RESEARCH ARTICLE

Association between Polymorphisms of Interleukin-17A and Interleukin-17F Genes and Silicosis Susceptibility in Chinese Han People

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Abstract

Background: To explore the relationship between polymorphisms of interleukin17 (IL-17) gene(A-832G 7488A/G) and the susceptibility to silicosis, a risk factor for lung cancer. Materials and Methods: A total of 113 silicosis patients and 116 workers without silicosis were enrolled in the case-control study. IL-17A A-832G and IL-17F 7488A/G polymorphisms were evaluated by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Results: The frequencies of AA,GG and AG of IL-17A A-832G locus in the case and control groups were 46.9%, 8.0%, 45.1%, and 49.2%, 7.6%, 43.2%, respectively, with no significant differences ($p>0.05$).The GG genotype in the IL-17F (7488A/G) locus was not found. The frequencies of AA and GA of IL-17F 7488A/G locus in the case and control groups were 84.1%, 15.9% and 66.4%, 33.6%, respectively ($p<0.05$). Analysis of combined effects showed that the individuals with GG+AG genotype of IL-17A and GG+GA genotype of IL-17F are protected against silicosis (OR=0.469). Conclusions: IL-17F 7488A/G is associated with susceptibility to silicosis, and G allele may have a protective effect. No relationship was found between IL-17A gene polymorphisms at A-832G and silicosis.

Keywords: Silicosis - IL-17A, IL-17F - polymorphism - disease susceptibility - combined effect

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Introduction

Pneumoconiosis is still one of the most serious occupational disease over the world, especially in developing countries (Naidoo et al., 2005; Chen et al., 2012; Oyunbileg et al., 2011; Pingle et al., 2012). By the end of 2012, the total of pneumoconiosis patients is 727148 in china, which is 76.74% of the total number of occupational disease. (http://www.niohp.net.cn/Contents/Channel23/2011/1227/16777 /content16777). Silicosis is one of the most common and serious pneumoconiosis, which is shown as a chronic inflammatory response leading to severe pulmonary fibrotic changes (Berran et al., 2001). Many cytokine involved in pulmonary fibrotic process and interleukin 17 is one of these cytokine. Interleukin17 (IL-17) is a primary effector secreted from Th17 cells which is considered to be a group of important induce inflammatory reaction cells (Gaffen et al., 2008). The important members of IL-17 family are IL-17A and IL-17F, and the main biological function of IL-17 is promoting the inflammatory reaction (Han et al., 2009; Zhang et al., 2012; Fang et al., 2012). IL-17 is not only associated with chronic inflammation, but also promots the formation of pulmonary fibrosis. (Simonian et al., 2009). In addition, animal studies have shown that IL-17 can promote the proliferation, transformation, and collagen synthesis of the pulmonary fibroblasts in the formation of pulmonary fibrosis (Dong et al., 2012). In conclusion, IL-17 plays an important role in the formation process of pulmonary fibrosis.

In this article, we analyzed two single-nucleotide polymorphisms (SNPs) in the IL-17A (A-832G) and IL-17F (7488A/G) to explore the relationship between polymorphisms of interleukin-17 (IL-17) gene and the susceptibility of silicosis. Up to date, no related studies about the association between polymorphisms of IL-17A and IL-17F genes and silicosis susceptibility have not been analyzed.

Materials and Methods

Study subjects

The subjects were obtained from one gold mine or steel enterprise and were the unrelated Han people in China. The case group consisted of 113 patients with stage I silicosis, who were diagnosed by pneumoconiosis diagnostic groups with confirmed qualification, and was matched with the control group (116 workers without silicosis), according to the age, sex, nationality, working place, exposure to dust. These patients did not have any

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Table 1. Primers of the SNPs at IL-17A A-832G and IL-17F 7488A/G

	IL-17A A-832G	IL-17F 7488A/G
Upstream primer	5'-TTACACTCCAGCCATTGAGTTG-3'	5'-ACCAAGGCTGCTCTGTTTCT-3'
Downstream primer	5'-TGAAAATGGGGATAGAGACTGG-3'	5'-GGTAAGGAGTGGCATTTCTA-3'

hereditary blood diseases.

Collection and preservation of the sample

The study was approved by the Medical Ethics Committee of Hebei United University (permit number 13057), and all subjects provided written informed consent.1.5ml peripheral venous blood was collected and mixed it with EDTA to prevent blood coagulating. All sample's genomic DNA was extracted from blood by salting out method and were kept in -20°C until used.

Genetic polymorphism analysis

The primer for IL-17A A-832G and IL-17F 7488A/G was designed by the gene pool of literature reports (Luo et al., 2010; Wu., 2010). The primer of sequence of each SNP was shown in Table 1. The PCR reactions were performed in a total volume of 20μl mixture containing: 8μl 2xTaq PCR MasterMix (BioTeke corporation, PR1701), 1μl genomic DNA, 1μl each primer, 9μl double-distilled water without bacteria.

PCR conditions were as follows: denaturation step at 95°C for 5min, followed by 35 cycles of 30s at 95°C, 30s at 58°C, 30s at 72°C, A final elongation at 72°C for 5min for IL-17A A-832G; denaturation step at 95°C for 5min, followed by 38 cycles of 30s at 95°C, 45s at 60°C, 45s at 72°C, and a final elongation at 72°Cfor 10 min for IL-17F 7488A/G. The PCR products were digested in 65°Cfor 30min by Taq I (New England Biolabs, Beverly, MA, USA, R0149S) for the A-832G, and digested in 37°C for 15 min by NlaIII (New England Biolabs, Beverly, MA, USA, R0125L) for the 7488A/G. All the digested PCR products were identified by 3% agarose gel electrophoresis and stained with ethidium bromide for visualization under UV light.

Statistical analysis

All statistical analyses were performed by the SPSS software 17.0. (SPSS Inc, Chicago, IL, USA) The quantitative data obeyed normal distribution was using the two independent sample t-test. The IL-17 allele and genotype frequencies in the two group by the χ^2 test. The combined effect of IL-17A and IL17F genotypes between cases and controls, the Hardy-Weinberg equilibrium (HWE) in all subjects using the χ^2 test. *p*-value was considered significant at a level of <0.05.

Results

Characteristics of participants

In this study, 229 Chinese Han subjects consisted of 113 silicosis patients and 116 silicosis-free controls were enrolled .The average ages was 58.79±7.72 years (mean±SD) and 57.36±7.61 years (mean±SD) in the two groups. The average ages exposed to dust was 29.08±6.31 years (mean±SD) and 29.84±6.08 years (mean±SD) in the two group. There were no statistically significant differences among the cases and controls with respect to the average ages and the average ages exposed to dust (p=0.161, p=0.351, respectively). In addition, the smoking rates were 69.91% and 69.83% in the cases and controls. There were no statistically significant differences among the two groups (p=0.989). The allele frequencies of the SNPs at A-832G and 7488A/G sites in cases and controls were tested in Hardy-Weinberg equilibrium. There were no statistically significant difference between cases and controls (p=0.820, p=0.105 respectively).

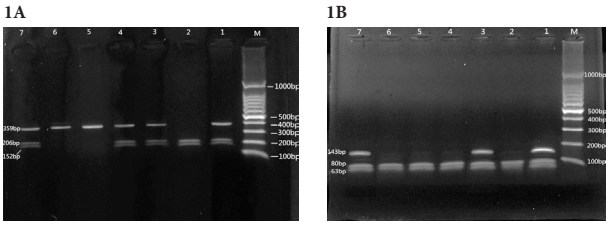

Figure 1. The PCR Electrophoregram of the SNPs of Human IL-17 DNA. A: (M:100bp DNA ladder Mark; 1,3,4,7: AG genotype; 2: GG genotype; 5,6: AA genotype); **B.** (M: 100bp DNA ladder Mark; 1, 3, 7: GA genotype; 2, 4, 5, 6: AA genotype)

Analysis of the SNPs at IL-17A A-832G and IL-17F 7488A/G sites

After digested with the corresponding enzyme, the PCR product at IL-17A (A -832G) site was digested into three types of fragments: 359bp, 206bp+152bp and 359bp+206bp+152bp, as shown in Figure 1A. PCR product at IL-17F (7488A/G) site was digested into three types of fragments: 143bp, 80bp+63bp and 143bp+80bp+63bp, as shown in Figure 1B. In this study, AA, GG and AG genotypes at IL-17A (A-832G) site were found. AA and GA genotypes at IL-17F (7488A/G) site were found, and GG genotypes was not found.

Allele and genotype distribution of the SNPs at A-832G, 7488A/G in silicosis cases and controls

The frequencies of AA, GG and AG of IL-17A (A-832G) locus in cases and controls were 46.9%, 8.0%, 45.1% and 49.2%, 7.6%, 43.2%, respectively, there was no significant difference between cases and controls (p>0.05). As shown in Table2. For the locus of IL-17F (7488A/G), GG genotype was not be found. The frequencies of AA and GA in the cases and controls were 84.1%, 15.9% and 66.4%, 33.6% respectively.

Significant difference was observed in the genotype distribution between cases and controls. (p>0.05). The GA genotype was statistically associated with the decreased risk of silicosis compared to AA genotype (p=0.002), and the risk of GA genotype individuals suffered from silicosis was lower than the AA genotype individuals. Allele analysis revealed that the G allele may be a protective factor of silicosis. (OR=0.428; 95%CI=0.237-0.774), as shown in Table3.

Table 2. Genotype and Allele Distribution of the SNPs at A-832G in Silicosis Patients and Controls

Genotype	Cases (%) n =113	Controls (%) n=116	χ^2	*p*	OR(95%CI)
AA	53(46.9)	57(49.2)			1
GG	9 (8.0)	9 (7.6)	0.02	0.886	0.930(0.343-2.519)
AG	51(45.1)	50(43.2)	0.113	0.737	0.912(0.531-1.565)
GG/AG	60(53.1)	59(50.8)	0.115	0.735	0.914(0.544-1.536)
Allele					
A	157(69.5)	164(70.7)	0.081	0.775	0.943(0.632-1.403)
G	69(30.5)	68(29.3)			

Table 3. Genotype and Allele Distribution of the SNPs at 7488A/G in Silicosis Patients and Controls

Genotype	Cases(%) n=113	Controls(%) n=116	χ^2	*p*	OR(95%CI)
AA	95(84.1)	77(66.4)			
GG	0(0)	0(0)			
GA	18(15.9)	39(33.6)			
GG/GA	18(15.9)	39(33.6)	9.583	0.002	0.374(0.198-0.705)
Allele					
A	208(92.0)	193(83.2)	8.221	0.004	0.428(0.237-0.774)
G	18 (8.0)	39(16.8)			

Table 4. Relationship between the Combined Effect of IL17A, IL17F Genotypes and Silicosis

Genotype		Cases	Controls	χ^2	*p*	OR(95%CI)
A-832G	7488A/G					
AA	AA	49	46			1
(GG+AG)	AA	46	31	1.146	0.284	1.393(0.759-2.558)
AA	(GG+GA)	4	11	3.220	0.073	0.341(0.101-1.148)
(GG+AG)	(GG+GA)	14	28	3.903	0.048	0.469(0.220-1.000)

The combined effect of IL-17A and IL-17F genotypes between cases and controls

The genotypes of IL-17A (A-832G) and IL-17F (7488A/G) were combined to analyze the relationship between the combined effect of IL17A, IL17F genotypes and silicosis. The individuals with AA genotype of IL-17A and AA genotype of IL-17F at the same time were regarded as reference. This result showed that the risk of the individuals (with GG+AG genotype of IL-17A and (GG+GA) genotype of IL-17F at the same time) suffered from silicosis was lower than the individuals (with AA genotype of IL-17A and AA genotype of IL-17F at the same time) (OR=0.469). As shown in Table4.

Discussion

Interleukin-17 (IL-17) is a relatively newly described cytokine that bridges the adaptive and innate immune systems. Many study showed that a large group of human fibrosis diseases have abnormally high IL-17 expression. IL-17 was found to stimulate fibroblasts to produce IL-6, IL-11, IL-8 (Mo et al., 2001; Ran et al., 2012). Majority of analyzes about genetic polymorphism of IL-17 were focus on a range of cancers, including breast cancer, gastric cancer and cervical cancer (Quan et al., 2005;Shibata et al., 2009; Shu et al., 2010;Wang et al., 2012). But no analysis about the relationship between IL-17 polymorphisms and silicosis has been reported. We explored the relationship between polymorphisms of IL-17 and the susceptibility of silicosis because IL-17 played an important role in silicosis. However, no relationship was found between IL-17A gene polymorphisms at A-832G and silicosis now. The frequencies of genetic polymorphisms often vary between ethnic groups (Spielman et al., 2007), In the present study, the G allele frequency of A-832G was 0.293 among 116 control subjects, which is accord with the Chinese Han population (0.244) in SNP DataBase, but significantly lower than that of Sub-SaharanAfrican (0.372) population and European (0.783) population. (http://ww w.ncbi.nlm.nih.gov/SNP).

Kawaguchi et al reported that the IL-17F 7488A/G variant, which causes a His-to-Arg substitution at amino acid 161 (H161R) variant, influences the risk of asthma (Kawaguchi et al., 2006). In this study, we found that GA genotype of IL-17F 7488A/G was significantly associated with an decreased risk for silicosis compared to AA carriers, and G allele may be a protective factor of silicosis. Some study showed that the frequency of the 7488A/G GG genotype was low (0%) (Arisawa et al., 2007; Bazzi et al., 2011). This might explain why there was no GG Genotype to be found in this study. Similar to this study, Arisawa et al showed that G allele has been associated with protective effects in Asian patients with inflammatory and autoimmune conditions (Arisawa et al., 2007; Arisawa et al., 2008). But in present study, the findings suggest that IL-17F 7488G allele is associated with increased lung cancer risk in the Tunisian population (Kaabachi et al., 2014). This opposite result might because the variance of Genotype frequency in different populations, or the different function of IL-17 in silicosis and lung cancer.

IL-17A and IL-17F genes are mapped on the same chromosome at position 6p12T. (Paradowska-Gorycka et al., 2010). So we investigated the relationship between the combined effect of IL-17A, IL-17F genotypes and silicosis. Our study showed that the risk of the individuals (with GG+AG genotype of IL-17A and (GG+GA) genotype of IL-17F at the same time) suffered from silicosis was lower than the individuals (with AA genotype of IL-17A and AA genotype of IL-17F at the same time). When the polymorphisms of IL-17A A-832G and the polymorphisms of IL-17F 7488A/G were analyzed together, we found that the risk of the individuals (with GG+AG genotype of IL-17A and GG+GA genotype of IL-17F at the same time) suffered from silicosis was higher than the individuals with GG+GA genotype of IL-17F alone (0.469>0.428). This might explained that GG+AG genotype of IL-17A weaken the protection of GG+GA genotype of IL-17F 7488A/G for silicosis.

Nowadays, A large of studies about Genetic polymorphisms (Fayaz;et al., 2013; Gao et al., 2013; Li et al., 2013) has been reported, but in these studies, the relationship between the combined effect of different Gene loci has not been researched.To our knowledge, we have demonstrated that the relationship between combined

effect of IL17A, IL17F genotypes and silicosis. And this is the first study shows that IL-17F 7488A/G polymorphism may contribute to the risk of silicosis. However, our results were obtained with a limited sample size, which weakens our ability to solidify statistic associations. Additional studies are needed to explore the association between the polymorphisms of IL-17 and the risk of silicosis in other ethnic populations.

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