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Original Article

Relationship of *IL27* gene polymorphisms with the risk of pulmonary tuberculosis in Chinese Han population

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Abstract: Objective: The aim of this study was to explore the effect of human interleukin-27 (*IL-27*) gene polymorphisms -964A/G and 2095T/G on the development of pulmonary tuberculosis (PTB), and its radiographic characteristics and severity. Methods: A case-control study was performed in 123 PTB patients and 110 healthy controls. Differences in genotype and allele distributions of the two polymorphisms were analyzed via chi-square test between PTB patients and controls, between patients with single- and multi-lobe involvement, and between patients with and without cavities. Results: Significant differences were found in the genotype and allele distributions of -964A/G ($P<0.05$), in which GG genotype carriers were more common in PTB patients. In addition, the -964AA genotype was more prevalent in patients with single-lobe involvement compare with those with multi-lobe involvement (51.355 vs. 30.30%, $P=0.035$), as well as the 2095TT genotype (89.19% vs. 71.21, $P=0.036$). But there were no significant differences between patients with or without cavitations in either polymorphisms ($P>0.05$). Conclusion: *IL-27* gene polymorphism -964A/G was correlated with PTB susceptibility, and G allele might be a risk factor for the disease onset. Furthermore, the -964AA genotype might be associated with a protective role that limits the intrapulmonary spread of TB in Chinese population. But the role of 2095T/G was not sure.

Keywords: *IL-27*, tuberculosis, polymorphisms

Introduction

Tuberculosis (TB) is an infectious disease with higher death rate worldwide, and has a long history in human society. TB remains to be a leading cause of morbidity and mortality in developing countries, although the first anti-tuberculosis drug has been introduced approximately 50 years ago [1, 2]. It is estimated that about 60% of the world's population have been infected with pathogen mycobacterium tuberculosis, however only almost 10% of these TB infected individuals can present clinical disease during their lifetime. The remarkable individual differences suggest that the pathogenic factors may relate to nutrition, constitution [3], specific and nonspecific resistance [4, 5] and genetic susceptibility [6, 7]. Therefore, identifying the host genes responsible for susceptibility and resistance to TB may lead us to a better understanding of the infection mechanism of TB and development of prophylactic or treatment strategies.

Interleukin-27 (IL-27) is a novel IL-12 family member, which is recently discovered. IL-27 is composed of two subunits, the Epstein-Barr virus-induced gene 3 protein (EBI3) and a novel IL-12 p35-related polypeptide p28 [8]. IL-27 is a multifaceted heterodimeric cytokine involved in the immune response as well as in the development of inflammation. IL-27 is produced by activated antigen-presenting cells, it acts as an early mediator of naive T-cell proliferation and is a pronounced inducer of interferon gamma (IFN-γ) production, particularly in synergy with IL-12 [9, 10]. The human IL-27 gene is located on chromosome 16p11, and several single nucleotide polymorphisms (SNPs) have been identified [11].

In the previous studies, several SNPs of *IL-27* gene have been found to be involved in immune-related disease, such as ulcerative colitis and Crohn's disease [12, 13]. As we all know, pulmonary TB (PTB) is one of the inflammatory lung disease with a cell-mediated immune response,

Table 1. Demographic and clinical characteristics of the study population

	Patients with PTB n=123 (%)	Healthy controls n=110 (%)
Male/female	43 (22-71)	38 (23-66)
Method of diagnosis	76 (61.8)/47 (38.2)	70 (63.6)/40 (36.4)
Bacteriological		
Positive TB-PCR	117 (95.1)	
Clinical diagnosis	1 (0.8)	
Radiographic characteristics	5 (4.1)	
Multi-lobe involvement, n/N (%)	66 (64.1)/103	
Presence of vacities, n/N (%)	39 (37.9)/103	

Note: PTB = pulmonary tuberculosis; PCR = polymerase chain reaction.

which promotes us to assess the influence of IL-27 on the development of PTB. In China population, several candidate gene polymorphisms have been identified to be associated with PTB risk, such as IL-17, IL-23 receptor (*IL-23R*) gene and so on [14, 15]. But few studies have been performed to explore the correlation of *IL-27* gene polymorphisms and PTB susceptibility.

Hence, the aim of our study was to investigate whether the *IL-27* gene polymorphisms affect the development of PTB in Chinese Han population. We selected two common SNPs of -964A/G and 2905T/G, and their genotype and allele distributions were analyzed detailedly.

Materials and methods

Study subjects

This case-control study was reviewed and approved by Ethics committee of Shaanxi Center for Tuberculosis Control and Prevention. Sample collection was based on ethics criteria of national human genome research. Written consents were obtained from all of the participants. All subjects were Chinese Han population, who had no genetic connections with each other.

A cohort of 233 participants were enrolled in this case-control study, including 123 patients with PTB and 110 health controls. All patients were diagnosed in Shaanxi Center for Tuberculosis Control and Prevention from Feb 2013 to Nov 2014, and were confirmed according to the following standard inclusion criteria: (1) culture positive for TB and/or (2) clinical TB diagnosed based on the clinical radiological and histological grounds from the International Union against TB and Lung Disease. Patients with evidence of human immunodeficiency virus infection or other immunodeficiency, or whose PTB status could not be confirmed were excluded. 110 healthy samples that had no history of chest disease were recruited as controls.

Sample collection and DNA extraction

3 ml peripheral venous blood were collected from each participant, anticoagulated by 0.5% EDTA (pH=8.0). Genomic DNA was extracted by Biospin Whole Blood Genomic DNA Extraction Kit (Bioer technology CO., LTD, China) according to the manufacturer's protocol, and stored at -20°C for standby application.

Genetic typing assay

For the SNP -964A/G, its genotypes were detected by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) method. Primer sequences were designed by Primer Premier 5.0, with forward primer: 5'-CCCGCCTGGTTTCT-ATCTCAC-3' and reverse primer: 5'-AGTGACCTGGGGCTTTGCTT-3'. The PCR amplification was performed in a total volume of 25 μl containing 2.5 μl 10 × Buffer, 1.5 μl $MgCl_2$, 3 μl template DNA, 0.5 μl upstream primer, 0.5 μl downstream primer, 0.3 μl Taq DNA polymerase, 2 μl dNTP, and 14.7 μl deionized sterile water. PCR were performed according to general procedures with annealing temperature of 63°C. Then the PCR products were digested with *XhoI* at 37°C water bath overnight. Finally, digested DNA products were then analyzed by 2% agarose gel electrophoresis and visualized by UV light. The SNP 2905T/G were genotyped by sequence-specific primer-PCR (SSP-PCR) [16].

Radiographic extent of tuberculosis

Simple chest X-rays (CXRs) were applied before the administration of anti-tuberculosis medications. The degree of TB involvement was confirmed by chest computed tomography scans.

Table 2. Genotype and allele distributions of *IL-27* gene -964A/G and 2905T/G polymorphisms in case and control groups

Genotype/Allele	Case *n*=123 (%)	Control *n*=110 (%)	X^2	*P*	OR (95% CI)
-964A/G					
AA	36 (29.27)	44 (40.00)	-	-	1
AG	52 (42.28)	46 (41.82)	1.145	0.285	1.382 (0.764-2.499)
GG	35 (28.45)	20 (18.18)	4.540	0.033	2.139 (1.058-4.325)
A	124 (50.41)	134 (60.91)	-	-	1
G	122 (49.59)	86 (39.09)	5.184	0.023	1.533 (1.060-2.216)
2905T/G					
TT	71 (57.72)	73 (66.36)	-	-	1
TG	39 (31.71)	31 (28.18)	0.774	0.379	1.294 (0.729-2.296)
GG	13 (10.57)	6 (5.45)	2.456	0.117	2.228 (0.802-6.184)
T	181 (73.58)	177 (80.45)	-	-	1
G	65 (26.42)	43 (19.55)	3.085	0.079	1.478 (0.954-2.289)

Two board-certified chest radiologists interpreted the images independently.

Measurement of serum IL-27 levels

Serum samples were collected before the treatment was initiated. IL-27 serum concentrations were analyzed and compared between patients with various genotype and disease status, with the latter defined according to the number of lobes affected by TB and presence or absence of cavitations.

Statistical analysis

All data analysis was performed by using PASW statistics 18.0 statistical software. Hardy-Weinberg equilibrium (HWE) was tested to assess the representativeness of control group. Chi-square test was carried out to evaluate the differences in the genotype and allele distribution of -964A/G and 2905T/G polymorphisms between various groups. The relative risk for disease was evaluated by odds ratios (ORs) and 95% confidence intervals (CIs). The differences had statistical significance when $P<0.05$.

Results

Participant demographics

The baseline characteristics of all participants were presented in **Table 1**. As shown, 123 patients with PTB and 110 healthy individuals were enrolled in this case-control study. The case group age ranged from 22 to 71 years old, while the controls aged 23-66 years old. The percentage of men in case group was 61.8% compared with 63.6% in control group. There were no significant differences between case and control group in age and gender ($P>0.05$). Of the 123 PTB patients, 117 (95.1%) were diagnosed bacteriologically, and 103 PTB patients were characterized radiographically. Among them, multi-lobe involvement were observed in 66 patients (64.1%), and 39 patients (37.9%) presented with cavitations.

Distributions of genotypes and alleles

The genotype and allele frequencies of *IL-27* gene -964A/G and 2905T/G polymorphisms were displayed in **Table 2**. Via chi-square test, genotypes distributions of the two polymorphisms were all according to HWE in control group, indicating the representativeness of participants. Besides, the -964GG genotype was more common in patients with PTB than that in controls (28.45% vs. 18.18%, $P=0.033$). And the -964G allele was more prevalent than the -964A allele in case group than that in control group (49.59% vs. 39.09%, $P=0.023$). All results suggested that *IL-27* gene -964A/G polymorphism was associated with PTB susceptibility, and G allele might be a risk factor for the onset of PTB (OR=1.533, 95% CI=1.060-2.216) (**Table 2**). However, no significant differences were found in the genotype and allele distributions of *IL-27* gene 2905T/G polymorphism between two groups ($P>0.05$), which demonstrated that there was a lack of association between *IL-27* gene 2905T/G polymorphism and PTB risk in our cohort.

Table 3. Genotype and allele distributions of *IL-27* gene -964A/G and 2905T/G polymorphisms in PTB cases according to lobar extent of involvement

Genotype/Allele	Single-lobe involvement *n*=37 (%)	Multi-lobe involvement *n*=66 (%)	*P*	OR (95% CI)
-964A/G				
AA	19 (51.35)	20 (30.30)	0.035	2.428 (1.057-5.575)
AG	15 (40.54)	36 (54.55)	-	1 (Reference+)
GG	3 (8.11)	10 (15.15)		
A	53 (71.62)	76 (57.58)	0.046	1.860 (1.008-3.429)
G	21 (28.38)	56 (42.42)	-	1 (Reference)
2905T/G				
TT	33 (89.19)	47 (71.21)	0.036	3.335 (1.039-10.708)
TG	4 (10.81)	18 (27.27)	-	1 (Reference+)
GG	0 (0)	1 (1.52)		
T	70 (94.59)	112 (84.85)	0.036	3.125 (1.025-9.523)
G	4 (5.41)	20 (15.15)	-	1 (Reference)

Note: Reference+ are respectively AG and GG, TG and GG.

Table 4. Genotype and allele distributions of *IL-27* gene -964A/G and 2905T/G polymorphisms in PTB cases with or without a cavitary lesion

Genotype/Allele	With cavity *n*=39 (%)	Without cavity *n*=64 (%)	*P*	OR (95% CI)
-964A/G				
AA	15 (38.46)	24 (37.50)	0.922	1.042 (0.459-2.365)
AG	20 (51.28)	32 (50.00)	-	1 (Reference+)
GG	4 (10.26)	8 (12.50)		
A	50 (64.10)	80 (62.50)	0.817	1.071 (0.597-1.923)
G	28 (35.90)	48 (37.50)	-	1 (Reference)
2905T/G				
TT	29 (74.36)	50 (78.13)	0.661	0.812 (0.320-2.061)
TG	9 (23.08)	13 (20.31)	-	1 (Reference+)
GG	1 (2.56)	1 (1.56)		
T	67 (85.90)	113 (88.28)	0.617	0.809 (0.351-1.863)
G	11 (14.10)	15 (11.72)	-	1 (Reference)

Note: Reference+ are respectively AG and GG, TG and GG.

We further compared the genotype and allele distributions in the PTB patients according to lobar extent of involvement, and with or without cavitary lesion. As shown in **Table 3**, the -964AA genotype carriers were significantly more than the -964AG and -964GG carriers in PTB patients with single-lobe involvement than in those with multi-lobe involvement (51.35% vs. 30.30%, *P*=0.035). In addition, the -964 A allele frequency increased significantly in cases with single-lobe involvement compared with those with multi-lobe involvement (71.62% vs. 424.42%, *P*=0.046). Similarly, significant differences were also identified in TT genotype and T allele distributions of the 2905T/G between groups (*P* <0.05).

From **Table 4**, no significant differences in the genotype and allele distributions of -964A/G and 2905T/G polymorphisms were observed between PTB patients with and those without cavitations (*P*>0.05).

Discussion

TB has been considered as a global disease with high morbidity particularly in Africa and Asia. Although it seems to be controlled after the improvement of sanitation condition and the development of anti-tuberculosis agent, drug resistant tuberculosis (DR-TB) particularly the emergence of multi-drug-resistant tuberculosis (MDR-TB) has become a major public health issue threatening human health nowadays, which is remarkable higher in China [17]. Therefore, there is an urgent need for TB diagnosis and treatment to be strengthened. Genetic factors have been identified to play crucial roles in TB development. Recent years, several candidate genes have been found to be correlated with TB, including the tumor necrosis factor alpha

(*TNF-α*) gene [18], immunity-related GTPase M (*IRGM*) gene [19], Toll-like receptor 2 (*TLR2*) gene [20] and so on. These reports suggest the important role of genetic factors in the development of TB. However, the genetic pathogenesis of TB is still a mystery.

IL-27, is one of the important cytokines, which regulates the human innate and adaptive immune system. It is produced from macrophages earlier than IL-12, which plays an important role in early efficient inducing of Th1 differentiation until sufficient IL-12 is produced [21]. It promotes both anti- and pro-inflammatory immune response [22]. Recently, IL-27 has been reported to play a crucial role in some immune-related disorders [23]. Furthermore, several polymorphisms of *IL-27* gene have been suggested to be associated with several autoimmune disease, such as ulcerative colitis (UC) and Allergic rhinitis (AR) [12, 24]. The -964A/G polymorphism is a common mutation of *IL-27* gene, which has been widely explored to be correlated with the susceptibility to several inflammatory diseases, including asthma and inflammatory bowel disease [11, 25]. PTB is one of the inflammatory lung diseases, but its association with *IL-27* gene polymorphisms has not been investigated in Chinese Han population.

In the present study, we investigated the distribution of *IL-27* gene polymorphisms in a Chinese Han population and explored its association with PTB susceptibility. The present stud demonstrated significant differences in *IL-27* alleles or genotypes between PTB patients and healthy controls, or between patients with single- and multi-lobe involvement. But the distributions of *IL-27* gene polymorphisms had no significant difference between patients with or without cavitary lesion. As shown, the -964GG genotype and G allele frequencies increased in case group compared with controls, and the differences were statistically significantly, which suggested that *IL-27* gene -964A/G polymorphism was correlated with PTB susceptibility and G allele might be a risk factor for the onset of PTB. Our study turned the attention to the potential role of *IL-27* gene polymorphism in patients with PTB in China. The local inflammation of PTB, accompanied by the destruction of lung tissue, is caused by the interaction between a potent immune response and a chronically persistent pathogen [26]. The extent of involvement may be regulated by the interaction between the host and the microorganism, which finally results in pathological changes of the lung parenchyma [6, 27]. In this study, the -964AA genotype and -964A allele occurred more commonly in patients with single-lobe involvement compared with patients with multi-lobe involvement, demonstrating that -964AA might limit the intrapulmonary spread of TB. Besides, the same phenomenon was observed in 2905TT genotype.

In conclusion, the -964A/G mutation in the *IL-27* promoter region was identified to be correlated with PTB susceptibility. Furthermore, the -964AA genotype was more prevalent in patients with single-lobe involvement than that in patients with multi-lobe involvement. All findings revealed that *IL-27* -964AA genotype may play a protective role in preventing the intrapulmonary spread of PTB in the Chinese Han population. Although it was the first time to explore the genetic association of *IL-27* gene with PTB susceptibility in Chinese population, some limitations still presented. Therefore, a lager or different population should be taken into account to confirm our results, and further studies with multivariate risk assessments should be warranted.

Disclosure of conflict of interest

None.

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