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Potential correlation between EDN1 gene polymorphisms with preeclampsia

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Abstract. – OBJECTIVE: To explore the potential correlation between endothelin 1 (EDN1) gene polymorphisms with preeclampsia (PE).

PATIENTS AND METHODS: The single nucleotide polymorphisms (SNPs) of 248 PE patients and 232 healthy controls were genotyped by Polymerase Chain Reaction (PCR). The possible association between EDN1 polymorphisms and PE was revealed through the *t*-test and the Chi-square test.

RESULTS: PE risk was significantly correlated with the C allele of polymorphism rs5370 in EDN1. The polymorphism rs5370 in EDN1 was remarkably associated with the onset of severe PE, rather than mild PE. The markedly increased risk of early-onset PE was related to the C allele of polymorphism rs5370 in EDN1, while no significant difference in the allele frequency of polymorphism rs1800541 was detected between the PE group and the control group. In the co-dominant model, the CC genotype of polymorphism rs5370 in EDN1 was associated with the increased PE risk. PE risk in the population carrying TC genotype was 1.59 times higher than those with TT/CC genotype, while polymorphism rs1800541 had no apparent association with PE risk. In the severe PE group, there was an evident difference in the genotype frequency between the dominant and over-dominant models of polymorphism rs5370. In the recessive model, the raised risk of early-onset PE was notably correlated with the TT/CC genotype compared with that of TT genotype. However, no evident association with the genotype frequency of polymorphism rs1800541 was observed between PE patients and controls.

CONCLUSIONS: EDN1 gene polymorphism rs5370 is correlated with the increased risk of PE.

Key Words:
Endothelin 1 gene, Polymorphism, Preeclampsia.

Introduction

Preeclampsia (PE), a multisystemic pregnancy disease, is the leading cause of perinatal morbidity and mortality in the world. In addition, PE pregnancies and their offsprings suffer higher risks of cardiovascular events, as well as metabolic and mental disorders[1]. The etiology and pathogenic mechanism of PE have not been completely elaborated yet. Currently, effective preventive and therapeutic methods for PE are lacked. Genetic susceptibility is considered as an important cause. Various genes have been reported to be related to PE risk[2,3], in which multiple genes or single nucleotide polymorphisms (SNPs) may contribute to the incidence and development of PE[4]. Therefore, genetics studies are necessary to sufficiently elaborate on the role of the genetic factors in the pathogenesis of PE. Endothelins (EDNs) are a peptide family that induces the deoxyribonucleic acid (DNA) replication and cell growth in diverse tissues, which mainly regulate vascular tone, angiogenesis, and mitosis[5,6]. Moreover, EDNs are involved in multiple aspects of tumor progression, such as cell apoptosis, epithelial-mesenchymal transition, stromal reaction, invasion, metastasis, and drug resistance[7]. The EDN family consists of three peptides, including EDN1, EDN2, and EDN3, among which, EDN1 is the most important mitogen and immunomodulator. EDN1 can exert mitogenic effects by binding to its receptor type A (EDNRA)[8]. The EDN1/EDNRA axis plays a key role in the incidence of a variety of cancers. Several SNPs of EDN1 and EDNRA genes can affect the expressions or functions of those peptides, but the role of the EDN1 gene polymorphisms in the progression of PE has not been investigated. Hence, the present study aims to explore the correlations of EDN1 gene polymorphisms (G5665T and T-1370G) with PE in Chinese Han pregnancies.

Patients and Methods

Patients

This case-control study was approved by the Ethics Committee of our hospital. All the par-

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ticipants provided the written informed consent. A total of 480 pregnant women were recruited from our hospital from January 2015 to December 2018. Inclusion criteria: subjects who were diagnosed with systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg, and proteinuria (>200 mg/24 h and/or urinary protein 1+) at least twice at 20 weeks after pregnancy. Severe PE was defined as systolic blood pressure ≥ 160 and/or diastolic blood pressure ≥ 110 mmHg, accompanied with evident proteinuria (>2 g/24 h and/or 2+ on test paper) or multiple organ dysfunctions, such as pulmonary edema, epilepsy attack, oliguria, thrombocytopenia, liver dysfunction, and central nervous system dysfunction. PE includes early-onset type (<34 weeks) and late-onset type (≥34 weeks). In this trial, a total of 248 pregnancies aged 19-45 years old [Mean ± standard deviation (SD): 30.26±5.79] were divided into two groups, including 197 severe cases and 51 mild cases. Moreover, 232 normal pregnancies aged 18-42 years old (Mean ± SD: 30.42±4.89) complicated with gestational hypertension and proteinuria during the same time period were recruited. Exclusion criteria: subjects with diabetes mellitus, heart disease, chronic hypertension, nephropathy, autoimmune disease, thrombophilia, multiple pregnancy, or fetal malformation.

DNA Extraction and Genotyping

DNA was isolated from the whole blood using DNA isolation and genotyping kit (Flexigene Kit, Qiagen, Hildren, Germany). The genomic DNA was extracted from 2 mL of ethylenediaminetetraacetic acid (EDTA)-anticoagulated peripheral blood using a DNA extraction kit (Bioteke, Beijing, China) according to the instructions. In short, a Polymerase Chain Reaction (PCR) system was prepared (25 μL), including 2.5 μL of 10× PCR buffer solution, 1.5 mmol/L $MgCl_2$, 0.15 mmol/L dNTP, 0.5 mmol/L primers, 100 ng genomic DNA, and 1 U Taq DNA polymerase. The primer sequences were shown in Table I. The PCR conditions were as follows: initial denaturation at 94°C for 4 min, followed by 33 cycles for denaturation at 94°C for 30 s, annealing of rs1126579 at 57°C for 30 s (annealing of rs2230054 at 60°C), and finally extension at 72°C for 10 min. The products of PCR were digested with specific restriction enzymes (Accl of rs1126579 and Pstl of rs2230054) in a reaction volume of 10 μL at 37°C overnight. The relative expression level of the target gene was expressed by the $2^{-\Delta\Delta Ct}$ method.

Restriction Fragment Length Polymorphism Analysis

The digestion products were separated using 6% polyacrylamide gel and stained with 1.5 g/L silver nitrate. For G5665T, the T allele could be cleaved into two fragments (18 bp and 233 bp), while C allele could not, with a 251 bp fragment. For T-1370G, C allele could be cleaved into two fragments (18 bp and 229 bp), while T allele could not, with a 247 bp fragment. For the purpose of quality control, about 10% of samples were randomly selected for repeat measurement, and the results were 100% consistent.

Statistical Analysis

The Statistical Product and Service Solutions (SPSS) 22.0 software (IBM Corp., Armonk, NY, USA) was used for statistical analysis. The allele distribution, genotype frequencies, and Pearson Chi-square test were applied to examine the Hardy-Weinberg equilibrium.

In the case-control mode of genotypic association test, the online software SNPStats was adopted to establish the codominant, dominant, recessive, over-dominant, or logarithmically convex genetic model. The Chi-square test was performed to assess the differences in the allele and genotype frequencies, and $p<0.05$ suggested that the differences were statistically significant.

Table I. Primers of gene polymorphisms for PCR amplification.

Polymorphism	Primer sequences	Annealing temperature	Restriction enzyme
EDN1 G5665T	F: 5'-CAAGATTCTAGCTATACATGGCTTG-3' R: 5'-AAGAACGTGGCCTCGTCT-3'	55	SphI
EDN1 T-1370G	F: 5'-TACTCATCCAATGTTAGCCC-3' R: 5'-CCAGGTTGTAGGGCAGCCTGC-3'	54	XmnI

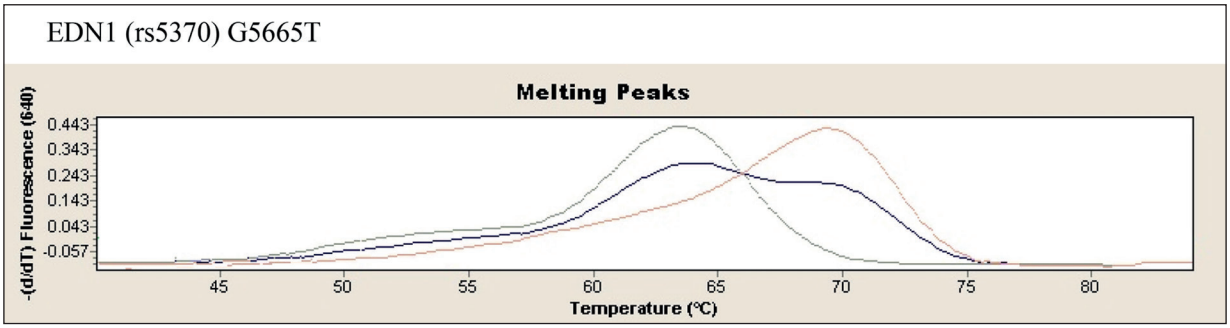

Figure 1. Melting curve analysis for EDN1 (rs5370) G5665T detection. According to the figure, the melting curves are converted into melting peaks by plotting the relationship between the fluorescent negative derivative and temperature, in which the first peak corresponds to the G allele, and the second peak corresponds to the T allele.

Results

PCR Analysis

The High Pure PCR Template Preparation Kit (Roche Diagnostics, Basel, Switzerland) was utilized to isolate the DNA in the leukocytes. Light SNiP assay (Tib MolBiol, Berlin, Germany) was adopted for SNP genotyping of EDN1 G5665T, EDN1 T-1370G, EDNRA C+70G, and EDNRA G-231A. The Light SNiP assay was based on melting curves, including premixed primers and probes. The melting curve analysis for EDN1 (rs5370) G5665T detection showed that the first peak corresponded to the G allele, and the second peak corresponded to the T allele (Figure 1). In the melting curve analysis for EDN1 (rs1800541) T-1370G detection, the first peak corresponded to the T allele, and the second peak corresponded to the G allele (Figure 2).

Associations of Allele Frequencies of Two SNPs with PE Risk

The genotyping of two complete SNPs was successfully conducted among 248 PE patients and 232 controls in the present study. The genotype distribution of the two polymorphisms was in line with the Hardy-Weinberg equilibrium in the control group (rs5370 p=0.66, rs1800541 p=0.054). As shown in Table II, the remarkably increased risk of PE was correlated with the C allele of polymorphism rs5370 in EDN1 [p=0.02, odds ratio (OR)=1.58, 95% confidence interval (CI)=1.13-1.89].

PE pregnancies were further divided into four subgroups: severe PE (n=196), mild PE (n=52), early-onset PE (n=89), and late-onset PE (n=107). Compared with that in control group, the association with EDN1 polymorphism rs5370 was observed in the women with severe PE (p=0.03, OR=1.48, 95% CI=1.14-1.92), while no significant association was observed in those with mild PE (p=0.95, OR=1.02, 95% CI=0.62-1.66). The markedly increased risk of the early-onset PE was related to the C allele of polymorphism rs5370 in EDN1 (p=0.006, OR=1.65, 95% CI=1.15-2.36). However, the data manifested no significant

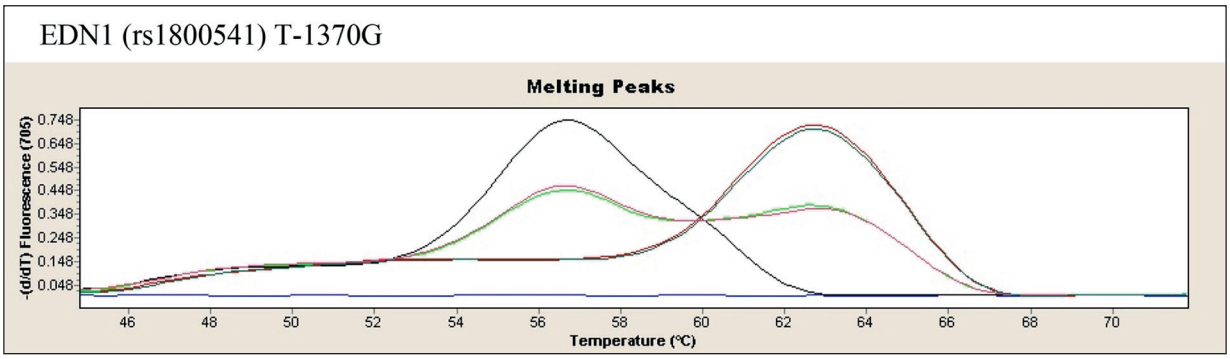

Figure 2. Melting curve analysis for EDN1 (rs1800541) T-1370G. According to the figure, the melting curves are converted into melting peaks by plotting the relationship between the fluorescent negative derivative and temperature, in which the first peak corresponds to the T allele, and the second peak corresponds to the G allele.

Table II. Allele frequencies of two SNPs in PE group and control group.

	Allele			
	T (%)	C (%)	OR (95% CI)	*p*-value
rs5370				
Control	315 (65.0)	160 (35.0)		
PE	268 (55.7)	221 (44.3)	1.58 (1.13-1.89)	0.02
PE				
SPE	212 (53.8)	182 (46.2)	1.60 (1.22-2.10)	0.001
MPE	53 (64.6)	29 (35.4)	1.02 (0.62-1.66)	0.78
SPE				
Early-onset	88 (53.0)	78 (47.0)	1.65 (1.15-2.36)	0.004
Late-onset	124 (54.4)	104 (45.6)	1.56 (1.32-2.15)	0.032
rs1800541				
Control	134 (28)	352 (72)		
PE	147 (31)	329 (69)	1.17 (0.84-1.55)	0.56
PE				
SPE	122 (31.0)	272 (69.0)	1.18 (0.88-1.58)	0.29
MPE	25 (30.5)	57 (69.5)	1.15 (0.69-1.92)	0.57
SPE				
Early-onset	51 (30.7) 115 (69.3) 1.17 (0.79-1.71) 0.4	115 (69.3)	1.17 (0.79-1.71)	0.44
Late-onset	71 (31.1)	157 (68.9)	1.19 (0.84-1.68)	0.33

Note: PE: preeclampsia, MPE: mild preeclampsia, SPE: severe preeclampsia.

difference in the allele frequency of polymorphism rs1800541 between the PE group and control group ($p>0.05$) (Table II).

Associations of Genotype Frequencies of SNPs with PE Risk in PE Group and Control Group

In the co-dominant model, the CC genotype of polymorphism rs5370 in EDN1 was associated with the raised risk of PE compared with TT genotype ($p=0.0019$, OR=2.21, 95% CI=1.19-3.36). In comparison with TT genotype, TC/CC genotype in the dominant model related with prominently increased risk of PE ($p=0.001$, OR=1.88, 95% CI=1.39-2.68). In addition, the risk of PE pregnancies carrying TC genotype was 1.59 times higher than those of TT/CC genotype (95% CI=1.04-2.12, $p=0.021$). However, no evident difference was detected between EDN1 polymorphism rs1800541 and PE risk ($p>0.05$, Table III).

Genotype Frequencies of SNP in Control Group and PE Group

In the severe PE group, there were significant differences in the genotype frequencies between dominant ($p<0.01$) and over-dominant ($p=0.022$) models of rs5370 (Table IV). The similar results were also observed in the late-onset group. In comparison with TT genotype, TC/CC genotype in the recessive model was correlated with the increased risk of early-onset PE ($p=0.013$, OR=1.97, 95% CI=1.14-3.42). Nevertheless, the genotype frequencies of rs1800541 exhibited no significant associations with PE risk ($p>0.05$, Table V).

Discussion

PE is a complex pregnancy-specific hypertension syndrome that seriously threatens the health of pregnant women and fetuses. The genetic factors are regarded to be related to the development of PE[9]. As a vital related peptide to normal cell cycle, EDN1 can enhance the proliferation of various somatic cells including thyroid cells. Previous investigations[10-13] have discovered that EDN1 directly stimulates the cell proliferation, migration, invasion, angiogenesis, and inhibits cell apoptosis. Previous investigations[14,15] have proposed that together with its mitogenic feature, EDN1 is closely associated with inflammations. EDN1 can promote the synthesis of many pro-inflammatory cytokines and nuclear factor-κB. Besides, upregulated EDN1 is one of the indexes of ICAM1 synthesis-endothelial dysfunction[16]. The present study aims to analyze the influences of

Table III. Associations of genotype frequencies with PE risk in two groups.

Genetic model	Genotype	PE group N = 238 (%)	Control group N = 243 (%)	Logistic regression or (95% CI)	*p*-value
rs5370					
Co-dominant	TT	65 (27.3%)	101 (41.6%)	1.00 (reference)	
	TC	135 (56.7%)	114 (46.9%)	1.87 (1.43-2.84)	
	CC	38 (16%)	28 (11.5%)	2.21 (1.19-3.36)	0.0019
Dominant	TT	65 (27.3%)	101 (41.6%)	1.00 (reference)	
	TC/CC	173 (72.7%)	142 (58.4%)	1.88 (1.39-2.68)	0.001
Recessive	HH/CC	200 (84%)	215 (88.5%)	1.00 (reference)	
	CC	38 (16%)	28 (11.5%)	1.46 (0.86-2.47)	0.16
Over-dominant	TC/CC	103 (43.3%)	129 (53.1%)	1.00 (reference)	
	TC	135 (56.7%)	114 (46.9%)	1.59 (1.04-2.12)	0.021
rs1800541					
Co-dominant	CC	101 (42.4%)	121 (49.8%)	1.00 (reference)	
	TC	127 (53.4%)	110 (45.3%)	1.38 (0.96-2.00)	
	TT	10 (4.2%)	12 (4.9%)	1.00 (0.42-2.41)	0.51
Dominant	CC	101 (42.4%)	121 (49.8%)	1.00 (reference)	
	TC/TT	137 (57.6%)	122 (50.2%)	1.35 (0.94-1.93)	0.41
Recessive	CC/TC	228 (95.8%)	231 (95.1%)	1.00 (reference)	
	TT	10 (4.2%)	12 (4.9%)	0.84 (0.36-1.99)	0.69
Over-dominant	CC/TT	111 (46.6%)	133 (54.7%)	1.00 (reference)	
	TC	127 (53.4%)	110 (45.3%)	1.38 (0.97-1.98)	0.176

Table IV. Comparison of genotype frequencies of rs5370 between the two groups.

	Genotype				
	TT	TC	CC	OR (95% CI)	*p*-value
PE					
SPE	49 (24.9%)	114 (57.9%)	34 (17.3%)	2.15 (1.42-3.24)	< 0.01
MPE	16 (39.0%)	21 (51.2%)	4 (9.8%)	1.11 (0.56-2.19)	0.46
SPE					
Early-onset	22 (26.5%)	44 (53.0%)	17 (20.5%)	1.97 (1.14-3.42)	0.023
Late-onset	27 (23.7%)	70 (61.4%)	17 (11.9%)	2.29 (1.39-3.78)	< 0.001
Control	101 (41.6%)	114 (46.9%)	28 (11.5%)		

Note: PE: preeclampsia, MPE: mild preeclampsia, SPE: severe preeclampsia.

Table V. Comparison of genotype frequencies of rs1800541 between the two groups.

	Genotype				
	CC	TC	TT	OR (95% CI)	*p*-value
PE					
SPE	84 (42.6%)	104 (52.8%)	9 (4.6%)	1.33 (0.91-1.95)	0.33
MPE	17 (41.5%)	23 (56.1%)	1 (2.4%)	1.40 (0.72-2.74)	0.74
SPE					
Early-onset	35 (42.2%)	45 (54.2%)	3 (3.6%)	1.36 (0.82-2.25)	0.63
Late-onset	49 (43.0%)	59 (51.8%)	6 (5.2%)	1.32 (0.84-2.06)	0.23
Control	121 (49.8%)	110 (45.3%)	12 (4.9%)		

Note: PE: preeclampsia, MPE: mild preeclampsia, SPE: severe preeclampsia.

EDN1 polymorphisms on the susceptibility to PE. Two possible mutation sites of EDN1 were identified, including a 3'-Untranslated region (rs5370) and an exon (rs1800541) sequence. It has been reported[17-19] that EDN1 polymorphism rs5370 is correlated with the susceptibility to lung cancer, septic shock, colorectal cancer, and biliary tract cancer. Besides, EDN1 polymorphism rs1800541 is associated with the risk of colorectal cancer. To our knowledge, the role of EDN1 polymorphisms in PE progression has not been explored yet. According to the present study, the markedly increased risk of PE was considered to have an association with the C allele of polymorphism rs5370 in EDN1. There were many researches supporting that the CC and TC/CC genotypes of polymorphism rs5370 in EDN1 were more common in patients with severe PE than TT genotype. Moreover, the TC/CC genotype (compared with TT genotype) and TC genotype (compared with TT/CC genotype) were more common in patients with severe PE or late-onset severe PE. Similarly, the TC/CC genotype (compared with TT genotype) and TC genotype (compared with TT/CC genotype) occurred more frequently in patients with early-onset severe PE. All these results demonstrated that EDN1 polymorphism rs5370 is a risk factor for severe PE, which was not reported before. In addition, previous investigations revealed that the CC genotype of polymorphism rs5370 in EDN1 has a correlation with the raised risk of lung cancer in the European population. The C allele of polymorphism rs5370 in EDN1 is also associated with the high risk of septic shock in the American and Japanese population. However, some investigations[20,21] have manifested that people carrying CC genotype of polymorphism rs5370 in EDN1 exhibit lower risks of colorectal cancer, hepatitis C virus infection, and stroke. On the contrary, other studies[22] have indicated that EDN1 polymorphism rs5370 had no association with the susceptibility to periodontitis in Chinese population. These inconsistent findings may be caused by racial difference and various types of diseases, and such genetic associations need to be verified by further research. No differences in the allele and genotype frequencies of EDN1 polymorphism rs1800541 were observed between PE patients and controls in this study. Currently, the research on the relationship between EDN1 polymorphism rs1800541 and PE still remains controversial. The data in this study were consistent with those in the analyses on polymorphism rs1800541 in the Czech population. However, some findings have proven that the Chinese carriers of TT genotype of polymorphism rs1800541 in EDN1 display elevated risks of cholangiocarcinoma and calculus. Such a difference may be explained by racial difference and various types of diseases. It is important to point out that the novelty of this study was the genetic homogeneity of the study population, because only individuals with the same genetic background of the Chinese Han population were enrolled. Nevertheless, some restrictive conditions in the present study require further exploration. For example, placental genotyping was not performed for enrolled pregnant women. Besides, the sample size was small, and the multicenter large-sample studies need to be conducted to validate our conclusions.

Conclusions

We detected that the genetic association of EDN1 gene polymorphism rs5370 is linked to the increased risk of PE, while polymorphism rs1800541 has no association with the disease.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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