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Original scientific paper

ASSOCIATION BETWEEN IL-4 GENE POLYMORPHISMS AND CHRONIC OBSTRUCTIVE PULMONARY DISEASE OF PATIENTS IN HEBEI PROVINCE, CHINA

Yumin CHI, Qingguo DI, Guangchao HAN, Min LI, Baohua SUN*

Respiratory Department, Cangzhou Central Hospital, Cangzhou 061001, Hebei Province, P.R. China

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We aimed to study the association between IL-4 gene polymorphisms and chronic obstructive pulmonary disease (COPD) of patients in Hebei Province. The blood samples of 62 COPD patients and 301 healthy subjects were collected. IL-4 gene polymorphisms were detected by PCR with sequence-specific primer typing. There were significant differences in the alleles of IL-4-33, IL-4-590 and IL-4-1098 between COPD patients and healthy subjects ($P<0.05$). IL-4-590/C ($P<0.001$, OR = 4.619, CI = 1.640-7.985) had the highest susceptibility, suggesting that people with this allele were 3.6 times more prone to COPD than those with IL-4-33-590/T allele. COPD of susceptible patients was positively associated with IL-4-33/C:C ($P<0.001$, OR = 5.3, CI = 0.939-7.714), IL-4-590/C:C ($P<0.001$, OR = 29.5, CI = 11.138-57.647) and IL-4-1098/T:T ($P<0.001$, OR = 3.3, CI = 1.016-7.862). According to the level of protectiveness, COPD of patients (protective) was negatively associated with IL-4-33/C:T ($P = 0.009$, OR = 0.109, CI = 0.015-0.809), IL-4-590/C:T ($P = 0.009$, OR = 0.397, CI = 0.195-0.810), IL-4-1098/G:G ($P<0.001$, OR = 0.133, CI = 0.054-0.331) and IL-4-1098/G:T ($P<0.001$, OR = 0.209, CI = 0.104-0.527). IL-4/TCT ($P<0.001$, OR = 35.500, CI = 11.30-111.515) was found in susceptible patients. Negative association was found between COPD and haplotypes: IL-4/TTC ($P<0.001$, OR = 0.061, CI=0.008-0.443) and IL-4/GCC ($P<0.001$, OR = 0.193, CI = 0.076-0.488). IL-4/GCT, IL-4/GTC

Corresponding author: Baohua Sun, Respiratory Department, Cangzhou Central Hospital, Cangzhou 061001, Hebei Province, P.R. China, E-mail: sunbhcch@hotmail.com

and IL-4/GTT were only found in healthy subjects. IL-4 gene polymorphisms (loci -1098, -590 and -33) were closely associated with COPD in Hebei Province.

Keywords: association, IL-4, genetic polymorphism, chronic obstructive pulmonary disease

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a lung disease characterized by incomplete reversible airflow limitation, with high prevalence and mortality rates. The incidence rate of COPD among Chinese people aged over 40 years old has been as high as 8.2%, leading to nearly 1 million deaths (VESTBO *et al.*, 2013).

In the pathogenesis of COPD, the roles of cytokines have attracted wide attention. They are polypeptides which are secreted and synthesized by various immune cells. With a wide variety of types and functions, cytokines affect and regulate each other to form a network that acts on related target cells and participates in the onset and progression of COPD. At present, the associations between pathogenesis of COPD and genetic polymorphisms of cytokines such as tumor necrosis factor α, interleukins and growth factors have been extensively studied (MALTAIS *et al.*, 2014).

Human interleukin-4 (IL-4) is mainly produced by activated T cells, mast cells and basophils, as a characteristic cytokine promoting humoral immunity and inhibiting cellular immunity. IL-4 can promote the production of IgE in B lymphocytes, so it was initially believed to play an indispensable role in the pathogenesis of asthma. Meanwhile, IL-4 may participate in the pathogenesis of COPD, and can directly or indirectly activate and stimulate inflammatory cell aggregation to induce airway hyperresponsiveness. Although IL-4 has been involved in the onset of COPD and asthma, in-depth studies are still in need. Meanwhile, sample size, variables and regional factors also result in distinct differences (LEUPPI *et al.*, 2013; PASCOE *et al.*, 2015). IL-4 haplotypes (-589C, -33C) may be related to the occurrence of COPD (VANFLETEREN *et al.*, 2013). Besides, the heterozygous genotype of -589C/T in IL-4 promoter region may be related to the pathogenesis of COPD (KÖHNLEIN *et al.*, 2014). The changes of IL-4 level in COPD patients have been widely explored, but the association between IL-4 gene polymorphisms and COPD especially that in a certain region, has seldom been reported. Thereby motivated, we herein aimed to investigate the association between IL-4 gene polymorphisms and COPD patients in Hebei Province, China.

MATERIALS AND METHODS

Subjects

A total of 62 COPD patients admitted in our hospital from 2015 to 2016 were selected. All patients received detailed inquiry of medical history, comprehensive physical examination, chest X-ray or chest CT examination, and pulmonary function test to exclude bronchial asthma, bronchiectasis, tuberculosis, lung mass, pulmonary interstitial fibrosis, thoracic malformations and other respiratory diseases. Patients were included in accordance with the diagnostic criteria of chronic obstructive pulmonary disease stipulated by the American Thoracic Society. There were 44 males and 18 females. Except for four patients, all the others were smokers.

Meanwhile, 301 healthy volunteers in Hebei Province, who had similar gender ratio and age to those of the COPD patients, were enrolled as a control group. They were not blood-related with the COPD group, without any chronic respiratory diseases. They had normal results of

physical examination, chest X-ray examination, pulmonary function test and electrocardiography.

This study has been approved by the ethics committee of our hospital. All enrolled subjects had signed informed consent.

Collection of Samples

Venous blood (5 mL) was taken from subjects. The blood samples were anti-coagulated with EDTA and stored in a -80°C refrigerator.

Extraction and Purification of Target Gene

Genomic DNA was extracted using a blood genomic DNA extraction kit.

IL-4 Genotyping

Cytokine genotyping was performed by polymerase chain reaction with sequence-specific primer (PCR-SSP) typing using a cytokine genotyping kit (Invitrogen, Brown Deer, WI, USA) to determine the alleles, genotypes, haplotypes and diplotypes of IL-4-1098, IL-4-590 and IL-4-33 polymorphisms. Lyophilized primer mixtures and reagents in the kit were developed by University of Heidelberg for the cytokine polymorphism component of the 13th International Histocompatibility Workshop (Seattle, USA). PCR amplification was carried out strictly according to manufacturer's manual using a PTC-100 thermal cycler (MJ Research, Inc., Waltham, MA, USA). Briefly, PCR-SSP typing was performed by using 48 PCR primer mixtures aliquoted in 96-well PCR trays (two typings per tray). Master mix consisting of $MgCl_2$, buffer, d-NTP's and glycerol, which was supplied along with the reagents, was mixed with 1.2-3.0 μg DNA and 20 U Taq polymerase and dispensed in 48 wells. Agarose gel (2%) electrophoresis revealed either a positive or a negative specific amplification for each well. Subsequently, the results were automatically analyzed by Cytokine-SCORE software. Manual interpretation was also possible according to the interpretation scheme provided along with the kit (WEDZICHA *et al.*, 2013).

Statistical Analysis

A population genetics analysis package developed by the Biostatistics Core for the Workshop, PyPop, was used to analyze cytokine data. Allele frequencies and expected Hardy Weinberg proportions (HWPs) for each single nucleotide polymorphism (SNP) were determined. The exact deviation of genotype frequency from HWP was calculated using the Arlequin implementation through PyPop.

SNPs that did not conform to HWP were evaluated to determine whether there were excessive homozygotes or heterozygotes, or if the frequencies of any genotypes were significantly different from the expected values by using the Chi-square test. Comparisons of frequencies for two groups were performed by the χ^2 test. Pearson's P values, crude odds ratio (OR) and Wald's 95% confidence interval (CI) were calculated to test the associations between IL-4 polymorphisms and COPD with GraphPad QuickCalcs. $P<0.05$ was considered statistically significant.

RESULTS

IL-4 Alleles

There were significant differences in the alleles of IL-4-33, IL-4-590 and IL-4-1098 between COPD patients and healthy subjects (P<0.05) (Table 1), so they may be candidates for such population. IL-4-590/C (P<0.001, OR = 4.619, CI = 1.640-7.985) had the highest susceptibility, indicating that people with this allele were 3.6 times more prone to COPD than those with IL-4-33-590/T allele.

Table 1. Frequencies of IL-4 alleles

IL-4 polymorphism	Allele	COPD (n=62)		Control (n=301)		Pearson P value	Odds ratio	Wald's 95% CI
		N	F	N	F			
IL-4-33	C	60	0.857	176	0.308	<0.001	2.712	0.590-6.496
	T	10	0.143	396	0.692			
IL-4-590	C	68	0.971	377	0.659	<0.001	4.619	1.640-7.985
	T	2	0.029	195	0.341			
IL-4-1098	G	66	0.943	409	0.715	<0.001	2.114	0.462-5.464
	T	4	0.057	153	0.198			

N: Absolute number; F: frequency; CI: confidence interval; COPD: chronic obstructive pulmonary disease.

IL-4 Genotypes

According to the level of susceptibility, COPD of susceptible patients was positively associated with IL-4-33/C:C (P<0.001, OR = 5.3, CI = 0.939-7.714), IL-4-590/C:C (P<0.001, OR = 29.5, CI = 11.138-57.647) and IL-4-1098/T:T (P<0.001, OR = 3.3, CI = 1.016-7.862).

According to the level of protectiveness, COPD of patients (protective) was negatively associated with IL-4-33/C:T (P = 0.009, OR = 0.109, CI = 0.015-0.809), IL-4-590/C:T (P = 0.009, OR = 0.397, CI = 0.195-0.810), IL-4-1098/G:G (P<0.001, OR = 0.133, CI = 0.054-0.331) and IL-4-1098/G:T (P<0.001, OR = 0.209, CI = 0.104-0.527) (Table 2).

Table 2. Frequencies of IL-4 genotypes in COPD patients and normal population

IL-4 polymorphism	Genotype	COPD (n=62)		Control (n=301)		Pearson P value	Odds ratio	Wald's 95% CI
		N	F	N	F			
IL-4-33	C:C	20	0.571	16	0.056	<0.001	5.3	0.939-7.714
	C:T	1	0.029	61	0.213	0.009	0.109	0.015-0.809
	T:T	14	0.400	209	0.731	0.049	0.532	0.301-1.008
IL-4-590	C:C	23	0.657	4	0.014	<0.001	29.5	11.138-57.647
	C:T	11	0.314	187	0.654	0.009	0.397	0.195-0.810
	T:T	1	0.029	95	0.332	0.511	2.074	0.225-19.093
IL-4-1098	G:G	4	0.114	174	0.608	<0.001	0.133	0.054-0.331
	G:T	2	0.057	1	0.004	<0.001	0.209	0.104-0.527
	T:T	29	0.829	111	0.388	<0.001	3.3	1.016-7.862

IL-4 Polymorphisms

As shown in Table 3, IL-4/TCT (P<0.001, OR = 35.500, CI = 11.30-111.515) is found in susceptible patients. Negative (protective) association was found between COPD and haplotypes: IL-4/TTC (P<0.001, OR = 0.061, CI=0.008-0.443) and IL-4/GCC (P<0.001, OR = 0.193, CI = 0.076-0.488). IL-4/GCT, IL-4/GTC and IL-4/GTT were only found in healthy subjects.

Table 3. IL-4 polymorphisms of COPD patients and normal population

IL-4 polymorphism	COPD (n=62)		Control (n=301)		Pearson P value	Odds ratio	Wald's 95% CI
	N	F	N	F			
GCC	5	0.071	163	0.285	<0.001	0.193	0.076-0.488
GCT	0	0	8	0.014	↑	↑	↑
GTC	0	0	4	0.007	↑	↑	↑
GTT	0	0	1	0.002	↑	↑	↑
TCC	34	0.486	202	0.353	0.030	1.730	1.050-2.850
TCT	14	0.200	4	0.007	<0.001	35.500	11.30-111.515
TTC	1	0.014	110	0.192	<0.001	0.061	0.008-0.443
TTT	16	0.229	80	0.140	0.049	1.822	0.944-3.340

DISCUSSION

A number of studies have been conducted both at home and abroad on COPD candidate genes. However, except relatively explicit results on the study of α-antitrypsin gene, the other candidate genes have great differences in different regions and races. Initially, human IL-4 can promote B lymphocytes to produce IgE, so it is believed that the substance plays an indispensable role in the pathogenesis of asthma disease. At the same time, the relevant research and experiments show that IL-4, which may participate in the pathogenesis of COPD, can directly or indirectly activate the stimulation of inflammatory cell aggregation induced airway hyperresponsiveness. Although foreign studies suggest that IL-4 is involved in the pathogenesis of COPD and asthma, but there is still few domestic researches on the field. Moreover, sample size, variable factors, geographical and other effects also lead to some differences in the results (BARNES, 2013; VOGELMEIER *et al.*, 2013; CELLI *et al.*, 2015). Genetic polymorphisms have been extensively studied at the current stage: SNPs of IL-4-33C/T, -589C/T and other loci. VANFLETEREN *et al.* (2013) found that IL-4 haplotype (-589C, -33C) may be related to the occurrence of COPD. KÖHNLEIN *et al.* (2014) reported that IL-4 promoter region -589C/T heterozygous genotype may be related to the incidence of COPD. There are relatively more studies on the changes of IL-4 levels in COPD patients domestically, but studies on IL-4 gene polymorphisms remain few, especially there is still lack of studies on large sample size of a certain regional variable. Foreign researches of the gene have also a variety of results, which may be due to the existence of regional differences (THOMSEN *et al.*, 2013; BARRECHEGUREN *et al.*, 2015).

In this study, the relationship between the IL-4-33, IL-4-590 and IL-4-1098 polymorphisms and COPD susceptibility in 62 COPD patients was analyzed. The results showed that there was a statistically significant difference in IL-4-33, IL-4-590 and IL-41098 alleles between COPD patients and normal Hebei people, so it can be inferred that IL-4-33, IL-4-590

and IL-4-1098 alleles may be candidates for COPD patients. IL-4-590/C (P<0.001, OR = 4.619, CI = 1.640-7.985) had the highest incidence of susceptibility, suggesting that patients with IL-4-590/C allele had 3.6 times higher risk of being affected with COPD than patients with IL-33-590/T allele. BRIGHTLING *et al.* (2014) studied the SNP of IL-4 -33C/T locus in 100 COPD patients. The results showed that no SNP difference was found in IL-4-33C/T locus between COPD patients and normal people, which can be inferred that IL-4-33C/T locus may not be the candidate of COPD. The reason for different results may be due to geographical differences. According to the level of susceptibility, we found that COPD of patients (susceptible to infection) and IL-4-33/C:C, IL-4-590/C:C and IL-4-1098/T:T were positively associated. According to the protective level, the association between COPD patients (protective) and IL-4-33/C:T, IL-4-590/C:T and IL-4-1098/G:G was negative. IL-4/TCT (P<0.001, OR = 35.500, CI = 11.30-111.515) is found in (susceptible) patients with positive COPD (based on susceptibility). IL-4/TTC (P<0.001, OR = 0.061, CI = 0.008-0.443); IL-4/GCC (P<0.001, OR = 0.193, CI = 0.076-0.488). However, IL-4/GCT, IL-4/GTC and IL-4/GTT were found only in the normal population.

As COPD is a complex disease with multiple genetic predisposition, the research of its susceptible genes is in the ascendant with the progress of science and technology and upgrading of new detection means. Due to ethnic and ethnic differences, the conclusions of different genes may differ in different countries, so disease gene screening should be made on susceptible population according to the differences between countries and regions, which is of important value for the early prevention for the disease onset.

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POVEZANOST IZMEĐU POLIMORFIZMA GENA IL-4 I HRONIČNE OBSTRUKTIVNE BOLESTI PLUĆA PACIJENATA U HEBEI PROVINCIJI, KINA

Yumin CHI, Qingguo DI, Guangchao HAN, Min LI, Baohua SUN*

Respiratorni departman, Cangzhou centralna bolnica, Cangzhou 061001, Hebei Provincija, Kina

Izvod

Cilj rada je bio proučavanje povezanosti između polimorfizma IL-4 gena i hronične obstruktivne bolesti pluća (COPD) pacijenata u Hebei provinciji. Sakupljeni su uzorci krvi 62 pacijenta sa COPD i 301 zdrave osobe. Polimorfizam IL-4 gena detektovan je PCR metodom sa sekvenca specifičnim prajmerima. Utvrđena je značajna razlika u alelima kod IL-4-33, IL-4-590 i IL-4-1098 između obolelih i zdravih osoba (P<0.05). IL-4-590/C (P<0.001, OR = 4.619, CI = 1.640-7.985) su imali najveću osetljivost, ukazujući da ljudi sa ovim alelom bili 3.6 puta više skloni COPD nego oni sa IL-4-33-590/T alelom. COPD osetljivih pacijenata je bio pozitivno povezan sa IL-4-33/C:C (P<0.001, OR = 5.3, CI = 0.939-7.714), IL-4-590/C:C (P<0.001, OR = 29.5, CI = 11.138-57.647) i IL-4-1098/T:T (P<0.001, OR = 3.3, CI = 1.016-7.862). Prema nivou zaštite, COPD pacijenata je bio negativno povezan sa IL-4-33/C:T (P = 0.009, OR = 0.109, CI = 0.015-0.809), IL-4-590/C:T (P = 0.009, OR = 0.397, CI = 0.195-0.810), IL-4-1098/G:G (P<0.001, OR = 0.133, CI = 0.054-0.331) i IL-4-1098/G:T (P<0.001, OR = 0.209, CI = 0.104-0.527). IL-4/TCT (P<0.001, OR = 35.500, CI = 11.30-111.515) je pronađen kod osetljivih pacijenata. Negativna povezanost je pronađena između COPD I haplotipova: IL-4/TTC (P<0.001, OR = 0.061, CI=0.008-0.443) i IL-4/GCC (P<0.001, OR = 0.193, CI = 0.076-0.488). IL-4/GCT, IL-4/GTC i IL-4/GTTsu pronađene samo kod zdravih osoba. Polimorfizam IL-4 gena (loci -1098, -590 and -33) bili su blisko povezani sa COPD u Hebei provinciji.

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