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Association of IL-18 polymorphisms with risk of polycystic ovary syndrome in a Han population of China.

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Abstract

We aimed to investigate the association of two SNPs in the promoter region of IL-18 (IL-18-607C/A rs1946518 and -137G/C rs187238) with the risk of PCOS in a Chinese Han population. A total of 210 patients with PCOS and 408 subjects without PCOS were selected between June 2015 and December 2016. The genotyping of IL-18-607C/A rs1946518 and -137G/C rs187238 polymorphisms was carried out by an iPlex GOLD SNP genotyping analysis using the Sequenom MassARRAY® System. Unconditional multivariate logistic regression analysis was taken to calculate the relationship of IL-18-607C/A rs1946518 and -137G/C rs187238 polymorphisms with risk of PCOS. We found that BMI ≥ 24 (OR=2.78, 95% CI=1.85-4.17), ever smoking (OR=2.51, 95% CI=1.08-5.79) and ever drinking (OR=3.64, 95% CI=2.33-5.69) were associated with higher risk of PCOS. Those carrying the CC genotype were associated with 2.40 fold risk of developing PCOS (OR=2.40, 95% CI=1.22-4.72), and the GC+CC genotype displayed an increased risk of PCOS (OR=2.34, 95% CI=1.23-4.48). We suggest that IL-18-137G/C could be considered as a predictive factor for the pathogenesis of PCOS in the Han population of China.

Keywords: IL-18, -607C/A, -137G/C, Polymorphism, Polycystic ovary syndrome.

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Introduction

Polycystic ovary syndrome is one of the most common endocrine malfunctions in child-bearing women, which is clinical manifestations of menstrual abnormalities, hair growth, obesity, high blood insulin, and insulin resistance [1]. It is estimated that the incidence of polycystic ovary syndrome is about 7.1%-11.2% in Chinese women aged 12-44 years [2]. The etiology of polycystic ovary syndrome is very complication and unclear, and many environmental and lifestyle factors greatly contribute to the pathogenesis of the polycystic ovary syndrome [3-6]. However, hereditary factors play a critical role in the pathogenesis of polycystic ovary syndrome. Currently, many studies have reported an association between genetic factors and risk of polycystic ovary syndrome [7-11].

Inflammatory cytokines may be important factors in the pathogenesis of polycystic ovary syndrome. There is convincing evidence describing the influence of low-grade inflammation and cytokines in polycystic ovary syndrome [12-16]. IL-18 is an 18 kDa cytokine, which belongs to the Interleukin-1 (IL-1) superfamily [17]. Two previous studies have indicated a significant association between IL-18 polymorphisms and risk of polycystic ovary syndrome in Asian population [18,19], but they only reported the role of IL-18-237G>C polymorphism in the risk of this disease. Therefore, we aimed to investigate the association of two SNPs in the promoter region of IL-18 (IL-18-607C/A rs1946518 and-137G/C rs187238) with the risk of PCOS in a Chinese Han population.

Methods

Ethics

All the investigated respondents voluntary taken part in the study after full understanding of the purpose, and signed informed consent forms before enrolment. The protocol of our study was approved by the ethics committee of Guizhou Provincial People's Hospital.

Patients and controls

During June 2015 and December 2016, a total of 210 Han patients with PCOS were enrolled from the Center for Reproductive Medicine in Guizhou Provincial People's Hospital. The diagnosis of PCOS was according to the criteria of Rotterdam PCOS consensus in 2003 [20].

Simultaneously, a total of 408 Han healthy individuals without PCOS were randomly selected from the Center for Reproductive Medicine in Guizhou Provincial People's Hospital. Two individuals were matched with one patient with PCOS by age (± 5 y). All the controls were diagnosed free of PCOS by B ultrasonic examination and reproductive hormone

tests. The exclusion criteria of controls were those with irregular menstrual periods, malignant tumors, autoimmune diseases and ovarian related diseases. The mean ages of patients with PCOS and controls were 28.63 ± 3.94 and 27.92 ± 4.28 y, respectively; the mean age of menarche were 13.07 ± 2.01 and 13.23 ± 1.86 y, respectively; the mean BMI were 22.98 ± 2.87 and 21.36 ± 2.73 kg/m^2, respectively.

Demographic and lifestyle characteristics were collected from the questionnaire survey, including weight, height, alcohol drinking and tobacco smoking habit and physical activity. The Body Mass Index (BMI) is defined as the body mass divided by the square of the body height.

The reproductive hormone indexes were tested by automatic chemiluminescence immune detection system, including Follicle-Stimulating Hormone (FSH), Luteinizing Hormone (LH), Estradiol (E2), Progesterone (P) and Testosterone (T).

Genotyping

Five ml of peripheral venous blood were obtained from each respondent. The blood samples were centrifuged to separate serum, and stored at -80°C until use. Genomic DNA was isolated from peripheral blood samples with Blood DNA extraction kit. The DNA samples were kept at -20°C until using. The genotyping of IL-18-607C/A rs1946518 and-137G/C rs187238 polymorphisms was carried out by an iPlex GOLD SNP genotyping analysis using the Sequenom MassARRAY® System (Sequenom, San Diego, USA). The primers used for amplification of IL-18-607C/A and-137G/C were designed by Sequenom Assay Design 3.1 software.

Then the SAP and iPLEX reactions were carried out to analyse the PCR amplification products.

The PCR products are then desalted and crystallized, and then analysed by SpectroCHIP and MALDI-TOF MS reaction. The mass spectra peak and genotypes of IL-18-607C/A and-137G/C was analysed by Typer 4.0 software.

Statistical analysis

Continuous variables were displayed as means ± Standard Deviations (SD), and categorical variables were shown as percentages and frequencies (%). Comparison of demographic, lifestyle and clinical characteristics between the PCOS patients and controls were calculated using Chi-square (χ^2) test or student t test. Chi-square (χ^2) test with one degree of freedom was used to estimate whether the IL-18-607C/A rs1946518 and-137G/C rs187238 genetic frequencies were in line with the Hardy-Weinberg equilibrium. Unconditional multivariate logistic regression analysis was taken to calculate the relationship of IL-18-607C/A rs1946518 and-137G/C rs187238 polymorphisms with risk of PCOS. Odds Ratios (ORs) and 95% Confidence Intervals (CIs) were used to express the results. Interaction between environmental factors and genetic polymorphisms of IL-18-607C/A rs1946518 and-137G/C rs187238 was estimated by spearman correlation analysis. All the data were analysed with the software IBM SPSS Statistics for Windows, Version 20.0. (IBM Corp., Armonk, NY, USA). Two tailed P<0.05 was regarded as statistical significant difference.

Results

Comparison with controls, patients with PCOS were more likely to have an early age of menarche, high level of BMI, FSH, LH and T, low level of P, and a habit of smoking and drinking (Table 1).

The genotype distributions of the IL-18-607C/A and-137G/C were according to the HWE in both patients (for-607C/A: χ^2=2.42, P=0.09; for-137G/C: χ^2=0.86, P=0.35) and controls (for-607C/A: χ^2=1.99, P=0.16; for-137G/C: χ^2=3.12, P=0.07) (Table 2).

By multivariate logistic regression analysis, we found that BMI ≥ 24 (OR=2.78, 95% CI=1.85-4.17), ever smoking (OR=2.51, 95% CI=1.08-5.79) and ever drinking (OR=3.64, 95% CI=2.33-5.69) were associated with higher risk of PCOS when compared with the reference group (Table 3). Compared with individuals carrying GG genotype of IL-18-137G/C, those carrying the CC genotype were associated with 2.40 fold risk of developing PCOS (OR=2.40, 95% CI=1.22-4.72), and the GC+CC genotype displayed an increased risk of PCOS (OR=2.34, 95% CI=1.23-4.48).

We found that IL-18-137G/C polymorphisms was associated with BMI ≥ 24 in the risk of PCOS, while the IL-18-137G/C polymorphism had no interaction with, age, age of menarche, smoking and drinking in the risk of PCOS (Table 4). Moreover, we observed that IL-18-137G/C polymorphism had no interaction with FSH, LH, E2, P and T levels in the risk of PCOS (Table 5).

Table 1. *Demographic and clinical characteristics of patients with PCOS and controls.*

Variables	Patients (%)	Controls (%)	χ^2 or t value	P value
	n=210	n=408		
Age, years	28.63 ± 3.94	27.92 ± 4.28	-1.01	0.32
Age of menarche, years	13.07 ± 2.01	13.23 ± 1.86	2.01	0.045
Weight, kg	58.38 ± 5.92	54.94 ± 5.57	7.13	<0.001
Height, m	1.60 ± 0.06	1.61 ± 0.06	-1.82	0.07
BMI, kg/m^2	22.98 ± 2.87	21.36 ± 2.73	6.84	<0.001
≥ 24	71 (33.81)	65 (15.93)	25.82	<0.001
Ever smoking	14 (6.67)	11 (2.94)	4.78	0.03
Ever drinking	65 (30.00)	72 (12.01)	30.24	<0.001
FSH	4.47 ± 2.28	3.68 ± 2.15	4.25	<0.001
LH	14.83 ± 4.54	11.33 ± 3.21	11.11	<0.001
E2	91.93 ± 21.21	91.10 ± 21.77	0.45	0.65
P	7.26 ± 2.92	8.11 ± 3.05	-3.35	0.001

T	0.76 ± 0.20	0.52 ± 0.21	13.71	<0.001

Table 2. *Genotype distributions of IL-18 -607C/A rs1946518 and -137G/C rs187238 between the two study groups.*

IL-18	Patients (%)	Controls (%)	χ^2 test	P value	HWE in patients		HWE in controls	
					χ^2	P value	χ^2	P value
-607C/A								
CC	73 (34.76)	135 (33.09)						
CA	87 (41.43)	187 (45.83)						
AA	50 (23.81)	86 (21.08)	1.19	0.55	2.42	0.09	1.99	0.16
-137G/C								
GG	89 (42.38)	194 (47.55)						
GC	100 (47.62)	191 (46.81)						
CC	21 (10.00)	23 (5.64)	4.53	0.1	0.86	0.35	3.12	0.07

Table 3. *Unconditional multivariate logistic regression analysis for the risk factors of PCOS.*

Variables	β	S.E.	Wals	Adjusted OR (95% CI)	P value
-607C/A					
CC				1.0 (Ref.)	
CA	-0.15	0.19	0.6	0.86 (0.59-1.26)	0.44
AA	0.07	0.23	0.1	1.08 (0.69-1.69)	0.75
CA+AA	-0.075	0.179	0.174	0.93 (0.65-1.32)	0.68
-137G/C					
GG				1.0 (Ref.)	
GC	0.04	0.19	0.04	1.04 (0.72-1.50)	0.85
CC	0.88	0.35	5.46	2.40 (1.22-4.72)	0.01
GC+CC	0.85	0.33	6.64	2.34 (1.23-4.48)	0.01

Table 4. *Interaction between environmental factors and IL-18 -137G/C in the risk of PCOS.*

Variables	Patients		χ^2 or t	P value	Controls		χ^2 or t	P value
	GG	GC+CC	GG		GC+CC			
Age (y)	28.63 ± 4.00	28.66 ± 3.53	-0.04	0.97	27.97 ± 4.28	27.16 ± 4.14	0.88	0.38
Age of menarche, years	13.04 ± 2.13	13.33 ± 1.27	-0.64	0.52	13.21 ± 1.87	13.51 ± 1.74	-0.74	0.46
BMI								
<24	120	19			325	18		
≥ 24	69	2	0.61	0.43	60	5	6.15	0.01
Smoking								
Never	176	20			374	22		
Ever	13	1	0.17	0.68	11	1	0.14	0.71
Drinking								
Never	130	17			338	21		
Ever	59	4	0.25	0.62	47	2	1.33	0.25

Table 5. *Interaction between clinical factors and IL-18 -137G/C in the risk of PCOS.*

Variables	Patients		t	P value	Controls	t	P value
	GG	GC+CC	GG		GC+CC		

FSH	4.40 ± 2.23	5.11 ± 2.67	-1.36	0.177	3.72 ± 2.16	3.03 ± 1.95	1.49	0.14
LH	14.90 ± 4.54	14.22 ± 4.57	0.65	0.514	11.35 ± 3.23	10.94 ± 2.89	0.6	0.55
E2	91.80 ± 20.97	93.10 ± 23.78	-0.27	0.79	90.53 ± 21.76	100.53 ± 20.14	-2.05	0.06
P	7.23 ± 2.93	7.54 ± 2.88	-0.47	0.641	8.12 ± 2.99	8.04 ± 3.95	0.11	0.91
T	0.77 ± 0.20	0.74 ± 0.24	0.49	0.622	0.52 ± 0.21	0.52 ± 0.15	0.08	0.94

Discussion

In the present study, we found that IL-18-607C/A polymorphism was significant associated with an increased risk of PCOS. Chronic low-grade inflammation is reported to be correlated with abnormal metabolism in PCOS patients, and it is resulted from abnormal endometrium implantation [15,16]. Liu et al. performed a prospective study on the association between serum levels of TSP-1, NF-κB and TGF-β1 and PCOS, and they found that the testosterone, Free Androgen Index (FAI), Luteinizing Hormone/Follicle-Stimulating Hormone (LH/FSH) ratio, HOMA-IR, TSP-1 and NF-κB were significantly higher in the PCOS groups than those in the control group [21]. Yang et al. performed a study on the association of serum IL-18 concentrations and PCOS, and they found that IL-18 level was increased in PCOS patients and correlated with insulin resistance, obesity and hyperandrogenism [22].

Three previous studies investigated the association between IL-18 polymorphisms and risk of polycystic ovary syndrome [18,19,23]. Yang et al. performed a study with 118 Chinese women with PCOS and 79 controls, and they found a significant different in the G and C allele frequencies of IL-18-137G/C between the two study groups, and those carrying the C allele of-137G/C may contribute to the risk of PCOS in a Chinese population [18]. Kim et al. performed a study in a Korean population with 126 women with PCOS and 113 controls, and they reported that the IL-18-137G allele could serve an important role in the predisposition to glucose intolerance in Korean women with PCOS [18]. Deligeoroglou et al. performed study in Greeks, and they found that IL-18 is elevated in lean patients and raised in the presence of obesity and insulin resistance [23]. However, some studies reported inconsistent results. Wu et al. performed a meta-analysis with eighteen studies, and they found no association between IL-18-607C/A and-137G/C polymorphisms and risk of PCOS [24]. In our study, we found that IL-18-137G/C polymorphism was associated with PCOS, while no correlation between IL-18-607C/A and this disease. Therefore, further studies are greatly needed to confirm our findings.

There are three limitations should be mentioned in the present study. First, since patients and controls were only enrolled from one place of China, these participants may not well represent individuals in other places, and the selection bias may be inevitable. Second, the sample size of patients and controls was relatively small, which may result in a low statistical power to identify differences between groups. Third, this study is a case-control study design, and the recall bias may influence the reliability of data.

In conclusion, this study provides a new insight in PCOS that IL-18-137G/C genotype is associated with the risk of PCOS. We suggest that IL-18-137G/C could be considered as a predictive factor for the pathogenesis of PCOS in the Han population of China.

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