS ELEVATED IN PREGNANCY AND PRETERM PREECLAMPSIA

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Abstract

Glycogen phosphorylase is a key enzyme in glycogenolysis. Released with myocardial ischemia, blood concentration of glycogen phosphorylase isoenzyme BB (GPBB) is a marker of acute coronary syndromes. Pregnancy imposes metabolic stress, and preeclampsia is associated with cardiac complications. However, plasma GPBB concentration during pregnancy is unknown. This study was conducted to determine maternal plasma GPBB concentration in normal pregnancy and in preeclampsia. Plasma samples from six groups (n=396) were studied: non-pregnant women and pregnant women with normal term delivery, term preeclampsia, term small-for-gestational-age neonates, preterm preeclampsia, and preterm small-for-gestational-age neonates. GPBB concentration was measured with a specific immunoassay. Placental tissues (n=45) obtained from pregnant women with preterm and term preeclampsia, spontaneous preterm delivery, and normal term cases were analyzed for potential GPBB expression by immunoblotting. Median plasma GPBB concentration was higher in pregnant women than in non-pregnant women (38.7 ng/ml versus 9.2 ng/mL, $P<0.001$), which remained significant after adjusting for age, race, and parity. Maternal plasma GPBB concentrations did not change throughout gestation. Preterm but not term preeclampsia cases had higher median plasma GPBB concentration than gestational-age-matched normal pregnancy cases (72.6 ng/ml versus 26.0 ng/ml, $P=0.001$). Small-for-gestational-age neonates did not affect plasma GPBB concentration. GPBB was detected in the placenta and was less abundant in preterm preeclampsia than in preterm delivery cases ($P<0.01$). There is physiologic elevation of plasma GPBB concentration during pregnancy; an increase in maternal plasma GPBB is a novel phenotype of preterm preeclampsia. It is strongly suggested that these changes are attributed to GPBB of placental origin.

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Disclosures

None.

Keywords

glycogen phosphorylase; hypoxia; placenta; preeclampsia; pregnancy

Introduction

Glycogen phosphorylase (GP) is an essential enzyme in the regulation of glycogen metabolism. It catalyzes and converts glycogen into glucose-1-phosphate in the first rate-limiting step of glycogenolysis.[1–5] Three different GP isoenzymes have been identified in humans – GP isoenzyme LL (GPLL, liver), GP isoenzyme MM (GPMM, muscle), and GP isoenzyme BB (GPBB, brain) – which have been named for the tissues in which they were initially identified.[1,2] Skeletal muscles solely contain GPMM; GPLL is present in all human tissues except in the brain, heart, and skeletal muscles; and GPBB is mainly detected in the brain but can also be found in high concentration in the heart muscle.[1,2] When there is tissue hypoxia and ischemia, glycogenolysis is initiated with the conversion of GP into its more active, soluble, and cytoplasmic form by phosphorylase kinase.[3] As cell membrane permeability also increases during hypoxia, a high GP concentration gradient in the sarcoplasmic reticulum compartment allows GP efflux into the bloodstream via the T-tubule system.[3] Based on the distribution of GPBB in the heart muscle and increased plasma concentration of GP during ischemia and hypoxia, several studies have demonstrated that plasma GPBB is a surrogate marker of acute coronary syndromes.[3–6]

Pregnant women experience dynamic and intense metabolic stress with increased cardiac output in compliance with rising demands of the growing fetus and placenta.[7] In addition, during pregnancy, hypoxia plays a crucial role not only in normal placental and embryonic development during the first trimester,[8–10] but also in the development of adverse obstetric outcomes including preeclampsia[11–13] and fetal growth restriction (FGR).[14] Furthermore, maternal cardiac complications such as myocardial infarction and heart failure are more frequent in cases with preeclampsia.[15] However, the plasma concentrations of GPBB in normal pregnancy and in pregnancy disorders have not been studied so far. We hypothesized that there is a change in plasma GPBB concentration during pregnancy and especially in association with preeclampsia.

The objective of this study was to determine the changes in maternal plasma GPBB concentrations for normal pregnant women and women with preterm preeclampsia or term preeclampsia.

Methods

Study Design

Plasma samples were collected from the following six groups classified according to the clinical circumstances at the time of blood sampling: non-pregnant women (n=33), pregnant women with a normal singleton pregnancy (n=151), patients with term preeclampsia (n=72), patients with term small-for-gestational-age (SGA) neonates and no preeclampsia (n=38), patients with preterm preeclampsia (n=74), and patients with preterm SGA neonates and no preeclampsia (n=28). Three serial blood samples obtained during each trimester throughout gestation (6–14 weeks, 15–24 weeks, and 37–42 weeks) were available in 32 of 151 normal pregnant women. Preeclampsia was diagnosed according to the diagnostic criteria of the American College of Obstetricians and Gynecologists:[16] in the presence of hypertension (elevated systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg at least twice, 4 hours to 1 week apart, after the 20th week of gestation) and of proteinuria (≥300 mg in a 24-hour urine collection, or 2 random urine specimens containing ≥1+ or more protein

by dipstick or 1 dipstick measurement ≥2+ protein). SGA pregnancies included 1) those of FGR fetuses detected by ultrasonography and confirmed SGA neonates after delivery in cases of > 5 days of blood-sampling-to-delivery intervals, and 2) SGA neonates at the time of delivery in cases of ≤ 5 days of intervals. The SGA neonate was defined when its birth weight was below the 10th percentile for gestational age according to the reference range proposed by Alexander et al,[17] and FGR was diagnosed when the estimated fetal weight proposed by Hadlock et al[18] was below the 10th percentile for gestational age. A normal pregnancy outcome was defined when patients met the following criteria: 1) no prior abnormal medical and surgical conditions, 2) no obstetrical, maternal, or fetal complications during pregnancy, and 3) delivery of a healthy neonate at term whose birth weight was appropriate for gestational age (AGA) (>10th percentile and <90th percentile). Placental tissues (n=45) were obtained from pregnant women with preterm preeclampsia (n=9), preterm labor and preterm delivery (n=9), term preeclampsia (n=9), and normal term delivery (n=18).

All women were enrolled at Hutzel Women's Hospital in Detroit, Michigan, and provided written informed consent for the collection and use of clinical data and biological materials. Plasma and tissue samples from all patients were retrieved from the Bank of Biological Materials of the Perinatology Research Branch, *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD), National Institutes of Health (NIH), U. S. Department of Health and Human Services (DHHS). This study was conducted under the ethical standards for human experimentation established in the Declaration of Helsinki. The Institutional Review Boards of Wayne State University and the NICHD/NIH/DHHS approved the collection and use of clinical data and biological materials for research purposes.

Enzyme-linked Immunosorbent Assay (ELISA) for GPBB

Blood samples, collected in EDTA tubes, were centrifuged at 1,300xg for 10 min at 4°C. The plasma samples were kept at −70°C until assay. Maternal plasma GPBB concentration was measured with GPBB ELISA Kits (Diagenics SE, Essen, Germany), according to the manufacturer's instructions. The sensitivity of the assay was 1.463 ng/ml, and the coefficients of intra-assay variation and inter-assay variation were 5.2% and 10.7%, respectively.

Immunoblotting

Villous placental tissue samples were pooled from five different sampling sites. An equal amount of placental villous tissues was taken from five sampling sites generated by a systematic random sampling method. After trimming the chorionic plate and the basal plate, the pooled samples were flash-frozen using liquid nitrogen and kept at −80°C until use. Proteins were extracted from liquid nitrogen-pulverized chorionic villous tissue using a radioimmunoprecipitation assay buffer (Sigma-Aldrich, St. Louis, MO) with a proteinase inhibitor mixture (Roche, Basel, Switzerland). Protein lysates were subjected to 4–15% SDS-PAGE gel (Bio-Rad, Hercules, CA), electrophoresed under reducing conditions, and followed by electro-transfer onto polyvinylidene difluoride (PVDF) membranes (GE Healthcare Bio-Sciences, Piscataway, NJ). Non-specific binding was blocked for 1 hour at room temperature with 5% nonfat dry milk in TBS containing 0.1% Tween 20 (0.1% TBS-T). After washing, membranes were incubated overnight at 4°C with primary antibodies specific to GPBB (mouse anti-human; SC-81751) and HPRT (rabbit anti-human; SC-20975) (1:200; Santa Cruz Biotechnology, Inc., Santa Cruz, CA). HRP-conjugated anti-mouse IgG (7076) and anti-rabbit IgG (7074) (1:1,500; Cell Signaling Technology, Inc., Danvers, MA) were used as secondary antibodies. Signals were detected using chemiluminescence (ChemiGlow West Kit; Alpha Innotech Corporation, San Leandro, CA), and the

densitometric analysis was performed using FluorChem SP densitometry (Alpha Innotech Corporation).

Immunofluorescence Staining

For double-label immunofluorescence staining, 5-μm-thick frozen sections of the placenta were used. These were fixed with 4% paraformaldehyde, permeabilized with 0.25% Triton X-100, and incubated with 5% BSA in PBS for 30 min at room temperature. Tissue sections were incubated with anti-GPBB (ab61036; 1:25; rabbit polyclonal; Abcam, Cambridge, MA) and cytokeratin-7 (M7018; 1:1,000; mouse monoclonal, Dako, Carpinteria, CA) in 1% BSA in PBS for 1 hour. Thereafter, the sections were incubated using Alexa Fluor® 488 donkey anti-rabbit IgG (A21206) and Alexa Fluor® 594 donkey anti-mouse IgG (A21203) as secondary antibodies in 1% BSA for 30 min and mounted in ProLong Gold antifade reagent with DAPI (Invitrogen, Carlsbad, CA). The stained sections were evaluated with a Leica TCS SP5 spectral confocal system (Leica Microsystems, Wetzlar, Germany).

Statistical Analysis

Statistical analysis was performed using SPSS version 15.0 (SPSS Inc., Chicago, IL). For continuous variables, after distributions were determined for normality using Kolmogorov-Smirnov tests, the Kruskal-Wallis analysis of variance was used with the Mann-Whitney U test or one-way ANOVA with post-hoc analysis. For categorical variables, proportions were compared using the χ^2 test or Fisher's exact test. For related variables, the Friedman test and the Wilcoxon signed rank test were performed to examine the change in maternal GPBB concentrations throughout gestation. Medians and ranges or interquartiles were calculated for continuous variables and frequencies and percentages were reported for categorical variables. Analysis of covariance and logistic regression analysis were conducted for comparison of maternal GPBB concentrations to adjust for maternal age, racial disparity, parity, baby gender, and gestational age at blood sampling. All *P* values are 2-sided, and a value of less than 0.05 is considered statistically significant.

Results

GPBB Plasma Concentration in Pregnancy and Preeclampsia

The analysis of plasma GPBB concentration in this study (Table 1 and Table 2) included 396 women. Table 1 shows the demographics and clinical characteristics of the study groups of non-pregnant women and pregnant women with normal term delivery. Maternal age, racial distribution, and parity were different between non-pregnant women and pregnant women with normal term delivery ($P<0.01$, for each). Pregnant women with normal term delivery included (1) 32 cases whose blood samples were drawn serially for the longitudinal analysis, (2) 65 cases presenting with term in labor, (3) 25 cases presenting with term no labor, and (4) 29 normal term delivery cases whose blood samples were collected before 37 weeks of gestation as preterm controls for preterm preeclampsia patients. Table 2 illustrates the demographics and clinical characteristics of the study populations including cases with preeclampsia or SGA neonates and their gestational age-matched controls both in term and preterm gestations. There were significant differences in parity, gestational age at blood sampling, gestational age at delivery, baby gender, the rates of SGA neonates, and Cesarean delivery among groups both in term and preterm gestations ($P<0.05$, for each).

The median GPBB plasma concentration was significantly higher in pregnant women with a normal pregnancy outcome (n=151) than in non-pregnant women (n=33) (median 38.7 ng/ml, range 2.8–825.4 ng/ml vs. median 9.2 ng/ml, range 1.5–61.8 ng/ml, $P<0.001$), which remained significant after adjusting for maternal age, race, and parity (Figure 1). In addition, an analysis of plasma samples serially obtained during each trimester from 32 women with a

normal pregnancy outcome, whose blood samples were drawn in the absence of labor, demonstrated no differences in the maternal plasma GPBB concentration as a function of advancing gestation (1st trimester: median 26.4 ng/ml, range 8.3–155.0 ng/ml; 2nd trimester: median 39.5 ng/ml, range 6.0–205.5 ng/ml; and 3rd trimester: median 27.5 ng/ml, range 6.8–88.5 ng/ml) (Figure 2).

Figure 3 displays the comparison of maternal plasma GPBB concentration according to the presence or absence of labor at term because it was shown that labor itself is associated with intermittent perfusion and oxidative stress of the placenta.[19] The comparison was conducted in patients at term with labor at the time of blood sampling (n=65) and those without labor at the time of blood sampling (n=57: last drawn samples of cases for the longitudinal analysis [n=32] and cases of additional term no labor at blood sampling [n=25]) (Table 1). There was no statistically significant difference in maternal plasma GPBB concentration between patients with and without labor (median 62.6 ng/ml, range 2.8–825.4 ng/ml vs. median 40.5 ng/ml, range 5.0–163.9 ng/ml, P=0.157).

Maternal plasma GPBB concentration was higher in preterm preeclampsia patients (n=74; median 72.6 ng/ml, range 1.6–428.8 ng/ml) than in gestational-age-matched pregnant women with a normal pregnancy outcome (n=33; median 26.0 ng/ml, range 6.8–205.5 ng/ml) and in patients with preterm SGA neonates and no preeclampsia (n=28; median 15.9 ng/ml, range 1.7–468.9 ng/ml) (P<0.01, for each), which remained statistically significant after adjusting for maternal age, gestational age at blood draw, race, baby gender, and parity (Figure 4A). Preterm SGA gestation without preeclampsia cases did not affect maternal plasma GPBB concentration. In term pregnant women, there was no difference in the maternal plasma GPBB concentration among preeclampsia cases (n=72; median 39.2 ng/ml, range 2.7–311.9 ng/ml), SGA gestation and no preeclampsia cases (n=38; median 67.2 ng/ml, range 3.3–158.4 ng/ml), and normal cases (n=122; median 45.2 ng/ml, range 2.8–825.4 ng/ml) (Figure 4B). In addition, preterm preeclampsia patients had a significantly higher median plasma GPBB concentration than term preeclampsia patients (P<0.05).

Subgroup analysis was performed to determine the relationship between maternal plasma GPBB concentration and the severity of preeclampsia. There were 82% (59/72) of term preeclampsia patients and 96% (71/74) of preterm preeclampsia patients who had severe symptoms and signs of preeclampsia based on the criteria of the American College of Obstetricians and Gynecologists.[16] There was no significant difference in the plasma GPBB concentration according to the severity of preeclampsia both in term preeclampsia cases (mild preeclampsia: median 21.0 ng/ml, range 6.1–147.9 ng/ml vs. severe preeclampsia: median 39.5 ng/ml, range 2.7–311.9 ng/ml, P=0.959) and preterm preeclampsia cases (mild preeclampsia: median 37.1 ng/ml, range 18.1–72.4 ng/ml vs. severe preeclampsia: median 72.8 ng/ml, range 1.6–428.8 ng/ml, P=0.331). However, severe preeclampsia cases at preterm had a higher median plasma GPBB concentration than severe preeclampsia cases at term (P<0.05). When subgroup analysis in the context of preeclampsia was conducted to determine whether there is an additional effect of SGA birth on maternal plasma GPBB concentration, preterm preeclampsia patients who delivered SGA neonates (n=37; median 63.0 ng/ml, range 1.6–263.7 ng/ml) and those who delivered AGA neonates (n=37; median 72.8 ng/ml, range 2.4–428.8 ng/ml) had higher median maternal plasma GPBB concentrations than gestational-age-matched pregnant women with a normal pregnancy outcome (n=33; median 26.0 ng/ml, range 6.8–205.5 ng/ml) (P<0.05, for each). However, there was no difference in maternal plasma GPBB concentration between SGA neonates and AGA neonates of preterm preeclampsia patients. There were also no differences in maternal plasma GPBB concentration among term preeclampsia patients who delivered SGA neonates (n=32; median 36.1 ng/ml, range 2.7–308.0 ng/ml) and AGA neonates (n=40;

median 45.0 ng/ml, range 3.2–311.9 ng/ml) and gestational-age-matched pregnant women with a normal pregnancy outcome (n=122; median 45.2 ng/ml, range 3.2–311.9 ng/ml).

Placental Expression of GPBB

The significant increase of GPBB concentration in normal pregnant women and particularly in women with preterm preeclampsia, which is characterized by uteroplacental hypoxia, led us to examine (1) whether GPBB is expressed in the placenta, and (2) whether there is a difference in GPBB expression in the placenta according to the presence or absence of preeclampsia and gestational age at delivery. When we conducted immunoblotting using the placental protein lysates of 45 cases (Table S1, please see http://hyper.ahajournal.org), GPBB was readily detected in the villous placenta of all cases (Figure 5A). Densitometric analysis of immunoblotting data showed a significant decrease in the expression of GPBB in preterm preeclampsia cases [n=9; median GPBB/HPRT ratio, 0.59 (range, 0.06–1.00)] than in cases of preterm labor and delivery [n=9; median GPBB/HPRT ratio, 1.18 (range, 0.61–2.64)] ($P<0.01$), while there was no difference in placental GPBB expression between term preeclampsia cases [n=9; median GPBB/HPRT ratio, 1.10 (range, 0.62–1.54)] and normal term delivery cases [n=18; median GPBB/HPRT ratio, 1.05 (range, 0.53–4.01)] (Figure 5B). Accordingly, placental GPBB in preterm preeclampsia cases was less abundant than in term preeclampsia cases ($P<0.01$). Labor itself did not change the GPBB expression in the placentas of cases delivered at term, which was similar to the result in maternal plasma GPBB concentration (Figure 5B). Immunofluorescence staining further demonstrated distinct GPBB immunoreactivity in the syncytiotrophoblast and cytotrophoblast (Figure 5C).

Discussion

This study reports for the first time profiles of plasma GPBB concentration in normal pregnancy and in pregnancy complicated with preeclampsia. The primary novel findings of this study are: (1) there is a physiologic increase in plasma GPBB concentration during pregnancy; (2) preterm but not term preeclampsia is characterized by a further increase in plasma GPBB concentration; (3) GPBB is readily detected in the placenta and its abundance is decreased in preterm preeclampsia cases; (4) SGA gestation does not affect plasma GPBB concentration; and (5) there was no difference in maternal plasma GPBB concentration between women with and without labor at term.

Normal tissues abundant in GPBB are the brain and the myocardium,[1] and the placenta turned out to be an additional source of the enzyme. While pregnancy is clearly a physiological state, the median plasma GPBB concentration in normal pregnant women is almost three times higher than the cut-off value (10 ng/ml) used for the diagnosis of acute coronary syndromes in non-pregnant patients.[5,6] Pregnancy remarkably alters cardiovascular functions with increased cardiac output, but electrocardiographic changes are restricted to only left-axis deviation, and there is no evidence that pregnancy increases the risk of ischemic myocardial damage.[7] Pregnancy can also change cerebrovascular blood flow, such as the decrease in the mean blood flow of the middle and the posterior cerebral arteries, but does not affect cerebrovascular autoregulation;[20] and the blood-brain-barrier (BBB) remains intact in healthy pregnant women.[21] Therefore, it is less likely that the increase in the plasma GPBB concentration during normal pregnancy is solely attributed to the GPBB release from the heart or the brain. Instead, additional GPBB release from the placenta would be a reasonable expectation.

Preeclampsia is a pregnancy-specific syndrome characterized by hypertension and proteinuria, and it occurs in 5–8% of all pregnancies worldwide.[22,23] Uteroplacental hypoxia[11,12] and anti-angiogenic conditions in maternal and fetal compartments[24–27] are reported as key steps for the occurrence of preeclampsia. It has been proposed that

preeclampsia is a two-stage disorder: stage 1 (placental preeclampsia): abnormal placentation leading to placental hypoxia, and stage 2 (maternal preeclampsia): maternal symptoms of preeclampsia originating from placenta-derived circulating factors such as soluble fms-like tyrosine kinase 1 (sFlt-1) and soluble endoglin (sEng).[28,29] Higher maternal plasma GPBB concentration and lower placental GPBB expression in preterm preeclampsia patients in the current study are very likely to be associated with uteroplacental hypoxia leading to the conversion of GPBB into soluble form and to the release of placental GPBB into the bloodstream. However, in contrast to normal pregnancy, increased GPBB released from the heart and the brain would also be possible in patients with preeclampsia. Hypertensive disorders in pregnancy are associated with the aberrations of cardiovascular functions such as hyperdynamic ventricular function and low intravascular blood volume.[30,31] Moreover, Ladner et al have reported that essential hypertension and preeclampsia are associated with the development of acute myocardial infarction during pregnancy.[32] Melchiorre et al also have reported that about 20% of patients with preeclampsia at term show evident myocardial damage.[33] In addition, increased BBB permeability and brain edema are found in both preeclampsia and eclampsia patients.[21,34] However, no patient of the current study was diagnosed with acute myocardial infarction or brain damage during the index pregnancy.

Studies about preeclampsia have shown that there are significant differences in the pathogenesis, blood biomarker profiles, and clinical presentations according to the gestational age at disease onset.[26,35–39] Preeclampsia developing before 37 weeks of gestation (preterm preeclampsia) is a more severe and complicated maternal phenotype of preeclampsia than that developing at term (term preeclampsia) in general.[39] The risk of the recurrence in later pregnancies,[35] the long-term risk of mortality from the following cardiovascular causes,[36] the proportion of SGA neonates,[37,38] and the concentrations of maternal serum liver enzymes[39] are higher in preterm preeclampsia patients than in term preeclampsia patients. Huppertz recently proposed that these features largely comprise the phenotype of early-onset but not late-onset preeclampsia.[40] Our observation of a significant increase in maternal plasma GPBB concentration in preterm preeclampsia, but not in term preeclampsia, further supports the opinion that preeclampsia can be subclassified according to the gestational age at disease onset.[41]

Pregnancies presenting SGA neonates and preeclampsia have been reported to share common features of shallow placentation and uteroplacental insufficiency.[42] However, maternal manifestation of these conditions differs from each other, and some investigators have proposed that they are biologically different disease entities.[43] In the present study, an additional effect of SGA birth on maternal GPBB concentration in preeclampsia cases was not found, which suggests that the increased maternal GPBB concentration is mainly related to preeclampsia. This finding was consistent with that from the analysis of GPBB concentration according to SGA births among normotensive pregnant women. Although an SGA gestation is one of the indicators for severity of preeclampsia,[16] and commonly associated with preeclampsia,[38,44] an SGA gestation alone was less likely to affect maternal GPBB concentration.

Previous studies of placental metabolism in preeclampsia have shown abnormalities in glycogen metabolism.[45–49] Bloxam et al demonstrated impaired glycolysis in the placentas of women with preeclampsia by showing decreased concentrations of pyruvate and lactate but not glycogen and glucose.[46] Arkwright et al also have shown that glycogen content is increased in the syncytiotrophoblast of preeclampsia cases, which was accompanied by 16-fold and 3-fold increases of glycogen synthase content and glycogen phosphorylase activity.[47] Increased placental glycogen phosphorylase activity in preeclampsia cases is quite consistent with our observation that release of GPBB is increased in pregnant women

with preeclampsia, which would lead to a higher plasma concentration of GPBB and less abundance in placental GPBB.

There are limitations in our study. As postpartum blood samples were unavailable, maternal GPBB concentration during the postpartum period could not be examined. The change in GPBB concentration after delivery would be a key piece of data in support of our hypothesis that increased GPBB concentration during pregnancy originates from placental tissue. Another limitation is that the blood samples and placental samples were not obtained from the same pregnant women and the analysis using the blood and placental samples from the same pregnant women would have been more relevant for the determination of placental origin of peripheral blood GPBB, although it was not feasible for us. In this study, there were differences of racial distribution and parity for each of the analyses such as (1) non-pregnant women vs. pregnant women with a normal pregnancy, (2) preeclampsia vs. SGA gestation vs. controls in preterm gestation, and (3) preeclampsia vs. SGA gestation vs. controls in term gestation. Although the differences in plasma GPBB concentration remained significant after adjusting for these confounding factors, future studies designed on a large scale are needed to address this issue.

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Perspectives

Maternal plasma GPBB concentration is increased during pregnancy, and its robust increase is a novel phenotype of preterm preeclampsia. The findings herein strongly suggest that maternal plasma GPBB can be a useful marker in the detection of early-onset (preterm) preeclampsia. Uteroplacental hypoxia precedes the clinical symptoms of preeclampsia,[28,29] and a future study on the predictive value of plasma GPBB concentration as a marker of preeclampsia or other obstetric complications, such as fetal death in asymptomatic pregnant women (i.e., during the mid-trimester), will be necessary.

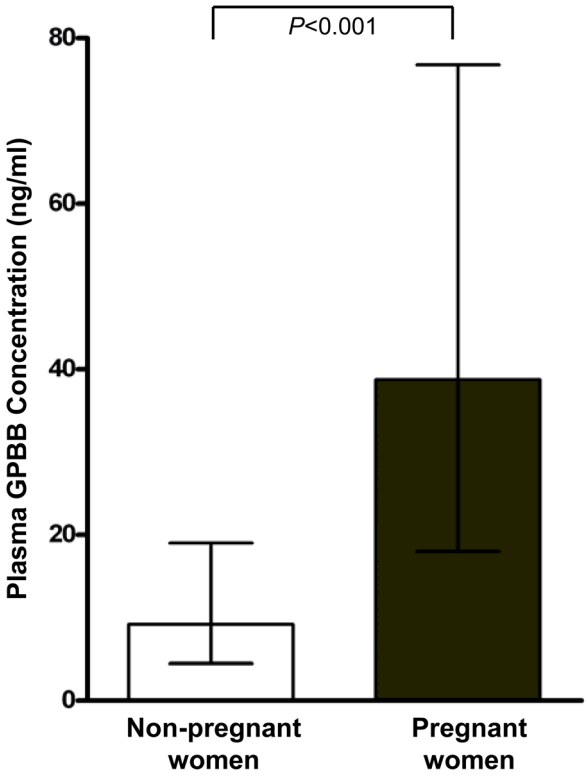

Figure 1.
Plasma GPBB concentration in pregnant women. Median GPBB plasma concentration was higher in pregnant women with a normal pregnancy outcome (n=151) than in non-pregnant women (n=33) (*P*<0.001). Plasma GPBB concentrations were shown as median and interquartile ranges.

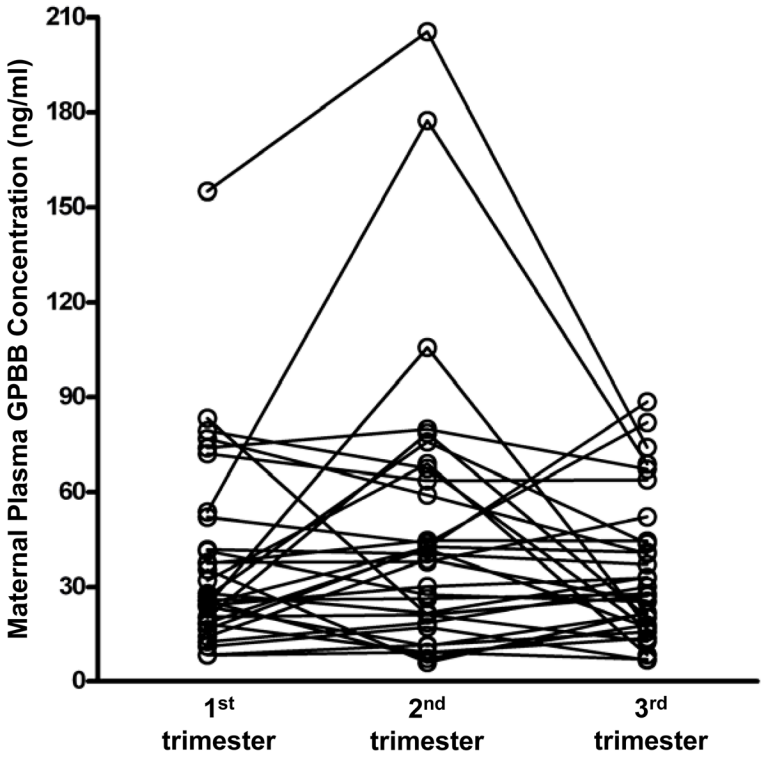

Figure 2.
Maternal plasma GPBB concentration as a function of gestational age. There was no difference in the maternal plasma GPBB concentration as a function of advancing gestation.

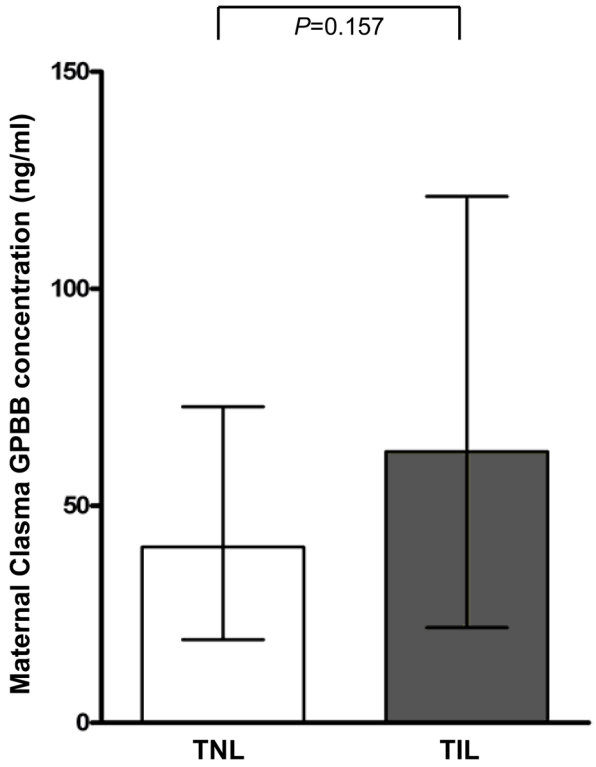

Figure 3.

Comparison of maternal plasma GPBB concentration according to the presence or absence of labor. Maternal plasma GPBB concentration tended to be higher in patients in labor at term (n=65) than in those not in labor at term (n=57), but the difference was not statistically significant (*P*=0.157). Maternal plasma GPBB concentrations were shown as median and interquartile ranges.

TNL: term not in labor, TIL: term in labor

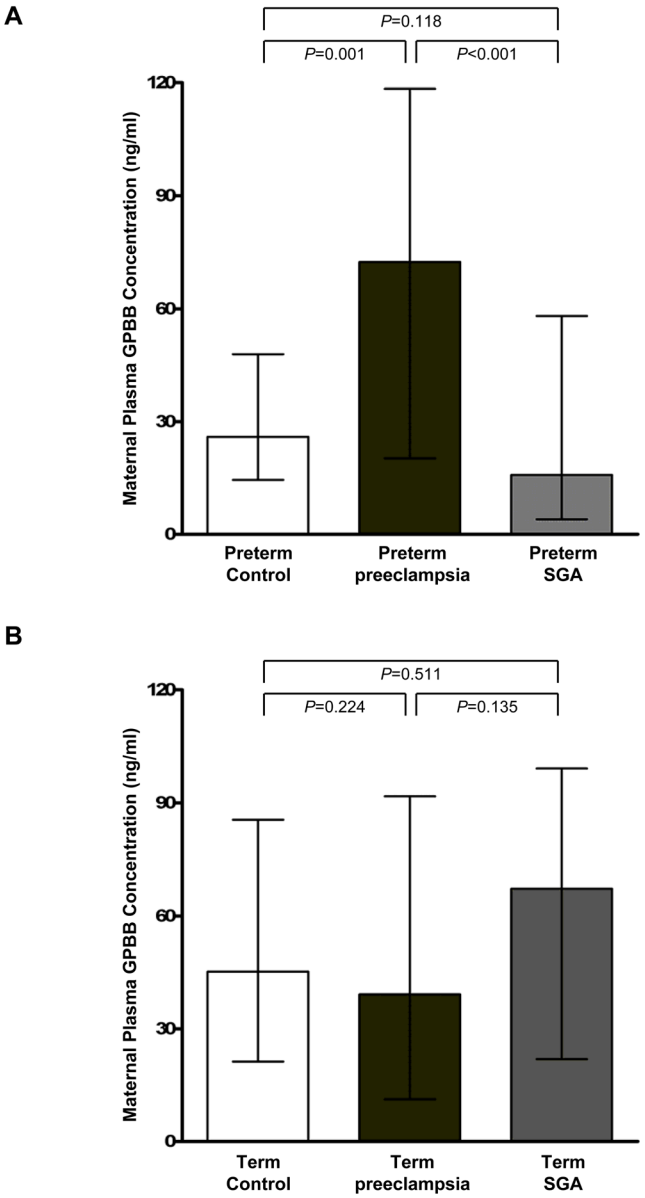

Figure 4.

Comparison of maternal plasma GPBB concentration according to preeclampsia and small-for-gestational-age (SGA) neonates in preterm and term gestations. A, Maternal plasma GPBB concentration was higher in preterm preeclampsia patients (n=74) than in gestational-age-matched pregnant women with a normal pregnancy outcome (n=33) (*P*=0.001), while there was no difference between patients with preterm SGA neonates and no preeclampsia (n=28) and preterm controls. B, There was no difference in the maternal plasma GPBB concentration among normal term cases (n=122), term preeclampsia cases (n=72), and patients with term SGA neonates and no preeclampsia (n=38). Maternal plasma GPBB concentrations were shown as median and interquartile ranges.

SGA, small-for-gestational-age

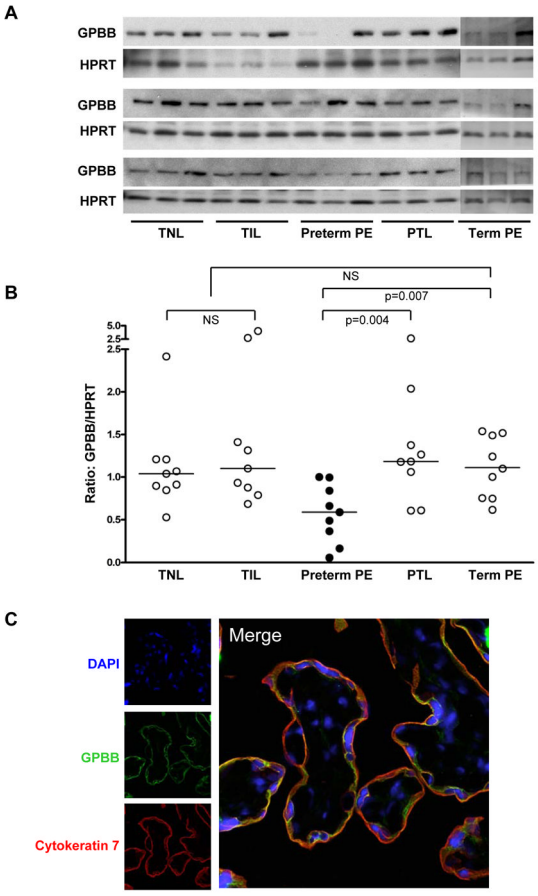

Figure 5.

Placental expression of GPBB. A, GPBB was readily detected in the villous placenta of all cases with immunoblotting. B, Placental GPBB was less abundant in preterm preeclampsia cases (n=9) than in preterm labor and delivery cases (n=9), in term preeclampsia cases (n=9), and in normal term delivery cases (n=18) (P<0.01, for each) as shown by densitometric analysis. C, Distinct GPBB immunoreactivity in the syncytiotrophoblast and villous cytotrophoblasts of a representative case of normal term delivery at 40 weeks. GPBB expression is observed in the syncytiotrophoblast and villous cytotrophoblasts.
TNL: term not in labor, TIL: term in labor, PE: preeclampsia, PTL: preterm labor.

Table 1

Demographics of the study population of non-pregnant women and pregnant women with normal term delivery

Group	Non-Pregnant	Normal Term Birth	*P* value	Normal term birth			
				Cases for the longitudinal analysis	Cases of additional term no labor at blood sampling	Cases of term in labor at blood sampling	Preterm control cases for preterm preeclampsia
	N=33	*N*=151		*N*=32	*N*=25	*N*=65	*N*=29
Maternal age (years) *	26 (18–49)	23 (15–40)	0.005	20.5 (15–31)	27 (15–40)	23 (16–35)	23 (15–34)
Race (%)			<0.001				
Black	39.4	90.7		93.8	80.0	92.3	93.1
White	45.5	2.6		0.0	4.0	4.6	0.0
Hispanic	0.0	0.7		0.0	4.0	0.0	0.0
Others	15.2	6.0		6.3	12.0	3.1	6.9
Para (Nullipara) (%)	63.6	31.8	<0.001	56.3	8.0	33.8	20.7
Gestational age at blood sampling (weeks) *				1st: 11.7 (7.1–13.9) 2nd: 17.6 (15.1–26.7) 3rd: 38.1 (37.0–40.6)	39.1 (37.3–40.9)	39.7 (37.0–41.9)	31.0 (24.6–36.9)
Gestational age at delivery (weeks) *		39.4 (37.0–42.0)		39.7 (38.0–42.0)	39.1 (37.3–40.9)	39.7 (37.0–41.9)	39.3 (37.6–41.6)
Baby gender (Male) (%)		49.0		50.0	64.0	46.2	51.7
SGA neonates (%)		0.0		0.0	0.0	0.0	0.0
Cesarean delivery (%)		29.8		31.3	100.0	3.1	27.6

*, median (range)

SGA, small-for-gestational-age

Table 2

Demographics of the study population of cases with preeclampsia and small-for-gestational-age neonates

Group	Term			Preterm		
	Control†	Preeclampsia	SGA	Control‡	Preeclampsia	SGA
	N=122	*N*=72	*N*=38	*N*=33	*N*=74	*N*=28
Maternal age (years) *	23 (15–40)	22.5 (16–44)	22.5 (16–37)	23 (15–34)	25.5 (15–43)	21.5 (16–39)
Race (%)						
Black	90.2	83.3	92.1	93.9	82.4	92.9
White	3.3	6.9	7.9	0.0	9.5	0.0
Hispanic	0.8	4.2	0.0	0.0	0.0	3.6
Others	5.7	5.6	0.0	6.1	8.1	3.6
Para (Nullipara) (%)	34.4	62.5	34.2	21.2	52.7	35.7
Gestational age at blood sampling (weeks) *	39.1 (37.0–41.9)	38.7 (37.0–42.4)	39.1 (37.1–41.6)	30.1 (20.3–36.9)	30.3 (22.6–36.7)	33.6 (20.3–36.7)
Gestational age at delivery (weeks) *	39.6 (37.0–42.0)	38.7 (37.0–42.4)	39.3 (37.1–41.6)	39.6 (37.6–42.0)	30.9 (22.9–36.7)	33.8 (20.9–36.9)
Baby gender (Male) (%)	50.8	44.4	26.3	51.5	51.4	17.9
SGA neonates (%)	0.0	44.4	100.0	0.0	50.0	100.0
Cesarean delivery (%)	30.3	45.8	26.3	27.3	83.8	17.9

*, median (range)

SGA, small-for-gestational-age

†, Term controls (n=122) for cases with term preeclampsia or term SGA neonates consisted of cases of term no labor (n=57) and those of term in labor at blood sampling (n=65).

‡, Preterm controls (n=33) for cases with preterm preeclampsia or preterm SGA neonates, matched with gestational age at blood sampling, consisted of preterm control cases for preterm preeclampsia (n=29) and 4 cases for longitudinal study (2nd drawn blood samples [n=4]).