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Influence of interleukin-17 gene polymorphisms on the development of pulmonary tuberculosis

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ABSTRACT. We conducted a case-control study in a Chinese population to examine the correlations between interleukin (IL)-17 gene polymorphisms and tuberculosis (TB) development. The study population included 336 TB subjects and 351 control subjects who were enrolled between June 2012 and June 2014. Genotyping analyses of IL-17A rs2275913 and rs3748067 and IL-17F rs763780 were analyzed using polymerase chain reaction-restriction fragment length of polymorphism. The genotype distributions of IL-17 rs2275913 were found to be in Hardy-Weinberg equilibrium in the controls, while the IL-17 rs3748067 and rs763780 were not. Based on unconditional logistic regression, individuals carrying the AA genotype and GA + AA genotype of rs2275913 were more likely to have a significantly increased risk of TB compared to subjects with the GG genotype. The ORs (95%CI) for the AA genotype and GA + AA genotype were 2.20 (1.35-3.60) and 1.52 (1.11-2.09), respectively. The CC genotype and TC + CC genotype of rs763780 were associated with increased risk of TB when compared with the TT genotype. The ORs (95%CI) for the CC genotype and TC + CC genotype were 1.99 (1.05-3.87) and 1.58 (1.07-2.33), respectively. In conclusion, rs763780 may play a critical role in the etiology of TB.

Key words: Interleukin-17; Polymorphism; Pulmonary tuberculosis

INTRODUCION

Tuberculosis (TB) is an infectious disease with the highest death rate worldwide, despite the fact that the first anti-tuberculosis drug was introduced approximately 50 years ago (Lawn and Zumla, 2011). It is estimated that 8.1 million people were diagnosed with active TB and 1.3 million died from it by the World Health Organization in 2011 (Lawn and Zumla, 2011). It is reported that about 60% of the world's population have been infected with pathogen mycobacterium tuberculosis, but less than 10% of these TB-infected cases will present clinical disease during their lifetimes.

Previous studies have reported that cytokine genes contribute to susceptibility of TB, such as interferon-1β, interleukin (IL)-3, IL-6, IL-10, and IL-12B IL-17 as well as IL-18 (Selvaraj et al., 2008; Abhimanyu et al., 2011; Meenakshi et al., 2013; Feng et al., 2014; Tiwari et al., 2014; Zhang et al., 2014). However, the results have been inconsistent.

Th17 cells are a unique subset of effector T helper cells that subvert the Th1 and Th2 lineages (Harrington et al., 2005). Increasing studies have reported that Th17 cells are the main IL-17-producing cells and contribute to protective immunity against *Mycobacterium tuberculosis* (Khader et al., 2007; Chen et al., 2009; Paidipally et al., 2009). Khader et al. (2007) reported that Th17 response had a role in protective immunity in *M. tuberculosis* infection, and *M. tuberculosis* infection was correlated with a decreased Th17 response because of suppressing Th1 cytokines (Khader et al., 2007). Therefore, Th17 cells not only contribute to the development of *M. tuberculosis*, but also protect against intracellular pathogens (Pitta et al., 2009).

Previous studies reported an association between genetic polymorphisms in IL-17 and the susecptibility of TB (Abhimanyu et al., 2013; Ocejo-Vinyals et al., 2013; Peng et al., 2013; Tiwari et al., 2014), but the results of previous studies are inconsistent. Therefore, we conducted a case-control study in a Chinese population to examine the correlations between IL-17 gene polymorphisms and TB development.

MATERIAL AND METHODS

Study population

A total of 336 TB subjects and 351 control subjects were collected from First Affiliated Hospital of Xinxiang Medical University between June 2012 and June 2014. TB patients were diagnosed based on X-rays and bacteriologically with both positive sputum smear examination and culture for *M. tuberculosis*. The excluded criteria for TB cases were those with extra pulmonary TB in organs other than the lungs, and with human immunodeficiency virus-positive. A total of 351 control subjects who had undergone routine healthy examination in the First Affiliated Hospital of Xinxiang Medical University during the same period. The inclusion criteria for the control subjects were free of TB and had no history of an inflammatory autoimmune disease.

A detained questionnaire was taken to collect the demographic information of TB patients and controls, including age, gender, tobacco smoking and severity levels. A written informed consent form was completed by all TB patients and controls before participating in the study. Our study protocol was approved by the ethics committee of the First Affiliated Hospital of Xinxiang Medical University.

Single nucleotide polymorphism selection and genotyping analysis

Three single nucleotide polymorphisms of IL-17A rs2275913 and rs3748067 and IL-17F

rs763780 were selected. Each TB patient and control subject provided 5 mL peripheral blood and stored at -20°C until use. Genomic DNA of IL-17A rs2275913 and rs3748067 and IL-17F rs763780 was isolated from the ethylenediaminetetraacetic acid (EDTA)-anticoagulated peripheral blood samples using a TIANamp blood DNA kit (Tiangen Biotech, Beijing, China) according to the manufacturer instructions. Genotyping analyses of IL-17A rs2275913 and rs3748067 and IL-17F rs763780 were analyzed using polymerase chain reaction-restriction fragment length of polymorphism. The positive and reverse primers of IL-17A rs2275913 and rs3748067 and IL-17F rs763780 were designed using the Primer 5.0 software (Premier Biosoft, Palo Alto, CA, USA). Polymerase chain reaction was performed using the following conditions: initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 45 s, annealing at 62°C for 60 s and extension at 72°C for 60 s, and final extension at 72°C for 10 min. The PCR products were visualized by 1.0% agarose gel electrophoresis with ethidium bromide staining and UV light.

Statistical analysis

Continuous variables are reported as means ±standard deviation (SD), and categorical variables were expressed as frequencies and percentage (%). Student *t*-test or χ^2-test was used to compare continuous variables and categorical variables between case and control groups. Hardy-Weinberg equilibrium for IL-17A rs2275913 and rs3748067 and IL-17F rs763780 among controls was compared using chi-squared goodness of fit test. Unconditional logistic regression analysis was performed to evaluate the effects of IL-17A rs2275913 and rs3748067 and IL-17F rs763780 polymorphisms on the risk of TB, with results expressed as odds ratios (ORs) and corresponding 95%CI. Homozygotes of the most frequent genotype were considered as the reference group for analysis. For all statistical analyses, values were considered to be statistically significant when $P < 0.05$.

RESULTS

This study included 336 TB cases (70.54% males and 29.46% females) and 351 controls (69.52% males and 30.48% females) (Table 1). The mean ages for TB cases and controls were 42.5 ± 10.6 and 41.3 ± 10.4 years, respectively. There were no significant differences in the distribution of gender, age, and tobacco smoking between TB cases and controls. Two hundred and seventy TB cases (80.36%) were mild-moderate level of TB and 66 (19.64%) were advanced level of TB.

Table 1. Basic characteristics of cases and controls.

Variables	Cases	%	Control	%	χ^2 value	P value
Age, years						
<50	240	71.43	243	69.23		
≥50	96	28.57	108	30.77	0.39	0.52
Gender						
Female	99	29.46	107	30.48		
Male	237	70.54	244	69.52	0.09	0.77
Tobacco smoking						
Never	197	58.63	229	65.24		
Ever	139	41.37	122	34.76	3.19	0.07
Severity levels						
Mild-moderate	270	80.36				
Advanced	66	19.64				

The genotype distributions of IL-17A rs2275913 and rs3748067 and IL-17F rs763780 were shown in Table 2. By χ^2-test, there were no significant differences in the genotype distributions of IL-17 rs2275913, rs3748067, and rs763780 between TB cases and controls. The genotype distributions of IL-17 rs2275913 were found to be in Hardy-Weinberg equilibrium in the controls, while the IL-17 rs3748067 and rs763780 were not. Minor allele frequencies in controls of the 3 single nucleotide polymorphisms in IL-17 were similar to the minor allele frequencies in NCBI.

Table 2. Allele frequencies of IL-17 genes in cases and control subjects.

SNP	Cases	%	Controls	%	χ^2 value	P value	Minor allele frequency in NCBI	Minor allele frequency in controls	P value for Hardy-Weinberg Equilibrium
rs2275913									
GG	129	38.39	171	48.72					
GA	143	42.56	142	40.46					
AA	64	19.05	38	10.83	12.19	0.002	0.2927	0.3105	0.30
rs3748067									
CC	277	82.44	303	86.33					
CT	40	11.90	37	10.54					
TT	19	5.66	11	3.13	3.09	0.21	0.0769	0.0840	<0.001
rs763780									
TT	250	74.40	289	82.34					
TC	54	16.07	44	12.54					
CC	31	9.23	18	5.13	6.92	0.03	0.0935	0.1766	<0.001

The associations between the IL-17A rs2275913 and rs3748067 and IL-17F rs763780 polymorphisms and risk of TB are shown in Table 3. Based on unconditional logistic regression, individuals carrying the AA genotype and GA + AA genotype of rs2275913 were more likely to have a significantly increased risk of TB when compared with the GG genotype. The odds ratio (95% confidence interval) for the AA genotype and GA + AA genotype of rs2275913 were 2.20 (1.35-3.60) and 1.52 (1.11-2.09), respectively. Moreover, the CC genotype and TC + CC genotype of rs763780 were associated with increased risk of TB when compared with the TT genotype. The odds ratio (95% confidence interval) for the CC genotype and TC + CC genotype were 1.99 (1.05-3.87) and 1.58 (1.07-2.33), respectively. However, we found no significant association between rs3748067 and TB development.

Table 3. Association of IL-17 rs2275913, rs3748067 and rs763780 polymorphisms with risk of TB.

Variables	Cases	%	Control	%	OR (95% CI)[1]	P value
rs2275913						
GG	129	38.39	171	48.72	Ref.	
GA	143	42.56	142	40.46	1.33 (0.95-1.87)	0.08
AA	64	19.05	38	10.83	2.20 (1.35-3.60)	<0.001
GA + AA	207	61.61	180	51.29	1.52 (1.11-2.09)	0.006
rs3748067						
CC	277	82.44	303	86.33	Ref.	
CT	40	11.90	37	10.54	1.18 (0.71-1.96)	0.49
TT	19	5.66	11	3.13	1.89 (0.84-4.47)	0.1
CT + TT	59	17.56	48	13.67	1.34 (0.87-2.08)	0.16
rs763780						
TT	250	74.40	289	82.34	Ref.	
TC	54	16.07	44	12.54	1.42 (0.90-2.24)	0.11
CC	31	9.23	18	5.13	1.99 (1.05-3.87)	0.02
TC + CC	85	25.30	62	17.67	1.58 (1.07-2.33)	0.01

[1]Adjusted for gender, age, tobacco smoking, and alcohol consumption.

DISCUSSION

In this study, we conducted a case-control study to evaluate the association between IL-17A rs2275913 and rs3748067 and IL-17F rs763780 polymorphisms and risk of TB in a Chinese population. We observed that individuals carrying the AA genotype and the GA + AA genotype of rs2275913 and the CC genotype and the TC + CC genotype of rs763780 were associated with increased risk of TB compared with the wide-type genotype, suggesting that IL-17 gene polymorphisms contribute to the susceptibility of TB.

Multiple previous studies have reported an association between IL-17 gene polymorphisms and autoimmune diseases (Jin et al., 2011; Zhang et al., 2013; Maalmi et al., 2014; Shen et al., 2015). Jin et al. (2011) reported an association between IL-17 gene polymorphisms and susceptibility to asthma in a Korean population, and suggested that the rs1889570 polymorphism was correlated with the development of asthma. Shen et al. (2015) reported that the IL-17 rs2275913 and rs3819024 variant alleles were associated with a decreased risk of rheumatoid arthritis, while the IL-17 rs3819025 and rs8193036 variant alleles were correlated with an increased risk of rheumatoid arthritis. Maalmi et al. (2014) conducted a study in a Tunisian population, and found that the IL-17A rs2275913 and IL-17F rs763780 were associated with an increased risk of asthma in children. Zhang et al. (2013) examined the association between IL-17 gene polymorphisms and inflammatory bowel disease, and reported that the IL-17 rs2275913 and rs763780 polymorphisms were associated with an increased risk of inflammatory bowel disease.

For the association between IL-17A and IL-17F gene polymorphisms and TB risk, 4 studies have reported an association, but the results of them were inconsistent (Abhimanyu et al., 2013; Ocejo-Vinyals et al., 2013; Peng et al., 2013; Tiwari et al., 2014). Ocejo-Vinyals et al. (2013) investigated an association between IL-17 rs2275913 polymorphism and susceptibility to TB, and suggested that the GG genotype of IL-17A rs2275913 was associated with an increased risk of pulmonary tuberculosis. Peng et al. (2013) conducted a study of a Chinese population and found that patients who carried the T allele of IL-17 rs763780 were more susceptible to TB compared to CC genotype, but no significant association was found between rs2275913 polymorphisms and TB risk. However, Tiwari et al. (2014) reported that the IL-17 gene polymorphisms were not associated with the development of TB. Abhimanyu et al. (2013) reported no association between IL-17A and IL-17F polymorphisms and risk of TB. In our study, we found that the CC genotype of rs763780 gene polymorphism was associated with an increased risk of TB. The discrepancies between these results may have been caused by differences in ethnicities, study design, and sample sizes.

There were three limitations to this study. First, genotype distributions of IL-17 rs3748067 and rs763780 were not in Hardy-Weinberg equilibrium in the control group. These results suggested that the controls did not represent the distribution of the general population. Therefore, some selection bias may have existed in this study. Second, the small sample size of this study may have limited the statistical power of identifying the difference between groups. Third, other genetic polymorphisms may influence the risk of TB, except for the inflammatory cytokines. Therefore, further large-sample studies are needed to investigate the association between inflammatory cytokines and susceptibility of TB.

In summary, we suggest that the AA genotype and GA + AA genotype of rs2275913 and the CC genotype and TC + CC genotype of rs763780 polymorphisms were associated

with an increased risk of TB, and rs2275913 and rs763780 contribute to the etiology of TB. Further large-sample studies are greatly needed to confirm these associations.

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