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Association between polymorphism in Cyclophilin A gene and its serum and placental expression in Han Chinese women with severe preeclampsia

Wenjuan Sun[a,b], Yongping Xu[b], Qian Xin[c], Yuan Zhang[d], Baoxia Cui[a,*], Fanzhen Hong[b,*]

[a] Department of Obstetrics and Gynecology, Qilu Hospital of Shandong University, Jinan, Shandong, PR China
[b] Department of Obstetrics, The Second Hospital of Shandong University, Jinan, Shandong, PR China
[c] Central Laboratory, The Second Hospital of Shandong University, Jinan, Shandong, PR China
[d] Center of Evidence-based Medicine, The Second Hospital of Shandong University, Jinan, Shandong, PR China

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ABSTRACT

Objectives: Cyclophilin A (CypA) plays important roles in inflammation and oxidative stress and is significantly increased in serum of preeclampsia (PE) patients. We aimed to investigate CypA genetic polymorphism and its serum and placenta expressions in severe PE of Han Chinese women.

Methods: A case-control study of 82 severe PE patients and 179 healthy pregnancies was conducted. Single-nucleotide polymorphism (SNP) sites of rs3735481, rs9638978 and rs11984372 were analyzed by TaqMan assay. CypA serum levels were determined by enzyme-linked immunosorbent assay (ELISA). CypA mRNA levels and protein expressions in placentas were assessed by quantitative real-time polymerase chain reaction (PCR), western blot, and immunofluorescence assay, respectively.

Results: There were significantly lower frequency of rs3735481 allele C (odds ratio (OR): 0.60, 95% confidence interval (CI): 0.36–0.98; $p = .04$), and significantly higher frequency of rs9638978 allele A in severe PE especially in early onset PE patients (OR: 2.23, 95% CI: 1.35–3.71, $p = .002$). Frequency of rs9638978 AA genotype was significantly higher in early onset PE ($p < .001$). CypA serum levels were significantly higher in severe PE especially in early onset PE ($p < .001$). Meanwhile, CypA serum levels were significantly lower in carriers with the AC genotype of rs3735481 ($p = .019$) and significantly higher in carriers with the AA genotype of rs9638978 ($p = .017$). CypA mRNA levels and protein expressions were found to be significantly increased in PE placentas (both $p < .01$).

Conclusions: The CypA genetic polymorphisms of rs3735481 and rs9638978 may be associated with severe PE, and rs9638978 AA genotype may be associated with an increasing risk of early onset severe PE in Han Chinese women. High CypA levels in serum and placenta may contribute to the pathogenesis of severe PE. Our results may provide a new clue for the etiology of severe PE.

1. Introduction

Preeclampsia (PE) is the most complicated type of pregnancy-related hypertensive disorder [1]. Three to 5% of pregnancies in the United States and up to 8% of pregnancies worldwide are reported affected by PE. Combined with other hypertensive disorders PE acts as a main cause of maternal and fetal mortality and morbidity [2,3]. The clinical manifestations of PE include severe high blood pressure, proteinuria, and complications such as renal and heart insufficiency, liver involvement, hematological disorders, preterm birth and fetal intrauterine growth restriction [4]. It has been reported that PE is associated with inherited susceptibility, oxidative stress, immune regulation, and superficial implantation of placenta [1,5,6], but the underlying mechanisms remain elusive.

The key consequences of PE are uterine spiral artery remodeling disorder and ischemic placental injury, which could activate the maternal immune and cardiovascular systems [7]. There are many common pathogenic factors and pathological features between PE and cardiovascular disease, such as systemic inflammation, oxidative stress, and vascular endothelium dysfunction [8]. Trophoblast function and spiral artery remodeling have been shown to be modulated by a number of factors, including transforming growth factors (TGF), insulin-like

* Corresponding authors at: Department of Obstetrics, The Second Hospital of Shandong University, NO. 247, Beiyuan Street, Jinan 250033, Shandong Province, PR China (F. Hong). Department of Obstetrics and Gynecology, Qilu Hospital of Shandong University, Cultural West Road, Jinan 250013, Shandong Province, PR China (B. Cui).
E-mail addresses: cuibaoxia@sdu.edu.cn (B. Cui), hongfz@sdu.edu.cn (F. Hong).

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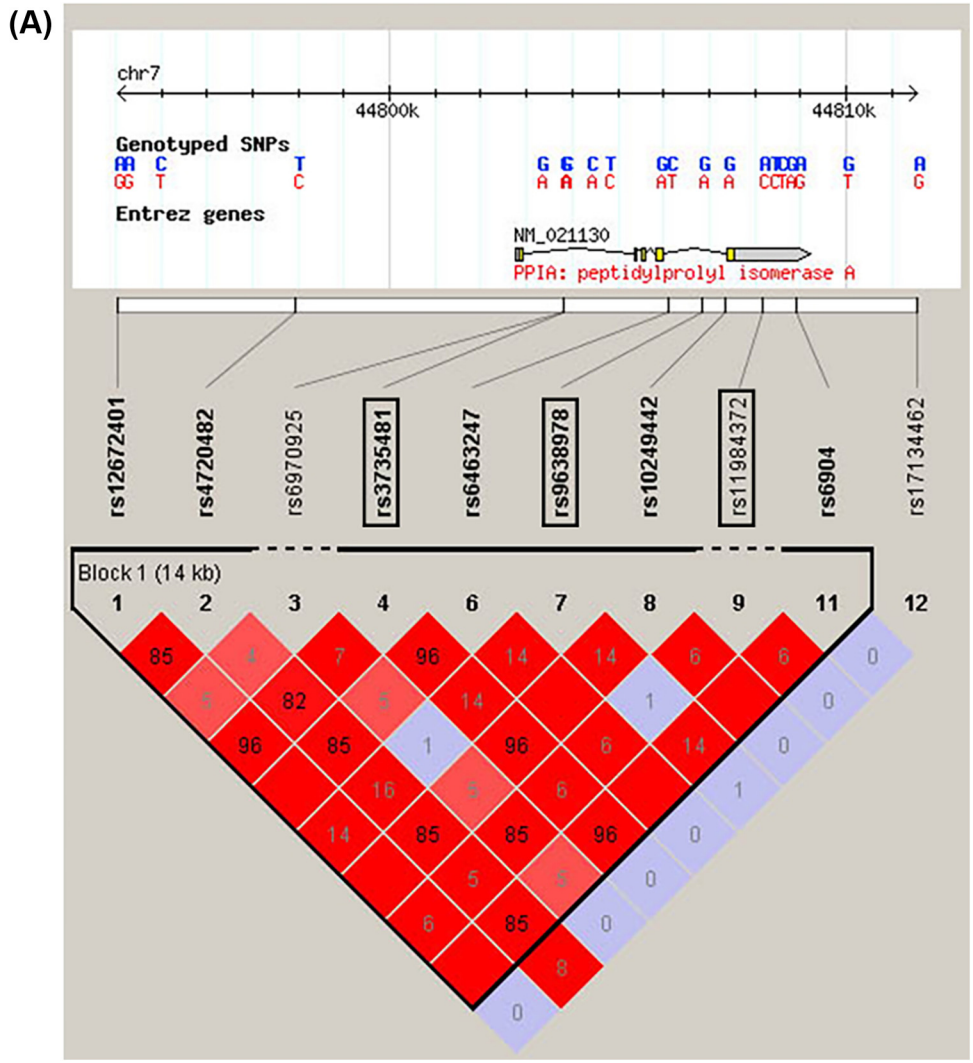

Fig. 1. (A). Linkage disequilibrium diagram of SNPs in PPIA gene. Three tagging SNP sites of rs3735481, rs11984372 and rs9638978 were chosen by the Tagger analysis method for analysis. (B). Comparisons of CypA levels in maternal serum. Serum CypA levels were significantly higher in severe PE patients especially in early-onset PE patients compared to the healthy controls ($p < .001$). (C) Comparisons of CypA serum levels in allelic-specific expressions of three SNPs. Serum CypA levels were significantly lower in carriers with the AC genotype of rs3735481 ($p < .02$), and significantly higher in carriers with the AA genotype of rs9638978 ($p < .02$). No significant difference was found in carriers with AA and AC genotypes of rs11984372 ($p > .05$).

growth factor (IGF), interleukins (ILs), and adhesion molecules [9]. Cyclophilin A (CypA), acting as an inflammatory mediator, has been proven to contribute to the pathogenesis of cardiovascular diseases [10]. It may be also involved in the pathogenesis of PE.

CypA, distributing ubiquitously in both intracellular and extracellular regions, is the most abundantly expressed family member of cyclophilins with peptidylprolyl isomerase (PPIase) activity [11]. It is secreted by vascular smooth muscle cells (VSMC), macrophages, endothelial cells (EC), and fibroblast-like synoviocytes, and participates in autocrine and paracrine signaling pathways [12–15]. Extracellular CypA (eCypA) plays potent chemotactic roles in apoptosis (EC), inflammation (EC, VSMC), proliferation and migration (cancer cell, VSMC), chemotaxis (leukocytes), matrix metalloproteinase (MMP) activation (monocytes), and platelet activation [11,16]. In cardiovascular diseases, serum CypA levels were significantly higher and closely related to the disease severity, making it a potential biomarker [10,17]. The serum CypA levels were also enhanced in patients with rheumatoid arthritis, type 2 diabetes, and chronic obstructive pulmonary disease [13,18,19]. Recently, it was reported that maternal serum CypA levels were elevated significantly in severe PE patients [20], and thus CypA and the inflammatory reactions mediated by it may be associated with the onset of PE.

PPIA (peptidylprolyl isomerase A), located in the 7p13 chromosome region, is the coding gene of CypA [21]. To date, few studies have investigated the genetic polymorphisms of CypA, mainly in viral infection and coronary artery disease (CAD) [21,22]. The relationship between CypA genetic polymorphism and severe PE remains unclear. Here, we investigated the associations between the CypA genetic polymorphisms and their maternal serum and placenta expression with severe PE in Han Chinese women.

2. Materials and methods

The study protocol was reviewed and approved by the ethics committee of the Second Hospital of Shandong University in accordance with the code of ethics of the World Medical Association. All pregnant women involved in the study were fully informed about the study and provided written informed consent.

2.1. Patients

Eighty-two patients diagnosed with severe PE and 179 healthy age-

matched pregnant women were recruited from the Department of Obstetrics in the Second Hospital of Shandong University between July 2015 and September 2017. All subjects were singleton pregnancy and Han Chinese. The inclusion criteria for the PE group was de novo hypertension with systolic blood pressure ≥ 160 mmHg or diastolic blood pressure ≥ 110 mmHg after 20 weeks of pregnancy, accompanied by proteinuria, or without proteinuria but with any new onset, including liver or renal dysfunction, visual involvement, thrombocytopenia, pulmonary edema, and cerebral symptoms [23]. Women who had histories of chronic hypertension, renal or cardiovascular diseases, diabetes, hematological diseases, hepatitis, intrapartum infections, or any other pregnancy complications were excluded from the study.

2.2. Collection of specimens

Venous blood was collected into tubes anticoagulated with ethylenediaminetetraacetic acid (EDTA) before delivery and centrifuged for 30 min to separated plasma and blood corpuscles. Placenta specimens were drawn in 5 min after delivery at the maternal surface near the umbilical cord and stored at −80 °C.

2.3. Genomic DNA isolation

DNA samples from blood specimens were extracted using the TIANamp genomic DNA kit (TIANGEN, China), and the concentrations were determined using a Nanodrop 2000c spectrophotometer (Thermo Fisher Scientific, USA).

2.4. Tagging single nucleotide polymorphisms (SNP) selection

The polymorphism disequilibrium of PPIA was analyzed by Haploview 4.2 software, and three tagging SNP sites (rs3735481, rs9638978 and rs11984372) were chosen for analysis using the Tagger analysis method (Fig. 1A).

2.5. Genotyping of SNPs

SNPs genotyping was conducted by TaqMan assays. TaqMan probes and primers for genotyping were synthesized by Invitrogen. Polymerase chain reaction (PCR) was run in a final volume of 10 μL solution containing 5 μL of 2 × Premix Ex Taq (Takara, Japan), 10–20 ng of genomic DNA, 0.05 μL of Taq-man probes, and DNase-free water. The application was performed in the LightCycler 480 Instrument II (Roche Applied Science, USA) under the following cycling conditions: 95 °C for 30 s; 30 cycles of 95 °C for 30 s (denaturation), 60 °C for 30 s (annealing), and 72 °C for 30 s (extension) and 72 °C for 5 min (final extension).

2.6. Enzyme-linked immunosorbent assay (ELISA)

Concentration of serum CypA was measured using human CypA ELISA kits (Cusabio, USA). The coefficient of variation values (CV) were < 8% for intra-assay precision and less than 10% for inter-assay precision across the standard curve.

2.7. Quantitative real-time PCR (qRT-PCR)

The total mRNA samples of placentas were isolated using the RNAsimple Total RNA Kit (TIANGEN, China). Reverse transcription reaction was performed according to the appendices of the PrimeScript™ RT Reagent Kit (Takara, China). qRT-PCR was carried out using a Roche LightCycler 480 Instrument II in a 10 μL volume, containing 1 μL of CypA or glyceraldehyde 3-phosphate dehydrogenase (GAPDH) primers, diluted cDNA templates and SYBR premix Ex Taq II (Takara, China). The amplification was run at using the following cycling parameters: 95 °C for 30 s, 40 cycles of 95 °C for 5 s, and 60 °C for 20 s, then by melting curve analysis (95 °C for 15 s, 60 °C for 15 s, and 95 °C for 15 s). Each cDNA sample was run in quadruplicate. The mRNA level of GAPDH was used for standardization. The unit value of relative CypA mRNA level was obtained by the $2^{-\Delta\Delta Ct}$ method. The primers for human CypA genes were 5′-GTCAACCCCACCGTGTTCTTC-3′ (sense) and 5′-TTTCTGCTGTCTTTGGGACCTT G-3′ (antisense), and for GAPDH genes were 5′-AGAAGGCTGGGGCTCATTTG-3′ (sense) and 5′-AGGGG CCATCCACAGTCTTC-3′ (antisense).

2.8. Western blot

Total proteins were extracted from placental tissues using radioimmunoprecipitation assay (RIPA) (Solarbio, USA) and phenylmethylsulfonyl fluoride (PMSF) (Meilunbio, China) premixture. Then, protein concentrations were measured using the BCA Protein Assay Kit (Thermo Fisher Scientific, USA) and detected at 595 nm by a NanoDrop 2000c ultra-micro spectrophotometer (Thermo Scientific, USA). After preparation of 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), protein extracts (40–50 μg) and protein ladders were added in each hole, respectively, for electrophoresis, then transferred onto a polyvinylidene fluoride (PVDF) membrane (Millipore, USA) and incubated with 5% skim milk in 1 × tris-buffered saline with Tween (TBST) at room temperature for 1.5 h. Immunoblotting was performed using 1 μg/mL of CypA or GAPDH antibodies (Abcam, UK) overnight at 4 °C. After washing three times with 1 × TBST, the PVDF membrane was incubated with horseradish peroxidase (HRP)-conjugated secondary antibody (Abcam, UK) at room temperature for 1 h. Then, the specific band with Pierce™ ECL western blotting substrate (Thermo Fisher Scientific, USA) was detected by the Bio-Rad electrophoresis image analyzer (Bio-Rad, USA). The gray value of CypA was analyzed with GAPDH as an internal control through the image analysis software and the results were presented as mean ± SEM.

2.9. Immunofluorescence staining

Fresh placenta samples were selected to prepare frozen sections (5 μm). Sections were fixed with 4% polyformaldehyde solution for 15 min at 4 °C and washed three times by 1 × PBST. Then, 0.2% Triton-X100 was used to permeate for 8 min at room temperature. After washing three times with 1 × PBST, sections were blocked up with normal sheep serum (Beyotime, China) for 30 min and then incubated with CypA antibody (Abcam, UK) overnight at 4 °C, washed, and incubated with species-specific Dylight 649 secondary fluorescent antibody (Abbkine, USA) for 1 h at room temperature. Then, sections were washed by 1 × PBST, incubated with 4′,6-diamidino-2-phenylindole (DAPI, Beyotime, China) for 5 min and subsequently the excess DAPI washed away. A Leica confocal microscope (Leica Instruments GmbH, Germany) was used for observation and image collection.

2.10. Statistical analysis

Statistical analysis was performed with SPSS Statistics 24.0 (IBM, USA) and GraphPad Prism 5.0 (GraphPad Software, USA). Comparisons of clinical characteristics were compared by *t*-test or chi-squared test. The chi-squared test was also applied to Hardy–Weinberg equilibrium (HWE) of genotype distribution and comparisons of SNP genotypes and allele frequencies. The odds ratios (ORs) and 95% confidence intervals (CIs) were analyzed in different genetic models. The correlation analysis of numerical variables obeying normal distribution was analyzed by Pearson correlation. *P*-value < .05 was considered to be statistically significant. The Bonferroni correction was applied to account for multiple comparisons ($P = .025$ for three SNPs, comparing two times; $P = .017$ for three subgroups, comparing three times).

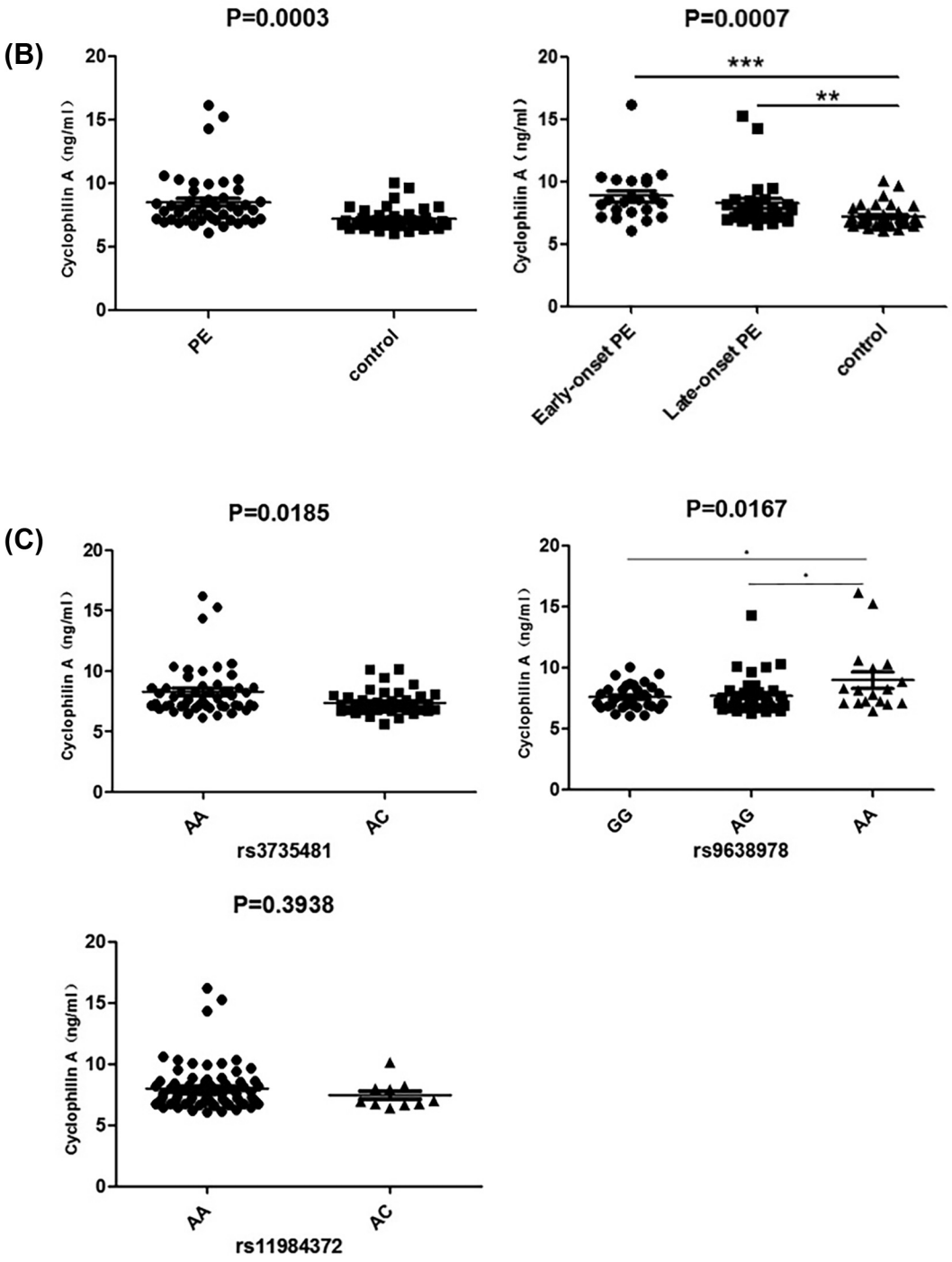

Fig. 1. (*continued*)

3. Results

3.1. Demographic and clinical characteristics

A total of 261 pregnant women were involved in this study, including 82 PE patients and 179 healthy pregnancies (Table 1). The mean maternal age of the PE group was 29.93 ± 4.73 y, with no significant difference compared to the healthy control group (29.07 ± 4.12 y, $p = .14$). Sixteen of 82 (19.51%) severe PE patients had a history of PE. No significant differences were found in factors of gravidity, cesarean section (CS) times, and abortion times ($p > .05$), but body mass index (BMI), systolic and diastolic blood pressure in the PE group were significantly higher than that in the control group ($p < .001$). All of the PE patients had proteinuria, of which 10.98% (9/82) of patients had 0.1–0.5 g/L, 39.02% (32/82) of patients had 0.5–2.0 g/L, and 50.00% (41/82) of patients had more than 2.0 g/L. The gestational age and fetal weight were significantly lower in the PE group ($p < .001$). CS delivery occurred in 92.68% (76/82) of PE patients, while others underwent induced labor because of dead fetus. The mean Apgar score at 1 min of the PE group was 9.57 ± 1.35, significantly lower than that of the control group (9.91 ± 0.52, $p = .04$), but no significant difference was found at 5 min ($p = .21$).

According to the time of onset, the severe PE patients were further divided into early onset (diagnosis of PE at < 34 weeks) PE subgroup (n = 38) and late onset (diagnosis of PE at ≥ 34 weeks) PE subgroup (n = 44). The data analysis showed BMI, gestational age, fetal weight,

Table 1
Clinical characteristics of PE patients and healthy pregnant women.

Characteristics	PE group (n = 82)	Control group (n = 179)	$t/\chi 2$	*P* value
Maternal age (y)	29.93 ± 4.73	29.07 ± 4.12	1.48	.14
BMI (kg m^{-2})	31.10 ± 3.56	28.68 ± 3.53	5.12	< .001*
History of preeclampsia (%)	16 (19.51)	0 (0.00)	37.21	< .001*
Gravidity (%)				
1	21 (19.51)	66 (36.87)	3.21	.20
2	31 (37.80)	57 (31.84)		
≥3	30 (36.59)	56 (31.28)		
CS times (%)				
0	61 (74.39)	126 (70.39)	0.89	.64
1	20 (24.39)	52 (29.05)		
≥2	1 (1.22)	1 (0.56)		
Abortion times (%)				
0	39 (47.56)	100 (55.87)	2.17	.34
1	27 (32.93)	44 (24.58)		
≥2	16 (19.51)	35 (19.55)		
Proteinuria (g/L)		(−)		
0.1–0.5	9 (10.98)	/		
0.5–2.0	32 (39.02)	/		
2.0–5.0 and ≥5	41 (50.00)	/		
Gestational age (weeks)	35.55 ± 3.24	39.13 ± 1.32	−9.64	< .001*
Systolic BP (mm Hg)	171.11 ± 13.09	122.66 ± 10.92	29.17	< .001*
Diastolic BP (mm Hg)	108.04 ± 10.46	76.81 ± 8.42	23.75	< .001*
Mode of delivery (CS, %)	76 (92.68)	141 (78.77)	7.77	< .01*
Fetal weight (g)	2329.82 ± 804.15	3379.60 ± 510.07	−9.98	< .001*
Apgar score – 1 min	9.57 ± 1.35	9.91 ± 0.52	−2.13	.04*
Apgar score – 5 min	9.84 ± 0.74	9.96 ± 0.33	−1.26	.21

Abbreviations: BMI, body mass index; y, years; CS, cesarean section; BP, blood pressure. The data are expressed as means ± SE. *: $P < 0.05$.

systolic and diastolic blood pressure in these two subgroups had significant difference compared to the control group ($p \leq .001$), meanwhile significant lower gestational age and fetal weight, and remarkable higher proteinura level were found in the early onset PE patients than that in the late onset PE patients ($p < .001$) (S. Table 1). No significant differences were found in the other factors.

3.2. Allelic and genotypic frequencies of SNPs

The genotypic distributions of SNPs rs3735481, rs9638978 and rs11984372 were conformed to HWE in the PE group, control group and subgroups ($p > .05$). In Table 2, frequency of rs3735481 allele C was significantly lower in the PE group than that in the control group (OR: 0.60, 95% CI: 0.36–0.98, $p = .04$), and frequency of rs9638978 allele A was significantly higher in the PE group than that in the control group (OR: 1.55, 95% CI: 1.05–2.29, $p = .03$), implying a protective role of rs3735481 allele C and a pathogenic role of rs9638978 allele A in the pathogenetic process of severe PE. There were no significant differences in allelic frequencies of rs11984372 ($p > .05$). In comparison of the three subgroups, frequency of rs9638978 allele A was found significantly higher in the early onset PE group (OR: 2.23, 95% CI: 1.35–3.71, $p = .002$), while no significant differences were found in allelic frequencies of the other two SNPs ($p > .05$, Table 3).

Furthermore, we evaluated genotype distribution of rs3735481 and rs9638978 in four genetic models (codominant, dominant, recessive, and overdominant) in order to find an association between genotype and disease. In comparison between early onset PE group and control group, there were significant differences in the codominant model (AA vs. GG), dominant model (AA + AG vs. GG), and recessive model (AA vs. GG + AG) of rs9638978 polymorphism (OR: 5.75, 95% CI: 2.01–16.48, $p < .001$; OR: 2.29, 95% CI: 1.09–4.82, $p = .03$; OR: 4.32, 95% CI: 1.67–11.17, $p = .001$; respectively) (Table 3). But no significant differences were found in any genetic models of rs3735481 polymorphism and the rest models of rs9638978 polymorphism ($p > .05$) (Tables 2 and 3). Because the frequency of CC genotype of rs11984372 was zero, we only made a codominant model of AA vs. AC, and no significant difference was found in the current study ($p > .05$).

3.3. Higher maternal serum levels of CypA in severe PE

To further determine the role of CypA in severe PE, we tested maternal serum CypA levels in severe PE patients and healthy pregnancies through ELISA assay. After a preliminary experiment, we chose 48 severe PE patients (22 early onset PE and 26 late onset PE) and 40 healthy pregnancies by age matching method from the samples for genotyping. The clinical data analysis of these two subgroups showed that factors of BMI, systolic and diastolic blood pressure, gestational age, fetal weight, and mode of delivery had significant differences ($p < .05$), and Apgar score at 1 min was significant lower in the PE group, while no significant differences were found in the other factors (S. Tables 2 and 3).

The results showed significantly higher CypA levels in severe PE patients especially in early onset PE patients compared to the healthy controls ($p < .001$), but no significant difference was found between the early onset PE subgroup and late onset PE subgroup ($p > .05$) (Fig. 1B). The serum CypA levels in different genotypes of rs3735481 and rs9638978 were further compared in our study (Fig. 1C). The serum CypA levels were significantly lower in carriers with the AC genotype (n = 37) than those with the AA genotype (n = 51) of rs3735481 ($p = .019$). For rs9638978, serum CypA levels in carriers with the AA genotype (n = 17) were significantly higher compared with carriers with the AG (n = 38) and GG (n = 33) genotypes ($p = .017$). There was no significant difference between the AG and GG genotypes of rs9638978 ($p > .05$) and between the AA and AC genotypes of rs11984372 ($p > .05$). These above results suggested that different genotypes of rs3735481 and rs9638978 may be associated with serum CypA concentrations.

3.4. Increased mRNA and protein expressions of CypA in placentas of severe PE

To further investigate the CypA expression in the placenta, tissues were collected from the same subjects for genotyping. The expression of CypA mRNA and protein in placenta were detected through qRT-PCR, western blot, and immunofluorescence, respectively. In the study, only a small amount of placenta tissues was collected and parts of the

Table 2
Comparison of the allele and genotype frequencies between PE group and control group.

SNPs	Location	Allele/Genotype	PE group (number, %)	Control group (number, %)	Odds ratio	95% CI	*P* value
rs3735481	44837322	Allele					
		A	140 (85.37)	278 (77.65)	1 (Reference)		
		C	24 (14.63)	80 (22.35)	0.60	0.36–0.98	.04*
		Codominant#					
		AA	59 (71.95)	107 (59.78)	1 (Reference)		
		AC	22 (26.83)	64 (35.75)	0.62	0.35–1.11	.11
		CC	1 (1.22)	8 (4.47)	0.23	0.03–1.86	.13
		Dominant					
		AA	59 (71.95)	107 (59.78)	1 (Reference)		
		AC + CC	23 (28.05)	72 (40.22)	0.58	0.33–1.02	.06
		Recessive					
		AA + AC	81 (98.78)	171 (95.53)	1 (Reference)		
		CC	1 (1.22)	8 (4.47)	0.26	0.03–2.15	.18
		Overdominant					
		AA + CC	60 (73.17)	115 (64.25)	1 (Reference)		
		AC	22 (26.83)	64 (35.75)	0.66	0.37–1.17	.15
rs9638978	44840337	Allele					
		G	103 (62.80)	259 (72.35)	1 (Reference)		
		A	61 (37.20)	99 (27.65)	1.55	1.05–2.29	.03*
		Codominant#					
		GG	32 (39.02)	92 (51.40)	1 (Reference)		
		AG	39 (47.56)	75 (41.90)	1.50	0.86–2.61	.16
		AA	11 (13.41)	12 (6.70)	2.64	1.06–6.56	.03
		Dominant					
		GG	32 (39.02)	92 (51.40)	1 (Reference)		
		AA + AG	50 (60.98)	87 (48.60)	1.65	0.97–2.81	.06
		Recessive					
		GG + AG	71 (86.59)	167 (93.30)	1 (Reference)		
		AA	11 (13.41)	12 (6.70)	2.16	0.91–5.12	.08
		Overdominant					
		AA + GG	43 (52.44)	104 (58.10)	1 (Reference)		
		AG	39 (47.56)	75 (41.90)	1.26	0.74–2.13	.39
rs11984372	44841646	Allele					
		A	158 (96.34)	348 (97.21)	1 (Reference)		
		C	6 (3.66)	10 (2.79)	1.32	0.47–3.70	.59
		Codominant					
		AA	76 (92.68)	169 (94.41)	1 (Reference)		
		AC	6 (7.32)	10 (5.59)	1.33	0.47–3.80	.59
		CC	0 (0.00)	0 (0.00)	/	/	

*: $P < 0.05$; # :The Bonferroni correction was account for multiple comparisons, *: $P < 0.025$.

samples were loosed during storage, thus the subgroups for placenta detection were relatively small. After a preliminary experiment, we chose 40 placenta tissues from 20 severe PE patients (11 early onset PE and 9 late onset PE) and 20 age matched healthy controls. The clinical data analysis showed that the BMI, systolic and diastolic blood pressure, gestational age, fetal weight, mode of delivery, and Apgar score at 1 min had significant differences between the two subgroups ($p < .05$), meanwhile significant lower gestational age and fetal weight were found in the early onset PE patients ($p < .01$), but no significant differences were found in the other factors ($p > .05$) (S. Tables 4 and 5).

The results showed dramatically increased CypA mRNA levels in placentas of the severe PE patients especially in placentas of the early onset PE patients compared to those of the healthy controls ($p < .01$) (Fig. 2A). CypA protein expressions in the placentas of severe PE patients were also markedly elevated according to western blot ($p < .01$) (Fig. 2B). The immunofluorescence staining showed that CypA (red color) was in the cytoplasm around the nucleus and the extracellular matrix, and DAPI (blue color) was located in the nucleus of villous trophoblasts (Fig. 2C). Consistent with the results of western blot, the expressions of CypA were also significantly higher in the placentas of severe PE patients. The upregulation of CypA in severe PE placentas indicated that CypA may be involved in the pathogenesis of PE.

3.5. Correlation analysis

Because statistically significant differences in BMI, systolic and diastolic blood pressure, gestational age, and fetal weight were observed between the two groups ($p < .001$, S. Table 2), correlation analyses were performed to test their influences on serum CypA levels (Table 4). The data analysis showed significant correlations between systolic blood pressure ($R = 0.37$, $p < .001$) and diastolic blood pressure ($R = 0.24$, $p = .02$) with serum CypA level, but no significant differences were found in the other factors ($p > .05$).

4. Discussion

Although both the etiological and clinical aspects of PE have been studied extensively, the incidence is still not improved [23]. PE remains a major cause of mortality and morbidity for both the mother and fetus [3]. Studies have shown that CypA played important roles in inflammatory conditions, oxidative stress, hypoxia, and endothelial dysfunction, which are also involved in the pathogenesis of PE; thus, it may also play a critical role in the etiology of PE [11].

Genetic susceptibility is an important risk factor for PE. Substantial evidence has shown high risks of some maternal genotypes for severe PE, including polymorphism of the coagulation factor V gene (F5), coagulation factor II gene (F2), leptin receptor gene (LEPR), and thrombophilic gene [5]. PPIA polymorphism rs6850 has been proven to be associated with an elevated risk of CAD [22], and the regulatory polymorphisms of PPIA accelerate the progression of human immunodeficiency virus (HIV)-1 pathogenesis [21]. In our study, we first demonstrated an association of CypA genetic polymorphisms with severe PE risk in Han Chinese women. The results showed significantly lower frequency of SNP rs3735481 allele C in the PE patients

Table 3
Comparison of the allele and genotype frequencies in early onset PE group, late onset PE group, and control group.

SNPs (location)	Allele/Genotype	Early onset PE group (n = 38, %)	Late onset PE group (n = 44, %)	Control group (n = 179, %)	Early onset PE group VS. Control group			Late onset PE group VS. Control group		
					Odds ratio	95% CI	*P* value	Odds ratio	95% CI	*P* value
rs3735481	Allele									
(44837322)	A	66 (86.84)	74 (84.09)	278 (77.65)	1 (Reference)			1 (Reference)		
	C	10 (13.16)	14 (15.91)	80 (22.35)	0.53	0.26–1.07	0.07	0.66	0.35–1.23	.18
	Codominant[#]									
	AA	28 (73.68)	31 (70.45)	107 (59.78)	1 (Reference)			1 (Reference)		
	AC	10 (26.32)	12 (27.27)	64 (35.75)	0.60	0.27–1.31	0.20	0.65	0.31–1.35	.24
	CC	0 (0.00)	1 (2.27)	8 (4.47)	/	/	0.15	0.43	0.05–3.58	.42
	Dominant									
	AA	28 (73.68)	31 (70.45)	107 (59.78)	1 (Reference)			1 (Reference)		
	AC + CC	10 (26.32)	13 (29.55)	72 (40.22)	0.53	0.24–1.16	0.11	0.62	0.31–1.27	.19
	Recessive									
	AA + AC	38 (100.00)	43 (97.73)	171 (95.53)	1 (Reference)			1 (Reference)		
	CC	0 (0.00)	1 (2.27)	8 (4.47)	/	/	0.18	0.50	0.06–4.08	.51
	Overdominant									
	AA + CC	28 (73.68)	32 (72.73)	115 (64.25)	1 (Reference)			1 (Reference)		
	AC	10 (26.32)	12 (27.27)	64 (35.75)	0.64	0.29–1.41	0.26	0.67	0.32–1.40	.29
rs9638978	Allele									
(44840337)	G	41 (53.95)	62 (70.45)	259 (72.35)	1 (Reference)			1 (Reference)		
	A	35 (46.05)	26 (29.55)	99 (27.65)	2.23	1.35–3.71	0.002*	1.10	0.66–1.83	.72
	Codominant[#]									
	GG	12 (31.58)	20 (45.45)	92 (51.40)	1 (Reference)			1 (Reference)		
	AG	17 (44.74)	22 (50.00)	75 (41.90)	1.74	0.78–3.87	0.17	1.35	0.68–2.66	.39
	AA	9 (23.68)	2 (4.55)	12 (6.70)	5.75	2.01–16.48	< 0.001*	0.77	0.16–3.70	.74
	Dominant									
	GG	12 (31.58)	20 (45.45)	92 (51.40)	1 (Reference)			1 (Reference)		
	AA + AG	26 (68.42)	24 (54.55)	87 (48.60)	2.29	1.09–4.82	0.03*	1.27	0.65–2.46	.48
	Recessive									
	GG + AG	29 (76.32)	42 (95.45)	167 (93.30)	1 (Reference)			1 (Reference)		
	AA	9 (23.68)	2 (4.55)	12 (6.70)	4.32	1.67–11.17	0.001*	0.66	0.14–3.07	.60
	Overdominant									
	AA + GG	21 (55.26)	22 (50.00)	104 (58.10)	1 (Reference)			1 (Reference)		
	AG	17 (44.74)	22 (50.00)	75 (41.90)	1.12	0.55–2.27	0.75	1.39	0.72–2.69	.33
rs11984372	Allele									
(44841646)	A	74 (97.37)	84 (95.45)	348 (97.21)	1 (Reference)			1 (Reference)		
	C	2 (2.63)	4 (4.55)	10 (2.79)	0.94	0.20–4.38	0.94	1.66	0.51–5.41	.40
	Codominant									
	AA	36 (94.74)	40 (90.91)	169 (94.41)	1 (Reference)			1 (Reference)		
	AC	2 (5.26)	4 (9.09)	10 (5.59)	0.94	0.20–4.47	0.94	1.69	0.50–5.67	.39
	CC	0 (0.00)	0 (0.00)	0 (0.00)	/	/		/	/	

*: $P < 0.05$; [#] :The Bonferroni correction was account for multiple comparisons, *: $P < 0.025$.

($P < .05$), and remarkably higher frequency of rs9638978 allele A in the PE patients especially early onset PE patients ($P < .01$). Allele C of rs3735481 may provide a protective effect (OR = 0.60), while allele A of rs9638978 may provide a pathogenic effect (OR = 1.55) on severe PE. Further analysis on polymorphisms of rs3735481 and rs9638978 in four genetic models showed significant differences in genotypic frequencies of rs9638978 codominant model (AA vs. GG), dominant model (AA + AG vs. GG), and recessive model (AA vs. GG + AG) between the early onset PE group and the control group ($p < .05$), but no significant differences were found in the other models of rs9638978 polymorphisms and rs3735481 polymorphisms ($p > .05$). Meanwhile, we found a significantly higher level in the AA genotype of rs9638978 in the early onset PE patients ($p < .001$), indicating that carriers with the AA genotype of rs9638978 may have a higher risk of early onset severe PE. There were also significantly lower serum CypA levels in the AC genotype of rs3735481 and significantly higher serum CypA levels in carriers with the AA genotype of rs9638978 ($p < .05$), indicating that the allele C of rs3735481 may exhibit a lower serum CypA level, while allele A of rs9638978 may cause higher serum CypA levels.

CypA, a highly conserved protein that is abundantly expressed in all mammalian cell types, played important roles in inflammatory stimuli and oxidative stress [12]. It acts as a pro-inflammatory cytokine with many cellular functions, such as promoting inflammation, proliferation, apoptosis, migration, and generation of reactive oxygen species (ROS), by activating different signal pathways [11,24]. By binding to its receptor, CD147, CypA could activate the phosphorylation of ERK, PI3K/Akt, and nuclear factor (NF)-kB, and promote platelet adhesion and inflammatory effects in monocytes and macrophages [10,16,25]. An enhanced serum CypA level has been proven to contribute to the pathogenesis of cardiovascular diseases via the EMMPRIN (CD147) signal pathway [10]. In vascular remodeling, a positive feedback mechanism was found between generation of ROS and CypA secreted by EC and VSMC [11]. This plays important roles in VSMC migration, proliferation, and accumulation of inflammatory cells in CypA knockout mice, manifesting with a decreased intimal and medial hyperplasia [15]. Enhanced CypA levels were also shown in inflammatory conditions, such as rheumatoid arthritis, diabetes mellitus, and chronic obstructive pulmonary disease (COPD) [13,18,19]. In our study, we found that the serum CypA levels were significantly higher in severe PE patients especially in early onset PE patients than that in the healthy pregnancies ($p < .001$). Consistent with our results, Wibowo et al. have also reported a higher maternal serum CypA levels in severe PE patients [20]. These data may imply that high serum CypA levels contribute to the pathogenesis of severe PE. Furthermore, for the first time, we detected significantly higher levels of CypA mRNA and protein in the placentas of severe PE patients compared to the healthy pregnancies ($p < .05$), indicating a pathological role of CypA in severe PE. The mechanism by which CypA mediated PE may include inflammatory reaction, increased apoptosis, endothelial cell injury, or oxidative stress.

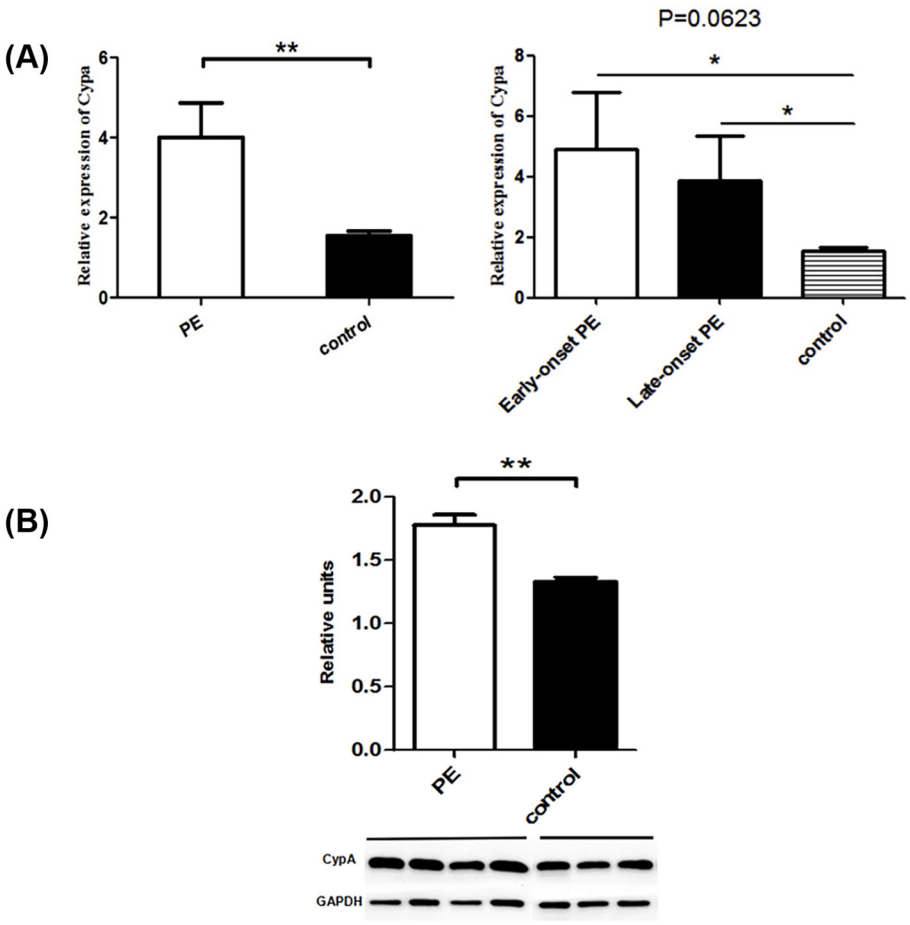

Fig. 2. (A). Comparison of CypA mRNA levels in placentas. The CypA mRNA levels were dramatically increased in placentas of severe PE patients especially in early-onset PE patients compared to the healthy controls ($p < .05$). (B). Comparison of CypA protein expressions in placentas. The CypA protein levels was significant higher in placentas of severe PE patients ($p < .01$). (C). CypA expressions in placentas by immunofluorescence staining (200×). Red color and blue color represented the staining of CypA and nucleus, respectively. Higher CypA expressions were found in placentas of severe PE patients. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 4
Correlation analysis between BMI, systolic BP, diastolic BP, gestational age, fetal weight and serum CypA level.

Factors	Correlation coefficient (r)	*P* value
BMI	0.18	.09
Systolic BP	0.37	< .001*
Diastolic BP	0.24	.02*
Gestational age	−0.16	.14
Fetal weight	−0.19	.07

* : $P < .05$.

Several critical risk factors for severe PE have been identified, including gestational hypertension history, chronic hypertension, diabetes, chronic kidney disease, and autoimmune disease [26]. However, the predictive values of these factors were limited in clinical settings. In CAD, CypA has been suggested as a predictor of its severity, with a prognostic value when combined with other traditional biomarkers [17,27]. In our present study, we found a significantly higher serum CypA levels in severe PE patients, making it a potential new predictor for the severity of PE. Meanwhile, systolic and diastolic blood pressure also had significant correlations with serum CypA level ($p < .05$), implying that the serum CypA level, in combination with systolic/diastolic blood pressure, may provide a predictive value for severe PE.

5. Conclusions

In summary, the role of CypA in severe PE was studied for the first time. The CypA genetic polymorphisms of rs3735481 and rs9638978 are preliminarily shown to be associated with severe PE in Han Chinese women. The AA genotype of rs9638978 may be associated with a higher risk of early-onset PE. The CypA levels were significantly elevated in maternal serum and placenta of severe PE patients especially in early onset PE patients, indicating that CypA may play an etiological role in severe PE and may be utilized as a novel biomarker for PE.

6. Disclosure

The authors declare no conflicts of interest.

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Authors' contributions

W.S., B.C. and F.H. designed the study; W.S., Y.X. and Q.X. collected the data and performed the experiments; Q.X. and Y.Z. performed the statistical analysis; W.S., Y.X. and Q.X. contributed to the writing of the manuscript; B.C. and F.H. were accountable for the final approval. All authors have seen and approved this final manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.preghy.2018.11.005.

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