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Original Article

Genetic association of *PON2* polymorphisms with susceptibility to preterm birth

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Abstract: Target: The objective of this study was to investigate the association of paraoxonase 2 (*PON2*) Ala148Gly (A148G) and Ser311Cys (S311C) polymorphisms with the susceptibility to preterm birth. Methods: In this case-control study, we genotyped *PON2* A148G and S311C polymorphisms of 110 premature infants and 121 normal infants using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The control group was matched with the case group by maternal gestational age. The distribution differences of genotypes and alleles between the two groups were checked by χ^2 test which was also used to evaluate the Hardy-Weinberg equilibrium (HWE). Odds ratio (OR) and 95% confidence interval (CI) were used to represent the relative risk of preterm birth. Results: The genotypes distributions of *PON2* A148G and S311C polymorphisms in the control group accorded with HWE. The genotype frequency of AA in A148G polymorphism was significantly higher in cases, compared with AG/GG (P=0.011); CC genotype of S311C polymorphism in infant obviously increased the risk of preterm birth and its genotype frequency was 2.169 times higher than the common genotype SS (OR=2.169, 95% CI=1.016-4.630). What's more, birth weight was associated with preterm birth and A148G polymorphism had an influence on birth weight obviously (P<0.05). Conclusions: In *PON2* gene, AA common genotype of A148G polymorphism and CC mutant genotype of S311C were associated with the risk of preterm birth development. In addition, A148G polymorphism plays an important in infant birth weight, but not S311C.

Keywords: Paraoxonase 2 (*PON2*), polymorphism, preterm birth

Introduction

Preterm birth is the leading cause of perinatal morbidity and mortality currently [1]. At present stage, about 70% of the neonatal diseases in the perinatal period occur in premature infants [2]. Thus the viability of infants are weak and they may be sick or disabled with various complications, which leads to high mortality, in addition, these effects on health can continue to adulthood [3, 4]. Over the past 20 years, regardless of many measures have been taken to prevent preterm birth, the achievement is unsatisfactory [5]. Epidemiological data revealed that preterm birth was related to various factors, such as reproductive history, lifestyle, psychological states, pregnant infection and heredity [6-9].

Therefore, exploring the pathology and etiology of preterm birth and preventing the occurrence of preterm birth to improve the population quality of the newborn are important directions of the current perinatology researches. However, at present, researches on the etiology of premature mainly focus on environmental factors and clinical factors, and the impact of genetic factors has been rarely studied.

Paraoxonase (PON) is a group of enzymes that are synthesized by the liver and combined with high density lipoprotein cholesterol (HDL-C). They are encoded by *PON* genes located in human chromosome 7q21.3-22.1, including *PON1*, *PON2* and *PON3* with a close homology in structure [10, 11]. Among of them, *PON2* contains 9 exons and 8 introns and has various types of mRNA [12]. There are two common polymorphisms of *PON2* in the coding area: A148G polymorphism is the replacement of alanine (A) with glycine (G) in codon 148 of intron 5, and serine (S) of S311C in codon 311 of exon

Table 1. Primer sequences for *PON2* A148G and S311C polymorphisms amplification

Locus		Primer sequence	Length
A148G	Forward	5'-CAACCCACCATAGGGATTGTTTG-3'	153 bp
	Reverse	5'-TATATACAGTGGAAATTTTTAAATTTGAAGCAG-3'	
S311C	Forward	5'-ACAAGGCTCTGTGGTATAAAGTGCC-3'	197 bp
	Reverse	5'-GTGACATGCATGTACGGTGGTCT-3'	

9 is replaced by cysteine (C). *PON2* expresses in many tissues, such as in brain, liver, kidney, heart, lung and testicle [13, 14]. In 1999, Busch et al. found that the G148A polymorphism of paraoxonase 2 (*PON2*) was significantly associated with low birth weight of the newborns [15]. It also has been found that *PON2* may be connected with preterm birth [16].

Therefore, this study aimed to investigate the relationship between *PON2* A148G and S311C polymorphisms and the risk of preterm birth in infants based on Chinese Han population.

Materials and methods

Detailed information of study subjects

In this case-control study, a total of 231 effective mother-newborn pairs were selected as study population. 110 of them were premature birth and the other were normal birth as the case and control groups, respectively. The cases and controls all hospitalized in the maternity department of Jinan Maternity and Child Health Care Hospital during October, 2013 and December, 2014. The related screening standards were as follows: (1) The preterm infants, whose parents of were living and well and accepted the investigation, were live births delivered under normal circumstances. They were not twins or multiples, and possibilities of artificial labor or accidental premature delivery were excluded. (2) The controls were delivered after 37 weeks of gestation without drug treatment in childbirth. The age difference between preterm and mature parturients was less than 4 years. The full-term infants were born in the same midwifery station with the preterm infants, and the birth date difference was less than 3 days. (3) Prolonged pregnancy parturient that had 42 weeks of gestation or more but were still not in labor were not enrolled in this study.

This study was approved by the Ethics Committee of Jinan Maternity and Child Health Care Hospital. The sample collection process was conducted under the national human genome research ethics guidelines. All of subjects were aware of the study contents and they offered their informed consent forms.

Questionnaire survey

A group of doctors who had been unified trained interviewed all enrolled parturients through face to face using unified questionnaires. The research content included: physical condition, daily life style, general healthy status and disease history, living circumstance, diet habit, reproductive history, menstruation and occupational history.

The birth weight of new infants was weighed using an electronic scale within an hour of their birth. The gestational weeks were calculated according to the date of the last menstrual period and the delivery date of the parturient and the birth records were noted thoroughly.

DNA extraction and genotyping

10 ml umbilical cord blood were collected from all enrolled infants and put into anticoagulative tubes with ethylenediamine tetraacetic acid tetrasodium salt (EDTA-Na4). The genome DNA was extracted with the whole blood genomic DNA kits.

The *PON2* A148G and S311C polymorphisms were genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The primers used for specific amplification were designed by Primer5.0 software and synthesized in Sangon Biotech (Shanghai) Co., LTD. The primer sequences were listed in **Table 1**.

The genome DNA was amplified in 20 μl PCR reaction systems, including template DNA 5 μl, 10×PCR buffer 1 μl, $MgCl_2$ 1 μl, each 1μl of forward and reverse primers, dNTPs 1 μl, Taq DNA polymerase 1 μl and sterilized deionized water 9 μl.

The PCR reaction conditions started with 15 min initial denaturation at 94°C, followed by 35 cycles of 45 s degeneration at 94°C, 45 s annealing at 58°C, 30 s extension at 72°C and finally the reaction was ended with 5 min extension at 72°C.

Table 2. General features of the parturient and infants

General feature		Case, n=110 (%)	Control, n=121 (%)	*P*
Parturient				
Age (x ± s)		26.9±5.81	25.1±5.55	*P*>0.05
Pregravidic body mass index (kg/m^2)		21.57±1.62	22.05±1.33	*P*>0.05
Reproductive history	No	98 (89.09)	107 (88.43)	*P*>0.05
	Yes	12 (10.91)	14 (11.57)	
Degree of education	Primary school or below	30 (27.27)	43 (35.54)	*P*>0.05
	Middle school	57 (51.82)	58 (47.93)	
	High school or above	23 (20.91)	20 (16.53)	
Infants				
Birth weight (x ± s)		2015.98±721.23	3311.35±435.15	*P*<0.05
Gender	Boy	57 (51.82)	62 (51.24)	*P*>0.05
	Girl	53 (48.18)	59 (48.76)	

Table 3. *PON2* A148G and S311C polymorphisms genotype and allele distributions

Genotype/Allele	Case, n=110 (%)	Control, n=121 (%)	χ^2 (*P*)	OR (95% CI)
A148G				
AG + GG	28 (25.45)	50 (41.32)	-	1.000 (Ref.)
AA	82 (74.55)	71 (58.68)	6.487 (0.011)	2.062 (1.177-3.615)
S311C				
SS	49 (72.73)	62 (56.20)	-	1.000 (Ref.)
SC	37 (25.45)	45 (33.88)	0.018 (0.893)	1.040 (0.586-1.847)
CC	24 (1.82)	14 (9.92)	4.095 (0.043)	2.169 (1.016-4.630)
S	135 (85.45)	169 (73.14)	-	1.000 (Ref.)
C	85 (14.55)	73 (26.86)	3.675 (0.055)	1.458 (0.991-2.144)

The A148G polymorphism of *PON2* presented a 153 bp fragment after the amplification. 2U restriction enzyme Fnu4 HI was used to digest PCR products in incubator at 37°C for 15 h. The enzyme-digested products were separated by 2.5% agarose gel electrophoresis. After that, the genotype determination process was conducted under ultraviolet lamp. In A148G polymorphism, the products were separated into 123 bp in AA wild homozygote, 153 bp in GG mutant homozygote and 123 bp, 153 bp in AG heterozygote.

The amplification fragment length of *PON2* S311C polymorphism was 197 bp. It was digested by 0.2 μl restriction enzyme Dde I n incubator at 37°C for 15 h. Then the enzyme-digested products were put into 3% agarose gel (including 0.5 μg/ml ethidium bromide) for 30 min electrophoresis at 250 V voltage. There were three genotypes in S311C polymorphism: SS homozygote with two stripes of 70 bp and 78 bp, CC homozygote with a stripe of 148 bp and CS heterozygote with three stripes of 70 bp, 78 bp and 148 bp.

Statistical analysis methods

We set up a database with Statistical Package for the Social Science (SPSS 18) to manage and statistically analyze the data. The measurement data were presented with x ± s and compared by t test. The enumeration data were presented as n (%) and compared by χ^2 test. Hardy-Weinberg equilibrium (HWE) in controls, genotype frequencies and allele frequencies in case and control groups were tested by χ^2 test, with statistical significance when *P*<0.05. The relative risk of preterm birth was represented by odds ratio (OR) and 95% confidence interval (CI).

Results

General status of the cases and controls

The general status of the parturient and newborns are given in **Table 2**. It seemed that there were 110 newborns in the case group (gestational age <37 weeks), including 57 boys (51.82%) and 53 girls (48.18%). Their average

Table 4. The genotypes distributions of *PON2* A148G, S311C polymorphisms based on birth weight of infants in two groups

Index	Birth weight		Case, n=110	Control, n=121	*P*	OR (95% CI)
A148G	+	+	17	28	-	1.000 (Ref.)
	+	-	12	18	0.846	1.098 (0.426-2.831)
	-	+	35	41	0.374	1.406 (0.662-2.985)
	-	-	46	34	0.034	2.228 (1.055-4.709)
S311C	-	+	29	40	-	1.000 (Ref.)
	-	-	20	22	0.565	1.254 (0.580-2.712)
	+	+	34	37	0.486	1.267 (0.650-2.470)
	+	-	27	22	0.161	1.693 (0.809-3.543)

Note: In the second column, "+" and "-" represent mutant and wild genotypes, respectively; "+" of the third column represent the birth weight ≥2500 g, "-" represent the birth weight <2500 g.

birth weight was 2015.98±721.23 g. In control group (gestational age ≥37 weeks), there were 121 newborns with 62 boys (51.24%) and 59 girls (48.76%). The average birth weight of the babies in control group was 3311.35±435.15 g. The ratio of the infants in case and control groups by sex was consistent (girl to boy as 1:1.08) and the difference was not statistically significant (P>0.05). But the difference of birth weight between two groups were statistically significant (P<0.05).

The gestational age status, average progestational BMI and reproductive history of the two groups were consistent and the differences had no statistical significance (P>0.05). Mother's education level was 27.27% of primary school or below, 51.82% of middle school and 20.91% of high school or above in cases. The control group was 35.54% of primary school or below, 47.93% of middle school and 16.53% of high school and the different was not significant in two groups (P>0.05).

HWE test

Analysis on the genotype and allele frequencies of PON2 A148G and S311C polymorphisms in case and control groups demonstrated that the genotype distributions in the control group were to be in accordance with HWE. And the result showed that the study samples could represent the general population.

Effects of PON2 polymorphisms on preterm birth

The AA genotype frequency of *PON2* A148G polymorphism was obviously higher in the case than control groups (74.55%, 58.68%) and the difference was significant (P=0.011), therefore, A148G polymorphism was related to the increased risk of preterm birth (OR=1.177, 95% CI=1.177-3.615). The results were listed in **Table 3**. Similarly, CC genotype also increased 1.169 times risk of preterm birth development, compared with the common genotype SS (OR=2.169, 95% CI=1.016-4.630). In addition, *PON2* A148G polymorphism was also found to be associated with the birth weight of new infants, but not S311C (OR=2.228, 95% CI=1.055-4.709) (**Table 4**).

Discussion

Preterm birth is defined as delivery with less than 37 weeks gestation by the world health organization (WHO). The mortality of preterm infants is 12~17 times higher than that of the normal new infants, except deformity, 75% of the perinatal deaths are related to preterm birth [17]. Those survival preterm infants also face many health problems, such as intraventricular hemorrhage, cerebral white matter damage and even cerebral palsy, progressive retardation, visual and auditory defects. Even though the progress of neonatal diagnosis and treatment technology largely improve the living status of preterm infants, the incidence of preterm birth is rising steadily. Related studies have also found that not all pregnant women exposed to the risk factors will occur preterm birth, which manifested that hereditary factor plays a role which don't be ignored in the process of preterm birth [18].

Encoding product of *PON2* gene is an intracellular protein that is widely expressed, containing two common polymorphisms. The replacement of G with C allele leads to the transformation from Ala to Gly in codon 148, namely A148G. And the replacement of Ser with Cys in codon 311, namely S311C. These two polymorphisms have been reported to be involved in various diseases. Janka et al. discover that the interaction between Ser311Ser homozygote of *PON2* and *EPOE4* gene had certain influences on progressive dementia and cerebral ischemic dementia. Liang et al. researched 194 mother-newborn pairs and found that newborns with I48AA and 311SS genotypes of *PON2* had obviously increased risk of preterm birth compared

to those with other genotypes [19]. The genetic polymorphisms of *PON2* are associated with many pathological and physiological states.

In present study, we discussed the relationship between *PON2* A148G and S311C polymorphisms and the susceptibility to preterm birth development. Through the analysis of genotype frequencies in 231 mother-newborn pairs, we discovered that compared to the mutant GG homozygote/AG heterozygote, the common AA homozygote was associated with preterm birth and might be an independent risk factor for the development of disease. In the contrary, CC mutant genotype in S311C polymorphism showed the relevance with preterm birth in new infants. In addition, *PON2* A148G polymorphism was found to be associated with the birth weight of new infants, this conclusion has been also proved by Busch et al. in 1999 [15].

Studies have found that the occurrence of preterm birth is largely affected by environmental factors. It was reported that parturient with below 20 years old or above 35 years old were more likely to cause preterm birth [20]. Study conducted by Savitz, Pastore and other scholars has proved that progestational low weight of parturients was highly related to preterm birth. But Cnattingius et al. ascertained a positive correlation between BMI value and the incidence and risk of preterm birth [21]. Besides, as a kind of wound surgical operation, artificial abortion operation can increase the risk of uterine cavity and reproductive tract infections, which has also been considered by more and more scholars as a main cause of preterm birth [22, 23]. Antenatal examination is an important means of health care during pregnancy. Regular antenatal examination is a positive effect to reduce the incidence of adverse pregnancy and prevent birth defects. Ovros et al. found through their research that the incidences of low birth weight and preterm birth were higher in group without prenatal care than in control group [24]. What's more, stress caused by negative events can increase the secretion of catecholamine and cortisol in pregnant women, then the corticotropin releasing hormone (CRH) in placenta will be activated to induce a series of biological responses, which leads to preterm birth [25]. Other studies have confirmed that the risk of preterm birth was higher in parturient with low income than high income [26]. Economic income may be indirectly associated with the education level, prenatal care consciousness, antenatal examination frequency, occupation and labor intensity, nutritional intake, gestational weight gain, reproductive system infection, psychological pressure and some unhealthy living habits of the parturient [27-31].

Based on the above research results, preterm birth is currently considered as a complex process which is affected by many kinds of factors. These factors play the role in preterm through the interaction each other. Hence, more aggressive researches should be carried out to effectively identify the high-risk populations, and find a research direction of the pathogenesis, which provides an opportunity for the prevention and cure of preterm birth in the future.

Disclosure of conflict of interest

None.

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