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Analysis on the correlations of ENOS and ET-2 gene polymorphisms with eclampsia

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Abstract. – OBJECTIVE: To explore the correlations of endothelial nitric oxide synthase (eNOS) G894T and endothelin-2 (ET-2) A985G gene polymorphisms with eclampsia.

PATIENTS AND METHODS: A total of 110 eclampsia patients in our hospital from July 2014 to August 2017 were enrolled as the observation group and 100 healthy pregnant women in the same period as the control group. The polymorphisms of eNOS G894T and ET-2 A985G genes in the two groups were analyzed *via* polymerase chain reaction (PCR), and their correlations with eclampsia risk were investigated.

RESULTS: The distribution frequency of eNOS G894T genotype TT and GT and T allele, as well as the ET-2 A985G genotype GG and AG and G allele, were evidently higher in the observation group than in the control group ($p<0.05$). ENOS G894T genotype TT and ET-2 A985G genotype GG were significantly associated with the occurrence of eclampsia.

CONCLUSIONS: The polymorphisms of eNOS G894T and ET-2 A985G genes are correlated with the occurrence of eclampsia.

Key Words:
Eclampsia, Endothelial nitric oxide synthase, Endothelin-2, Gene polymorphism.

Introduction

Eclampsia, the most severe symptom of hypertensive disorders during pregnancy, refers to the convulsion based on maternal hypertensive disorders, which cannot be explained by other reasons. Eclampsia occurs at any stage of the perinatal period and remains one of the leading causes of maternal and neonatal deaths[1-3]. The pathogenesis of eclampsia has not been fully clarified yet, and accumulated evidence has revealed that eclampsia was closely associated with heredity. Recent studies have reported[4-6] that endothelial nitric oxide synthase (eNOS) G894T gene polymorphisms might be one of the candidate risk factors of eclampsia. Endothelin (ET), discovered in 1988, is secreted by vascular epithelium, heart and kidney cells and acts as one of the crucial factors regulating blood pressure[7]. Sharma et al[8] found that A985G base mutation and ET-2 gene polymorphism could serve as an independent predictor for hypertension patients. There have been many studies on the correlation of eNOS allele mutation with hypertension home and abroad, but the relationship between its gene polymorphism and eclampsia has not been reported so far. The present study aimed to detect the gene polymorphisms of eNOS G894T and ET-2 A985G using chip test technique and then analyze their correlations with eclampsia in 110 eclampsia patients and 100 healthy controls.

Patients and Methods

Patients

A total of 110 eclampsia patients diagnosed in our hospital from July 2014 to August 2017 were enrolled as the observation group, and 100 healthy pregnant women in the same period were selected as the control group. There were no significant differences in general information between the two groups ($p>0.05$) (Table I). This study was approved by the Ethics Committee of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology. Signed written informed consents were obtained from all participants before the study.

Collection of Samples

Collection of serum sample: a total of 3 mL blood median cubital veins were collected from each patients before the administrated with any drug. The same volume of blood was obtained from the median cubital veins of healthy non-pregnant women receiving physical examinations. Peripheral venous blood was collected from the women in the control group within 3 h after their admittance to the hospital. The blood

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Table I. Comparisons of the general information of the two groups of patients.

Parameter	Observation group (n=110)	Control group (n=100)	*p*
Age (Years old)	28.4±4.3	29.5±5.7	0.318
Gestational age	34.2±2.8	36.1±2.3	0.524
History of hereditary diseases			
Yes	7 (6.4%)	3 (3%)	0.256
No	103 (93.6%)	97 (97%)	
Smoking			
Yes	28 (25.5%)	23 (23%)	0.680
No	82 (74.5%)	77 (77%)	
Drinking			
Yes	42 (38.2%)	33 (33%)	0.442
No	68 (61.8%)	67 (67%)	

samples were allowed to stand for 1-2 h at room temperature and centrifuged at 1,000 g (or 3,000 rpm) for 15 min to extract the serum. The serum was then sub-packaged and stored at -80°C for detecting the levels of eNOS and ET-2 in serum.

Single Nucleotide Polymorphism (SNP) Genotyping via Polymerase Chain Reaction (PCR)

Oligo 6.0 was employed to design the primer sequences at SNP sites and their TaqMan probe sequences (Table II). The primers were then synthesized by Sangon Biotech (Shanghai, China Co., Ltd). The PCR reaction system was prepared with 17.8 μL TransStart Probe quantitative PCR (qPCR) SuperMix (Beijing TransGen Biotech Co., Ltd. Beijing, China), 1 μL DNA solution and 1.2 μL mixed primer solution (forward primers, 0.4 μL; reverse primers, 0.4 μL; probe, primers, 0.4 μL). Then, the amplification reaction and genotyping were performed using the CFX96 fluorescence qPCR instrument (Bio-Rad, Hercules, CA, USA). The reaction condition was as follows: 94°C for 5 min, 94°C for 10 s and 60°C for 30 s, for 40 cycles. Each sample was put into 3 duplicated wells. The diethyl pyrocarbonate (DEPC)-treated water (Beyotime, Shanghai, China) was selected as the negative control and the plasmid containing the designed sequences (Shanghai Sangon Biotech Co., Ltd. Shanghai, China) was used as the positive control. Determination of genotype was as follows: the genes near FAM horizontal ordinate were identified as wild type, the genes near VIC vertical ordinate were considered as homozygous type and the rest near 45° line were taken as heterozygous type.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 20.0 software (IBM, Armonk, NY, USA) was utilized to analyze data. Hardy-Weinberg equilibrium with $p>0.05$ was adopted to check the representativeness of the sample. Chi square test was employed to compare the distribution frequency of gene polymorphisms. $p<0.05$ suggested the difference was statistically significant.

Results

Hardy-Weinberg Analysis on the Distributions of G894T and A985G Genotypes

Genetic equilibrium test was performed for the distributions of eNOS G894T and ET-2 A985G genotypes in the two groups. The measured values of the genotype distributions were then compared with the estimated values. At the same time,

Table II. Primer sequences.

Gene	Primer sequence	Probe sequence
ENOS	Forward: 5′-CAGCTCTGCATTCAGCACGGC-3′ Reverse: 5′-AGGGGCACCTCAAGGACCAG-3′	FAM: 5′-AGATGAGCCCCCAGAACTCTTCC-3′ VIC: 5'- AGATGATCCCCCAGAACTCTTCC-3′
ET-2	Forward: 5′- GGCTACAAACCAGGAGCAAC-3′ Reverse: 5′-CACAGCCAGCCAGGATGCCA-3′	FAM: 5′-AGCCCTGGAGACTGGATGGCT-3' VIC: 5′- AGCCCTGGAGGCTGGATGGCT -3'

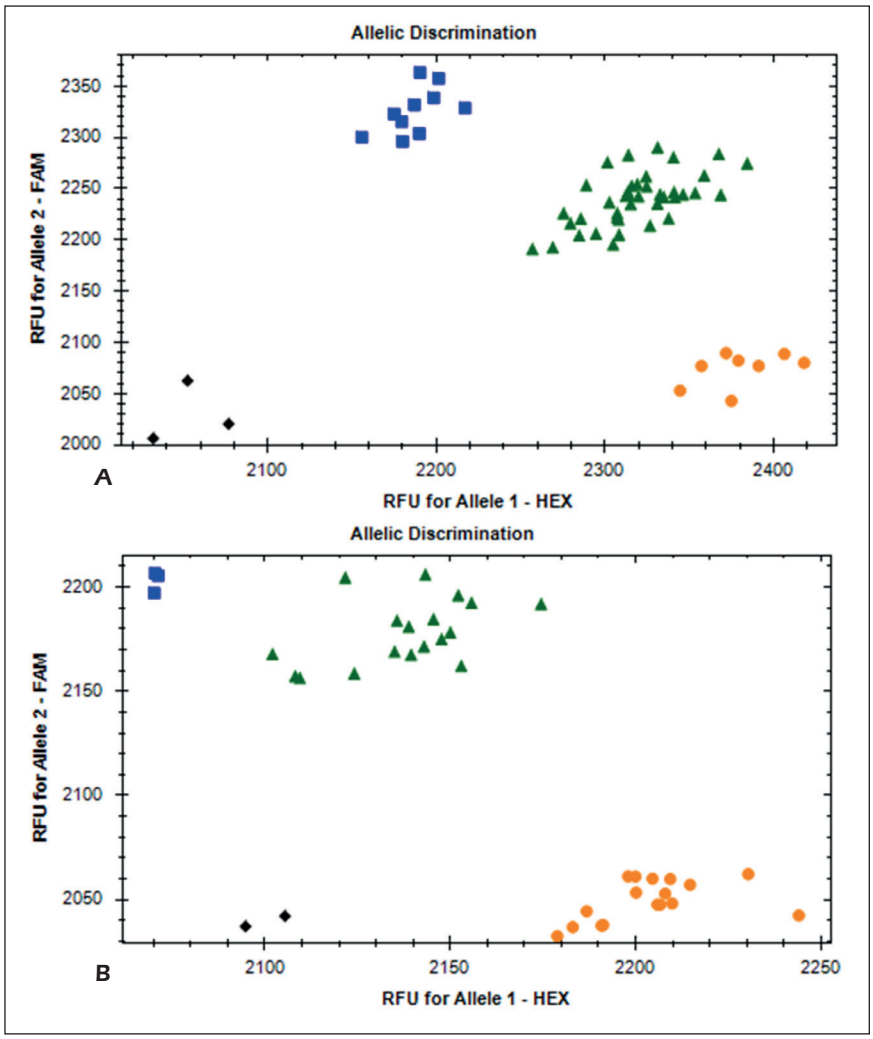

Figure 1. ***A***, PCR detection result of eNOS G894. ***B***, PCR detection result of ET-2 A985G.

the results of our chi square test revealed that the genotype distributions of eNOS G894T and ET-2 A985G in the two groups met the Hardy-Weinberg equilibrium law ($p>0.05$) (Figure 1).

Genotype and Allele Distributions of eNOS G894T Mutation Site

The genotype frequency difference of eNOS G894T mutation site was compared between the two groups, and the results showed that the observation group had substantially higher genotype TT, genotype GT and T allele distribution frequency than the control group, with a statistically significant difference ($p<0.05$) (Table III).

Genotype and Allele Distributions of ET-2 A985G Mutation Site

The genotype frequency difference of ET-2 A985G mutation site was compared between the two groups, and the results manifested that the observation group had substantially higher genotype GG, genotype AG and G allele distribution frequency than the control group, and the difference was statistically significant ($p<0.05$) (Table IV).

Logistic Regression Analysis on the Correlations of the Two Genotypes of eNOS G894T and ET-2 A985G with the Incidence Rate of Eclampsia

Taking the genotypes of eNOS G894T and ET-2 A985G as the independent variables, we conducted the logistic regression analysis to determine the correlation of gene polymorphisms and eclampsia risk. According to the results, eNOS genotype TT and ET-2 genotype GG were significantly related to the incidence of eclampsia ($p<0.05$) (Table V).

Table III. Genotype and allele distributions of eNOS G894T mutation site.

Genotype	Observation group [n=110, (n)%]	Control group [n=100, (n)%]	*p*
TT	7 (6.36)	3 (3)	0.031
GT	37 (33.6)	19 (19)	0.045
GG	56 (50.9)	78 (78)	0.105
G	93 (84.5)	97 (97)	0.232
T	44 (40)	22 (22)	0.006

Table IV. Genotype and allele distributions of ET-2 A985G mutation site.

Genotype	Observation group [n=110, (n)%]	Control group [n=100, (n)%]	*p*
GG	84 (76.4)	57 (57)	0.021
AG	23 (20.9)	35 (35)	0.048
AA	3 (2.7)	8 (8)	0.100
G	107 (97.3)	92 (92)	0.033
A	26 (23.6)	43 (43)	0.143

Analysis on the Correlations of the Frequency Distributions of the Two Genotypes of eNOS G894T and ET-2 A985G with the Onset Risk of Eclampsia

Chi square test was performed to analyze the frequency distributions of eNOS G894T and ET-2 A985G genotypes, as well as their correlations with eclampsia risk. We found that the *p*-value of the distributions of eNOS G894T genotype TT frequency and ET-2 A985G genotype GG was less than 0.05 (Table VI). Our results suggested that eNOS G894T genotype TT and ET-2 A985G genotype GG are notably correlated with eclampsia.

Discussion

ENOS G894T gene is located on the exon of chromosome 7 and its polymorphism exactly refers to the polymorphism of this exon region. ENOS G894T affects the production of glutamic acid, and lacked glutamic acid will be refilled by L-aspartic acid[9]. Currently, no definite conclusion on the relationship between eNOS and eclampsia has been reported. Ma et al[10] revealed that G894T T and G alleles were correlated with the occurrence of gestational hypertension among Asians. Based on the meta-analysis on eclampsia, Zeng et al[11] found that eNOS G894T gene was related to eclampsia risk, and genotype TT markedly increased the risk of eclampsia. Shaamash et al[12] also pointed out that there was no statistical difference between the gene polymorphism of eNOS and the susceptibility to eclampsia. In the present study, the frequency of G894T genotype TT and GT and allele T was higher in the observation group than in the control group. Our data indicated that the genotype TT and GT and al-

Table V. Logistic regression analysis on the correlations of the two genotypes of eNOS G894T and ET-2 A985G with the incidence rate of eclampsia.

Genotype		Single-factor		Multi-factor	
		95% confidence interval (CI)	*p*	95% CI	*p*
ENOS G894T	TT	0.899 (0.824-1.236)	0.522	0.864 (0.781-1.259)	0.000
	GT	0.943 (0.749-1.186)	0.614	0.987 (0.783-1.243)	0.910
	GG	0.630 (0.434-0.914)	0.115	0.725 (0.497-1.057)	0.195
ET-2 A985G	GG	0.870 (0.697-1.086)	0.219	0.687 (0.356-1.124)	0.000
	AG	0.652 (0.461-0.923)	0.216	0.731 (0.514-1.039)	0.180
	AA	0.838 (0.713-0.985)	0.032	0.893 (0.758-1.052)	0.177

Table VI. Analysis on the correlations of the frequency distributions of the two genotypes of eNOS G894T and ET-2 A985G with the onset risk of eclampsia.

Site	Genotype	Observation group	Composition proportion (%)	Control group	Composition proportion (%)	χ^2	p	OR	95% CI
ENOS	TT	7	6.36	3	3	0.846	0.000	1.000	0.589-1.457
G894	GT	37	33.6	19	19	0.040	0.850	1.028	0.411-2.568
T	GG	56	50.9	78	78	0.030	0.925	0.957	0.604-1.516
ET-2	GG	84	76.4	57	57	0.956	0.000	1.234	0.702-1.014
A985	AG	23	20.9	35	35	0.031	0.781	1.384	0.556-1.464
G	AA	3	2.7	8	8	0.028	0.655	1.520	0.631-1.882

lele T might be correlated with the incidence of eclampsia, which was consistent with the report of Srivastava et al[13]. The eNOS G849T polymorphism was also previously reported to be related to multiple diseases, such as cardio-cerebrovascular disease, diabetes and placental abruption[12,14-18]. The results of this study also revealed that allele T was an independent risk factor for eclampsia. ET is a kind of peptide-like substances and mainly secreted by vascular endothelial cells. ET controls the opening of calcium channel and raises the extracellular concentration of ionized calcium by binds to its corresponding receptor. Meanwhile, ET can enhance the vasoconstrictive effects of norepinephrine, increase the peripheral vascular resistance, and thus raise the blood pressure. Serrano et al[19] reported that ET-2 A985G gene was remarkably related to the occurrence of eclampsia. According to the results of this study, the frequency of ET-2 A985G genotype GG and AG and allele G in the observation group was higher than that in the control group, indicating that ET-2 might be the susceptibility gene of eclampsia. Logistic regression analysis also displayed that ET-2 A985G and eNOS G894T genotypes were the independent risk factors for eclampsia.

Conclusions

Eclampsia is a complex disease caused by the interactions of various factors. Due to the limitations of current study methods and techniques, there is not a clear consensus on whether eNOS G894T and ET-2 A985G are correlated with the eclampsia. The present study demonstrated the remarkable correlations of ET-2 A985G and eNOS G894T gene polymorphisms with the onset of eclampsia. However, the studies on gene polymorphisms are quite different among differences races. Therefore, such correlations need to be further verified with a higher number of data.

Conflict of Interests

The authors declared no conflict of interest.

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