BDNF Val66Met was associated with the susceptibility of PE but irrespective of neonatal poor prognosis in a Han population:a case-control study

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Abstract

Objective: Brain-Derived Neurotrophic Factor (BDNF) plays a role in placental development and is involved in the pathogenesis of preeclampsia (PE). In this study, we aimed to investigate the correlation of Val66Met variation in BDNF and PE and further explore the possible relationship between Val66Met and neonatal poor prognosis. Methods: TaqMan probe fluorescent PCR was used to analyze the genotypic and allelic frequency of BDNF Val66Met in 1138 cases of the PE group and 1342 cases of the control group. Besides, 200 pairs pregnant women with PE and their newborn, along with 208 pairs healthy women and their newborn were enrolled to evaluate the mother-fetal transmission effect. Furthermore, we detected the expression level of BDNF in placental tissue at the RNA and protein levels in 21 PE patients and 21 healthy pregnant women. Results: We found that the allele distribution was significantly difference in case group and control group (2=4.657, P=0.031, OR=0.884, 95%CI=0.791-0.989), suggesting BDNF Val66Met may be related to PE susceptibility. While the genotype distribution and additive gene have no significant difference. The analysis of maternal-fetal pairing showed the transitivity of BDNF Val66Met have no significant difference between the case group and the control group. Besides, the mRNA and protein expression level of BDNF in PE group was significantly lower than that in control group. Conclusion: We found that BDNF Val66Met may be associated with the occurrence of PE, and allele G may play a protective role. However, this locus may be not related to the poor neonatal prognosis.

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Key words:

Preeclampsia; Brain-Derived Neurotrophic Factor; Gene expression; Gene polymorphism; Maternal-fetal pairing analysis

1.Introduction

Preeclampsia (PE) is a kind of pregnancy complications diagnosed with newly occurring hypertension and proteinuria after 20 gestation weeks[1], which is a primary reason that lead to the death of pregnant women, fetus and newborn and is also related to fetal growth retardation and premature births. As the pathogenesis of PE has not been fully interpreted, the current understanding is still based on abnormal placenta and inflammation[2]. Under normal physiological conditions, placentation is closely correlated with spiral artery formation and placental trophoblasts cells invasion. In the process of PE, when the adaptation process of

spiral arteries cannot be performed ideally, manifesting an obvious decline in the proportion of vascular remodeling which may result obstructive lesions. Besides, the insufficient trophoblastic invasion will cause the hypoxia and inflammation reaction, which in turn promote the trophoblastic apoptosis.

Under physiological conditions, placental trophoblast cells located at the maternal-fetal interface produce a variety of growth factors, such as neurotrophic factors[3]. Studies have indicated that neurenergen contributes to angiogenesis and energy homeostasis, probably playing a important role in early vascular development of fetus[4, 5]. Brain-derived neurotrophic factor (BDNF) , belonging to the member of neurotrophin family, plays a leading part in the formation of new vessels with the placental environment from the early phase of embryo development by binding the tyrosine kinase receptor B(TkrB)[6]. The TrkB signaling pathway is a new paracrine pathway that can regulate the growth of human trophoblast and promote trophoblast differentiation, proliferation and survival, so this signaling pathway plays an important role in the growth of human trophoblasts. Besides, BDNF can penetrate the maternal-fetal barrier, contributing to fetal phylogeny[7]. Previous study[8, 9] have demonstrated that there is an association between BDNF and PE. The level of expression of BDNF showed an obvious difference between PE pregnant women and healthy women[8, 10].

With the understanding of the PE pathogenesis, many researchers begin to focus on the important role of single-nucleotide polymorphism (SNP) in process of PE. Previous studies have indicated that certain candidate genetic loci SNPs associated with thrombosis, inflammation, oxidative stress and the renin angiotensin system[11, 12] are related to the occurrence of PE[13]. BDNF Val66Met (rs6265)(G to A) may cause transfer of amino acid residues from valine (Val) in position 66 (nucleotide 196) to Methionine (Met) at the anterior region of BDNF, which have a functional effect on the BDNF activity[14]. In this study, we focus on exploring the relationship between BDNF Val166Met site and occurrence of PE, meantime initially discuss the transmission from mother to infant of this SNP and the connection between BDNF Val66Met and new born poor prognosis associated with PE.

Subjects And Methods

Patients

2.1.1Peripheral blood collection

The research objects obtained from PE patients who were admitted from January 2014 to December 2017 to the obstetrics of public hospitals in Qingdao, Jinan, Binzhou and other regions. We used R i386 3.4.3 software to select 1138 cases as the case group and 1342 cases as the control group from PE sample bank, to explore the possible relationship between the SNP site Val166Met in *BDNF* and the occurrence of PE. At the same time, screening 200 pairs pregnant women with PE and their fetuses and 208 pairs healthy pregnant women and their fetus from pregnant women who admitted to the obstetrics department of Qingdao University Affiliated Hospital from January 2018 to June 2018. Collecting their peripheral blood to proceed the study of maternal-fetus paring.

Case group follow the diagnosis criteria of PE that were SBP[?]140mmHg, DBP[?]90mmHg, proteinuria [?]300mg/24h appearing after 20 gestation weeks[15]. Besides, cases group excluded following situations: gestational diabetes mellitus, thyroid disease, connective tissue diseases, hepatic and renal dysfunction and other complications and required the age of PE women over 25. The enrollment criteria of healthy control are as follows: (1) age[?]25 (2) gestation weeks [?]30 (3) normal blood pressure during pregnancy and no history of hypertension (4) normal result of blood and urine tests and normal hepatorenal function during the pregnancy (5) There is no other complications and some pathological conditions like placenta implantation, placenta previa, placental abruption, premature rupture of membranes and so on. This study was approved by the Ethics Committee of the Affiliated Hospital of Qingdao University and obtained the written informed consent from all participants.

2.1.2 Placenta collection

We screened the 21 PE patients and 21 healthy pregnant women who admitted to the Affiliated Hospital of Qingdao University from June 2017 to June 2018 to evaluate the expression level of BDNF. Placental

tissues were obtained from normal or PE pregnancies immediately after cesarean section. Square segments (estimate as 1×1×1 cm) were removed from maternal side and avoided parts of blood clots and infarction. The tissues were rinsed with saline, quickly snap-frozen in liquid nitrogen, and stored at -80 °C for further processing.

1. **Taqman real-time fluorescence quantitative PCR**
2. Use whole blood genomic DNA extraction kit to extract the peripheral blood DNA, then detect the DNA concentration and purity by ultraviolet spectrophotometer. Use designed probes to perform the fluorescence quantitative polymerase chain reaction (PCR). The PCR amplification volume is a total of 7ul, including 1 ul template DNA, 0.05 ul 20× Taqman probe stock solution, 4.2 ul autoclaved deionized water, 1.75ul 2×MIX. Amplification process using TIB-8600 fluorescence quantitative PCR instrument: denaturation at 95 for 10min, annealing at 95 for 15s, extension at 60 for 1min, 45 cycles, reading results after extension at 60. Performing the sequencing test to verify the accuracy of Taqman probe real-time PCR results.

2.3 Reverse transcription PCR

Total mRNA were extracted from placental tissue using Trizol method, and reversely transcribed into cDNA using TAKALA reverse transcription kit. Then, TransStart Tip Green qPCR SuperMIX amplification system and gene-specific oligonucleotide primers were used to quantify mRNA expression of BDNF by normalizing the β-action. Each experiment were performed at least for three times.

2.4 Statistical analysis

SPSS 24.0 software was used for statistical analysis. Genetic balance was analyzed by Hardy-Weinberg equilibrium test. It was considered that P> 0.05 indicated that the genotype distribution conformed to the HW balance; Independent sample t test and chi-square test were performed for statistical analysis of clinical data and experimental results. P <0.05 represents statistical significance. The 95% confidence interval (CI) and odds ratio (OR) were used for risk analysis.

3 Results

3.1 Clinical characteristics of PE and control groups.

By comparatively screening the clinical data of pregnancy women (such as BMI index, age, number of pregnancy and abortion, etc.), we enrolled 1138 cases as case group and 1342 cases as control group . There is no statistical difference in the above characteristics between the two groups (P > 0.05). However, the systolic and diastolic blood pressure were higher in the case group than in the control group, and the difference was statistically significant (P <0.05); The admission pregnancy week, gestation week to terminate pregnancy and fetal birth weight were significantly lower in the case group than in the control group (P <0.05). See Table 1.

3.2 Genetic analysis

The genotype distribution of the site conformed to genetic balance and had a better population representation through Hardy-Weinberg equilibrium test. As Table 2 showed,the locus allele of BDNF Val66Met was significantly different between the case group and the control group, and the difference was statistically significant(χ^2=4.657, P=0.031, OR=0.884, 95%CI=0.791-0.989), suggesting that allele G may play a protective role in the occurrence of PE. However, the genotype distribution is no significant difference(χ^2=4.555, P=0.103) between two groups. There was no significant difference between the two groups in additive gene analysis (χ^2 = 2.682, P = 0.102, OR = 0.856, 95% CI = 0.710-1.031).

3.3 Analysis of maternal-fetal paired single nucleotide polymorphisms

In order to figure out whether mother with Val66Met mutation will effect the SNP of fetus, we performed mother-fetal pairing to explore whether the site was transferred from mother to fetus. Tthe results of SNP analysis in paired pregnant women were basically consistent with the results obtained from large

samples. The difference between the BDNF gene Val66Met locus allele case group and the control group was statistically significant (χ2=9.054, P=0.003, OR=0.630, 95%CI=0.466-1.852). However, there was no significant difference in genotype (χ2 = 0.634, P = 0.728) and additive genes (χ2 = 0.477, P = 0.49, OR = 0.846, 95% CI = 0.525-1.362)(Table 3). Besides, Val66Met loci in BDNF gene of newborns in paired samples showed no significant difference in genotype distribution (χ^2 = 1.883, P = 0.390), alleles (χ^2 = 1.791, P = 0.181, OR = 1.208, 95% CI = 0.916-1.592) and additive genes (χ^2 = 1.407, P = 0.235, OR = 1.369, 95% CI = 0.814-2.304) between the two groups(Table 4).

We wondering whether Val166Met may have a potential impact on the occurrence of new born poor prognosis associated with PE. So, we performed pairing classification for maternal-fetal BDNF Val66Met locus phenotype. However, the results showed that there was no significant difference between the case group and the control group in the maternal-fetal transmission. (χ^2 = 4.896, P = 0.086). See Table 5.

3.4 The expression of BNDF was decreased in PE placentas

Finally, we compared the placental expression between PE and control group. The result of PT-PCR showed that BDNF mRNA in PE group was obviously decreased compared with control group (BDNF mRNA level: PE group vs. Control group, 0.6936±0.1472, n=21 vs. 1.350±0.2392, n=21, P=0.0245; Figure 1).

In accordance,the expression of BDNF protein in PE placentas was significantly lower than that in the control ones (p = 0.0011), as shown in Figure 2,3 .

4 Discussion

PE is a serious pregnancy complication that endangers the safety of mother and fetus and the quality of life. Though the exact pathogenesis of PE is still not clear, different pathogenesis finally showed a same pathological manifestations: poor placental perfusion and deficient trophoblast cells invasion, which eventually may result in placental dysfunction in the development of PE. Early blockage of trophoblasts may protect the embryo from high concentrations of oxygen. However, when the trophoblast cells are insufficiently infiltrated, the placenta will prematurely loss its blockage effect causing the occurrence of PE[16]. In recent years, genetics have also been considered as one of the causes of PE[17].

Neurotrophins are considered to be new pre-angiogenic factors that can affect endothelial cells and work through myeloid progenitor cells[18]. BDNF, as the one of member of Neurotrophins family, participates in neovascularization and contributes to the development of placenta through activating the expression of TrkB receptors[19]. The activated TrkB receptors conferred an antiapoptotic effect in the process of oxidative stress, contributing to resist adverse conditions[5]. Animal experiments[20]also showed that the BDNF / TrkB signaling system is implicated in embryo implantation, placental development and fetal growth. Before embryo implantation , BDNF can facilitate the growth of blastocyst and resist the apoptosis of embryonic cell during embryonic development[19]. Many studies proved there is a difference in the level of BDNF between PE and healthy pregnant women, suggesting that *BDNF* emits its effect in the occurrence and development of PE [5, 8, 21-23]. As a functional SNP in *BDNF* , Val66Met first described by Egan et al.[24] in 2003 and quickly regarded as a common*BDNF* mutation that can affect the functional expression of BDNF and suppress the transport and release of BDNF-containing vesicles in synapses[25]. In addition, MF et al. found cells transfected with Met alleles of BDNF can alter the activity-dependent secretion of BDNF[14]. This polymorphism has since been thought to be associated with many diseases, such as schizophrenia[14], neurodegenerative disease[26]and so on.

In this study, we first screened the matching samples from PE patients and analyzed the alleles of BDNF Val66Met locus in PE patients based on large sample research objects. Although there was no significant difference in genotypic distribution of BDNF Val66Met polymorphism in the PE group compared with the healthy control group, we found that the allele was significantly statistical difference. The ratio of allele G to A in PE group as a protective factor in the onset, suggesting that allele A may increase the risk of PE. This is the first description that BDNF Val66Met locus is associated with occurrence of PE. Besides, we detected the level of BDNF in the placenta tissue, and found a lower expression of BDNF in the placenta

of PE patients, further confirming the role of BDNF placental development and indicating the depression of BDNF may be related to the occurrence of PE. Vandita et al. [8]indicated maternal BDNF levels, cord BDNF levels and *BDNF* mRNA level are lower in PE group than control group in 61 PE pregnant women and 89 healthy women. They also found the marternal BDNF in case group have a statistical reduce in the another study[10] of 106 PE pregnant women and 95 healthy women. Kamini et.al[27]also have proved this result in a study that there are 72 PE women and 102 healthy pregnant women. These studies are consistent with our results. However, some reports have shown that the BDNF level are higher in PE group than the control group. J. Bienertova-Vasku et.al [23] measured BDNF levels of cord blood in 12 cases PE patients and 34 healthy controls, and suggested BDNF have an obvious increase in PE group. The BDNF levels in maternal plasma have also been observed to be elevated in 15 PE women in case group [5], inferred that small sample size may be the reason for the results that BDNF levels showed an increasing trend in case group. Therefore, these controversial results drive us to further investigate the role of *BDNF* in PE and explore probable reason for the low BDNF expression of PE placentas.

Then, we conducted a mother-fetal pairing study to further explore the mother-to-fetal transmission of BDNF Val66Met and anticipate to find out whether this variants is associated with the poor neonatal prognosis related to PE. As we all known, PE not only threatens the life and health of pregnant women, but also it has a severe adverse prognosis for newborns. By large sample clinical data analysis, we found pregnant women with PE have lower gestational age and birth weight of fetus. The higher incidence of premature birth and lower birth weight will necessarily affect newborn quality of life and survival. BDNF can act on embryos TrkB in preimplantation, promoting early embryo development and inhibiting embryo apoptosis in a paracrine / autocrine manner[28]. BDNF can play an important role in placental development and fetal growth through the TrkB signaling system and penetrate the uterus-placenta barrier in animal models[7, 29]. Here, we analyzed the BDNF Val66Met polymorphism of the newborn, and performed mother-fetal pairing to analyze the transmission trend of the allele between the mother and the fetus. However, the results showed that there was no significant difference in the BDNF Val66Met allele and genotype between the two groups, and there was no statistical difference in maternal-fetal transmission of the locus between the two groups, suggesting the Val66Met variant carrying by women with PE dose not transfer to her fetus. Therefore, we can concluded that the poor prognosis of neonates associated with PE may not be related to fetus' Val66Met polymorphism and we still need to do related research to explore the possible mechanism.

In this study, we found that the SNP at the Val66Met site of *BDNF* may be related to PE susceptibility. The pregnant women occurring G to A mutation at position 196 have a higher PE susceptibility. Besides, we also proved that BDNF Val66Met may be not related to the poor prognosis of neonates associated with preeclampsia. Therefore, we can conclude that the SNP at the Val66Met may have a potential impact in the occurrence of PE, which provide a possible direction for the treatment,while the reason for poor prognosis of neonates associated with PE still need further study.

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Conflicts of Interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

Authorship

Shiguo Liu designed this experiment. Chengcheng Guan and Meiyan Zhang conducted this experiment and wrote the body of this manuscript. Lu Zhang read and collected much literature about this manuscript. Bo Hou, Yan Zhang and Hong Jiang reviewed and corrected this manuscript.

Ethics statement

The present study was approved at 2017.12 by the ethics committee of the Affiliated Hospital of Qingdao

University.The ethic number is qdfy-302276.

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Table 1 Analysis of clinical data of case group and control group

Index	Case group (N=1138)	Control group(N=1342)	P value
BMI index(kg/m^2)	23.99±2.64	24.00±3.00	0.942
age(year)	31.60±5.52	31.67±4.56	0.741
gravidity(times)	2.58±1.38	2.56±1.31	0.710
Number of abortions(times)	1.40±0.55	1.50±0.54	0.751
Systolic pressure(mmHg)	163.52±18.68	117.15±10.33	0.000[a]
Diastolic pressure(mmHg)	105.77±12.62	74.27±7.49	0.000[a]
Admission pregnancy week(week)	34.88±3.88	39.16±1.36	0.000[a]
Gestation week(week)	35.57±3.38	39.25±1.24	0.000[a]
Fetal birth weight(g)	2468.44±757.83	3383.79±1.24	0.000[a]

Note: [a] means the difference is statistically significant, P <0.05

Table 2 Analysis of Val66Met locus of BDNF gene in large sample PE group and control group

Genotype	Case group(N=1138)	Control group(N=1342)	χ^2	P	OR	95%CI
AA	281	294				
AG	556	648				
GG	301	400	4.555	0.103		
Additive gene						
AA	281	294				
AG+GG	857	1048	2.682	0.102	0.856	0.710-1.031
allele						
A	1118	1236				
G	1158	1448	4.657	0.031[a]	0.884	0.791-0.989

Note: [a] indicates that the difference is statistically significant, P <0.05.

Table 3 Analysis of Val66Met loci of BDNF gene of maternal cases group and control groups in paired samples

Genotype	Case group	Control group	χ^2	P	OR	95%CI
AA	45	41				
AG	198	102				
GG	57	65	0.634	0.728		
Additive gene						
AA	41	45				
AG+GG	167	155	0.477	0.49	0.846	0.525-1.362
Allele						
A	108	184				
G	216	232	9.054	0.003[a]	0.630	0.466-0.852

Note: [a] indicates that the difference is statistically significant, P <0.05

Table 4 Analysis of Val66Met loci of BDNF gene of newborn cases group and control groups in paired samples

Genotype	Case group	Control group	χ^2	P	OR	95%CI
AA	30	39				
AG	102	103				
GG	76	66	1.883	0.390		
Additive gene						
AA	30	39				
AG+GG	178	169	1.407	0.235	1.369	0.814-2.304
Allele						
A	162	254				
G	181	235	1.791	0.181	1.208	0.916-1.592

Table 5 Analysis of Maternal-fetal Transmission of BDNF Gene Val66Met

Transmit type	Case group	Case group	χ^2	P
AA-AA/GG-GG	33	48		
AA-AG/GG-AG	100	83		

AG-AG	67	77	4.896	0.086

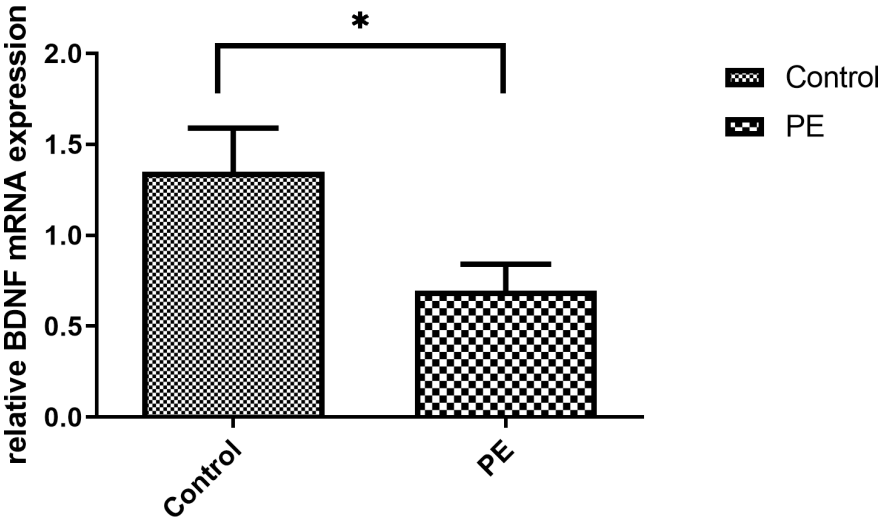

Figure 1 Comparison of BDNF mRNA expression level between PE group and control group

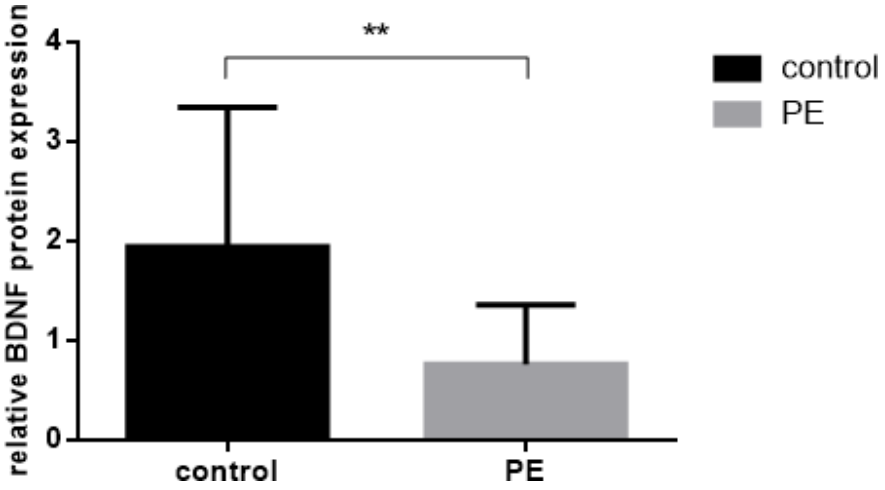

Figure 2 Analysis of BDNF protein expression levels in PE and control groups

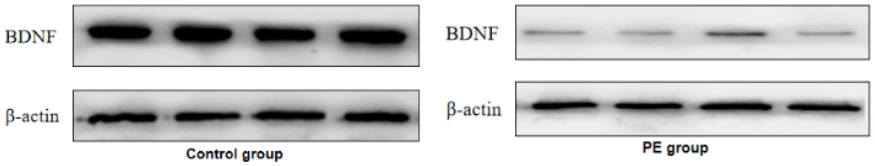

Figure 3 Analysis of BDNF protein expression levels in PE and control groups