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Association between COMT-287A/G (rs2075507) Polymorphism and Preeclampsia in a Chinese Han Population

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Abstract: Previous study indicated that *COMT* might play a significant role in the development of preeclampsia （PE）. The purpose of our study is to investigate the association of *COMT* -287A/G polymorphism (rs2075507) with PE susceptibility in Chinese han population. 505 PE patients and 824 normal pregnant women were enrolled in our research. All the subjects were detected the genotype of *COMT* -287 loci by Polymerase chain reaction (PCR)–restriction fragment length polymorphism (RFLP) method. Demographics and clinical data were performed using t test or Mann-Whitney test. The frequencies of the allelic and genotypic distribution between PE patients and healthy controls were analyzed by X^2 test. No significant difference in the genotypic distribution was found between the patients and controls (x^2 =0.13, p=0.94), while frequency of the allele showed a unstatistical significant difference (x^2=0.07, p=0.79). The *COMT* -287A/G polymorphism might be associated with the PE in Chinese women. Further researches are yet needed to validate the relationship between the *COMT* -287A/G and the PE in different ethnic groups.

Keywords: preeclampsia ; *COMT*-287A/G; polymorphism

Introduction

Preeclampsia (PE) is a common and severe compilation of pregnant women, which is a leading cause of perinatal morbidity and mortality. In recent years, the incidence of PE presents an upward trend, from 3% in 2002[1] increasing to 10% in 2009[2]. In spite of its fairly high incidence, the exact etiology of PE remains enigmatic. A lines of researches showed that PE is a complicated pregnancy-specific disease, with multiple genes, environmental and social factors acting in conjunction to cause, among which the genetic susceptibility plays an important role in this

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disorder. Up to now, several candidate genes have been studied, such as *CC*, *MHHFR*, *IL-1*β**a**nd so on[3-5]. However, some verdict is incompatible and specific causative genes involved in PE still remain to be identified.

The Catechol-O-methyltransferase (*COMT*) gene, located on chromosome 22q11.21, is a key enzyme metabolizing monoamine neurotransmitters and involves in the degradation of catecholamine and catechol estrogens, including dopamine, epinephrine, norepinephrine, and catechol estrogens whose degradation products was 2-methoxyoestradiol (2-ME) [6]. Distributed in the placenta, *COMT* was thought to be associated with obstetric diseases. Previous study indicated that *COMT* might play a significant role in the development of PE. Studies have shown that patients with PE were in the state of sympathetic overactivity [7] , and catecholamine level in plasma, especially norepinephrine and epinephrine levels were significantly higher in normotensive pregnant women[8] , while *COMT* activity was found to be lower in the placentas of patients with severe PE [9]. Kanasaki et al. constructed PE mouse model by knocking out *COMT*, which showed a PE like phenotype resulting from an absence of 2-ME, including hypertension, proteinuria and vascular and placental lesions [9]. In addition, women with homozygous for the variant *COMT* allele (158Met) had a grown risk of PE in a Korean cohort [10].

To identify the global association of other functional variants of *COMT* and PE development, the possible relevance needs to be confirmed by independent researches in different ethnic groups. The objective of this present study was to assess the genetic association between polymorphism of -287A/G (rs2075507) in the promoter of *COMT* and PE in Chinese women.

Materials and Methods

Subjects

505 PE patients and 824 normal pregnant women were recruited from Affiliated Hospital of Qingdao University Medical College and Linyi People's Hospital. According to the International Society for the Study of Hypertension in Pregnancy , PE was defined as blood pressure of ≥140/90 with proteinuria C≥300 mg in 24 h or≥2 + dipstick after 20 weeks of gestation[11]. Exclusion criteria of PE patients included diabetes mellitus, Rh incompatibility, bleeding disorders, systemic lupus erythematosus, polyhydramnios, and pregnancies complicated by fetal abnormalities. Pregnant women on any medication or chronic disorders affecting cardiovascular system, liver, kidneys, endocrine organs, and metabolic disorders were also excluded from the study. The average age and gestational week of PE patients were 30.41±5.27, 34.97±4.25 respectively. The control pregnant women matched with the research group according to the age, whose average is 30.44±4.33. The control group were healthy pregnant without diseases and did not include premature rupture of membranes, placenta previa, threatened abortion, artificial insemination, twins, fetal macrosomia and premature birth. Neither PE patients nor normal pregnancy subjects were smokers.

In addition, all the subjects that we have studied were inpatients, so we can make a comprehensive and detailed understanding of the patients' condition. Not only demographic data including age, gestational age, gravidity, parity and the number of previous abortions but also a detailed medical history was recorded. Besides those systemic physical and pelvic examinations, complete blood counts, routine urinary and biochemical analysis were performed. The study protocol was approved by the local ethics committee. All participants have given written informed consent.

Genotyping

Genomic DNA was isolated from peripheral blood Leukocyte by DNA extraction kit. The *COMT*-287A/G polymorphism was performed by Polymerase chain reaction (PCR)–restriction fragment length polymorphism (RFLP). Two oligonucleotide primers (Forwards:

5‘TAGTAACAGACTGGCACGAA3’ and Rewards: 5‘GTTCAAAGGGCATTTATCATG3’) were used to amplify the corresponding DNA fragment by the PCR. The amplification system was performed in a 10μL volume, containing 3.5μL ultra pure water, 5μL mix, 0.25μL forward primers, 0.25μL reverse primers and 1μl extracted of DNA. The amplification protocol is as follows: initial denaturation at 94°C for 5 min; 30 cycles of denaturation at 94°C for 30 s, annealing at 60°C for 30 s and extension at 72°C for 30s and final tension at 72°C for 7 min. Then，the amplification products was examined by electrophoresis on a 1.5% agarose gel. The 350bp PCR fragments were digested with HindⅢ enzyme at 37°C for 24h in a reaction volume of 10μL. Digested samples were separated by electrophoresis on a 2.5% agarose gel and visualized by goldview staining. The *COMT*-287A/G is digested into 3 expected results: AA showing one band with 350bp, GG showing two bands with 276bp and 74bp, AG showing three bands with 350bp, 276bp and 74bp.In order to ensure the accuracy of the results, each gel was read by two observers unaware of the subjects' status.

Statistical analysis

Statistical analysis was carried out using the SPSS V 22.0 software. Results were reported as mean±SD. The differences between case-control groups were examined by X^2 test or an independent t test whenever it was appropriate. Demographics data consisting of age, gestational age, number of gravidity, abortion and mean arterial pressure was performed using t test or Mann-Whitney test. The frequencies of allelic and genotypic distribution between PE patients and healthy controls were analyzed by X^2test for categorical data, which was used to test the Hardy-Weinberg equilibrium of the genotypic distribution. P value＜0.05 was considered statistically significant.

Results

Demographic and clinical characteristics of the study population

Demographic and clinical characteristics of the PE cohort and normal pregnant women are summarized in Table 1. There was no difference in maternal age, gravidity and number of abortions among PE and normal controls (all P＞0.05). As shown in table 1, the PE patients had a higher prevalence of preterm birth, whose gestational age of delivery was lower compared with normal controls (p＜0.01). The birth weight of neonates from the case group was lower compared with the neonates’ from healthy pregnant women(p＜0.01). As expected, the mean systolic blood pressure of the patient group was greatly increased, significantly higher than the healthy groups, so did the average diastolic blood pressure.

The distribution of the genotypes and the allele frequency

The distributions of the genotypic and allelic frequency are given in Table 2. The *COMT* -287A/G genotype polymorphism in case and control groups was conformed in Hardy-Weinberg equilibrium. In table 2, the percentage of genotypes were 57.82% for AA, 35.64% for AG and 6.53% for GG in case population. On the other hand, in healthy pregnant women the genotypic frequencies were 57.40% for AA, 35.56% for AG and 7.04% for GG. No significant difference in the genotypic distribution was found between the patients and controls (x^2=0.13, P=0.94). Further calculation the value of OR found that there was no association between AA genotypes versus the AG and GG genotype in the two groups(x^2=0.02, P=0.88, OR =1.02, 95%CI=0.81-1.27). The A and G allele frequency were 75.64% and 24.36% respectively in PE, while 75.18% and 24.82% respectively in control group. There was a statistical significant difference in allele frequency between PE patients and healthy control (x^2=0.07, P=0.79)，which indicated that the pregnant women carrying the G allele had 0.98 times the risk of PE than the pregnant women with the G allele (x^2=0.07, P=0.79, OR =0.98, 95%CI=0.81-1.17F).

Discussion

The PE is a devastating complication and severely threatens the lives of mothers and fetus. In addition, it

has been notified as a global health problem of women by the World Health Problem Orgnazation (WHO) [12-13]. Although many investigations have been done to study the pathogenesis of the PE, the precise pathogenesis of the disease remain unknown. In order to decrease the morbidity and mortality of the pregnant and children, it is necessary to do further research on the etiology of the PE.

COMT was distributed in many different body tissues and thought to protect against cardiovascular diseases, degenerative neurological conditions and estrogen-induced cancers [14]. *COMT* activity changes will cause a change of catecholamines and catechol estrogens, which leads to the disease. Catecholamine is a psychoactive substance containing catechol and amine. The chief physiological functions of catecholamine is to excite α receptor of the vascular, resulting in the vascular contraction and blood pressure change. 2-ME, a metabolite of estrogen, is generated by *COMT* which induces invasion of cytotrophoblasts into a naturally-derived, extracellular matrix [15].

Since *COMT* was firstly noted to be related with hypertension in pregnant [16], increasing evidence supports the role of *COMT* in human pregnancy. Barn et al.[16] primarily indicated that the *COMT* is associated with the PE, and found *COMT* activity lower in the placentas of patients in PE. Another study also demonstrated that the levels of *COMT* were significantly lower in women with severe PE [9]. In addition, many researches have been carried out to evaluate the association of *COMT* and the PE, occasionally indicating the inconsistent results. Some researchers suggested that SNPs (single nucleotide polymorphisms) in the *COMT* had been shown to significantly affect enzyme activity[17-19]. However, Hill et al. found no relationship between single *COMT* SNPs and PE in a Chilean population [4]. It was reported that women with homozygous for the variant *COMT* allele (158Met) in a Korean cohort had a grown risk of PE [10]. Similarly, the Val158Met polymorphism was also association with the PE in Han population [21]. In addition, Roten et al. studied the relationship between the low *COMT* activity haplotype and the recurrent PE in Norwegian population, demonstrating that a low *COMT* activity haplotype was associated with the recurrent PE [22].

In our present study, we intended to explore the relationship between the -287A/G (rs2075507) polymorphism of *COMT* and the PE. No statistical significant difference was found in genotype distribution among case-control groups. Similarly, the allelic frequency of *COMT* -287A/G showed no difference between the PE and non-preeclampsia groups. Another research indicated that Val158Met functional polymorphism was associated with the PE in Han population [21]. Inspired by Liang`s study results, on the basis of the difference of the allelic frequency, we can speculate that the diversity of the genotype distribution may not be showed between the patients and normal pregnant women because of the limitation of the sample size.

Our experiments may also exist in the following shortage, which influences the research results. Firstly, the number of specimens might lead to the negative results of genotypic distribution. Secondly, *COMT* -287A/G polymorphism might be diverse in different ethnic groups. In addition, the PE was regarded as a multifactorial disease, environmental risk factor such as diet, obesity stress, smoking and other social elements during pregnancy whose complex interaction could lead to our negative results of the distribution of the genotype. And the population stratification can also influence our laboratory results. Hence, it is worthwhile to do more analysis with a large sample size and more effectively methods to quantify the link among *COMT* -287A/G polymorphism and the PE.

In summary, we found no statistical significant difference between the genotype distribution and the PE, while the frequency of allele frequency was related to the PE. At present, the effective PE therapy is to end the pregnant, which brings great harm to

maters and neonates, even leading to the death. It is essential to do further researches to validate the relationship between *COMT* -287A/G and the PE, to explore the association between other susceptibility genes and the PE. It is possible that these findings may help us to diagnosis and treatment the PE and ensure the health of maters and fetuses.

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Table 1 Demographic and clinical characteristics of the preeclampsia cohort and normal pregnant women

	Preeclampsia	control	X^2	P
Maternal age(years)	30.41±5.27	30.44±4.33	0.214	0.831*
Gestational age（weeks）	34.97±4.25	38.58±2.12	12.423	p＜0.01*
Gestational age of at delivery（weeks）	36.55±2.97	39.35±1.47	11.048	p＜0.01*
gravidity	2.31±1.24	2.29±1.22	0.166	0.868
Number of abortions	0.87±1.03	0.78±0.95	1.015	0.310
Birth weight（g）	2731.13±916.83	3408±492.99	8.81	p＜0.01*
Systolic blood pressure(mmHg)	147.27±24.89	112.55±10.49	11.306	p＜0.01*
Systolic blood pressure(mmHg)	97.71±17.94	74.06±8.50	15.705	p＜0.01*

* Mann-Whitney test.

Table2 Distribution of the genotypes and the allele frequency of the *COMT* -287A/G polymorphism between case and control groups

Genotype Allele	case	control	x^2	P	OR	95%CI
AA	292(57.82%)	473(57.40%)				
AG	180(35.64%)	293(35.56%)				
GG	33(6.53%)	58(7.04%)	0.13	0.94		
AA	292(57.82%)	473(57.40%)				
AG+GG	213(42.18%)	351(42.60%)	0.02	0.88	1.02	0.81-1.27
total	505	824				
A	764(75.64%)	1239(75.18%)				
G	246(24.36%)	409(24.82%)	0.07	0.79	0.98	0.81-1.17
total	1010	1648				