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The association between maternal and foetal *REN* gene polymorphisms and preeclampsia/eclampsia: A hybrid design study

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ABSTRACT

Objectives: Preeclampsia (PE)/eclampsia (E) is an important cause of foetal and maternal morbidity and mortality, and its aetiology is poorly understood. Good evidence suggests that renin (*REN*) might be associated with PE/E. The risk of PE/E is determined by both maternal and foetal genes, but most previous studies have focused on maternal contributions. This study aimed to explore the association of maternal and foetal *REN* polymorphisms with PE/E in pregnant Han Chinese women.

Methods: A case-parents/mother-control study including 347 PE/E patients with their partners and offspring and 700 control mothers with their offspring was conducted. A log-linear model was used to investigate the association between maternal and foetal *REN* SNPs and PE/E simultaneously, as well as the interaction of *REN* SNPs and environmental factors on PE/E.

Results: The foetal *REN* rs5707 AC genotype in combination with a pre-pregnancy BMI ≥ 24 kg/m^2 was significantly associated with an increased risk of PE/E, with an OR of 2.75 (95%CI = 1.50–5.06). Maternal and foetal rs5707 were significantly associated with an increased risk of PE/E under the recessive model (AA + AC/CC). In haplotype analyses, foetal CCT (in the order of rs2368564, rs5707, rs5705) and TAT genotypes were positively associated with the risk of PE/E. There was no significant association between maternal and foetal *REN* SNP genotypes and PE/E in the transmission disequilibrium test (TDT) and log-linear model analysis.

Conclusions: Findings from this study indicate that foetal rs5707 polymorphisms may play a significant role in PE/E development, especially among overweight or obese pregnant women in China.

1. Introduction

Preeclampsia (PE), characterised by new-onset hypertension and organ dysfunction most commonly indicated by proteinuria, is a pregnancy-related complication that affects 3–5% of pregnancies [1]. In China, the prevalence of PE is approximately 2.9% [2]. Eclampsia (E), defined as one or more seizures or convulsions resulting from PE, is a serious complication of PE [3]. PE/E account for 15% of maternal mortality and 20–25% of foetal mortality worldwide [4]. Women with PE/E and who lack proper treatment have a higher risk of liver rupture, stroke, pulmonary edema, or kidney failure [5,6]. Furthermore, their babies have an increased risk of low birth weight, neonatal asphyxia, and perinatal foetal death [7,8].

Although numerous studies have investigated this topic, the exact aetiology of PE/E remains unclear. The currently accepted explanation is that insufficient uteroplacental perfusion caused by an abnormal invasion of spiral arterioles results in PE/E [9]. The renin-angiotensin system (RAS) is pivotal in regulating blood pressure and volume [10]. In the gravid state, in addition to the kidney-derived RAS, there is a tissue-based RAS in the uteroplacental unit, which may regulate regional maternal intervillous blood flow, contributing to pregnancy-associated vascular remodelling [11]. The RAS is dysregulated in PE/E women compared to that of healthy pregnant women [12]. Therefore, the RAS is considered one of the main causes of PE/E. The RAS regulates blood pressure through physiologically active angiotensin II, which is produced by a cascade of angiotensinogen (AGT) cleavage [12]. Angiotensin II is a powerful vasoconstrictor that also promotes aldosterone secretion and sodium reabsorption, thereby increasing blood pressure [13]. Renin catalyses the first step in the activation

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pathway of angiotensinogen and affects the production of Ang II [13]. Previous studies have shown that women with PE/E have a lower level of renin and Ang II, but a higher sensitivity to Ang II also exists, which enhances the vasoconstrictive effect of Ang II and leads to further elevation of blood pressure in PE [12,14]. Variation in plasma renin concentration is related to the *REN* gene polymorphism [15], but only a few studies have reported the association between *REN* single-nucleotide polymorphisms (SNPs) and PE/E. Elevated blood pressure is a typical symptom of PE/E, and women with PE/E have an increased risk of hypertension in the future [16,17]. It was previously reported that three SNPs (rs5705, rs5707, rs2368564) are associated with increased risk of hypertension [18–20]. Therefore, in the current study, we explored whether these three selected *REN* SNPs were also associated with PE/E.

All components of the RAS exist both in the maternal uterine decidua and foetal placental tissues [21]. The risk of PE/E is not only associated with maternal genetic contributions but also with the effects of foetal genes inherited from the father, as reported in previous studies [22,23]. Therefore, the effects of both maternal and foetal genes on PE/E should be explored simultaneously. Despite this, most current studies exploring the genetic mechanisms of PE/E have been based on a case-control study design that cannot distinguish between maternal and foetal gene effects. The case-parents/mother-control design, which combines the case-parents design with the case-control design, can explore maternal and foetal gene effects simultaneously and with higher statistical power [24]. Therefore, in the current study, we used the case-parents/mother-control study design to explore the association of PE/E with maternal and foetal *REN* SNPs (rs5705, rs2368564, rs5707), as well as the potential interaction between maternal and foetal *REN* SNPs and environmental factors on PE/E.

2. Materials and methods

2.1. Study settings and participants

A hybrid study design consisting of case-parent triads and control-mother dyads was applied in the current study. Research subjects were recruited from two Maternal and Child Care Hospitals in Anyang and Yichang city (in Henan and Hubei provinces, respectively, both of which are located in central China) from January 2008 to October 2015. The diagnostic criteria for PE were as follows: BP ≥ 140/90 mm Hg (measured twice at least 6 h apart) and proteinuria (24-h urinary protein ≥ 300 mg or urine dipstick protein ≥ +) after 20 weeks of gestation [25]. Eclampsia was defined as the occurrence of a seizure in the mother that was associated with PE, in the absence of any other causes for seizures [25]. Healthy, normotensive pregnant women who delivered in the same hospital were recruited into the control group. Participants were excluded for the following reasons: multiple pregnancies, other pregnancy complications, previous hypertension, diabetes mellitus, renal and cardiovascular disease, or a serious abnormality in their neonate. Overall, 347 PE/E patients with their partners and offspring, and 700 controls with their offspring, all of Han ethnicity, were recruited, as shown in Fig. S1.

2.2. Data collection

The demographic characteristics of participants were collected by a unified questionnaire, and face-to-face interviews with participants were conducted by professional medical staff. The questionnaire included the following components: maternal age, gestational age at delivery, blood pressure, educational background, parity, family history of hypertension, pre-pregnancy body mass index (BMI), foetal birth weight, partner smoking status during pregnancy, and stress during pregnancy. Of these, a pre-pregnancy BMI ≥ 24 kg/m^2 was defined as overweight or obese.

2.3. SNP selection and genotyping assays

All *REN* SNPs retrieved from the HapMap website (http://hapmap.ncbi.nlm.nih.gov/) were imported into Haploview software. The screening procedure for SNPs was as follows: First, we filtered these SNPs by Minor Allele Frequency (MAF) of Han Chinese in Beijing (CHB) > 20%, which resulted in 8 SNPs for inclusion. Second, we tested the linkage disequilibrium (LD) among these SNPs with an R^2 cutoff of 0.8 and found that they were classified into three blocks. Third, we selected a SNP of each block based on previously reported associations between the SNP and hypertension or cardiovascular diseases. In total, 3 SNPs (rs5705, rs5707 and rs2368564) were ultimately included in the current research.

In the case group, 5 ml venous blood of the PE/E patients and their partners, and 5 ml umbilical cord blood of the foetuses were drawn into EDTA-containing tubes; 5 ml of venous blood from the control pregnant women and 5 ml of umbilical cord blood from their babies were collected in the same way. DNA was extracted from blood leukocytes, using the Puregene® Blood Kit (QIAGEN, Germantown, MD, USA) and was stored at −80 °C. A NanoDrop™ 2000 spectrophotometer (Wilmington, DE, USA) was used to evaluate the quality and quantity of DNA. SNPs were genotyped using TaqMan™ SNP Genotyping Assays (Applied Biosystems [ABI], Foster City, CA, USA) and the 7900HT Fast Real-Time PCR System (ABI). All experiments were conducted based on corresponding guidelines and regulations. The genotyping success rates for rs5705, rs5707, rs2368564 were 84%, 88.3%, 82.1%, respectively.

2.4. Sample size

The sample size was calculated by the snp SampleSize function of the HAPLIN package in the R 3.1.1 software. The values of the parameters involved in the function were set as follows: the minor allele frequency (MAF) = 0.1, the relative risks (RR) = 1.5, the test power = 0.8, the significance level (α) = 0.05, and the ratio of the case and control = 0.5. This showed a need for 262 PE/E and 532 controls.

2.5. Statistical analysis

We used a log-linear modelling approach to estimate the simultaneous effects of maternal and foetal genotypes in disease based on a case-parents/mother-control hybrid design [24]. This modelling method expected counts of each possible genetic mating type combination under the assumption of Mendelian inheritance. The log-linear model included 30 mating type combinations (15 for the case, 15 for the control). There were two key assumptions for the case-parents/control-mother design: mating symmetry, the test for which was based on the control-mother dyads [24], and no population stratification. To estimate the effects of the mother and offspring genotypes in PE/E, the data of control pairs were integrated into a log-linear model by expectation-maximization. Then, the maximum likelihood method was used to iteratively fit the actual frequency with the theoretical frequency. The P-values were determined by likelihood ratio tests, the statistics of which were referred to as the chi-squared distribution. It was constructed as 2 times the difference of the saturated model minus the reduced model, with df equal to the difference in the number of parameters being fitted [24]. Gene-environment interaction analyses were based on the case-parent triads [26]. Log-linear Expectation Maximization (LEM) software was used to fit these models (examples of the LEM scripts are available at http://www.niehs.nih.gov/research/resources/software/biostatistics/lem/index.cfm).

The differences in the distributions of demographic characteristics, including genotype frequency between the cases and the controls, were evaluated by Student's *t*-tests or Pearson's $\chi 2$ test, where appropriate. The goodness-of-fit $\chi 2$ test was used for evaluating the Hardy-Weinberg equilibrium (HWE) for all genotypes in the control group. The low-frequency alleles of each SNP were considered as the mutant type, and the most frequently homozygous parental genotypes were regarded as the reference genotypes. Family-based association analyses were performed using the transmission disequilibrium test (TDT) [27]. The haplotypes of maternal and foetal *REN* genes were constructed by Phase

Table 1
Demographic characteristics of participants.

Variables	Cases $\bar{x}$ ± SD[a]/n (%)	Controls $\bar{x}$ ± SD/n (%)	*P*
Maternal age (years)	28.5 ± 5.7	27.5 ± 4.7	0.006*
Systolic BP (mm Hg)	148.7 ± 14.6	113.0 ± 10.9	< 0.001*
Diastolic BP (mm Hg)	100.0 ± 10.0	72.5 ± 7.8	< 0.001*
Maternal educational background			< 0.001*
Junior middle school and lower	197(58.5)	257(39.5)	
High school and above	140(41.5)	394(60.5)	
Family history of hypertension			0.007*
Yes	89(81.0)	121(73.4)	
No	246(19.0)	517(26.6)	
Pre-pregnancy BMI[b] (kg/m^2)			< 0.001*
< 24	211(67.0)	501(83.1)	
≥24	104(33.0)	102(16.9)	
Foetal birth weight (kg)	3.0 ± 0.7	3.3 ± 0.5	< 0.001*
Parity			0.495
Primipara	197(58.1)	393(60.6)	
Multipara	142(41.9)	256(39.4)	
Gestational age at delivery (week)	37.0 ± 4.2	38.6 ± 3.4	< 0.001*
Partner smoking during pregnancy			< 0.001*
Yes	177(52.7)	214(32.8)	
No	159(47.3)	439(67.2)	
Stress during Pregnancy			0.004*
Slight	223(70.3)	481(80.2)	
Medium	82(25.9)	104(17.3)	
High	12(3.8)	15(2.5)	

[a] SD, standard deviation.
[b] BMI, body mass index.

2.1 software [28]. Statistical results were adjusted for multiple testing using Bonferroni correction [29]. A 2-tailed *P*-value < 0.05 was considered statistically significant. Other statistical analyses were performed with SPSS (Version 21.0; SPSS, Chicago, IL, USA).

3. Results

3.1. The characteristics of the study subjects

The demographic characteristics of the 347 PE/E patients and 700 controls are presented in Table 1. Pregnant women with a pre-pregnancy BMI ≥ 24 kg/m^2 accounted for a larger proportion in the case groups compared with the control groups (33.0% vs. 16.9%, $P < 0.001$). The proportion of patients with high school education or above in the PE/E groups was lower than in the control groups (41.5% vs. 60.5%, $P < 0.001$). Compared with the control groups, a higher proportion of the partners of PE/E patients reported smoking during the pregnancy (52.7% vs. 32.8%, $P < 0.001$). Additionally, differences between the cases and controls in maternal age, gestational age at delivery, systolic and diastolic BP values, family history of hypertension, foetal birth weight and stress during pregnancy were statistically significant ($P < 0.05$). There was no difference in maternal parity between the two groups.

3.2. TDT analyses in case-parent triad families

TDTs were performed to test the linkage between *REN* SNPs and PE/E and to exclude spurious associations due to population stratification. Among all of the heterozygous parents, we identified the alleles of the three *REN* SNPs that were transmitted or untransmitted from the parents to the offspring. The results showed that there was no association between the three *REN* SNPs and PE/E (Table 2).

Table 2
Results of TDT analyses of the SNPs in case-parent triad families.

SNP	Transmitted allele	Non-transmitted allele		TDT	*P*
		A1	A2		
rs5705	A1	437	55		
	A2	41	11	2.04	0.153
rs5707	A1	246	130		
	A2	116	60	0.80	0.371
rs2368564	A1	313	92		
	A2	95	24	0.05	0.823

A1 and A2 represent the two alleles of each SNP.

3.3. Association analyses of maternal and foetal REN SNPs with PE/E

The distribution of the three selected *REN* SNP genotypes is shown in Table 3. All genotypes of the three SNPs in the control groups were consistent with HWE. Families in which one or more members failed to be genotyped and those inconsistent with Mendelian inheritance were excluded. A log-linear approach was employed to explore the association between maternal and foetal genes and PE/E simultaneously. In the current study, there was mating symmetry and no population stratification, as shown in supplementary Tables S1 and S2. For rs5707, compared with AA genotypes, a mother and foetus carrying CC genotypes showed a significant association with an increased risk of PE/E, with ORs of 1.64 (95%CI = 1.01–2.72) and 1.55 (95%CI = 1.02–2.44), respectively. However, this association disappeared after Bonferroni corrections (the corrected $\alpha = 0.05/3 = 0.017$). As shown in Table 4, maternal and foetal rs5707 polymorphisms in the recessive model (AA + AC/CC) were also found to increase the risk of PE/E, with ORs of 2.31 (95%CI = 1.41–3.79) and 2.27 (95%CI = 1.45–3.57). Furthermore, this association still existed after Bonferroni correction.

3.4. Maternal and foetal gene-environment interaction analyses

Gene-environment interactions based on the case-parent triad design were evaluated by a log-liner model in which the cases were divided into exposed and non-exposed groups. Therefore, only those dichotomous variables that were determined as the meaningful in Table 1 could be used to evaluate the gene-environment interaction under this model. These included educational background (below or above high school), pre-pregnancy BMI (< 24 kg/m^2 or ≥ 24 kg/m^2), and partner smoking status (yes or no). The results in Table 5 showed that pre-pregnancy BMI had a significant interaction with maternal rs5705 ($P_{LRT} = 0.013$) and foetal rs5707 ($P_{LRT} = 0.003$). The detailed interaction effects are shown in Table 6. A mother carrying the *REN* rs5705 TG genotype and a foetus carrying the *REN* rs5707 AC genotype in combination with a pre-pregnancy BMI ≥ 24 kg/m^2 was significantly associated with an increased risk of PE/E, with ORs of 2.23 (95%CI = 1.18–4.22) and 2.75 (95%CI = 1.50–5.06), respectively. However, the association between maternal *REN* rs5705 and a pre-pregnancy BMI ≥ 24 kg/m^2 disappeared after Bonferroni correction.

3.5. REN haplotypes and the risk of PE/E

REN haplotypes with a frequency exceeding 5% were included in our study. Thus, four common haplotypes containing the three selected SNPs were included and are shown in Table 7. Treating the CAT haplotype (in the order of rs2368564, rs5707, rs5705) as a reference, the haplotypes CCT and TAT in foetuses were significantly associated with an increased risk of PE/E, with ORs of 1.42 (95%CI = 1.15–1.75) and 1.45 (95%CI = 1.10–1.41).

Table 3
Genotype frequencies of rs5705, rs5707 and rs2368564 in REN and their association with PE/E risk.

SNPs	Genotype	Maternal				Foetal			
		Case/n (%)	Control/n (%)	OR[a] (95% CI[b])	*P*	Case/n (%)	Control/n (%)	OR(95% CI)	*P*
rs5705	TT	226(83.1)	485(79.8)	Reference	0.220	225(82.7)	490(80.6)	Reference	0.793
	TG	41(15.1)	119(19.6)	0.77(0.52–1.15)		42(15.5)	111(18.3)	1.13(0.76–1.67)	
	GG	5(1.8)	4(0.6)	1.79(0.56–5.71)		5(1.8)	7(1.1)	1.10(0.38–3.18)	
rs5707	AA	136(49.3)	342(52.8)	Reference	0.025	140(50.7)	333(51.4)	Reference	0.035
	AC	107(38.8)	270(41.7)	0.84(0.62–1.15)		96(34.8)	270(41.7)	0.89(0.66–1.20)	
	CC	33(11.9)	36(5.6)	1.64(1.01–2.72)		40(14.5)	45(6.9)	1.55(1.02–2.44)	
rs2368564	CC	157(60.0)	363(60.7)	Reference	0.511	156(59.5)	365(61.0)	Reference	0.853
	CT	96(36.6)	206(34.4)	1.04(0.77–1.41)		93(35.5)	208(34.8)	1.09(0.81–1.46)	
	TT	9(3.4)	29(4.9)	0.67(0.32–1.42)		13(5.0)	25(4.2)	1.05(0.55–2.00)	

[a] OR, odds ratio.
[b] CI, confidence interval.

4. Discussion

In this current study, we conducted a case-parents/ mother-control study design to explore the association of potentially functional polymorphisms in *REN* with the risk of PE/E. A log-linear model was used to investigate the effects of maternal and foetal genotypes on PE/E simultaneously. The results showed that maternal and foetal rs5707 had a significant association with the risk of PE/E under the recessive model. Meanwhile, a significant interaction was observed between foetal rs5707 and pre-pregnancy BMI in the development of PE/E. The haplotypes CCT and TAT in foetuses were also associated with PE/E risk.

PE is characterized by new-onset hypertension and organ dysfunction [1]. Previous studies reported that the insufficient uteroplacental perfusion caused by an abnormal invasion of spiral arterioles might result in PE [9,11]. Renin catalyses the first step in the activation pathway of angiotensinogen—a cascade that can result in aldosterone release, vasoconstriction, and increase in blood pressure [12]. In addition, renin secreted from the foetal-placental tissues and maternal uterine decidua tissue may participate in vascular remodelling in pregnant women, consequently leading to the development of PE/E [11]. Therefore, we assumed the three selected SNPs of *REN* that were reported in association with the risk of hypertension may also contribute to PE/E development due to the similar mechanisms.

In the recessive model of the present study, maternal and foetal rs5707 were observed to increase the risk of PE/E. To date, no researchers have explored the association of rs5707 with PE/E, but some have investigated its association with hypertension. A report published in 2008 showed that genotype GG of the rs5707 polymorphism was significantly associated with higher blood pressure levels and an increased risk of hypertension in Spanish women aged 40–70 [20]. This study supported our finding to some extent, in that mothers carrying CC genotypes, compared with AA/AC genotypes, have a higher risk of PE/E. The mechanism underlying the association of rs5707 with PE/E is yet to be determined. Nevertheless, considering the function of *REN* in

Table 5
Gene-environment interactions between maternal and foetal *REN* SNPs and environmental factors.

SNP	Environmental factor	Maternal		Foetal	
		χ^2	P_{LRT}[a]	χ^2	P_{LRT}
rs5705	Education background	0.02	0.992	5.24	0.073
	Pre-pregnancy BMI	8.73	0.013*	3.03	0.985
	Partner smoking	0.33	0.848	1.36	0.507
rs5707	Education background	0.24	0.887	0.11	0.946
	Pre-pregnancy BMI	2.70	0.259	1.78	0.003*
	Partner smoking	5.66	0.059	0.52	0.772
rs2368564	Education background	1.11	0.574	2.95	0.229
	Pre-pregnancy BMI	1.39	0.500	3.35	0.648
	Partner smoking	0.32	0.850	0.88	0.643

[a] P_{LRT}, *p*-value constructed by LRT (likelihood ratio test).

Table 6
The results of the interaction between maternal rs5705 and foetal rs5707 and pre-pregnancy BMI.

SNPs	BMI	Genotype	OR (95% CI)	*P*
Maternal *REN* rs5705	Non-exposed ($< 24\ kg/m^2$)	TT	Reference	0.435
		TG	1.34(0.40–1.28)	
		GG	2.81(0.19–11.08)	
	Exposed ($\geq 24\ kg/m^2$)	TT	Reference	0.046
		TG	2.23(1.18–4.22)	
		GG	–	
Foetal *REN* rs5707	Non-exposed ($< 24\ kg/m^2$)	AA	Reference	0.521
		AC	0.94(0.64–1.38)	
		CC	1.26(0.72–2.22)	
	Exposed ($\geq 24\ kg/m^2$)	AA	Reference	0.004*
		AC	2.75(1.50–5.06)	
		CC	1.48(0.63–3.51)	

"–" indicates that the result could not be calculated because of limited counts.

Table 4
Association of *REN* SNPs with PE/E under dominant and recessive genetic models.

SNPs	Model	Maternal		Foetal	
		OR (95% CI)	*P*	OR (95% CI)	*P*
rs5705	Dominant[a]	0.80(0.55–1.17)	0.249	0.87(0.60–1.26)	0.455
	Recessive[b]	2.82(0.75–10.60)	0.124	1.61(0.51–5.11)	0.421
rs5707	Dominant	1.15(0.87–1.53)	0.330	1.03(0.87–1.53)	0.853
	Recessive	2.31(1.41–3.79)	$9.277 \times 10^{-4*}$	2.27(1.45–3.57)	$3.720 \times 10^{-4*}$
rs2368564	Dominant	1.03(0.77–1.39)	0.830	1.06(0.79–1.43)	0.680
	Recessive	0.70(0.33–1.50)	0.356	1.20(0.60–2.38)	0.608

[a] Dominant, heterozygous + homozygous mutant type vs. homozygous wild type.
[b] Recessive, homozygous mutant type vs. homozygous wild type and heterozygous.

Table 7
Distribution of *REN* haplotype frequencies among cases and controls and their association with PE/E.

Haplotype[a]	Maternal				Foetal			
	Case/n (%)	Control/n (%)	OR[b] (95% CI)	*P*	Case/n (%)	Control/n (%)	OR (95% CI)	*P*
CAT	292(42.1)	605(43.2)	Reference		263(37.9)	631(45.1)	Reference	
CCT	236(34.0)	429(30.6)	1.14(0.92–1.41)	0.225	248(35.8)	420(30.0)	1.42(1.15–1.75)	0.001*
TAT	97(13.9)	199(14.2)	1.01(0.76–1.34)	0.945	112(16.1)	186(13.3)	1.45(1.10–1.90)	0.009*
TAG	44(6.4)	104(7.4)	0.88(0.60–1.28)	0.496	40(5.8)	101(7.2)	0.95(0.61–1.41)	0.801

[a] The haplotypes in the order of rs2368564, rs5707, rs5705 were analysed by PHASE 2.1 software and haplotypes with frequencies < 5% are not shown in the table.
[b] ORs and 95% CIs were calculated by unconditional logistic regression.

blood pressure regulation, we proposed a hypothesis that the polymorphic rs5707 CC genotype might be associated with high plasma renin activity [19]. Contradicting these results, two other case-control studies conducted in Japan [30] and northern China [31] found no association between rs5707 and hypertension. In addition to the differences in study design and sample size, these inconsistent results may be due to these studies focusing on all hypertensive patients, while our study and the Spanish study only concerned female patients. In addition, as to PE/E, the case-parent/mother-control design in our study is more powerful than the case-control design used in the above-mentioned reports [24].

Additionally, in the current study, it was observed that foetuses carrying rs5707 CC genotypes, compared with AA/AC, had increased risk of PE/E. Few studies have reported the association between foetal *REN* polymorphism and PE/E. A meta-analysis study of three European genome-wide association studies from the European Genome-phenome Archive [32] showed that foetal rs5707 and rs2368564 were not associated with PE, but foetal rs5705 had a significant association with PE. These inconsistent results may be due to different populations and different tests for the association analysis. All subjects of this meta-analysis study were from Europe. The association analyses in the three GWAS were tested by logistic regression that was implemented in SNPTEST. Our finding of the association between foetal rs5707 and PE/E provides further evidence for the paternal genetic contribution to PE/E, as previous studies reported that foetal genes partly inherited from the father can contribute to PE/E pathogenesis [22,23]. No significant association was observed between rs5705 and rs2368564 and PE/E in our study, but research conducted in Mexico [19] found that the *REN* rs5705 polymorphism increased the risk of developing hypertension. Therefore, well-designed research is still needed to confirm the association between rs5705 and PE/E.

A gene-environment interaction between foetal rs5707 and pre-pregnancy BMI was found in our study. The risk of the mother being overweight or obese on PE/E has been reported in previous studies [33,34]. When the patients were stratified by maternal BMI in the current study, the rs5707 AC genotype in babies of overweight or obese women was associated with an increased risk of PE/E compared with the AA genotype. An interaction between RAS-associated genes and pre-pregnancy BMI on the risk of preeclampsia was found in previous studies [35,36], but only a few studies reported the interaction of pre-pregnancy BMI with the foetal genotype. Overweight or obese women have increased or abnormally distributed adipose tissue, which is an important source for components of the RAS, especially angiotensin II [37]. Adipose tissue-derived angiotensin II might play a role in elevated blood pressure. However, the mechanism underlying the interaction between foetal rs5707 and pre-pregnancy BMI is not clear, and more studies are needed. Additionally, the association between the foetal rs5707 AC genotype and PE/E was not observed in all mothers, only in overweight or obese mothers, suggesting that lower maternal BMI may reduce the risk of foetal rs5707 on PE/E.

In our haplotype analysis, compared with the CAT haplotype, the foetal CCT and TAT haplotypes showed a highly increased risk of PE/E. A study from Mexico [19] including two *REN* gene polymorphisms (rs5705 and rs5707) reported an association between the TC haplotype and an increased risk of hypertension, a finding that was consistent with our results. Foetal rs5707 CC and rs2368564 TT genotypes were not independently associated with PE/E. However, compared with the reference haplotype CAT, the CCT and TAT haplotypes, which only differ in the polymorphisms rs5707 and rs2368564, respectively, were shown to significantly associate with PE/E. These results indicated that one or several *REN* polymorphisms or minor alleles of the haplotypes can modulate the activity.

There were some strengths in our study. First, we used a hybrid case-parent/mother-control study that avoids bias caused by population stratification. The test power of this study design is higher than that of traditional case-parents design and case-control design [24]. Second, as far as we are aware, this is the first study to explore the effects of maternal and foetal *REN* polymorphisms on PE/E simultaneously. However, some limitations also existed in our study. The main shortcoming of our study is that it did not include a validation experiment to clarify the mechanism underlying the ability of *REN* gene polymorphisms to confer susceptibility to PE/E. In addition, the subjects of our study were mainly from central China and were all of Han ethnicity, which limits the extrapolation of the study.

5. Conclusion

In summary, this study used a novel design to disentangle maternal and foetal genotypic effects of *REN* genes on PE/E. Our findings suggest that the foetal rs5707 SNP might be closely associated with PE/E development, especially in overweight or obese women. It is the first study to report the association of foetal rs5707 with PE/E, promoting the understanding of the role of *REN* genetic variation in the pathogenesis of PE/E. However, more well-designed research studies on the mechanism underlying the association between foetal rs5707 and PE/E are needed, which might contribute to the prevention, diagnosis and treatment of PE/E.

Ethics approval

The study was approved by the Institutional Review Board of Tongji Medical College, and written informed consent was obtained from all subjects before participating in the study.

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Declaration of Competing Interest

The authors declare that they have no conflict of interest.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.preghy.2019.09.011.

References

[1] B.W.J. Mol, C.T. Roberts, S. Thangaratinam, L.A. Magee, C.J.M. de Groot, G.J. Hofmeyr, Pre-eclampsia, The Lancet. 387 (2016) 999–1011.
[2] C. Ye, Y. Ruan, L. Zou, G. Li, C. Li, Y. Chen, et al., The 2011 survey on hypertensive disorders of pregnancy (HDP) in China: prevalence, risk factors, complications, pregnancy and perinatal outcomes, PLoS One 9 (2014) e100180.
[3] B.M. Sibai, Diagnosis, prevention, and management of eclampsia, Obstet. Gynecol. 105 (2005) 402.
[4] L. Duley, The global impact of preeclampsia and eclampsia, Semin Perinatol. 33 (2009) 130–137.
[5] J.P. Souza, A.M. Gulmezoglu, J. Vogel, G. Carroli, P. Lumbiganon, Z. Qureshi, et al., Moving beyond essential interventions for reduction of maternal mortality (the WHO Multicountry Survey on Maternal and Newborn Health): a cross-sectional study, Lancet 381 (2013) 1747–1755.
[6] H.D. O'Connor, M.P. Hehir, E.M. Kent, M.E. Foley, C. Fitzpatrick, M.P. Geary, et al., Eclampsia: trends in incidence and outcomes over 30 years, Am. J. Perinatol. 30 (2013) 661–664.
[7] T. Chaiworapongsa, P. Chaemsaithong, L. Yeo, R. Romero, Pre-eclampsia Part 1: current understanding of its pathophysiology, Nat. Rev. Nephrol. 10 (2014) 466–480.
[8] S. Swain, K.N. Ojha, A. Prakash, B.D. Bhatia, Maternal andperinatal mortality due to eclampsia, Indian Pediatr. 30 (1993) 771–773.
[9] F. Herse, R. Dechend, N.K. Harsem, G. Wallukat, J. Janke, F. Qadri, et al., Dysregulation of the circulating and tissue-based renin-angiotensin system in pre-eclampsia, Hypertension 49 (2007) 604.
[10] J. Yang, J. Shang, S. Zhang, H. Li, H. Liu, The role of the renin-angiotensin-aldosterone system in preeclampsia: genetic polymorphisms and microRNA, J. Mol. Endocrinol. 50 (2013) R53–R66.
[11] P.S. Leung, S.J. Tsai, G. Wallukat, T.N. Leung, T.K. Lau, The upregulation of angiotensin II receptor AT(1) in human preeclamptic placenta, Mol. Cell Endocrinol. 184 (2001) 95–102.
[12] D.M. Shah, Role of the renin-angiotensin system in the pathogenesis of pre-eclampsia, Am. J. Physiol. Renal. Physiol. 288 (2005) F614–F625.
[13] T.H. Le, T.M. Coffman, Targeting genes in the renin-angiotensin system, Curr. Opin. Nephrol. Hypertens. 17 (2008) 57–63.
[14] P. August, T. Lenz, K.L. Ales, M.L. Druzin, T.G. Edersheim, J.M. Hutson, et al., Longitudinal study of the renin-angiotensin-aldosterone system in hypertensive pregnant women: deviations related to the development of superimposed pre-eclampsia, Am. J. Obstet. Gynecol. 163 (1990) 1612–1621.
[15] T.A. Kotchen, J.M. Kotchen, C.E. Grim, V. George, M.L. Kaldunski, A.W. Cowley, et al., Genetic determinants of hypertension: identification of candidate phenotypes, Hypertension 36 (2000) 7–13.
[16] V.D. Garovic, S.R. Hayman, Hypertension in pregnancy: an emerging risk factor for cardiovascular disease, Nat. Clin. Pract. Nephrol. 3 (2007) 613–622.
[17] G.C. Smith, J.P. Pell, D. Walsh, Pregnancy complications and maternal risk of ischaemic heart disease: a retrospective cohort study of 129,290 births, Lancet 357 (2001) 2002–2006.
[18] D.N. Parchwani, D.D. Patel, J. Rawtani, N. Dikshit, Association of Mbo I-RFLP at the Renin Locus (rs2368564) with Essential Hypertension, Indian J. Clinical Biochem. : IJCB. 31 (2016) 431–438.
[19] J.M. Fragoso, E. Alvarez-Leon, H. Delgadillo-Rodriguez, M. Arellano-Gonzalez, F.C. Lopez-Pacheco, D. Cruz-Robles, et al., The C4280A (rs5705) gene polymorphism of the renin (REN) gene is associated with risk of developing coronary artery disease, but not with restenosis after coronary stenting, Exp. Mol. Pathol. 99 (2015) 128–132.
[20] M.L. Mansego, J. Redon, R. Marin, V. Gonzalez-Albert, J.C. Martin-Escudero, M.J. Fabia, et al., Renin polymorphisms and haplotypes are associated with blood pressure levels and hypertension risk in postmenopausal women, J. Hypertens. 26 (2008) 230–237.
[21] D.M. Shah, The role of RAS in the pathogenesis of preeclampsia, Curr. Hypertens. Rep. 8 (2006) 144–152.
[22] S. Cnattingius, M. Reilly, Y. Pawitan, P. Lichtenstein, Maternal and fetal genetic factors account for most of familial aggregation of preeclampsia: a population-based Swedish cohort study, Am. J. Med. Genet. 130a (2004) 365–371.
[23] L. Morgan, S. Crawshaw, P.N. Baker, J.F. Brookfield, F. Broughton Pipkin, N. Kalsheker, Distortion of maternal-fetal angiotensin II type 1 receptor allele transmission in pre-eclampsia, J. Med. Genet. 35 (1998) 632–636.
[24] S.H. Vermeulen, M. Shi, C.R. Weinberg, D.M. Umbach, A hybrid design: case-parent triads supplemented by control-mother dyads, Genet. Epidemiol. 33 (2009) 136–144.
[25] ACOG practice bulletin, Diagnosis and management of preeclampsia and eclampsia. Number 33, January 2002, Obstet. Gynecol. 99 (2002) 159–167.
[26] D.C. Thomas, Case-parents design for gene-environment interaction by Schaid, Genet. Epidemiol. 19 (2000) 461–463.
[27] D.J. Schaid, Likelihoods and TDT for the case-parents design, Genet. Epidemiol. 16 (1999) 250–260.
[28] M. Stephens, N.J. Smith, P. Donnelly, A new statistical method for haplotype reconstruction from population data, Am. J. Hum. Genet. 68 (2001) 978–989.
[29] Y. Benjamini, D. Drai, G. Elmer, N. Kafkafi, I. Golani, Controlling the false discovery rate in behavior genetics research, Behav. Brain Res. 125 (2001) 279–284.
[30] B. Hasimu, T. Nakayama, Y. Mizutani, Y. Izumi, S. Asai, M. Soma, et al., Haplotype analysis of the human renin gene and essential hypertension, Hypertension 41 (2003) 308–312.
[31] L. Wang, B. Zhang, M. Li, C. Li, J. Liu, Y. Liu, et al., Association between single-nucleotide polymorphisms in six hypertensive candidate genes and hypertension among northern Han Chinese individuals, Hypertens. Res. : Off. J. Japanese Soc. Hypertens. 37 (2014) 1068–1074.
[32] European Genome-phenome Archive. https://ega-archive.org/studies/EGAS00001001048. Accessed 18-Nov 2018.
[33] E. Pare, S. Parry, T.F. McElrath, D. Pucci, A. Newton, K.H. Lim, Clinical risk factors for preeclampsia in the 21st century, Obstet. Gynecol. 124 (2014) 763–770.
[34] M. Taebi, Z. Sadat, F. Saberi, M.A. Kalahroudi, Early pregnancy waist-to-hip ratio and risk of preeclampsia: a prospective cohort study, Hypertens. Res. : Off. J. Japanese Soc. Hypertens. 38 (2015) 80–83.
[35] G. Kobashi, A. Hata, K. Shido, K. Ohta, H. Yamada, S. Fujimoto, et al., The M235T variant of the angiotensinogen gene and the body mass index are useful markers for prevention of hypertension in pregnancy: a tree-based analysis of gene-environment interaction, Semin. Thromb. Hemost. 28 (2002) 501–506.
[36] A. Zhou, G.A. Dekker, E.R. Lumbers, S.Y. Lee, S.D. Thompson, L.M. McCowan, et al., The association of AGTR2 polymorphisms with preeclampsia and uterine artery bilateral notching is modulated by maternal BMI, Placenta 34 (2013) 75–81.
[37] C. Karlsson, K. Lindell, M. Ottosson, L. Sjostrom, B. Carlsson, L.M. Carlsson, Human adipose tissue expresses angiotensinogen and enzymes required for its conversion to angiotensin II, J. Clin. Endocrinol. Metabol. 83 (1998) 3925–3929.