

# Metabolomic Analysis of Serum and Placenta in Preeclampsia

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# Abstract

**Background:** Preeclampsia (PE) is one of the main causes of maternal and fetal morbidity and mortality worldwide. This study was aimed to explore the potential metabolic alterations in women diagnosed with PE and reveal the underlying pathogenesis of disease.

**Methods;** Healthy pregnant women and patients diagnosed with PE were recruited from August 2017 to February 2018. The metabolomic analysis of serum (n=90) and placenta (n=9) samples collected from the two groups were performed with the high performance liquid chromatography coupled with quadrupole-time-of light mass spectrometry (HPLC-QTOF-MS).

**Results:** In serum, 16 metabolites that were present in different concentrations between the two groups were identified, of which pyroglutamic acid (pGlu), methionine, glutamine and taurocholic acid may be used as potential PE diagnosis biomarkers with the area under ROC curve of 0.901, 0.909, 0.892 and 0.873 respectively. Furthermore, the metabolic pathways analysis with differential metabolites in serum and placenta samples showed that linoleic acid and alpha- linolenic acid metabolism, phenylalanine, tyrosine and tryptophan biosynthesis, D-glutamine/D-glutamate metabolism, phenylalanine metabolism, glutathione metabolism and tryptophan metabolism were significantly altered and might be involved in PE pathogenesis.

**Conclusions:** These results showed the altered metabolic pathways could contribute to the pathophysiologic mechanisms of PE.

## Background

Preeclampsia (PE) is a obstetric disorder characterized by hypertension and proteinuria greater than 300 mg/day after 20 weeks of gestation, which affects about 3–5% of all pregnancy [1]. It is one of the main causes of maternal and fetal morbidity and mortality during pregnancy [2]. Mothers who suffered PE present a higher risk of chronic hypertension, cardiovascular disease and diabetic mellitus type-2; their offspring also exhibits an elevated risk of cardiovascular disease and stroke. [3, 4]. The pathogenesis of PE has not been fully defined and it is generally believed that the placental ischemia plays a key role in the development of this disorder. In normal pregnancy, cytotrophoblasts of placenta invade uterine spiral arteries to increase the supply of oxygen and nutrients to fetus. In preeclampsia, however, this remodeling is defective, thus resulting in uteroplacental hypoperfusion [5].

Metabolomics, as a high-throughput technique, is widely used in the systematic study of metabolites in biological samples such as plasma, serum, urine, saliva, tissue and exhaled breath [6]. It has been shown to be a valid and powerful research tool in elucidating the pathogenesis of PE. For instance, it was found that branched-chain amino acids were significantly reduced in the serum of PE patients, which was correlated with intrauterine growth restriction in PE patients [7]. The decrease of arginine in PE could result in dysfunctional synthesis of nitric oxide, a key vasodilator and contribute to hypertension [8]. The decreased level of placental taurine was reported in PE and it might impair placental trophoblast invasion

of uterine spiral arteries [9]. Histidine and histidine-rich glycoprotein, which take part in coagulation system and angiogenic pathway, were found decreased in PE patients [10].

However, the results derived from the previous PE metabolomic studies typically showed poor reproducibility, which could be attributed to biological variation, external experimental conditions and availability of metabolome databases [11–14]. Moreover, no metabolomics studies have analyzed serum and placenta simultaneously with the liquid chromatography-mass spectrometry platform, which provides a more accurate and comprehensive way to reveal the metabolomic changes in the patients developing PE. In the present study, we aimed to identify the potentially PE-associated pathogenesis pathways with metabolomic analyses of both serum and placenta samples from women with PE.

## Methods

### Study population

The singleton pregnant women diagnosed with PE visiting Beijing Obstetrics and Gynecology Hospital were recruited and had their venous blood drawn before treatment from August 2017 to February 2018. The PE diagnosis was determined with the diagnostic criteria proposed by the International Society for the Study of Hypertension in Pregnancy (ISSHP), which defines PE as gestational hypertension (systolic/diastolic blood pressure  $\geq 140/90$  mmHg) in previously normotensive women accompanied by proteinuria (urine protein  $\geq 300$  mg/24 hours) [15]. Pregnant women with previous PE pregnancies, gestational mellitus, cardiovascular disease, hypertension, renal disease, autoimmune disease and metabolic disorders were excluded. The maternal age and gestational age matched healthy pregnant women were enrolled as controls during the study period. The study protocol was approved by the Ethics Committee of Beijing Obstetrics and Gynecology Hospital and the informed consents were obtained from all the participants.

### Sample preparation for metabolomics

The maternal blood samples from each participant (3 ml) were drawn from PE patients and healthy control matched for gestation age, left to clot for 30 min, and centrifuged for 10 min at 3500 rpm. The serum aliquots (1 ml) were separated and stored at  $-80^{\circ}\text{C}$ . The placenta tissue samples (100 mg) were collected at a maximum depth of 5 mm from the maternal central side of placenta (near the cord insertion) immediately after delivery and kept frozen at  $-80^{\circ}\text{C}$  [14].

For sample processing, 100 ml of each serum sample was mixed with 200 ml of acetonitrile: methanol (1:1) solution and vortexed for 30 sec, followed by 10 min ultrasonication. Then the mixture was centrifuged at 12,000 rpm for 15 min at  $4^{\circ}\text{C}$  and the supernatant was transferred into glass sample vials with screwed caps and stored at  $-80^{\circ}\text{C}$  until metabolomics analysis. As for the placenta, approximately 50 mg of placenta tissue from each patient was homogenized in 1 ml of cold mass spectrometry grade water with a plastic pestle. Then 200 ml of homogenate was mixed with 800 ml acetonitrile: methanol (1:1) solution, followed by vortex and ultrasonication. The mixture was subsequently centrifuged at 12,000

rpm for 15 min at 4 °C. The supernatant was dried in vacuum, reconstituted with 100 ml of acetonitrile: water (1:1) solution and stored at -80°C until metabolomics analysis.

### **LC-QTOF/MS analysis**

In our study, the serum and placenta metabolic fingerprinting was acquired with the AB SCIEX Triple TOF 5600 mass spectrometry (MS) system. The Acquity UPLC HSS T3 C18 column (2.1 mm × 100 mm, 1.8 mm, Waters, Milford, MA) was used in the sample separation step with column temperature maintained at 40°C. The mobile phase consisted of ultrapure water with 0.1% v/v formic acid (phase A) and acetonitrile with 0.1% v/v formic acid (phase B). The following elution gradient program was applied in the liquid chromatography: 5% B for 0-1 min; 5-95% B for 1-14 min; 95% B for 14-17 min; re-equilibration for 3 min. The sample injection volume was 5 ml and the flow rate was 0.3 ml/min.

The MS analysis was performed in both positive and negative ion modes and the conditions of ion source and gas for ionization were as follows: ion source voltage, 5500V (4500V in negative ion mode) ; gas temperature, 550 °C; curtain gas, 35 psi; gas1 (nebulizing gas), 50 psi; gas2 (heater gas), 55 psi. The declustering potential was set at 80 V on the orifice and the collisional energy was set between 20-50 V. The scan range was 100-1000 m/z in MS<sup>1</sup> and 50-1000 m/z in MS<sup>2</sup> respectively.

### **RNA isolation and quantification by RT-qPCR**

With the metabolomics analyses in our study, 8 genes involved in glutathione metabolism were chosen to test their expression levels in placenta. The real-time quantitative reverse transcription polymerase chain reaction (RT-qPCR) was performed and optimized as described previously [16]. Briefly, 20 tissues samples from PE (n=10) and normal pregnancies (n=10) were placed in liquid nitrogen and ground thoroughly with a mortar and pestle. The total RNA samples were isolated using TRIzol Reagent (Lot: 15596026, Thermo fisher, Carlsbad, CA) according to the manufacturer's instructions. The RNAs were dissolved in diethylpyrocarbonate-treated water and reversely transcribed by the SuperScript III First Strand Synthesis Super Mix Kit (Lot: 18080051, Thermo fisher, Carlsbad, CA). The cDNA was quantified with quantitative reverse transcription PCR (RT-qPCR) using the Luna Universal qPCR Master Mix (M3003L, NEB). The relative quantification of the PCR products was performed after normalization against the Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression, using the comparative cycle threshold method. The qPCR primers sequences of glutathione cysteine ligase catalytic subunit (GCLC), glutathione cysteine ligase modulate subunit (GCLM), glutathione synthase (GSS), glutathione reductase (GSR), glutathione peroxidase-1 (GPx-1), GPx-4, cyclooxygenase-1 (COX-1) and COX-2 were provided in **Supplementary Table S1**. RT-qPCR reactions were performed on 96-well plates and run in the CFX 96 system (Bio-Rad Laboratories Inc), the relative expression was analyzed using Bio-Rad CFX Manager Software.

### **Data processing**

The raw data of metabolic was collected and analyzed with the MassLynx software (Waters, Milford, MA). All the differential metabolites were identified by the in-house library with the aid of the reference standards and the open database of metabolic reaction network (MRN)-based recursive algorithm (MetDNA) [17]. The multivariate pattern recognition analysis was performed with the SMICA 14.1 software (Umetrics, Umea, Sweden). The principal component analysis (PCA) and the orthogonal partial least squares-discriminant analysis (OPLS-DA) were used in this study to discriminate the metabolic patterns between PE and healthy groups after pareto scaling. A permutation test was carried out to avoid OPLS-DA model over-fitting. The variable importance in the projection (VIP) values for the metabolites could be statistically identified with loading plots in OPLS-DA. Student's t test was performed to calculate the P values. The corresponding fold change showed how the identified metabolites of diseased individuals varied from that of the healthy controls. The differential metabolites were chosen with VIP >1 and p <0.05.

The clinical performance of selected potential metabolic biomarkers in PE was assessed by receiver operating characteristic (ROC) curves with MedCalc v11.4.2 (MedCalc Software, Ostend, Belgium). In addition, the metabolic pathway analysis of differential metabolites in serum and placenta was conducted by the web-based MetaboAnalyst 4.0 (<https://www.metaboanalyst.ca>) The gene expression of the enzymes involved in glutathione metabolism and cyclooxygenase enzymes were analyzed by student's t test and p <0.05 was considered statistically significant.

## Results

### Characteristics of study population for metabolomic analysis

Demographic characteristics of our study population are shown in Table 1. In sum, 60 women diagnosed with PE and 30 healthy pregnant women were recruited for the metabolomic study. The serum samples collected from all the PE subjects (n = 60) and controls (n = 30) were further analyzed in the metabolomic study. For the tissue study, 10 patients of each group agreed to donate their placenta for the present research. Of the collected placenta tissue, 5 of each group were applied in the metabolic study. However, due to insufficient tissue quality and quantity, 4 PE and 5 control placenta tissues were eventually applied for the metabolomics analysis.

Table 1  
Demographic characteristics of the study population

		PE	Control	p value
serum	n (samples)	60	30	—
	maternal age (years)	33.2 ± 4.6	31.7 ± 4.0	0.11
	gestational age at sampling(weeks)	32.5 ± 8.0	32.5 ± 0.5	0.98
placenta	n (samples)	4	5	—
	maternal age (years)	30.0 ± 0.8	31.4 ± 1.7	0.17
	gestational age (weeks)	34.5 ± 3.0	39.0 ± 1.2	0.02

Data are presented as mean ± standard deviation. Statistical p value is computed by student's t test.

No significant difference of maternal age or gestational age at sampling was observed between the PE group and the control group. Compared with the control group, the gestational age at delivery was significantly decreased in the PE group ( $p = 0.02$ ).

## Identification Of The Differential Metabolites

With the in-house metabolomic library, 31 peaks in serum were identified in the positive ion mode and 33 peaks identified in the negative ion mode. The PCA scores plot revealed a trend that all subjects could be separated into two clusters (Fig. 1A, Fig. 1B). The OPLSDA score plot demonstrated that metabolomics could be used to efficiently discriminate the PE and control groups (Fig. 1C, Fig. 1D). The permutation test showed that the Q2 regression line has a negative intercept, indicating the OPLSDA model was not over-fitting (Fig. 1E, Fig. 1F). A total of 16 differential metabolites identified in the serum samples with  $VIP > 1$  and  $p < 0.05$  were listed in Table 2. Interestingly, the levels of all these metabolites were up-regulated in PE group.

Table 2  
Serum differential metabolites between PE and control groups based on the in-house library

No	mode	VIP	p value	fold change	metabolite
1	Pos	1.1	<0.001	1.7	Acetylcarnitine
2	Pos	1.2	0.005	1.5	Methionine
3	Pos	1.8	0.001	1.5	Alloisoleucine
4	Pos	1.8	0.001	1.5	Isoleucine
5	Pos	1.8	0.001	1.5	Leucine
6	Pos	1.8	0.001	1.5	Norleucine
7	Pos	1.1	<0.001	1.4	Glutamine
8	Pos	1.3	<0.001	1.4	Tyrosine
9	Pos	1.3	<0.001	1.3	Tryptophan
10	Pos	1.2	<0.001	1.3	Pyroglutamic acid
11	Neg	1.8	<0.001	9.3	Taurocholic acid
12	Neg	2.2	<0.001	2.3	Elaidic acid
13	Neg	2.2	<0.001	2.3	Oleic acid
14	Neg	2.2	<0.001	2.3	Vaccenic acid
15	Neg	2.0	<0.001	2.0	Linoleic acid
16	Neg	2.7	<0.001	1.8	Phenylalanine

## Roc Analysis Of The Differential Metabolites

In the ROC analysis, the metabolites including pyroglutamic acid, methionine, glutamine and taurocholic acid in the serum, with the area under ROC curve values of 0.901, 0.909, 0.892 and 0.873 respectively, were shown to have the best performance in distinguishing the PE patients from the healthy pregnancy controls (Fig. 2).

## Metabolic Pathway Analysis

To obtain a wide range of differential metabolites in the PE patients and the healthy controls, only VIP > 1 was used as the criteria of differential metabolites. Based on the MetDNA identified metabolites (**Supplementary Table S2 and Supplementary Table S3**), the analysis of relevant pathways and networks of PE was further performed by MetaboAnalyst 4.0. Consequently, potential target metabolic pathway

analysis revealed that the differential metabolites identified in serum were closely associated with phenylalanine, tyrosine and tryptophan biosynthesis, linoleic acid and alpha-linolenic acid metabolism, D-glutamine/D-glutamate metabolism, and phenylalanine metabolism (Fig. 3A). The results also demonstrated that the relative metabolites identified in placenta played important roles in PE and were responsible for the changed metabolism of linoleic acid, alpha-linolenic acid, glutathione and tryptophan (Fig. 3B).

## Glutathione And Cox Enzyme Levels In Placentas

The levels of glutathione pathway enzymes and COX mRNA were investigated in the placentas of PE and normal pregnancies (Fig. 4). Although not statistically significant (possibly due to the relatively small sample size of placentas used in the study), the overt tendency of decreased GPx-1 expression and elevated GCLM, GSR expression were observed in the PE group. By contrast, the expression of placenta COX-1 and COX-2 was significantly higher in the PE patients when compared with the healthy pregnant group.

## Discussion

As a severe pregnancy complication, the pathophysiology of preeclampsia is not fully understood and the only effective treatment is delivery [10]. In the present study, we applied high performance liquid chromatography coupled with quadrupole-time-of light mass spectrometry (HPLC-QTOF-MS) to investigate the metabolic changes in women with preeclampsia. Ninety serum samples and nine placentas tissue were used in the above metabolomic analyses. Sixteen metabolites in serum were identified as the differential metabolites and the area under ROC curves suggested that pyroglutamic acid (pGlu), methionine, glutamine and taurocholic acid were potentially valuable for PE diagnosis. Furthermore, metabolic pathways analysis was performed on web-based Metaboanalyst4.0 and it revealed that the metabolisms of linoleic acid and alpha-linolenic acid, phenylalanine, tyrosine and tryptophan biosynthesis, D-glutamine/D-glutamate, phenylalanine, glutathione, tyrosine and tryptophan were significantly altered and might be involved in the PE pathogenesis.

Pyroglutamic acid, a natural amino acid derivative, can be synthesized in living cells enzymatically and non-enzymatically. It has been reported that pGlu could efficiently inhibit the catalytic activity of human angiotensin-converting enzyme (ACE) [18]. For instance, at the concentration of 20  $\mu\text{g}/\text{mL}$ , pGlu was found to inhibit 98.2% of the activity of human ACE *in vitro*. ACE plays a central function of converting angiotensin I (Ang I) to Ang II and it has been shown to contribute to hypertension via the renin-angiotensin system (RAS) [19–21]. However, the circulating and intrarenal RAS was supposed to be down-regulated to compensate the up-regulated local uteroplacental RAS in preeclampsia [22, 23]. In our study, we found that the serum pGlu was increased in PE group with a fold-change value of 1.3, which may be associated with the downregulation of intrarenal RAS in preeclampsia.

As an essential amino acid, methionine is required for protein synthesis. In the methionine cycle, it can be regenerated from homocysteine (Hcy) and transformed into S-adenosylmethionine (SAM), which is the universal methyl donor in many cellular methylation reactions [24]. In a case-control study, the SAM level was increased in the PE group although this difference was not statistically significant [25]. In another study with 32 PE patients and 64 controls, maternal plasma Hcy and folate were significantly elevated in patients in the third trimester [26]. In our analysis, the levels of methionine were much higher in PE patients than those in normal pregnancy. In addition, among those identified differential metabolites, the methionine showed the best performance for PE diagnosis with the AUC of 0.909. However, an opposite change of methionine has been reported in another metabolomics study [27]. Therefore, further studies are warranted to better understand the role of methionine in the PE pathogenesis.

Similarly, the glutamine serum level was also increased in the PE group. In a metabolomic study with placental tissues, it was reported that the concentrations of glutamine were elevated in severe PE patients [28]. Interestingly, there were other studies in which the glutamine in both placenta and serum were found at a lower concentration in PE women [29, 30]. What's more, the low concentration of glutamine may increase the expression of intercellular cell adhesion molecules-1 in human umbilical vein endothelial cells, enhance migration of neutrophils across the endothelial cells, and cause tissue destruction eventually [31].

Emerging metabolomic studies suggested that the dysregulation of lipid metabolism played an important role in the development of preeclampsia [28, 32, 33]. The lipid metabolism changes in PE could be characterized by increased levels of serum triglyceride (TG), low-density lipoprotein (LDL), and circulating free fatty acids (FFAs), and accompanied with decreased level of high-density lipoprotein (HDL). In a study focused on the components of esterified and free fatty acids, it has been reported that the levels of palmitic, oleic and linoleic acids were significantly increased in women with PE [34]. In the present study, the linoleate which could be consumed to derive linoleic acid was decreased and the metabolic product of alpha-linolenic acid such as (9Z,12Z,15Z)-octadecatrienoic acid was severely increased in the placenta of PE women. What was more, the levels of arachidonic acid and its derivative such as 5,6-epoxyeicosatrienoic acid (EET) in placenta was higher in the PE group compared with the normal pregnancy in our study. Linoleic acid is the precursor of endogenous arachidonic acid (AA) which could be further converted to EETs by the cytochrome P-450 (CYP) epoxygenase. Herse et al. reported that the EETs including 5,6-EET, 14,15-EET, and the dihydroxyeicosatrienoic acids, were elevated in the preeclamptic women due to the up-regulated expression of the CYP subfamily 2J polypeptide 2 (CYP2J2) [35]. More importantly, the supplement of linoleic acid during pregnancy has been reported to be beneficial to the prevention and management of PE [36].

This was the first metabolomics study of human placenta which reported the levels of glutathione (GSH), its oxidized form (glutathione disulfide, GSSG) and the GSSG/GSH ratio were all increased in PE patients. The change of placental GSH was consistent with previous study conducted by Knapen et al [37]. The decrease of GSH levels in placenta and serum was also has been reported in several studies [38–40]. As

observed in our study, it has been reported that the GSSG/GSH ratio was significantly increased in the placenta of PE patients in other researches [39, 41].

In order to better understand the GSH metabolism disturbance in PE, the RT-qPCR experiments were performed to examine the gene expression level of relevant enzymes in the pathway, such as the expression of GCLC, GCLM, GSS, GPx1, GPx4 and GSR. Although not statistically significant, the increased expression of GCLM may have reflected the cellular reducing power demands. However, excessive production of reactive oxygen species (ROS) depleted the GSH pool and resulting in high-level GSSG/GSH ratio (51.8 in PE vs 17.3 in control, extracted from raw data of MetDNA analyses with placenta) and decreased overall antioxidants. Interestingly, the GPx-1 mRNA expression showed a mild reduction in PE in our study. Bilodeau et al proposed that GPx-1/3/4 deficiency might promote the synthesis of vasoconstrictive eicosanoids such as F2-isoprostanes and thromboxanes, which are known to be up-regulated in PE placentas [42]. The mRNA expression of COX-1 and COX-2 that are directly involved in the production of thromboxanes [43], was significantly elevated in the PE placenta (Fig. 4). The induction of the COX enzymes has been reported closely related to excessive oxidative stress in rat cytotrophoblast, spongiotrophoblast and glycogen cells and might be regulated through activation of the p38MAPK and the NF- $\kappa$ B transcription factor [44].

As a part of tryptophan metabolic pathway, the major catabolic route is the oxidation of tryptophan to kynurenine by the hepatic enzyme tryptophan 2,3-dioxygenase or ubiquitous indoleamine 2,3-dioxygenase (IDO) [45]. In normal pregnancy, the IDO is highly expressed within placental and contributed to an increase of plasma kynurenine/tryptophan ratio. In preeclampsia, the levels of plasma tryptophan and kynurenine/tryptophan ratio were vastly decreased compared with normal pregnancy due to reduction of placental IDO expression [46]. Santillan et al. found that the IDO deficiency could lead to pathognomonic renal glomerular endotheliosis, proteinuria, pregnancy-specific endothelial dysfunction, intrauterine growth restriction, and mildly elevated blood pressure in IDO knockout mice models [47]. Thus, it was clear that the downregulation of tryptophan metabolism and reduction of IDO activity were involved in the pathogenesis of PE.

## Conclusions

In summary, the metabolomic profiling of serum and placenta by HPLC-QTOF-MS revealed key metabolites and metabolic pathways. Not only has our study provided potential metabolic biomarkers for PE diagnosis, and it also contributed to better understanding of the pathophysiologic mechanisms of PE. However, there are a few of limitations in our study. Firstly, because placenta samples are not available until delivery and women with PE have a higher risk of preterm delivery, it is difficult for us to collect gestational age matched placenta tissues for the metabolomics study. The metabolic profiling of placenta tissues could be potentially biased due to the gestational week difference. Secondly, as the number of placenta samples is relatively small, the biological variation could not be ignored during analysis. Last but not least, it would be helpful to validate the promising metabolic biomarkers for PE

diagnosis or even prediction with isotope-labeled standards on the LC-MS platform to examine and confirm their clinical utility.

## Abbreviations

PE

preeclampsia; ISSHP:International Society for the study of Hypertension in Pregnancy; MS:mass spectrometry; RT-qPCR:real-time quantitative reverse transcription polymerase chain reaction; GAPDH:Glyceraldehyde-3-phosphate dehydrogenase; GCLC:glutathione cysteine ligase catalytic subunit; GCLM:glutathione cysteine ligase modulate subunit; GSS:glutathione synthase; GSR:glutathione reductase; GPx:glutathione peroxidase; COX:cyclooxygenase; MRN:metabolic reaction network; MetDNA:metabolic reaction network-based recursive algorithm; PCA:principal component analysis; OPLS-DA:orthogonal partial least squares-discriminant analysis; VIP:variable importance in the projection; ROC:receiver operating characteristic; HPLC-QTOF-MS:high performance liquid chromatography coupled with quadrupole-time-of light mass spectrometry; pGlu:pyroglutamic acid; ACE:angiotensin-converting enzyme; Ang I:angiotensin I; RAS:renin-angiotensin system; Hcy:homocysteine; SAM:S-adenosylmethionine; TG:serum triglyceride; LDL:low-density lipoprotein; FFAs:circulating free fatty acids; HDL:high-density lipoprotein; EET:epoxyeicosatrienoic acid; AA:arachidonic acid; CYP:cytochrome P-450; GSH:glutathione; GSSG:glutathione disulfide;IDO:indoleamine 2,3- dioxygenase.

## Declarations

### Ethics approval and consent to participate

The study protocol was approved by the Research Ethics Committee of the Beijing Obstetrics and Gynecology Hospital. Written or verbal consent was obtained from all subjects from whom the serum samples or placenta tissues were collected.

### Consent for publication

Not applicable.

### Availability for data and materials

The relative peak areas of identified metabolites based on the in-house library in the serum of participants are available in the **supplementary Table S4**. Alternatively, the supplementary files including Table S1, Table S2, Table S3 and Table S4 are available in the Open Science Framework Repository ([www.osf.io](http://www.osf.io), DOI 10.17605/OSF.IO/XMBPA)

### Competing interests

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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## Authors' contributions

All authors have certified the author list and the contribution description. All authors have read and approved the submitted manuscript and any substantially modified version of the manuscript. Contribution to work: Y.D., X.L, S.Z., Y.Z. and Z.C. were involved in recruiting objectives and collecting samples. C.L., D.S., J.C., J.W., L.C. and S.H. were involved in performing experiments, acquisition of data, analysis and interpretation of data; Y.D. and Z.C. were involved in drafting of the article and critical approval of the final article. J.L., G.T., Y.L. and X.Y. were involved in the statistical analysis and figure preparation.

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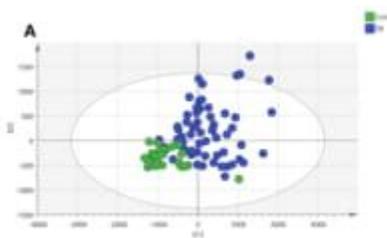
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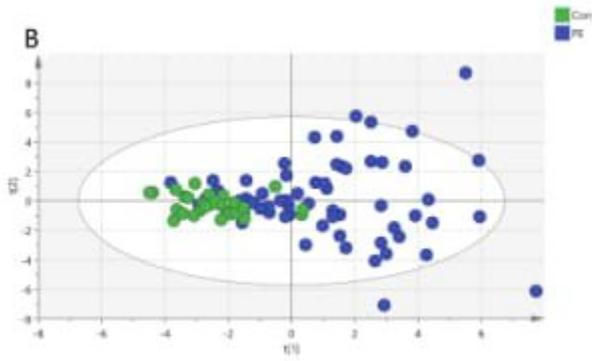
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## Figures



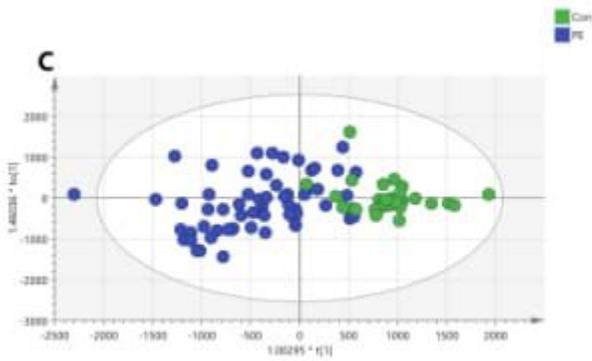
**Figure 1**

Multivariable analysis of serum samples. (A) Principle component analysis (PCA) score plot in positive ion mode



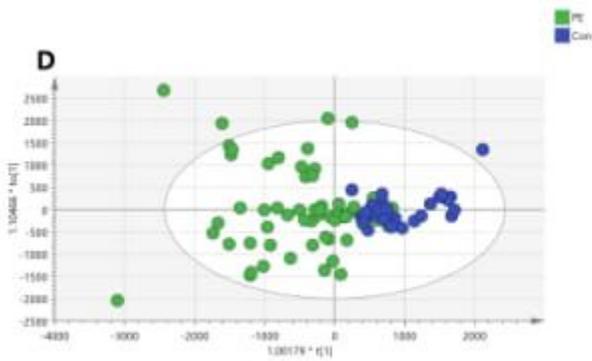
**Figure 2**

Multivariable analysis of serum samples. (B) PCA score plot in negative ion mode;



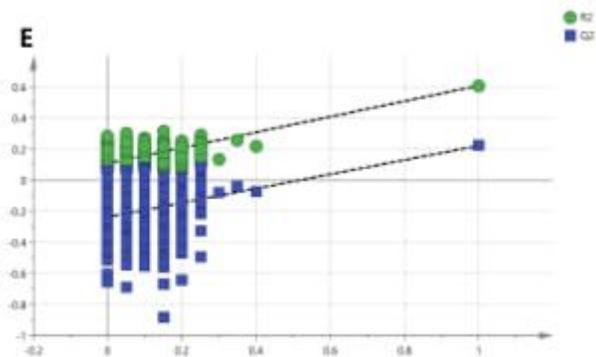
**Figure 3**

Multivariable analysis of serum samples. (C) Orthogonal partial least squares-discriminant analysis (OPLS-DA) score plot in positive ion mode



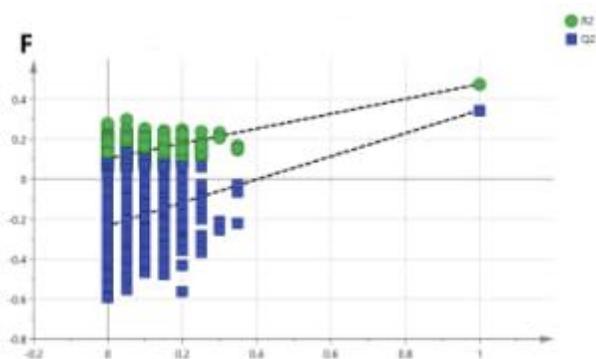
**Figure 4**

Multivariable analysis of serum samples.(D) OPLS-DA score plot in negative ion mode



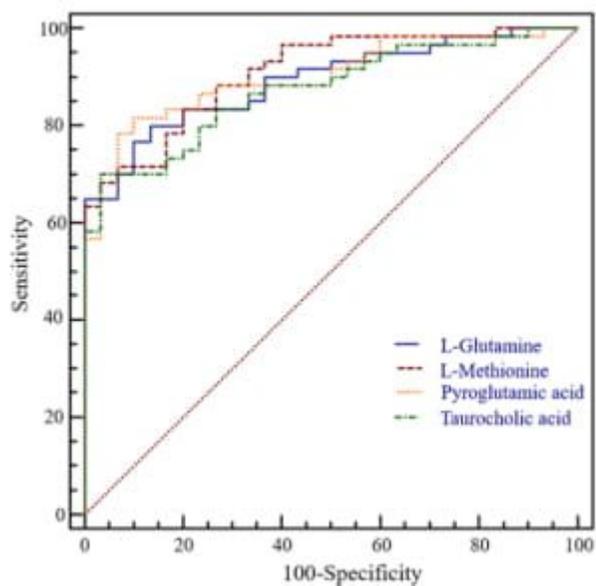
**Figure 5**

Multivariable analysis of serum samples. (E) Permutation test for the OPLS-DA model in positive ion mode. The Y-axis intercepts: R2= (0.0, 0.107), Q2= (0.0, -0.239);



**Figure 6**

Multivariable analysis of serum samples. (F) Permutation test for the OPLS-DA model in negative ion mode. The Y-axis intercepts: R2= (0.0, 0.104), Q2= (0.0, -0.233).



**Figure 7**

Receiver operating characteristic (ROC) curve analysis of the potential serum metabolites for PE diagnosis.

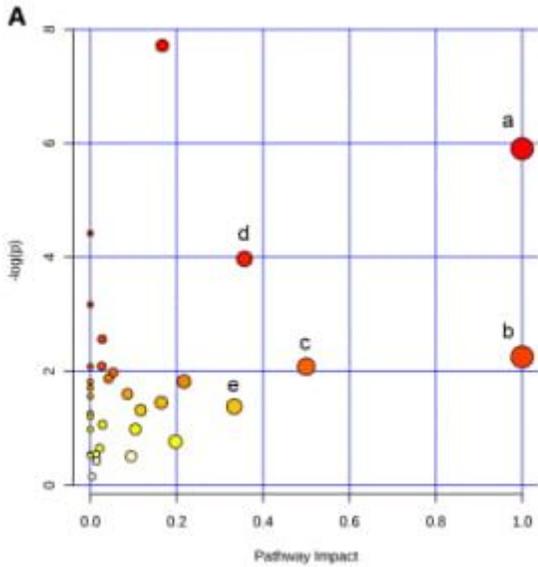


Figure 8

Summary of metabolic pathway analysis. (A) Altered metabolic pathway in serum samples between PE and control groups. a, phenylalanine, tyrosine and tryptophan biosynthesis; b, linoleic acid metabolism; c, D-glutamine/D-glutamate metabolism; d, phenylalanine metabolism; e, alpha-linolenic acid metabolism.

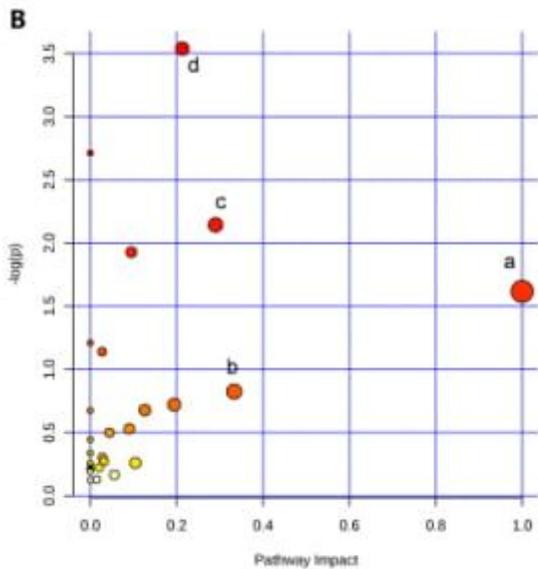
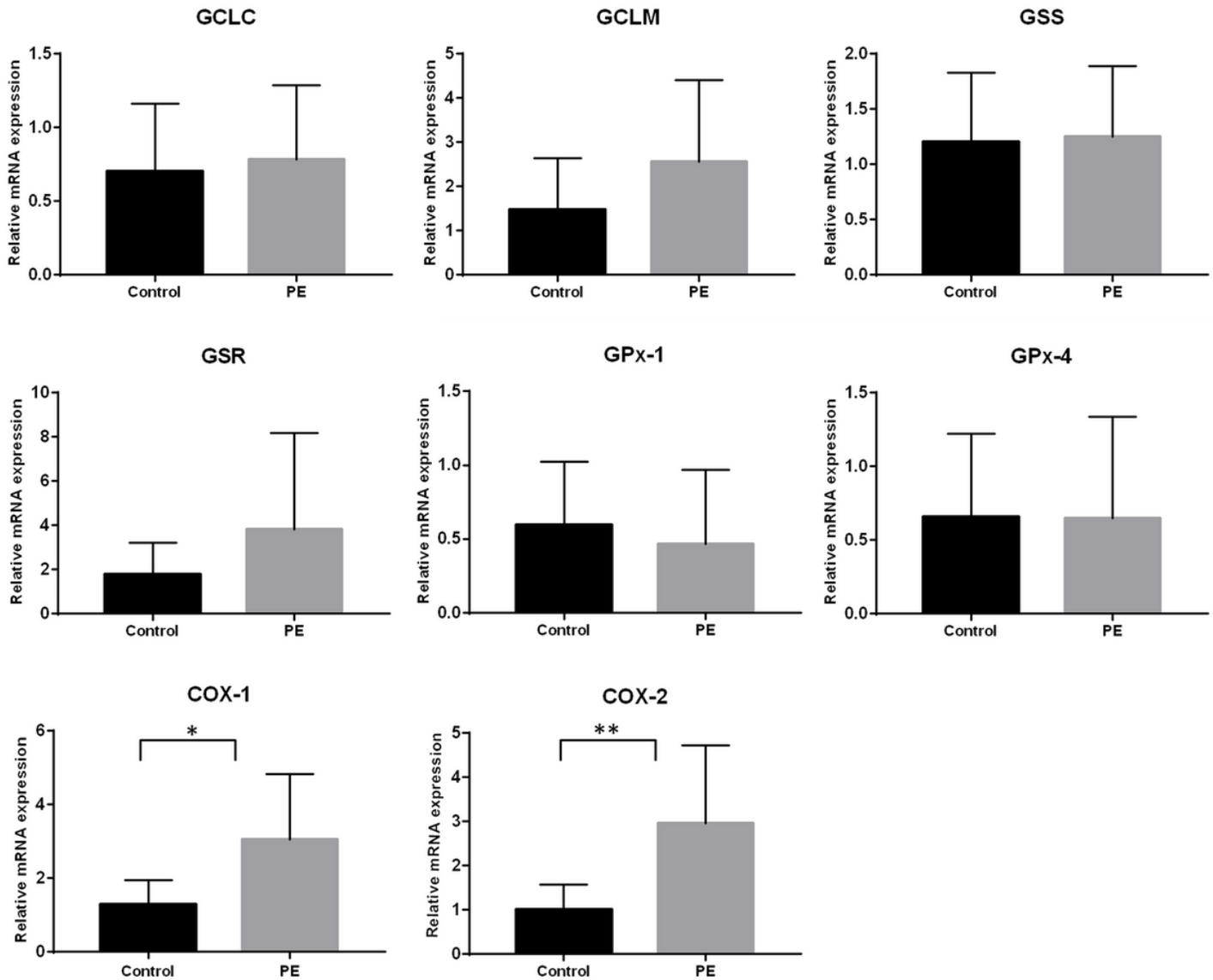


Figure 9

Summary of metabolic pathway analysis. (B) Altered metabolic pathway in placenta samples between PE and control groups. a, linoleic acid metabolism; b, alpha-linolenic acid metabolism; c, glutathione metabolism; d, tryptophan metabolism.



**Figure 10**

Gene expressions of glutathione pathway enzymes and COX enzymes in PE (n=10) and control (n=10) groups. GCLC, glutathione cysteine ligase catalytic subunit; GCLM, glutathione cysteine ligase modulate subunit; GSS, glutathione synthase; GSR, glutathione reductase; GPx, glutathione peroxidase; COX, cyclooxygenase. (\*p < 0.05; \*\*p < 0.01)

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryTables.xlsx](#)