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Original Article

The genetic analysis of β2 adrenergic receptor gene polymorphisms and preterm birth risk in a Chinese population

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Abstract: Background: Preterm birth is a leading cause of perinatal morbidity and death with sequelae in adult. Genetic factors are proved to play an important role in the onset of preterm birth. In this study, the association of β2 adrenergic receptor gene (*ADRB2*) polymorphisms with preterm birth was investigated in a Chinese population. Methods: 104 premature infants and 117 normal infants were included and were genotyped by the method of ploymerase chain reaction (PCR) based on *ADRB2* rs1042713, rs1042714 polymorphisms. Hardy-Weinberg equilibrium (HWE) was detected by χ^2 test in the control group. The frequencies of genotype, allele and haplotype between two groups were also compared using χ^2 test as well as the basic indexes of subjects. Linkage disequilibrium (LD) of two polymorphisms was analyzed with Haploview software. Odds ratio (OR) with 95% confidence interval (95% CI) was calculated to show the relative risk of preterm birth. Results: The heterozygous genotype CG of rs1042714 in cases accounted for lower ratio than that in controls (*P*=0.03), but not homozygous GG genotype. What's more, rs1042714 also showed the significant association with preterm birth risk under the model of dominance and allele (dominance: OR=0.52, *P*=0.03; allele: OR=0.59, *P*=0.04). Two polymorphisms of *ADRB2* existed the LD (D'=1.0) and haplotype G-G decreased the occurrence risk of preterm birth (OR=0.58, *P*=0.04). Conclusion: *ADRB2* rs1042714 polymorphism contributes to the susceptibility to preterm birth in Chinese population, but not rs1042713. And these two polymorphisms existed the interaction.

Keywords: *ADRB2*, polymorphisms, preterm birth, haplotype

Introduction

Preterm birth is a common and complex pregnancy complication, which is a primary cause of perinatal infant morbidity and death [1, 2]. The mortality of infants is about 3.1 million annually in the world and preterm birth complications account for 35% of causes [3]. In clinical, preterm birth is divided into two types: controlled and spontaneous preterm birth. The former is mainly caused by infection, preeclampsia, fetal distress, gestational diabetes and hypertension and so on [4-6] and the latter is the most predominant type with a sudden onset of labor [7]. Epidemiological investigation shows that it is multifactorial disease and the ethology still remain unknown [8]. Genetic and environmental factors are paid attention widely in the developmental of preterm birth. The latter has been proved, such as maternal age, education level and infection, but the role is weak. The genetic factors are not ignored and single nucleotide polymorphism (SNP) as a effective means is used to explore the pathology of disease [9].

The β2 adrenergic receptor (ADRB2) is a member of ADRBs subfamily, belonging to G protein-coupled receptors superfamily [10]. It is expressed ubiquitously in human tissues and organs, including the smooth muscle of the cervix and uterine corpus [11, 12]. The stimulation of ADRB2 by endogenous and exogenous agonists causes smooth muscle relaxation, which may affect cervical resistance to mechanical distension [13]. Study in vitro shows that ADRB2 stimulation plays an inhibitory role in cervical contractile activity [14]. ADRB2 receptor protein is encoded by *ADRB2* gene located on chromosome 5q31-32 with the length of 1.8 kb [15]. It

Table 1. The detailed information of study subjects

Index		N (%)		*P*
		Premature infant	Control	
Total/n		104	117	
Infant gender	Boys	61 (58.7)	65 (55.6)	>0.05
	Girls	43 (41.3)	52 (44.4)	
Birth weight/g	Mean value	2413.6±589.1	3684.3±478.5	<0.01
Maternal age	Mean age	27.4±4.1	26.8±2.9	>0.05
Mother's BMI	Pre-pregnancy	23.8±5.2	23.5±4.7	>0.05
Mother's education/years	≤12	41 (39.4)	29 (24.8)	<0.05
	>12	63 (60.6)	88 (75.2)	
GDM	Yes	13 (12.5)	11 (9.4)	>0.05
	No	91 (87.5)	106 (90.6)	
Family history	Yes	16 (15.4)	7 (6.0)	<0.05
	No	88 (84.6)	110 (94.0)	
Delivery history	0	51 (49.0)	63 (53.8)	>0.05
	1	39 (37.5)	43 (36.8)	
	≥2	14 (13.5)	11 (9.4)	

Note: BIM: body mass index; GDM: gestational diabetes mellitus.

only consists of exons and included multiple SNPs. Among them, two missense mutations Arg16Gly (rs1042713) and Gln27Glu (rs1042714) are studied and proved to be involved in various diseases, such as asthma, ischemic stroke, hypertensive [16-18]. However, these two polymorphims in *ADRB2* are rarely studied the association with preterm birth in the Chinese population.

Therefore, in present study, the correlation of *ADRB2* rs1042713, rs1042714 polymorphisms with preterm birth risk were explored and the linkage of these two polymorphisms was also analyzed to explain the etiology of preterm birth.

Materials and methods

Study population

This study adopted a population-based case-control design, 104 premature infants and 117 full-term infants were selected from the obstetrical department of Guizhou People's Hospital as the case and control groups from May, 2012 to August, 2013. Preterm birth was defined that gestational weeks of live births at normal delivery were more than 28 weeks but less than 37 weeks, and twins, polyembryony were excluded. Furthermore, all cases belonged to idiopathic preterm birth. In the control group, gestational weeks of infants were no less than 37 weeks and the date of birth difference with the cases was not more than 3 days, meanwhile, the mother's age difference was less than 4 years. They were all Chinese Han population without any blood relationship to each other. This proposal was supported by the Ethics Committee of Guizhou People's Hospital and the subjects' parents signed the written consent before collecting sample.

We also surveyed the basic characteristics of all mothers and infants using unified questionnaire and recorded the detailed information into the Excel form. The investigation content included infants' sex, birth weight and mother's age, body mass index (BMI) (pre-pregnancy), education level, delivery history and family history of preterm birth and so on, which uniformly summarized in **Table 1**.

Sample collecting and DNA extraction

10 ml umbilical cord blood were collected from every subject and put into blood collection tube with EDTA-2Na anticoagulation. Genomic DNA was extracted using blood genomic DNA extraction Kit purchased by TIANGEN BIOTECH (BEIJING) CO., LTD, according to the manufacturer's instructions. The samples were stored at -20°C.

Table 2. The genotype and allele distributions of *ADRB2* polymorphisms between the case and control groups

SNPs	MAF (%)		OR (95% CI)	*P*	Genotype frequency (%)			OR (95% CI)	*P*	P_{HWE}
	Case	Control				Case	Control			
rs1042713 A>G	35.50	44.02	0.76 [0.52, 1.12]	0.16	AA	39.42	28.20	1.00 [Ref.]	-	0.17
					AG	46.16	55.56	0.59 [0.33, 1.07]	0.08	
					GG	14.42	16.24	0.64 [0.28, 1.44]	0.28	
					AG/GG	60.58	71.80	0.60 [0.34, 1.06]	0.08	
rs1042714 C>G	12.98	20.09	0.59 [0.35-0.99]	0.04	CC	76.92	63.25	1.00 [Ref.]	-	0.68
					CG	20.19	33.33	0.50 [0.27, 0.92]	0.03	
					GG	2.89	3.42	0.69 [0.15, 3.20]	0.64	
					CG/GG	23.08	36.75	0.52 [0.29, 0.93]	0.03	

Note: SNP: single nucleotide polymorphism; MAF: minor allele frequency; HWE: Hardy-Weinberg equilibrium.

Genotyping

The genotyping of *ADRB2* rs1042713, rs1042714 polymorphisms was conducted in the cases and controls by ploymerase chain reaction (PCR) and sequencing. Firstly, PCR primers were designed using Primer Premier 5.0 software according to *ADRB2* gene sequences extracted from GeneBank of NCBI website and were synthesized in Shanghai Sangon Biotech Co., Ltd. The primer sequences were as follows: 5'CACGCAGCAAAGGGACGAG3' (forward), 5'CACAGGCCAGTGAAGTGATGAA3' (reverse). The PCR system was a volume of 25 μl solution, consisting of 1.0 μl template DNA, each 0.5 μl of forward and reverse primers, 12.5 μl PCR Master Mix and 10.5 μl aseptic ddH_2O. PCR amplification condition was as follows: initial denaturation at 95°C for 3 min; 35 cycles of denaturation at 94°C for 30s, annealing at 62°C for 45 s, extension at 72°C for 30 s; and final extension at 72°C for 7min and stored at 4°C. The quality and concentration of PCR amplification products were checked using 1.0% agarose gel electrophoresis and NanoDrop 2000c NanoVue Plus (Thermo Scientific, America).

The applicable PCR products were sequenced in Shanghai Sangon Biotech Co., Ltd to determine the genotypes of *ADRB2* two polymorphisms in a total of 221 subjects.

Statistical analysis

The comparisons of all data were conducted using PASW Statistics 18.0 software and all data were showed in this article in the form of $\bar{x}$±s and %. Firstly, the genotype distributions of *ADRB2* polymorphisms in the control group were detected whether conformed to Hardy-Weinberg equilibrium (HWE) by chi-square test. What's more, the χ^2 test was also used to compare the differences of genotype, allele and haplotype as well as the basic indexes of subjects between the case and control groups. The linkage disequilibrium (LD) between *ADRB2* polymorphisms was tested by Haploview software. The relative risk of preterm birth was represented with odds ratio (OR) with 95% confidence interval (95% CI). $P<0.05$ is a standard to determine the significant difference.

Results

The basic characteristics of study population

The detailed information of included infants and their mothers were displayed in **Table 1**. A total of 221 infants were enrolled, including 104 premature infants and 117 full-term infants. In the former, boys and girls accounted for 58.7% and 41.3% respectively and these percentages were 55.6% and 44.4% in the latter, which showed that there was no significant difference between two groups by gender. But the premature infants had the obviously lower birth weight than the controls ($P<0.05$). Mother's age, BMI (pre-pregnancy) and delivery history was no significantly associated with preterm birth in this study population and whether mother suffered from gestational diabetes mellitus or not had no influence on preterm birth ($P>0.05$). However, the mother's education level impacted on preterm birth and mother with a low education easily occurred preterm birth ($P<0.05$). Family history was also a risk factor for the onset of preterm birth ($P<0.05$).

Table 3. The haplotype analysis of *ADRB2* polymorphisms based on preterm birth

rs1042713-rs1042714	Haplotype frequency (%)		OR (95% CI)	*P*
	Case	Control		
A-C	62.50	55.98	1.00 [Ref.]	-
G-C	24.52	23.93	0.92 [0.59, 1.44]	0.71
G-G	12.98	20.09	0.58 [0.34, 0.99]	0.04

The genotypes and allele distributions ADRB2 ploymorphisms between two groups and the genetic association with preterm birth

The genotype and allele frequencies of *ADRB2* rs1042703, rs1042714 polymorphisms in two groups were showed in **Table 2**. Firstly, the genotype distribution of two polymorphisms both conformed to HWE in the control group (*P*>0.05). The minor allele frequencies of rs1042713 were 35.50% and 44.02% in cases and controls and there was no marked difference between two groups (*P*>0.05). Similarly, there was no significant difference among the genotypes of rs1042713 in two groups (*P*>0.05). Referring to rs1042714, we detected the obvious difference between the frequency of minor allele (12.98% & 20.09%, *P*=0.04). The heterozygous genotype CG in cases showed the significantly lower frequency than that in controls, and so was genotype with G allele (*P*<0.05). Therefore, people carrying CG genotype, G allele and genotype with G allele had the protective roles in resisting the occurrence of preterm birth (G vs. C: OR=0.59, 95% CI=0.35-0.99; CG vs. CC: OR=0.50, 95% CI=0.27-0.92; CG/GG vs. CC: OR=0.52, 95% CI=0.29-0.93).

The haplotype analysis of ADRB2 polymorphisms in preterm birth

Through the analysis of Haplotype software, we found the LD between *ADRB* rs10427-13, rs1042714 polymorphisms (D'=1.0, r^2= 0.159), which was consisted of three haplotypes, respectively A-C, G-C, G-G (**Table 3**). Their frequencies in the case and control groups were 62.50%, 24.52%, 12.98% and 55.98%, 23.93%, 20.09% respectively. G-G haplotype in cases was lower frequency than that in controls and may be a protective factor to against the onset of preterm birth (*P*=0.04, OR=0.58, 95% CI=0.34-0.99).

Discussion

According to the research data at home and abroad, preterm birth is a leading cause of perinatal onset and death with a creasing incidence rate in China annually. All the time, although the morbidity of preterm birth in China is lower than that in developed countries, such as America [3], due to the big population base, the number of premature infants is large in our country, which causes the heavy burden for family and even society. Recently, with the popularization of intensive care unit in newborns, such as perinatal care, mechanical ventilation, nutritional therapy, the survival rate of premature infants obviously increase, but the survivors usually carry the serious sequela in the nerve system [19, 20]. Therefore, exploring the etiology of preterm birth is an extremely important to timely find the susceptible population. As a multifactorial disease, preterm birth is influenced by genetic and environmental factors based on previous study [21].

Multiple environmental factors are associated with the development of preterm birth, which is proved by epidemiology. The influence factors of preterm birth among different countries and regions are not alike completely, but the ensure factors include the income of parents, maternal age, psychology and clinical factors. The study of Auger *et al.* find that gestational age and education level are related with preterm birth, that is, advanced maternal age with low education has a higher morbidity of preterm birth than that eligible maternal age with high education [22]. Clinical factors of gravidae accounts for a majority of preterm birth, especially intrauterine infection [23]. However, even though environmental factors are controlled, black women are 3.5 times higher risk to suffering from idiopathic preterm birth than white [24]. What's more, premature birth may occur in 80% of pregnant women who have the family history of preterm birth or are premature infants [25]. Therefore, genetic factors play a fatal role in the onset of preterm birth.

ADRB2 belongs to G protein-coupled receptor family and contains 413 amino acid residues. It is expressed in uterus, blood vessel, bronchia and hepar. ADRB2 stimulation of myometrium

activates adenylate cyclase to translate triphosadenine into cyclic adenosine monophosphate and then decreases the activity of myogen light-chain kinase and calcium concentration, which causes intramuscular spectrin loss of its function to inhibit uterine contraction. Meanwhile, the spasmolysis of vascular smooth muscle and the increasing blood flow of placenta result in decreased blood pressure, increased pulse pressure and improving the oxygen supply in utero. So far, research data show that *ADRB2* in humans exists genetic polymorphism, especially SNPs and they affect the biological characteristics of acceptor through modifying the structure of *ADRB2* gene or expression level of ADRB2 protein [26]. In code region of *ADRB2*, 9 mutations have been found. Among them, the common mutation loci are the 46th, 79th, which leads to the mutation of the 16th, 27th amino acids in ADRB2 protein. These two SNPs have been studies in a variety of diseases, but the effect on preterm birth is rarely paid attention, especially in Chinese population.

In present study, we revealed the relationship of *ADRB2* rs1042713, rs1042714 polymorphisms with preterm birth susceptibility in a Chinese Han population. Firstly, the basic characteristics of relative subjects were recorded and analyzed between the case and control groups. The results showed that mother's education and family history were the risk factors for preterm birth in this study population, but not mother's BIM, GMD and delivery history, what's more, the birth weight of premature infants was markedly lower than that of full-term infants. This is consistent with the previous study [25, 27]. *ADRB2* rs1042714 polymorphism was detected a protective role to against the occurrence of preterm birth, but the other polymorphism rs1042713 was not significantly associated with preterm birth. However, this results were inconsistent with the studies of Miller *et al.* and Suh *et al.* in Korean women [13, 28]. This discrepant result may be result from the different distribution of gene polymorphism and preterm birth in different races, small study population. In addition, there was the LD between rs1042713, rs1042714 polymorphisms and G-G haplotype decreased the risk of preterm birth in carriers.

All in all, preterm birth is an important factor to affect the quality of newborns and genetic factors play a fetal role in development of preterm birth. Although we obtained some achievements in present study for explaining the etiology of preterm birth, it is far away from clarifying the pathomechanism. Therefore, more relative studies are needed with well-designed and enough large sample size in the future.

Disclosure of conflict of interest

None.

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