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Correlation of IL-33 gene polymorphism with chronic obstructive pulmonary disease

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Abstract. – OBJECTIVE: To explore the correlation between interleukin (IL)-33 gene polymorphism with chronic obstructive pulmonary disease (COPD).

PATIENTS AND METHODS: A total of 210 COPD patients (observation group) and 180 healthy people receiving physical examinations (control group) were included in this study. Clinical information of each subject was collected. Relative levels of inflammation-related factor IL-33 and pulmonary function indexes were determined. Moreover, the polymorphism of IL-33 rs1891385 was detected with the TaqMan-minor groove binder (MGB) probe.

RESULTS: Observation group had a higher level of IL-33 than that of control group ($p<0.01$), and the forced expiratory volume in 1 second (FEV_1)/forced vital capacity (FVC) ratio (%) and FEV_1/the predicted value ratio (%) in observation group were lower than those in control group ($p<0.05$). There were significant differences in the distribution frequencies of genotypes and alleles between the two groups ($p<0.05$), and genotype AA exhibited a higher level of IL-33, but a lower FEV_1/FVC ratio (%) and FEV_1/the predicted value ratio (%) than those of genotypes AC and CC ($p<0.05$).

CONCLUSIONS: IL-33 and pulmonary function test can be used to effectively evaluate the progression of COPD, and the polymorphism of IL-33 rs1891385 is correlated with the onset of COPD.

Key Words:
Chronic obstructive pulmonary disease, Interleukin-33, Pulmonary functions, Single nucleotide polymorphism.

Introduction

Chronic obstructive pulmonary disease (COPD) is a common pulmonary disease with the leading manifestations of respiratory inflammations and irreversible airway obstructions. The global epidemiological surveys reported[1-3] that the incidence and mortality rates of COPD remain high throughout the world. Scholars[4-6] have revealed that T-lymphocytes are involved in regulating the occurrence and development of respiratory inflammations in COPD patients. Interleukin (IL)-33 is a cytokine secreted by T-lymphocytes, and it participates in the development of various inflammations and cardiopulmonary diseases by binding to the corresponding receptors[7-10]. In this study, COPD patients were enrolled from our department to detect the polymorphism of IL-33 rs1891385 with the TaqMan-minor groove binder (MGB) probe. We investigated the correlation of IL-33 gene polymorphism with COPD, hoping to provide theoretical support for the genetic polymorphism of COPD.

Patients and Methods

Patients and Healthy People

COPD patients admitted to the Respiratory and Critical Care Medicine Department of The First Affiliated Hospital of Zhengzhou University from January 2016 to January 2018 were selected. Inclusive criteria were as follows: (1) Patients conforming to the diagnostic criteria for COPD in 2016 GOLD Guidelines and (2) those with favorable compliance and complete information. Exclusion criteria: (1) Patients with infectious diseases in other systems, (2) patients affected by mental diseases or other cognitive dysfunctions and who could not cooperate in this study, or (3) patients suffered from dysfunctions of heart, kidney, liver or other major organs. Based on the above criteria, this study included 210 COPD patients as observation group, consisting

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of 120 males and 90 females, with the mean age of (55.80±6.80) years old. Besides, 180 healthy people receiving physical examinations in the same period were selected from the Medical Center of our hospital as control group. Among them, there were 102 males and 78 females, aged (54.90±6.46) years old on average. All subjects were unrelated Chinese Han individuals and signed the informed consent. This investigation was approved by the Ethics Committee of The First Affiliated Hospital of Zhengzhou University. The signed written informed consents were obtained from all participants.

Collection of General Clinical Information

Baseline characteristics of subjects were recorded, including name, age, sex, symptoms, signs, clinical test, and examination reports. 5 mL of venous blood was taken from the elbows of the subjects and centrifuged for 5 min at 800 g and 4°C. The serum was sub-packaged into 0.5 mL Eppendorf (EP) tubes (200 μL/tube) and stored at -80°C for later use. Serum level of IL-33 was measured *via* enzyme-linked immunosorbent assay (ELISA; R&D Systems, Minneapolis, MN, USA). All subjects underwent pulmonary function tests. The attending physicians from the Respiratory Department carried out the above operations.

Extraction of Deoxyribonucleic Acid (DNA)

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

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 20.0 software (IBM, Armonk, NY, USA) was used for statistical analysis. Measurement data were expressed as ($\bar{x} \pm s$). The independent-samples *t*-test was conducted for the inter-group comparisons of measurement data. The chi-square (χ^2)-test was adopted to compare 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 comparisons of genotype and allele frequencies in both groups. $p<0.05$ suggested that the difference was statistically significant.

Results

Comparison of IL-33 Level Between the Two Groups

The serum level of IL-33 was higher in observation group than that of control group ($p<0.01$). There were no differences in sex and age between the two groups ($p>0.05$) (Table II).

Comparisons of Pulmonary Function Indexes Between the Two Groups

The forced expiratory volume in 1 second (FEV_1)/forced vital capacity (FVC) ratio (%) and FEV_1/the predicted value ratio (%) in observation group were lower than those in control group ($p<0.05$) (Table III).

Genetic Equilibrium Test

The actual and theoretical frequencies of three genotypes in both observation group and control group were subjected to the likelihood-ratio χ^2-test. The results showed that the distributions of IL-33 rs1891385 genotype frequencies in the two groups were consistent with the Hardy-Weinberg equilibrium law ($p>0.05$) and comparable (Table IV).

Table I. TaqMan®-MGB probe information of IL-33 rs1891385.

SNP reference	rs1891385
Assay ID	C___2762163_10
SNP type	Intron
Context sequence	TATGAAATTGAGACAGTGGGATTTG[A/C]AAAGATTTTAATACTAACCCAAAAT

Table II. Comparison of IL-33 level between the two groups.

Group	No.	Sex (male/female)	Age (year old)	IL-33 (pg/L)
Observation group	210	120 (57.14)/90 (42.86)	55.80 ± 6.80	105.20 ± 6.44
Control group	180	102 (56.67)/78 (43.33)	54.90 ± 6.46	4.42 ± 1.60
t		0.009	1.542	13.831
p		0.925	0.526	0.01

Table III. Comparisons of pulmonary function indexes between the two groups.

Group	No.	FEV1/FVC ratio (%)	FEV1/the predicted value ratio (%)
Observation group	210	57.25 ± 20.48	68.01 ± 14.28
Control group	180	86.18 ± 13.78	95.68 ± 36.78
t		4.661	5.561
p		0.036	0.026

Comparison of Genotype Distribution Frequency

The distribution frequencies of genotypes AA, AC, and CC in observation group were 58.10%, 32.86%, and 9.04%, respectively, while those in control group were 63.33%, 34.45%, and 2.22%, respectively. The genotype distribution frequencies varied between the two groups ($p<0.05$) (Table V).

Comparison of Allele Distribution Frequency

The distribution frequencies of A and C alleles were 74.52% and 25.48%, respectively, while those in control group were 80.56% and 19.44%, respectively. The comparison revealed differences in allele distribution frequencies between the two groups ($p<0.05$) (Table VI).

Comparisons of IL-33 Levels and Pulmonary Functions Among Different Genotypes of IL-33 rs1891385 in Observation Group

The correlations of three genotypes with IL-33 level and pulmonary functions were further analyzed based on the results of comparisons of IL-33 level, pulmonary functions, and genotypes in observation group. The COPD patients with genotype AA exhibited a higher level of IL-33, but lower FEV_1/FVC ratio (%) and FEV_1/the predicted value ratio (%) than those with genotypes AC and CC ($p<0.05$) (Table VII).

Table IV. Genetic equilibrium test of IL-33 rs1891385 genotype.

Group	No.	AA		AC		CC		χ^2	*p*
		Actual frequency	Theoretical frequency	Actual frequency	Theoretical frequency	Actual frequency	Theoretical frequency		
Observation group	210	122	116.63	69	79.74	19	13.63	3.81	0.15
Control group	180	114	116.81	62	56.39	4	6.81	1.78	0.41

Table V. Comparison of IL-33 rs1891385 genotype distribution between the two groups.

Group	No.	Genotype [no. (%)]			χ^2	*p*
		AA	AC	CC		
Observation group	210	122 (58.10)	69 (32.86)	19 (9.04)	8.168	0.017
Control group	180	114 (63.33)	62 (34.45)	4 (2.22)		
Observation group	210	122 (58.10)	69 (32.86)	19 (9.04)	8.168	0.017
Control group	180	114 (63.33)	62 (34.45)	4 (2.22)		

Table VI. Comparison of IL-33 rs1891385 A/C allele distribution between the two groups [no. (%)].

Group	No.	Allele [no. (%)]		χ^2	*p*
		A	C		
Observation group	210	313 (74.52)	107 (25.48)	4.020	0.045
Control group	180	290 (80.56)	70 (19.44)		

Discussion

With economic development and exaggerated air pollution, the incidence and mortality rates of COPD increase year by year, which seriously affects the life quality and physical health of COPD patients. Several studies have reported that the onset of COPD is associated with a series of inflammatory responses following bacterial infections. Inflammatory factors were identified to be closely related to the occurrence and development of COPD. Their contents in organisms accordingly increase as COPD aggravates[11-13]. Hence, relative levels of inflammatory factors can assess the progression of COPD. This study showed that the serum level of IL-33 in observation group was higher than that in control group. It is indicated that the increase in the IL-33 level was correlated with the progression of COPD, and IL-33 can serve as an important serum marker for judging COPD. IL-33, a member from the IL-1 family, is highly expressed mainly in the nucleus. Once binding to the corresponding receptors, it can assemble intracellular myeloid differentiation factor 88 and IL-1 receptor-associated kinase 1 (IRAK1) and IRAK4 to form a complex, which in turn activates NF-κB and mitogen-activated protein kinase signaling pathways. Besides, the secretion of IL-5 and IL-3 in organisms are stimulated, thereby accelerating the progression of airway inflammatory responses[14-17]. It has been reported[18-20] that the level of IL-33 in patients with exacerbated COPD is significantly higher than that in healthy controls, and it is significantly lower after the conditions are improved through treatment than that before treatment. Furthermore, IL-33 can be applied to reliably judge the disease change of COPD. Both FEV_1/FVC ratio (%) and FEV_1/the predicted value ratio (%) are the indicators reflecting pulmonary obstructive ventilation dysfunction. The pulmonary functions of the two groups of subjects in this study were tested and compared. The observation group had a lower FEV_1/FVC ratio (%) and FEV_1/the predicted value ratio (%) than control group, suggesting that the pulmonary function decline in COPD patients and a correlation between pulmonary functions and the development of COPD exists. Therefore, pulmonary function tests can be combined with IL-33 level monitoring to evaluate the change of COPD, thus raising the diagnostic accuracy.

In recent years, as genomics and molecular biology develop, more and more studies[21,22] have reported the close correlation between the onset of COPD and abnormal gene expressions. Located on chromosome 9p24.1, human IL-33 gene with the relative molecular mass of 30 kDa encodes 270 amino acid polypeptides, and it is mainly expressed in epithelial cells and endothelial cells. At the stationary phase, IL-33 only acts as a transcription factor in nucleus. After inflammation stimuli, it will bind to its receptor SY2 to stimulate the downstream signaling pathways and induce Th2 cytokine, thereby mediating the inflammatory responses and im-

Table VII. Comparisons of IL-33 levels and pulmonary functions among different genotypes of IL-33 rs1891385 in observation group.

Item	AA	AC	CC	χ^2	*p*
IL-33 (pg/L)	107.22 ± 8.82	104.34 ± 7.01	98.67 ± 6.32	6.340	0.042
FEV_1/FVC ratio (%)	55.82 ± 21.74	57.64 ± 20.12	59.77 ± 20.10	6.471	0.041
FEV_1/the predicted value ratio (%)	65.74 ± 15.20	68.24 ± 14.24	69.10 ± 15.21	6.668	0.036

mune regulation. Therefore, it is presumed that the protein generated through the transcription and translation of IL-33 gene may be correlated with the occurrence and development of COPD. Moreover, the IL-33 polymorphic site rs1891385 (A/C) was selected in this study to analyze the genotype and allele frequencies in both observation group and control group with the TaqMan-MGB probe. The results showed that they were different between the two groups, indicating that the polymorphism of IL-33 rs1891385 (A/C) was correlated with the onset of COPD. Based on the results of the comparisons of IL-33 level, pulmonary functions and genotypes between the two groups, the correlations of three genotypes of IL-33 rs1891385 (A/C) with IL-33 level and pulmonary functions were further analyzed in observation group. According to the results, COPD patients with genotype AA exhibited a higher IL-33 level, but lower FEV_1/FVC ratio (%) and FEV_1/the predicted value ratio (%) than those with genotypes AC and CC. It is indicated that the mutation of IL-33 rs1891385 genotype AA may be associated with the elevated IL-33 level and lowered FEV_1/FVC ratio (%) and FEV_1/the predicted value ratio (%). The mutation of IL-33 rs1891385 genotype AA in COPD patients possibly causes the increase in IL-33 expression to enhance inflammatory responses in organisms, thus inducing the onset of COPD and dysfunction of pulmonary ventilation.

Conclusions

We showed that IL-33 and pulmonary function test can be used to effectively evaluate the progression of COPD, and the polymorphism of IL-33 rs1891385 is correlated with the onset of COPD.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Acknowledgements

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