European Review for Medical and Pharmacological Sciences 2019; 23: 8109-8114

Correlations of IL-6 and CRP gene polymorphisms with pulmonary heart disease

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Abstract. – OBJECTIVE: To explore the correlations of interleukin-6 (IL-6) and C-reactive protein (CRP) gene polymorphisms with pulmonary heart disease (PHD).

PATIENTS AND METHODS: A total of 98 patients with PHD and 102 healthy persons receiving physical examinations were enrolled. Their general clinical information was collected, and the levels of IL-6 and CRP in the plasma were determined. The pulmonary functions and blood gas were detected, and the TaqMan-minor groove binder (MGB) probe was used to detect the polymorphisms of IL-6 rs1800796 and CRP rs1800796.

RESULTS: Observation group had higher levels of IL-6 and CRP than control group ($p<0.05$). The forced expiratory volume in 1 second (FEV_1) (%), FEV_1/forced vital capacity (FVC) ratio (%), and arterial partial pressure of oxygen (PaO_2) in observation group were lower than those in control group ($p<0.05$), but the arterial partial pressure of carbon dioxide ($PaCO_2$) was higher than that in control group ($p<0.05$). There were differences in the distribution frequencies of the genotypes and alleles of IL-6 rs1800796 and CRP rs1800796 between the two groups ($p<0.05$).

CONCLUSIONS: IL-6 and CRP are correlated with the onset of PHD, and there are also correlations between the polymorphisms of IL-6 rs1800796 and CRP rs2794521 and the disease.

Key Words:
Interleukin-6, C-reactive protein, Pulmonary heart disease, Single nucleotide polymorphism.

Introduction

Clinically, pulmonary heart disease (PHD) is a common chronic respiratory disease, and its major pathologic process is that the pulmonary arterial hypertension and increased pulmonary circulation resistance, caused by chronic lesions in the thorax, pulmonary artery, and bronchus-lung tissues, develop into heart disease, which is often complicated with right heart failure in severe cases. The studies conducted in recent years have revealed that the changes in inflammatory factors are closely associated with the onset of PHD. The inflammatory cells in pulmonary tissues release inflammatory factors to aggravate the disease in patients. Interleukin-6 (IL-6), a kind of important cytokine, can participate in the occurrence of airway inflammations in PHD[1-3]. C-reactive protein (CRP), an acute-phase protein, will be significantly elevated when inflammations occur in organisms[4-6]. We selected the two sites of IL-6 and CRP genes for a genetic test, and explored the correlations of IL-6 and CRP gene polymorphisms with PHD, hoping to provide a theoretical support for its genetic polymorphism.

Patients and Methods

Patients

The PHD patients who were treated from January 2016 to January 2018 in the Respiratory Department of Ningxia Hui Autonomous Region People's Hospital were selected. They met the following criteria: 1) patients who satisfied the criteria formulated on the National Chronic Pulmonary Heart Disease Professional Conference, 2) those able to tolerate the specified related examinations and tests, and 3) those with favorable compliance and complete information. Exclusion criteria: 1) patients with heart disease due to other causes, 2) patients who were complicated with mental diseases or other cognitive dysfunctions and could not cooperate in the study, or 3) those who suffered from dysfunctions of heart, kidney, liver or other major organs. This study included 98 patients with PHD as observation group based on the above criteria,

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Table I. TaqMan®-minor groove binder (MGB) probe information of IL-6 rs1800796 and CRP rs2794521.

SNP reference	rs1800796	rs2794521
Assay ID	C__11326893_10	C____318207_10
SNP type	Intron	Intron
Context sequence	ATGGCCAGGCAGTTCTACA ACAGCC[C/G]CTCACAGGG AGAGCCAGAACACAGA	CAATTCCCATCTATGAG AGAACA[C/T]GCGGTGTTT GGTTTTTTGCATGGAC

and they consisted of 54 males and 44 females, with the mean age of (58.6±5.4) years old. Meanwhile, 102 healthy people receiving physical examinations in the same period were selected from the Medical Center of our hospital as control group, and among them, there were 55 males and 47 females, with the mean age of (58.2±5.2) years old. All study patients were unrelated Chinese Han individuals and signed the informed consent. This study was approved by the Ethics Committee of Ningxia Hui Autonomous Region People's Hospital.

Collection of General Clinical Information

The name, age, sex, symptoms, signs, test and examination reports of the study subjects were collected. After 3 mL venous blood was taken from the elbows of the study subjects, the levels of plasma IL-6 and CRP were determined *via* enzyme-linked immunosorbent assay (ELISA; R&D Systems, Minneapolis, MN, USA). The percentage of forced expiratory volume in 1 second (FEV_1)/the predicated value (FEV_1%) and FEV_1/forced vital capacity (FVC) ratio (FEV_1/FVC) were employed. Without oxygen absorption, the blood-gas analyzer was utilized to detect the arterial partial pressure of oxygen (PaO_2) and arterial partial pressure of carbon dioxide ($PaCO_2$). The above operations were conducted by the attending physicians from the Respiratory Department.

Extraction of Deoxyribonucleic Acid (DNA)

After 1 mL venous blood was drawn from the elbows of the study subjects, DNA was extracted using a medium-amount whole blood genomic DNA extraction kit (BioTeke Corporation, Beijing, China) according to the instructions in the kit. Moreover, the TaqMan® single nucleotide polymorphism (SNP) genotyping assay kit (Thermo Fisher Scientific, Waltham, MA, USA) was employed to detect and analyze the genotypes of the samples (the probe information is shown in Table I).

Statistical Analysis

SPSS 20.0 software was used for statistical analysis. Measurement data were expressed as ($\bar{\chi}$±s), and the independent-samples *t*-test was employed for the comparisons of measurement data between the two groups. The Chi-square (χ^2)-test was adopted to compare the count data between the two groups. The likelihood-ratio χ^2-test was performed to analyze whether the genotype distribution met the Hardy-Weinberg equilibrium law. R×C χ^2-test was applied for the comparison of the frequency of genotypes and alleles in each group. $p<0.05$ suggested that the difference was statistically significant.

Results

Comparisons of the General Information and the Levels of IL-6 and CRP Between the two Groups

There were no differences in sex and age between the two groups ($p>0.05$), and observation group had higher levels of IL-6 and CRP than control group ($p<0.05$) (Table II).

Comparisons of pulmonary function test indexes

Observation group had lower FEV_1%, FEV_1/FVC ratio (%) and PaO_2, but higher $PaCO_2$ than control group ($p<0.05$) (Table III).

Genetic Equilibrium Test

The likelihood-ratio χ^2-test was conducted for the actual and theoretical frequencies of three genotypes in observation group and control group. The distributions of IL-6 rs1800796 and CRP rs2794521 genotype frequencies in both groups were consistent with the Hardy-Weinberg equilibrium law ($p>0.05$) and comparable (Tables IV and V).

Comparisons of Genotype Distribution Frequencies Between the Two Groups

The distribution frequencies of IL-6 rs1800796 genotypes CC, CG, and GG in observation group

Table II. Comparisons of the general information and the levels of plasma IL-6 and CRP between the two groups.

Group	No.	Sex (male/female)	Age (years old)	IL-6 (ng/L)	CRP (mg/L)
Observation group	98	54 (55.10)/44 (44.90)	58.6±5.4	25.63±4.80	42.32±11.80
Control group	102	55 (53.92)/47 (46.08)	58.2±5.2	6.21±2.32	2.81±0.71
χ^2/t		0.028	1.642	3.842	3.831
p		0.867	0.525	0.037	0.036

Table III. Comparisons of pulmonary functions between the two groups.

Group		FEV_1%	FEV_1/FVC ratio (%)	PaO_2 (mmHg)	$PaCO_2$ (mmHg)
Observation group	98	36.41±6.82	42.11±5.14	36.51±5.34	58.45±6.34
Control group	102	80.03±6.31	81.32±5.91	68.33±4.40	30.53±5.11
χ^2		5.323	3.132	4.534	4.443
p		0.027	0.043	0.038	0.037

Table IV. Genetic equilibrium test of IL-6 rs1800796 genotype.

		CC		CG		GG			
Group	No.	Actual frequency	Theoretical frequency	Actual frequency	Theoretical frequency	Actual frequency	Theoretical frequency	χ^2	*p*
Observation group	98	32	34.33	52	47.35	14	16.33	0.95	0.62
Control group	102	49	52.25	48	41.51	5	8.25	2.49	0.29

Table V. Genetic equilibrium test of CRP rs2794521 genotype.

		TT		TC		CC			
Group	No.	Actual frequency	Theoretical frequency	Actual frequency	Theoretical frequency	Actual frequency	Theoretical frequency	χ^2	*p*
Observation group	98	78	75.47	16	21.06	4	1.47	5.66	0.06
Control group	102	65	62.75	30	34.51	7	4.75	1.74	0.42

were 32.65%, 53.06%, and 14.29%, respectively, while those in control group were 48.04%, 47.06%, and 4.90%, respectively. There were differences in genotype distribution frequencies between the two groups ($p<0.05$) (Table VI).

The distribution frequencies of CRP rs2794521 genotypes TT, TC, and CC in observation group were 79.59%, 16.33%, and 4.08%, respectively, while those in control group were 63.73%, 29.41%, and 6.86%, respectively. The genotype distribution frequencies were different between the two groups ($p<0.05$) (Table VI).

Comparisons of Allele Distribution Frequencies

The distribution frequencies of IL-6 rs1800796 C and G alleles in observation group were 59.18% and 71.57%, respectively, while those in control group were 40.82% and 28.43%, respectively. The comparison revealed differences in allele distribution frequencies between the two groups ($p<0.05$) (Table VII).

The distribution frequencies of CRP rs2794521 T and C alleles in observation group were 87.76% and 12.24%, respectively, while those in control

Table VI. Comparisons of genotype distribution frequencies between the two groups [n (%)].

Gene	SNP	Observation group			Control group			χ^2	*p*
IL-6	rs1800796	CC	CG	GG	CC	CG	GG		
		32 (32.65)	52 (53.06)	14 (14.29)	49 (48.04)	48 (47.06)	5 (4.90)	7.914	0.019
CRP	rs2794521	TT	TC	CC	TT	TC	CC		
		78 (79.59)	16 (16.33)	4 (4.08)	65 (63.73)	30 (29.41)	7 (6.86)	6.183	0.045

Table VII. Comparisons of genotype distribution frequencies between the two groups [n (%)].

Gene	SNP	Observation group		Control group		χ^2	*p*
IL-6	rs1800796	C	G	C	C		
		116 (59.18)	80 (71.57)	146 (40.82)	58 (28.43)	6.785	0.009
CRP	rs2794521	T	C	T	C		
		172 (87.76)	24 (12.24)	160 (78.43)	44 (21.57)	6.159	0.013

group were 78.43% and 21.57%, respectively. The comparison revealed differences in allele distribution frequencies between the two groups ($p<0.05$) (Table VII).

Discussion

As the environmental pollution and the aging of population are worsened in recent years, the incidence rate of PHD is increasing year by year[7-9]. Patients with chronic PHD suffer from ventilation dysfunction, and such pathological changes as histohypoxia, pulmonary arterial hypertension, increased blood viscosity, slow blood flow in pulmonary tissues and vascular endothelial injuries, thus leading to significantly raised cardiac load and progressive pulmonary function deterioration and ultimately resulting in heart failure and respiratory failure. Therefore, the early diagnosis and accurate assessment for PHD have become the focuses of clinical studies.

IL-6, a kind of glycoprotein produced by numerous cells, such as macrophages, T cells, and B cells, can induce fibrinogens to generate and initiate coagulation factors, thereby causing fibroblast proliferation and collagen deposition at inflammatory sites. Additionally, it can give rise to the infiltration of inflammatory cells, thus causing damage to the alveolar membrane. The infections, common causes of PHD, can also contribute to the increased secretion of IL-6[10,11]. CRP is a kind of inflammatory factor due to the activation of liver cells *via* IL-6 and a typical marker of acute-phase reactive protein. Its content is low in the serum of normal people, while the level of CRP will be significantly elevated once organisms are stimulated by inflammations[12-14]. The detection of IL-6 and CRP in the subjects of this study manifested that observation group had higher levels of IL-6 and CRP than control group, which is similar to the reports of other scholars[15-17]. Moreover, the pulmonary functions were detected and blood gas was analyzed in the present work, and it was found that FEV_1%, FEV_1/FVC ratio (%) and PaO_2 in observation group were lower than those in control group, but $PaCO_2$ was higher than that in control group, indicating that with the increase in the levels of IL-6 and CRP in plasma, airway obstruction may be exacerbated and respiratory functions may become poorer, further suggesting that infections enable PHD to aggravate airway inflammations. Ultimately, the worsened inflammations increase the levels of IL-6 and CRP. Hence, IL-6 and CRP are correlated with the occurrence and development of PHD, and they are of great significance for understanding the condition and prognosis of PHD.

We found that PHD is a disease associated with multiple-factor inheritance. Human IL-6 gene, located on chromosome 7, contains 5 exons and 4 introns, and according to the study reports, IL-6 gene polymorphism is related to the onset of respiratory diseases, such as sepsis and respiratory virus[18,19]. Located on human chromosome 1q13.2, CRP gene with 6.8 kb in length contains 2 exons and 1 intron, and studies[20,21] have demonstrated that CRP gene polymorphism is also closely

correlated with pulmonary infection and tumor. In this study, IL-6 rs1800796 (C/G) and CRP rs2794521 (T/C) were selected, and the TaqMan-MGB probe was used to analyze the genotype and allele frequencies in both observation group and control group, so as to clarify the correlations of IL-6 and CRP with PHD. The results of this study were subjected to the Hardy-Weinberg satisfaction test, and the two groups of genotypes met the Hardy-Weinberg equilibrium law, namely there were no significant differences in the observation value and expectancy value of the genotype frequencies between the two groups, suggesting that the distributions of the allele frequencies in the two groups can represent the distributions among their respective groups. There were differences in the frequencies of IL-6 rs1800796 (C/G) and CRP rs2794521 (T/C) genotype and allele distributions between the two groups, indicating that the polymorphisms of IL-6 rs1800796 (C/G) and CRP rs2794521 (T/C) were correlated with the onset of PHD.

Conclusions

We showed that IL-6 and CRP are correlated with the onset of PHD, and there are also correlations between the polymorphisms of IL-6 rs1800796 and CRP rs2794521 and the disease.

Conflict of Interests

The Authors declare that they have no conflict of interests.

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