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Independent and joint effects of the *IL-6* and *IL-10* gene polymorphisms in pulmonary tuberculosis among the Chinese Han population

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ABSTRACT. We investigated the association between interleukin (IL)-6 and IL-10 gene polymorphisms and the susceptibility to pulmonary tuberculosis (PTB). DNA samples were obtained from 191 Han Chinese patients with PTB and 191 healthy control subjects. *IL-6* (-572, -174, -597) and *IL-10* (-1082, -819) polymorphisms were analyzed using polymerase chain reaction-restriction fragment length polymorphism. The *IL-6* -572 C/C and *IL-10* -819 T/T genotypes were observed less frequently in the case group than in the control group, with crude odds ratios of 0.591 [95% confidence interval (CI) = 0.381-0.917] and 0.401 (95%CI = 0.257-0.627), respectively. A significant association remained after adjusting for environmental factors in multivariate logistic analysis. The homozygote genotypes

of *IL-6* -572 and *IL-10* -819 had an adjusted OR of 0.565 (95%CI = 0.356-0.898) and 0.341 (95%CI = 0.210-0.553), respectively. These results indicate that the mutant heterozygote *IL-10* -1082 A/G+G/G genotype and the homozygote *IL-10* -819 T/T genotype have a combined effect on PTB. These results suggest that the *IL-6* -572 C/C and *IL-10* -819 T/T genotype polymorphisms are protective factors against PTB.

Key words: Genetic polymorphism; Interleukin-6; Interleukin-10; Pulmonary tuberculosis

INTRODUCTION

One-third of the world's population is estimated to be infected with *Mycobacterium tuberculosis*, which can lead to pulmonary tuberculosis (PTB). However, only one-tenth of those infected with the bacteria manifest the clinical disease (Dolin et al., 1994), strongly indicating that inherited genetic factors play an important role in the development of PTB. Individual susceptibility to PTB is determined by the host genome. Among the numerous cytokines, 2 candidate genes, interleukin (IL) genes *IL-6* and *IL-10*, have been examined in recent studies (Jang et al., 2004; Amirzargar et al., 2006; Oral et al., 2006). The frequencies of the *IL-6* -174 and *IL-10* -1082, -819, and -592 genotypes were found to be significantly higher among PTB patients (Amirzargar et al., 2006; Oral et al., 2006). IL-6 and IL-10 are secreted by Toll-like receptor 2-expressing cells in response to the presence of *M. tuberculosis* early in infection and are involved in anti-tuberculosis immunity in the body (Jang et al., 2004). The aim of this study was to determine whether *IL-6* and *IL-10* gene polymorphisms are independently associated with susceptibility to PTB in the Chinese Han adult population and how they together influence PTB susceptibility.

MATERIAL AND METHODS

Cases and controls

New PTB patients (N = 191) diagnosed in the Tangshan Tuberculosis Hospital between August 2008 and July 2009 were selected as the case group. Inclusion criteria were determined based on The Classification of Chinese Tuberculosis (Tuberculosis Branch of Chinese Medical Association, 1998). The following inclusion criteria were used: sputum-positive patients with characteristic lesions based on chest X-rays, sputum-negative patients with signs of active PTB based on chest X-ray, or patients with a strongly positive purified protein derivative (PPD) test with significant clinical signs of TB of Chinese Han ethnicity at least 18 years old and newly confirmed cases. The control group included 191 healthy Han Chinese adults that were nonnegative for *M. tuberculosis* infection. The inclusion criteria were as follows: no history of TB, *M. tuberculosis* infection confirmed by PPD test, Chinese Han ethnicity, at least 18 years old, and no blood relationship with any of the subjects in either the study group or the control group. Exclusion criteria used in the control group were also used in

the study group, included the patients with pneumonia, lung cancer, pneumoconiosis, diabetes, HIV infection, the long-term use of adrenocortical hormone and other immunosuppression. This study was part of a molecular epidemiology study for tuberculosis conducted by the Department of Epidemiology, School of Public Health. Approval from the Ethics Committee of Hebei United University was obtained for the study (IRB No. 10-008).

Genotyping of *IL-6* and *IL-10*

First, 3 mL venous blood was collected from each research subject and ethylenediaminetetraacetic acid was added as an anticoagulant. The salting-out method was used to extract genomic DNA. Primers were designed using the Primer Premier 5.0 software (Beijing SBS Genetech Co., Ltd.; Bejing, China).

For the *Nla*III, *Mbi*I, and *Fok*I polymorphisms, the primers 5'-TTGTCAAGACATGC CAAAGTG-3' and 5'-TCAGACATCTCCAGTTCCTATA-3' were used to amplify the *IL-6* -174 locus gene. The *IL-6* -572 and -597 locus genes were amplified using the primers 5'-GGAGACGCCTTGAAGTAACTGC-3' and 5'-GAGTTTCCTCTGACTCCATCGCAG-3'. The *Eco*RII and *Eco*RV polymorphisms were amplified using the primers 5'-AAGACAACA CTACTAAGGCTTCTTTGGGCC-3' and 5'-CCAGCACATAGAATGAAACCTT-3'. The *IL-10* -1082 locus gene was amplified using the primers 5'-GGCAGTGGTGTACCCTTGTACA GGTGATGTGA-3' and 5'-TCCTTTACCCCGATTTCATTA-3'.

Statistical analysis

Non-conditional univariate logistic regressions were conducted for univariate or multivariate factor analyses using SPSS 11.5 (SPSS, Inc.; Chicago, IL, USA) to determine the relationship between *IL-6* and *IL-10* gene polymorphisms and susceptibility to TB. The occurrence of TB was the dependent variable, the genotypes were independent variables, and significant risk factors identified in univariate analyses were covariates. Individual and joint effects of *IL-6* and *IL-10* single-nucleotide polymorphisms were investigated using fixed and random effects logistic regression models. Interactions were studied both on a multiplicative and an additive scale. Multivariate logistic regression analysis with Bonferroni's correction was conducted using 2-tailed test statistics at $\alpha = 0.025$.

RESULTS

Univariate analysis of the general status of cases and controls

Table 1 shows that the history of exposure to TB (0 = no history of exposure, 1 = with history of exposure), family history of TB (0 = no family history, 1= with history of exposure), and body mass index <20 kg/m^2 were risk factors for TB. Furthermore, having the Bacillus Calmette-Guérin (BCG) scar (0 = no BCG scar, 1= with BCG scar) was found to be a protective factor. No significant difference was observed for educational background (0 = illiteracy, 1 = elementary school, 2 = middle school, 3 = junior college and above), occupation (0 = worker, 1 = farmer, 2 = cadre), smoking (0 = non-smokers, 1 = smokers), and drinking (0 = not drinking, 1 = drinking) between cases and controls (Table 1).

Table 1. Non-conditional univariate logistic regression analysis for the effect of different factors on tuberculosis.

Factors	Degree	Case	Control	Wald χ^2	P	OR	95%CI
Contact history	1	93	51	19.215	<0.001	2.605	1.698-3.997
	0	98	140				
Family history	1	56	25	14.400	<0.001	2.754	1.632-4.648
	0	135	166				
Low BMI	1	46	24	8.219	0.004	2.207	1.285-3.793
	0	145	167				
BCG scar	1	76	102	7.066	0.008	0.577	0.384-0.865
	0	115	89				
Smoking	1	66	75	0.910	0.340	0.817	0.539-1.238
	0	125	116				
Drinking	1	70	88	3.485	0.062	0.677	0.450-1.020
	0	121	103				

BMI = body mass index; BCG = Bacillus Calmette-Guérin; OR = odds ratio; 95%CI = 95% confidence interval.

Correlation of *IL-6* and *IL-10* gene site polymorphism and the occurrence of TB

The *IL-6* -572 (χ^2 = 2.920, P = 0.087), *IL-10* -1082 (χ^2 = 3.319, P = 0.068), and *IL-10* -819 (χ^2 = 2.671, P = 0.102) loci were consistent with Hardy-Weinberg equilibrium. This result indicates that the control population was in equilibrium and that the sample was representative of the population.

The mutation rates of the *IL-10* -1082 and -819 loci and *IL-6* -572 locus were 7.85, 67.02, and 81.68%, respectively, and the corresponding mutation rates of the controls were 5.76, 59.42, and 78.27%. Since only the wild-type homozygous GG genotype was observed at the *IL-6* -174 and -597 loci, the relationship with TB was not analyzed.

Compared with the wild-type genotype CC, the *IL-10* -819 homozygous TT genotype showed a lower risk of TB. The *IL-6* -572 homozygous CC genotype also showed a low risk for TB compared with the wild-type GG genotype. Thus, the *IL-10* -819 homozygous TT and *IL-6* -572 homozygous CC genotypes appear to be protective against TB (Table 2).

Table 2. Non-conditional univariate logistic regression analysis of the association between gene polymorphisms and tuberculosis occurrence.

Genotype		Case	Control	Wald χ^2	P	OR (95%CI)
IL-6	-572 GG	10	5			1.000 (ref.)
	GC	50	73	0.940	0.332	1.725 (0.573-5.196)
	CC	131	113	5.519	0.019	0.591 (0.381-0.917)
IL-10	-1082 AA	164	171			1.000 (ref.)
	AG	24	18	0.237	0.627	0.639 (0.105-3.876)
	GG	3	2	0.015	0.903	0.889 (0.134-5.888)
	-819 CC	31	26			1.000 (ref.)
	CT	64	103	0.702	0.402	0.770 (0.418-1.419)
	TT	96	62	16.070	<0.001	0.401 (0.257-0.627)

To control for confounding factors, multivariate logistic regression was performed in which variables showing statistical significance in univariate analyses were considered as covariates, the genotypes of *IL-6* and *IL-10* as independent variables, and the occurrence of TB as the dependent variable. The *IL-10* -819 homozygous TT and *IL-6* -572 homozygous CC genotypes showed a significant association with the occurrence of TB after adjusting for the

effects of history of TB exposure, family history of TB, low body mass index, and presence of BCG scar. Both genotypes were protective factors against TB (Table 3).

Table 3. Non-conditional multivariate logistic regression analysis for risk factors of tuberculosis.

Model	Variable	β	SE	Wald χ^2	P	OR (95%CI)
IL-6	-572 GG					
-572 model	GC	0.285	0.583	0.239	0.625	1.330 (0.424-4.171)
	CC	-0.571	0.236	5.843	0.016	0.565 (0.356-0.898)
	Contact history	0.751	0.238	9.944	0.002	2.119 (1.329-3.380)
	Family history	0.671	0.299	5.042	0.025	1.956 (1.089-3.512)
	Low BMI	0.638	0.300	4.542	0.033	1.893 (1.053-3.406)
	BCG scar	-0.702	0.222	10.28	0.002	0.496 (0.321-0.765)
IL-10	-1082AA					
-1082 model	A/G	-0.635	0.959	0.438	0.508	0.530 (0.081-3.474)
	G/G	0.181	1.001	0.033	0.857	1.198 (0.168-8.527)
	Contact history	0.869	0.242	12.850	<0.001	2.3851 (1.483-3.836)
	Family history	0.738	0.293	6.336	0.012	2.093 (1.178-3.719)
	Low BMI	0.715	0.300	5.672	0.017	2.045 (1.135-3.685)
	BCG scar	-0.593	0.225	6.937	0.008	0.553 (0.355-0.859)
IL-10	-819 C/C					
-819 model	CT	-0.348	0.334	1.085	0.298	0.706 (0.367-1.359)
	TT	-1.076	0.247	19.005	<0.001	0.341 (0.210-0.553)
	Contact history	0.730	0.242	9.105	0.003	2.075 (1.291-3.333)
	Family history	0.751	0.302	6.186	0.013	2.119 (1.173-3.828)
	Low BMI	0.771	0.311	6.142	0.013	2.163 (1.175-3.981)
	BCG scar	-0.785	0.228	11.881	0.001	0.456 (0.292-0.713)

BMI = body mass index; BCG = Bacillus Calmette-Guérin; OR = odds ratio; 95%CI = 95% confidence interval.

The combined action of the *IL-10* -819 site mutation homozygous TT genotype and the -1082 site mutant AG+GG genotype on the development of TB was statistically significant. The odds ratio (OR) of the occurrence of TB decreased from 0.454 to 0.150, indicating that the *IL-10* -1082 site variant AG+GG genotype enhances the protective effect of the *IL-10* -819 site TT genotype (Table 4).

Table 4. Logistic regression analysis of gene-gene combined action.

Genotype IL-10-1082	Genotype IL-10-819	Case	Control	OR (95%CI)
AA	CC	28	21	1.000 (ref.)
	CT	56	91	0.983 (0.509-1.899)
	TT	80	59	0.454 (0.283-0.728)
AG+GG	CC	3	5	1.000 (ref.)
	CT	9	12	0.120 (0.018-0.797)
	TT	15	3	0.150 (0.033-0.680)

DISCUSSION

IL-10 is an important multifunctional cytokine that shows immunomodulatory effects and immune stimulation. Its main biological function is to inhibit macrophage antigen presentation; TB immunologic deficiency is associated with IL-10 expression level (Sánchez et

al., 1994). A link exists among the *IL-10* -819C/T, -592C/A, -819T, and -592A sites (Zhang et al., 2002; Wang et al., 2006). *In vitro* experiments confirmed that different genotypes result in different expression levels of IL-10 (Turner et al., 1997; Crawley et al., 1999). Compared with the *IL-10* -1082A, -819T, and -592A alleles, the -1082G, -819C, and -592C sites alleles result in higher IL-10 expression (Hoffmann et al., 2001).

However, studies in different geographic regions showed different results. For example, in Cambodia, the -1082 A>G polymorphism was found to be associated with susceptibility to TB (Delgado et al., 2002; Ben-Selma et al., 2011), whereas no significance could be inferred from the -819 C>T gene polymorphism (Ma et al., 2010). The *IL-10* gene polymorphism was considered to be a potential risk factor for TB in the Hong Kong Chinese population (Tso et al., 2005). However, this was not observed in studies of Gambian (Delgado et al., 2002), Egyptian children (Mosaad et al., 2010), and Macedonian (Trajkov et al., 2009) populations. Furthermore, the allelic and genotypic frequencies of *IL-10* -1082 A>G did not differ significantly between normal healthy subjects and patients in India (Selvaraj et al., 2008) and Turkey (Akgunes et al., 2011). Different findings were reported in studies conducted in Hong Kong and Mainland China (Ma et al., 2007).

In this study, the frequency of the *IL-10* -1082 locus G allele was very similar to that reported for Mainland Chinese (Ma et al., 2007) and Japanese (6.5%) (Ide et al., 2002) populations, which largely differ from that in Cambodians (48%) (Delgado et al., 2002) and Turks (37.7%) (Oral et al., 2006). The frequency of the *IL-10* -1082 locus G allele mutation was reported to be higher among Chinese individuals (25%) in one study, but the sample size was small and the variation was relatively large.

A few studies have examined the relationship between *IL-6* gene polymorphisms and the occurrence of TB (Zhang et al., 2012). After Bonferroni correction to adjust for multiple testing as well as controlling for the effects of environmental factors such as the history of exposure to TB, family history of TB, and BCG scar in this study, the *IL-6* -572 locus was still significantly related to the occurrence of TB and was found to be a protective factor, which is in contrast to the results of a previous study of Turks (Oral et al., 2006). High IL-6 expression levels are thought to regulate the immune balance of Th1/Th2 and prevent *M. tuberculosis* infections from developing into TB because of immune imbalance. The different results observed may have resulted because of the different races of the subjects, different gene polymorphisms in different populations, and different genetic backgrounds, leading to different phenotypic effects for the same mutation. The development of TB is a multi-step process involving multiple factors, including a number of genetic factors. The coexistence of a variety of "susceptible" genotypes plays an important role. Thus far, no definite conclusions and general models have been obtained from various polygene studies in several countries. Different conclusions were reached in some studies regarding the relationship between cytokine gene polymorphisms and TB. In the present study, we found a protective effect of the *IL-10* -819 TT genotype and that the *IL-10* -1082 AG+GG genotype enhances this protective effect, therefore demonstrating combined action of the two polymorphisms.

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