

Original Paper

Maternal PCaaC38:6 is Associated With Preterm Birth – a Risk Factor for Early and Late Adverse Outcome of the Offspring

Jian Li^a Yong Ping Lu^{b,c} Christoph Reichetzeder^c Philipp Kalk^b Burkhard Kleuser^d
Jerzy Adamski^e Berthold Hocher^{a,c,f}

^aDepartment of Clinical Medicine, Medical college of Hunan Normal University, Changsha 410006, China; ^bDepartment of Nephrology, Charité - Universitätsmedizin Berlin, Campus Benjamin Franklin, Berlin; ^cInstitute of Nutritional Science, University of Potsdam, Potsdam-Rehbrücke; ^dInstitute of Nutritional Science, Department of Toxicology, University of Potsdam, Potsdam; ^eInstitute of Experimental Genetics, Helmholtz Zentrum München, Neuherberg; ^fIFLB, Institut für Laboratoriumsmedizin Berlin GmbH, Berlin, Germany

Key Words

Metabolomics • PCaaC38:6 • Biomarker • Preterm birth

Abstract

Background/Aims: Preterm birth (PTB) and low birth weight (LBW) significantly influence mortality and morbidity of the offspring in early life and also have long-term consequences in later life. A better understanding of the molecular mechanisms of preterm birth could provide new insights regarding putative preventive strategies. Metabolomics provides a powerful analytic tool to readout complex interactions between genetics, environment and health and may serve to identify relevant biomarkers. In this study, the association between 163 targeted maternal blood metabolites and gestational age was investigated in order to find candidate biomarkers for PTB. **Methods:** Five hundred twenty-three women were included into this observational study. Maternal blood was obtained before delivery. The concentration of 163 maternal serum metabolites was measured by flow injection tandem mass spectrometry. To find putative biomarkers for preterm birth, a three-step analysis was designed: bivariate correlation analysis followed by multivariable regression analysis and a comparison of mean values among gestational age groups. **Results:** Bivariate correlation analysis showed that 2 acylcarnitines (C16:2, C2), 1 amino acids (xLeu), 8 diacyl-PCs (PCaaC36:4, PCaaC38:4, PCaaC38:5, PCaaC38:6, PCaaC40:4, PCaaC40:5, PCaaC40:6, PCaaC42:4), and 1 Acylalkyl-PCs (PCaaC40:5) were inversely correlated with gestational age. Multivariable regression analysis confounded for PTB history, maternal body mass index (BMI) before pregnancy, systolic blood

Jian Li and Yong Ping Lu contributed equally to this study and therefore share first authorship.

Prof. Dr. Berthold Hocher

Department of Clinical Medicine, Medical college of Hunan Normal University, Changsha, (China) and Institute of Nutritional Science, University of Potsdam, Potsdam-Rehbrücke, Arthur-Scheunert-Allee 114-116, 14558 Nuthetal (Germany)
E-Mail hocher@uni-potsdam.de

pressure at the third trimester, and maternal body weight at the third trimester, showed that the diacyl-PC PCaaC38:6 was the only metabolite inversely correlated with gestational age. **Conclusions:** Maternal blood concentrations of PCaaC38:6 are independently associated with gestational age.

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Introduction

Preterm birth (PTB), defined as birth before 37th week of gestation, affects 5 to 18% of pregnancies worldwide. Evidence suggests that PTB not only significantly influences mortality and morbidity of the offspring in early life [1-7] but also causes serious long-term consequences in later life [8-11]. Systematic reviews and meta-analyses including large populations and different separate cohorts demonstrated that PTB increased the risk of metabolic syndrome at later childhood and adulthood [8-10]. Sensitive, effective, and non-invasive new biomarkers to screen and diagnose PTB before birth could lead to treatment options that prevent poor birth outcomes associated with PTB. Pregnancy is a very complex process, influenced by a plethora of physiological and pathophysiological factors. It is known that environmental factors like maternal infections, nutrition, and stressful events can be associated with changes in the serum metabolite profile. Metabolomics provides a powerful analytic tool to get insights into the complex interaction of genetics, environment and health and may serve to identify relevant predictive biomarkers for PTB. Previous work from *Romero et al.* [12] and *Menon et al.* [13] showed that the metabolomic profile of amniotic fluid is a good biomarker to assess the risk of preterm delivery. However, amniotic fluid sample collection is an invasive procedure with potential risk for adverse outcomes for both mother and child [14,15]. Blood plasma and (or) urine can be collected much easier and therefore should be targeted in the search of potential biomarkers. One recent study demonstrated that the maternal urinary metabolic profile in early pregnancy can be helpful to identify PTB [16]. So far, there is no publication about the metabolomic profile of maternal plasma and PTB. In this study, a targeted metabolomics approach was performed, measuring 163 metabolites which were analyzed for associations with gestational age. The detection of new biomarkers predictive for PTB could help to better understand the underlying pathomechanisms of PTB and to create new preventive strategies for PTB.

Materials and Methods

Clinic data collection

This observational study was approved by the local Ethics Committee. A total of 550 pregnant women who delivered their babies at the Charité obstetrics department in Berlin, Germany between January 2007 and December 2008 were invited to participate. As 27 mothers were excluded from the study because they delivered twins, 523 women entered the study. The majority of the mothers (n = 471) were of caucasian ethnicity, the others had an African, Asian, or Arabic background.

After written consent was obtained, a structured medical history was taken. The following data were extracted into our database: age, ethnicity, body height, body weight before pregnancy, gravidity, parity, diabetes mellitus and hypertension during pregnancy, smoking status before and during pregnancy, systolic and diastolic blood pressure (BP) measurements recorded during pregnancy and the mode of delivery. Biometric data of the newborn were collected during the routine postnatal examination: birth weight, birth length, head circumference, child sex, and Apgar score 5 minutes postnatally and Apgar score 10 minutes postnatally. Gestational age at delivery was based on last menstrual period, anamnesticly assessed during the first pregnancy examination.

Sample collection, blood metabolomics compounds assay

Midwives collected maternal blood from a cubital vein in the delivery room or on the ward prior to

birth, before the usage of oxytocin, or analgesics. Blood was centrifuged at 2750 g immediately after its taking and the obtained serum was stored at -80 °C until measurements were performed.

163 targeted small metabolites were quantified simultaneously in 10 µL of serum using the Absolute IDQTM-kit p150 (Biocrates Life Sciences AG, Innsbruck, Austria). The assay procedures were the same as previously described [17]. Concentrations of these targeted metabolites were recorded in µM. These metabolites included 14 amino acids, 1 sugar, 1 carnitine, 26 acylcarnitines, 14 hydroxy and dicarboxy-acylcarnitines, 10 sphingomyelins, 5 hydroxy-sphingomyelins, 38 diacyl-phosphatidylcholines (diacyl-PCs), 39 acyl-alkyl-phosphatidylcholines (Acylalkyl-PCs) and 15 lysophosphatidylcholines.

Statistical analysis

Data were analyzed with SPSS version 17.0. Results of quantitative data were expressed as arithmetic mean ± standard error (SE). Bivariate Correlation Analysis was applied to detect correlations of metabolites and gestational age. Additionally, certain factors (PTB history, maternal BMI before pregnancy, systolic blood pressure at the third trimester, maternal body weight at the third trimester) were used as confounders to calculate and adjust putative predictive metabolites in multivariable linear regression models. Unpaired t-test was used for comparison of continuous variables between two groups. A *p*-value less than 0.05 was considered significant.

Results

Description of the cohort

Descriptive data of the study population are given in table 1. The study population represented a typical German birth cohort in regards to key characteristics like maternal age, ethnicity, BMI before pregnancy, gravidity, parity, biometric data of the newborns like birth weight, birth length, child sex, and Apgar score (for more details, see Table 1). The distribution of gestational age is given in Figure 1.

Bivariate correlation analyses of maternal serum metabolites and gestational age

Bivariate correlation analyses showed that 2 acylcarnitines (C16:2, C2), 1 amino acid (xLeu), 8 diacyl-PCs (PCaaC36:4, PCaaC38:4, PCaaC38:5, PCaaC38:6, PCaaC40:4, PCaaC40:5, PCaaC40:6, PCaaC42:4), and 1 acylalkylphosphatidylcholine (PCaeC40:5) were significantly negatively correlated with gestational age (for more details, see table 2).

Multivariable regression analyses of maternal blood metabolites and gestational age

Significant results obtained by bivariate correlation analysis were subsequently analy-

Table 1. Detailed Descriptive Data of the Mother/Child Pairs (n = 523)

Variable	Mean±SE/%
Maternal age, y	30.78±0.26
Maternal height, cm	166.62±0.29
Maternal weight before pregnancy, kg	62.99±0.59
Maternal BMI before pregnancy, kg/m ²	22.66±0.20
Primigra/pluripara, %	37.37/62.63
Smoking before/during pregnancy, %	43.07/16.18
Hypertension before/during pregnancy, %	4.40/10.33
Diabetes mellitus before/during pregnancy, %	0.96/8.60
Mean weight 1 st half of pregnancy, kg	66.12±0.70
Mean weight 2 nd half of pregnancy, kg	69.11±0.63
Mean weight 3 rd half of pregnancy, kg	76.19±0.62
Mean systolic BP 1 st half of pregnancy, mm Hg	113.20±0.63
Mean systolic BP 2 nd half of pregnancy, mm Hg	112.58±0.53
Mean systolic BP 3 rd half of pregnancy, mm Hg	114.23±0.47
Mean diastolic BP 1 st half of pregnancy, mm Hg	68.42±0.45
Mean diastolic BP 2 nd half of pregnancy, mm Hg	67.40±0.35
Mean diastolic BP 3 rd half of pregnancy, mm Hg	69.26±0.33
Gestational age at delivery, weeks + days	38+3
Child sex, male/female, %	52.18/47.82
Child birth weight, g	3257.53±28.21
Child birth length, cm	50.03±0.18
Head circumference, cm	34.53±0.08
Apgar score at 5 min	9.21±0.05
Apgar score at 10 min	9.48±0.04
Data are given as mean ± SE or %	

zed by multivariable regression analysis considering PTB history, maternal BMI before pregnancy, systolic blood pressure at the third trimester, maternal BMI at the third trimester in model B. Analysis showed that PCaaC38:6 was the only metabolite significantly inversely correlated with gestational age (for more details, see table 3).

PCaaC38:6 serum concentrations in relation to gestational age

2 gestational age groups were stratified as follows: gestational age < 37 weeks as group 1 (PTB group), ≥ 37 weeks as group 2. The PTB group displayed significantly higher concentrations of PCaaC38:6 in comparison to group 2 ($100.09 \pm 4.22 \mu\text{M}$ vs. $89.66 \pm 1.17 \mu\text{M}$, $P = 0.023$);

4 gestational age groups were stratified as follows: gestational age < 35 weeks as group 1 (severe PTB group), 35~37 weeks as group 2 (moderate PTB group), 38~39 weeks as group 3, ≥ 40 weeks as group 4. PCaaC38:6 showed a trend to decrease with increasing gestational age (for more details, see figure 2).

Discussion

In the current birth cohort study, the association of 163 maternal serum metabolites and gestational age before delivery was investigated. Diacyl phosphatidylcholine PCaaC38:6 was strongly and inversely correlated with gestational age. Concentration of PCaaC38:6 was significantly higher in serum of mothers with PTB than in the serum of mothers who gave birth between 37th and 40th or above 40th week of gestation. This indicates that unsaturated diacyl-PCs (PCaaC38:6) might be candidate biomarkers for PTB screening and monitoring.

In recent years, elaborate molecular biologic methods like genomics, transcriptomics, and proteomics have been used to study normal pregnancy and pregnancy complications. Yet, only two studies have reported the use of metabolomics in PTB. Romero et al. [12]

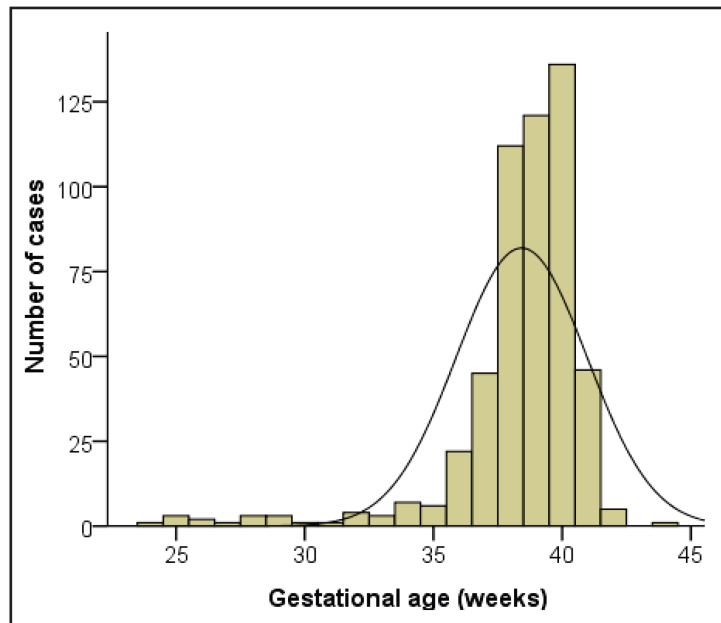


Fig. 1. Distribution of gestational age.

Table 2. Maternal serum metabolites related to gestational age in bivariate correlation analysis

	Variable	Pearson Correlation	P value
Acylcarnitines	C16:2	-0.163	1.189×10^{-4}
	C2	-0.122	0.004
Amino acids	xLeu	-0.091	0.033
Diacyl-PCs	PCaaC36:4	-0.104	0.014
	PCaaC38:4	-0.111	0.009
	PCaaC38:5	-0.088	0.040
	PCaaC38:6	-0.122	0.004
	PCaaC40:4	-0.089	0.037
	PCaaC40:5	-0.094	0.027
	PCaaC40:6	-0.120	0.005
Acylalkyl-PCs	PCaaC42:4	-0.084	0.048
	PCaaC40:5	-0.108	0.012

For each maternal serum metabolite, the bivariate correlation analysis was performed, with the maternal serum metabolite values being the dependent and each of the maternal gestational age being the independent variable. Only the 12 significant correlated metabolites are shown here. Diacyl-PCs: diacyl-phosphatidylcholines; Acylalkyl-PCs: acyl-alkyl-phosphatidylcholines

Table 3. Maternal serum metabolites related to gestational age in multivariable linear regression analysis

Variable	B	t	P	95.0% Confidence interval for B
PCaaC38:6	-0.04	-2.19	0.029	-0.072~-0.004
PTB history	-15.98	-4.14	4.06×10 ⁻⁵	-23.56~-8.40
Maternal BMI before pregnancy	-0.51	-2.93	0.017	-0.93~-0.09
Systolic blood pressure at the third trimester	-0.27	-4.32	4.09×10 ⁻⁵	-0.39~-0.14
Maternal body weight at the third trimester	0.31	3.98	7.83×10 ⁻⁵	0.16~0.46

Considering PTB history, maternal BMI before pregnancy, systolic blood pressure at the third trimester, maternal body weight at the third trimester, the 4 metabolites shown in table 2 (P<0.01) being the independent variable and gestational age being the dependent variable. Only PCaaC38:6 was significant correlated with gestational age. Shown are the B, t, and P-values for the maternal gestational age. B: non-standardized regression coefficient.

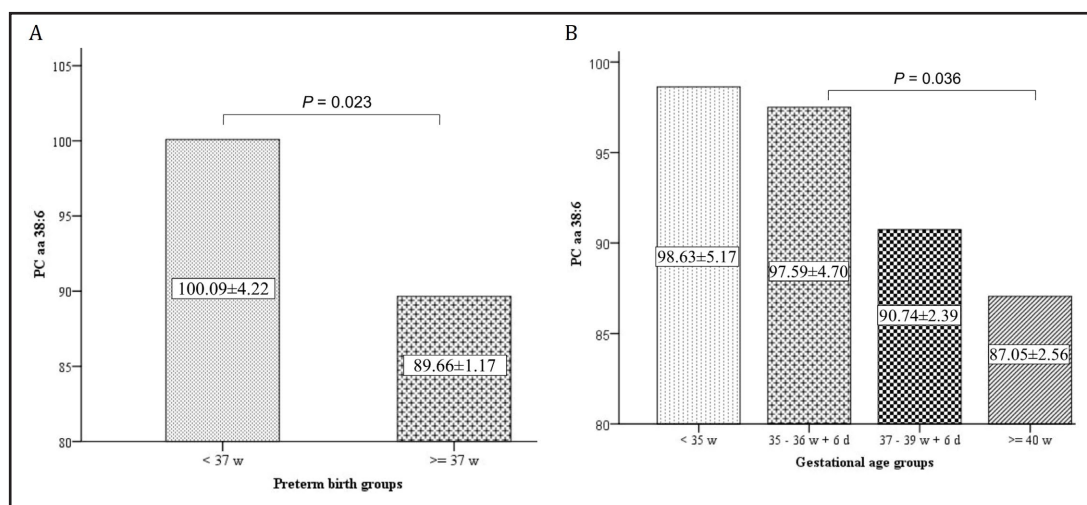


Fig. 2. Mean maternal PCaaC38:6 according to gestational age groups. A: 2 gestational age groups were stratified as follows: <37 weeks with 57 cases, ≥ 37 weeks with 466 cases. B: 4 gestational age groups were stratified as follows: <35 weeks with 29 cases, 35~37 weeks with 73 cases, 38~39 weeks with 233 cases, ≥ 40 weeks with 188 cases.

collected amniotic fluid from transabdominal amniocentesis at the time of diagnosis for preterm labor and showed that mothers without intraamniotic infection/inflammation who delivered preterm had a relative decrease in carbohydrates and amino acids. In contrast, mothers with intraamniotic infection/inflammation had a more substantial decrease in compounds of the carbohydrate cluster, and a relative increase in amino acids. Menon et al. [13] collected amniotic fluid from transvaginal amniocentesis samples taken prior to delivery during active labor in an African-American population and found that the global metabolite profiles differed significantly between normal and preterm birth. Many of the significantly altered metabolites in the PTB group reflected liver metabolism. Different to previous studies, the current study investigated maternal metabolites in serum obtained before delivery in a typical German cohort. Statistical analyses showed that maternal plasma phospholipids (six Diacyl-PCs and two Acylalkyl-PCs), especially PCaaC38:6, are relevant to PTB.

Glycerophospholipids make up the main structural lipids of cellular membranes, and phosphatidylcholine (PC) accounts for >50% of the glycerophospholipids in most eukaryotic membranes [18, 19]. PC is the main component of circulating lipoprotein classes in the human plasma [20]. In addition, PC is particularly essential for hepatic secretion of

triglyceride-rich very low density lipoprotein (VLDL) and high density lipoprotein (HDL) [19]. PC is essential for proper cell division and thus plays a very important role during fetal intrauterine growth. Furthermore, PC is the main source of choline which is essential for fetal brain and neurocognitive development [21,22]. Bernhard et al. [23] showed that in preterm infants, choline concentrations are lower in postnatal plasma than in cord plasma. La et al [24] conducted an S-plot for biomarkers of gestational age (PCs, PEs, and SMs) and showed that they had a weak, negative correlation with gestational age. Till now, there is no data available that links diacyl-PCs to gestational physiological or pathological conditions.

The most common phenotype of PTB is spontaneous PTB of unknown etiology. Risk factors of PTB include malnutrition, obesity, intra-amniotic infection (IAI) and inflammation, cigarette smoking, alcohol intake, drug use, antioxidant deficient diets, physiologic and psycho-social stressors, environmental pollutants, genotoxic agents, geographic location [25]. All of the PTB risk factors are capable of causing redox imbalances, leading to the production of superoxide, hydrogen peroxide, hydroxyl ions and nitric oxide that can damage collagen matrix and consume antioxidant defenses. These events can trigger uterine contractions (labor), leading to PTB and placing these infants at a higher risk of injury [25]. Recently, a large cohort study with 18,079 participants (KORA study) and seven years follow-up [26] showed that compared with former smokers and never smokers, current smokers had higher concentrations of unsaturated diacyl-PCs but lower concentrations of saturated diacyl-PC. It has been shown that unsaturated fatty acids are more vulnerable to lipid peroxidation [27-29]. Furthermore, polyunsaturated diacyl-PCs can promote the oxidation and fragmentation of γ -hydroxyalkenals [29]. Oxidative stress (OS) can occur early in pregnancy [30], induce pregnancy-related disorders like preeclampsia [31-33] and preterm premature rupture of membranes [34-36], and is considered a detrimental factor in preterm birth pathology [25]. Results of the current study showed that unsaturated diacyl-PCs (PCaaC36:4, PCaaC38:4, PCaaC38:5, PCaaC38:6, PCaaC40:4, PCaaC40:5, PCaaC40:6, PCaaC42:4), especially PCaaC38:6, were negatively correlated with gestational age. Higher unsaturated diacyl-PCs may be indicative of a higher level of OS. Future studies, including animal experiments and human epidemiological studies are needed to confirm the relationship between diacyl-PCs and OS.

One limitation of the study is that maternal serum metabolites were only measured prior to birth. Given that a predictive biomarker for PTB could be of great clinical significance, the current results should stimulate studies, which focus on measuring serum metabolites at multiple and/or earlier occasions in order to consider a potential gestational age dependent secretion of the metabolomic biomarkers. In particular the lipid biomarkers identified in the current study need confirmation in a second independent prospective study. Moreover, our work should stimulate preclinical work using specific tools to block and up-regulate the biological action of PCaaC38:6 to reveal causality and biological relevance between PCaaC38:6 and PTB.

Conclusion

Maternal blood PCaaC38:6 was clearly correlated with gestational age. PCaaC38:6 may be a candidate biomarker for PTB.

Disclosure Statement

The authors of this manuscript state that they do not have any conflict of interests and nothing to disclose.

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