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Original Article

StIL-17 gene polymorphisms in the development of pulmonary tuberculosis

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Abstract: We conduct a case-control study to explore the possible association between IL-17 gene polymorphisms and development of TB. Methods: The study population comprised 428 TB subjects and 428 control subjects between January 2013 and June 2014. Genotyping analyses of IL-17A rs2275913 and rs3748067 and IL-17F rs763780 and rs9382084 were analyzed using polymerase chain reaction-restriction fragment length of polymorphism (PCR-RFLP). Results: The TB cases were more likely to have a habit of smoking when comparing with controls. By conditional logistic regression analysis, individuals carrying CC genotype of rs763780 were more likely to have a significantly increased risk of TB when compared with TT genotype. The OR (95% CI) for CC genotype of rs763780 was 2.98 (1.58-5.92). Conclusion: In conclusion, we suggest that rs763780 play a critical role in the etiology of TB. These findings could be helpful in identifying individuals at increased risk for developing TB.

Keywords: IL-17, polymorphism, pulmonary tuberculosis

Introduction

Tuberculosis (TB) is the single infectious disease to cause highest death worldwide, despite the first anti-tuberculosis drug is used about 50 years ago [1]. It is estimated that 60% of the world's population are infected with Mycobacterium tuberculosis, and there were about 8.1 million new cases with active TB annually by World Health Organization in 2011. However, approximately 5-10% of these IB infected cases will develop clinical disease. Many risk factors have been associated with developing TB infection, including genetic and environmental factors [2, 3].

It is reported that the immune response to TB is usually influenced by interactions between antigen-presenting cells, lymphocytes, macrophages and monocytes as well as immune mediators [4]. Previous studies reported that cytokine genes play an important role in the development of TB, including IFN-α, IL-6, IL-10 and IL-12B as well as IL-17 etc. [5-7]. However, the results are inconsistent; some studies reported no association between cytokine genes and susceptibility of TB [6].

Th17 cells are a subset of T helper cells, and they are a unique subset of effector T helper cells to subvert the Th1 and Th2 lineages era [8]. Increasing evidences have shown that Th17 cells are the major IL-17-producing cells and participate in protective immunity against M. tuberculosis [9-11]. Khader et al. reports M. tuberculosis infection was associated with a decreased Th17 response, and can suppress Th1 cytokines [10]. Th17 cells have an important role in the development of autoimmune and inflammatory disease [12].

Previous studies reported the association between genetic polymorphisms of IL-17 and development of TB [6, 13, 14]. However, the results are inconsistent. Therefore, we conduct a case-control study to explore the possible association between IL-17 gene polymorphisms and development of TB.

Materials and methods

Study population

The study population comprised 428 TB subjects who were enrolled at the Huaihe Hospital

Table 1. Basic characteristics of the cases and controls

Variables	Cases	%	Control	%	χ^2 value	*P* value
Age, years						
< 50	178	41.59	186	43.46		
≥ 50	250	58.41	242	56.54	0.31	0.58
Gender						
Female	131	30.61	131	30.61		
Male	297	69.39	297	69.39	0.00	1.00
Tobacco smoking						
Never	220	51.40	249	58.18		
Ever	208	48.60	179	41.82	3.97	0.04
Alcohol drinking						
Never	321	75.00	309	72.20		
Ever	107	25.00	119	27.80	0.87	0.35

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

SNP	Minor allele frequency in NCBI	Minor allele frequency in controls	*P* value for Hardy-Weinberg equilibrium
rs2275913	0.2927	0.3075	0.23
rs3748067	0.0769	0.092	< 0.05
rs763780	0.0935	0.1005	< 0.05
rs9382084	0.4062	0.3965	0.56

of Henan University between January 2013 and June 2014. Diagnosis of TB was confirmed through X-rays and bacteriologically with both sputum smear examination and culture. Patients with extra pulmonary tuberculosis in organs other than the lungs, HIV-positive, and with other infectious diseases or with immunosuppressive conditions were excluded from our study. 428 control subjects were collected from health check center of the Huaihe Hospital of Henan University, who came to receive health examination in our hospital. All the control subjects were confirmed to be absence of TB, and no history of an inflammatory autoimmune disease and chronic infectious disease. The control subjects were matched to cases by sex and age.

The written informed consent form was obtained from all TB patients and controls before participating into study. The protocol of our study was approved by the ethics of the Huaihe Hospital of Henan University.

SNP selection and genotyping analysis

Four SNPs of IL-17A rs2275913 and rs3748067 and IL-17F rs763780 and rs9382084 were selected. Each TB patient and control subject was agreed to provide 5 ml peripheral blood and kept in -20°C until use. Genomic DNA of IL-17A rs2275913 and rs3748067 and IL-17F rs763780 and rs9382084 was isolated from peripheral blood and genotyped using a TIANamp blood DNA kit (Tiangen Biotech, Beijing, China). Genotyping analyses of IL-17A rs2275913 and rs3748067 and IL-17F rs763780 and rs9382084 were analyzed using polymerase chain reaction-restriction fragment length of polymorphism (PCR-RFLP). The primers and probes for IL-17A rs2275913 and rs3748067 and IL-17F rs763780 and rs9382084 were designed using Primer 5.0 software (PREMIER Biosoft, Palo Alto, CA) according to the manufacturer instructions. The PCR procedure was performed with an initial melting step of 5 minutes at 95°C, followed by 35 step cycles of denaturation at 95°C for 30 s, annealing at 62°C for 30 s, 72°C for 30 s, and a final extension at 72°C for 10 min.

Statistical analysis

Continuous variables and categorical variables were expressed as the mean ± SD and N (%), respectively. Student t test or χ^2-test was taken to compare the difference between case and control groups. The chi-squared goodness of fit test was taken to assess the genotype distributions were in Hardy-Weinberg equilibriums of IL-17A rs2275913 and rs3748067 and IL-17F rs763780 and rs9382084. The odds ratios (OR) and corresponding 95% confidence intervals (CIs) were calculated by conditional logistic regression analysis and utilized the association between genotype frequencies of IL-17A rs2275913 and rs3748067 and IL-17F rs763780 and rs9382084 and risk of TB. For all the statistical analyses, the significance level was set at *P* value less than 0.05.

Table 3. Genotype distributions of IL-17 genes between cases and controls

Variables	Cases	%	Control	%	OR (95% CI)[1]	*P* value
rs2275913						
GG	184	42.99	210	49.07	Ref.	
GA	188	43.93	172	40.19	1.25 (0.93-1.68)	0.13
AA	56	13.08	46	10.75	1.39 (0.88-2.21)	0.14
rs3748067						
CC	344	80.37	365	85.28	Ref.	
CT	58	13.55	48	11.21	1.28 (0.83-1.98)	0.23
TT	26	6.07	15	3.50	1.84 (0.92-3.80)	0.06
rs763780						
TT	319	74.53	357	83.41	Ref.	
TC	69	16.12	56	13.08	1.38 (0.92-2.06)	0.09
CC	40	9.35	15	3.50	2.98 (1.58-5.92)	< 0.05
rs9382084						
GG	146	34.11	159	37.15	Ref.	
GT	206	48.13	199	46.50	1.13 (0.83-1.53)	0.43
TT	78	18.22	70	16.36	1.21 (0.80-1.83)	0.33

[1]Adjusted for sex, age, tobacco smoking and alcohol drinking.

Table 4. Interaction between IL-17 rs2275913 gene polymorphism and demographic characteristicsin the risk of TB

Variables	IL-17 rs2275913				OR (95% CI)[1]	*P* value
	Cases		Controls			
	TT		TC + CC		TC + CC vs TT	
Gender						
Male	205	45	218	24	1.99 (1.14-3.55)	0.01
Female	114	64	139	47	1.66 (1.03-2.68)	0.03
Age						
< 50	107	24	115	16	1.61 (0.77-3.43)	0.17
≥ 50	212	85	242	55	1.76 (1.18-2.65)	0.004
Tobacco smoking						
Never	168	52	205	44	1.44 (0.90-2.32)	0.11
Ever	151	57	152	27	2.13 (1.24-3.69)	0.003
Alcohol drinking						
Never	235	86	245	64	1.40 (0.95-2.07)	0.07
Ever	84	23	112	7	4.38 (1.71-12.60)	< 0.05

[1]Adjusted for sex, age, tobacco smoking and alcohol drinking.

Results

The basic characteristics of the cases and controls are shown in **Table 1**. This study included 428 TB cases and 428 controls (69.39% males and 30.61% f females). The mean ages for cases and controls were 54.7 ± 11.4 years and 54.2 ± 12.8 years, respectively. There were no significant difference in the distribution of sex and age between TB cases and controls since the frequency matching between the two groups. The TB cases were more likely to have a habit of smoking when comparing with controls ($P < 0.05$).

The allele and genotype distributions of IL-17 rs2275913 and rs9382084 were found to be in line with Hardy-Weinberg in the controls, while the IL-17 rs3748067 and rs763780 was not (**Table 2**). The Minor allele frequencies in controls of the four SNPs in IL-17 were similar to MAF in NCBI.

The association between IL-17 rs2275913, rs3748067, rs763780 and rs9382084 polymorphisms and risk of TB was shown in **Table 3**. By conditional logistic regression analysis, individuals carrying CC genotype of rs763780 were more likely to have a significantly increased risk of TB when compared with TT genotype. The OR (95% CI) for CC genotype of rs763780 was 2.98 (1.58-5.92). However, we did not find significant association between rs3748067 and rs763780 and development of TB.

We further analyzed the association between IL-17 rs2275913 polymorphism and risk of TB stratified by sex, age, tobacco smoking and alcohol drinking (**Table 4**). We found IL-17 rs2275913 polymorphism have association with higher age, tobacco smokers and alcohol drinkers in the risk of TB, and the ORs (95% CI) were 1.76 (1.18-2.65), 2.13 (1.24-3.69) and 4.38 (1.71-12.60), respectively.

Discussion

Identification of genes involved in the genetic predisposition of disease plays an important role in both clinical practice and basic medicine

research. As proinflammatory cytokines, IL-17A and IL-17F, expressed by Th17 cells, play a role in coordinating local tissue inflammation. Previous studies have reported that IL-17 is an inflammatory cytokine involved in inducing chemokines gradients and initiating inflammation in the lung and IL-17 can promote the accumulation of both polymorphic and mononuclear cells during the mycobacterial infection [10]. In the present study, we found individuals carrying CC genotype of rs763780 were more likely to have a significantly increased risk of TB when compared with TT genotype, suggesting that IL-17 gene polymorphism play an important role in the development of TB.

Recently, increasing studies reported the association between IL-17 gene polymorphisms and autoimmune diseases [15-18]. Jin et al. conducted a study in Korean population, and found that IL-17 rs1889570 polymorphism was associated with susceptibility to asthma [15]. Another study in Chinese population reported that A allele of IL-17 rs2275913 was associated with increased risk of several asthma-related traits and confers genetic susceptibility to childhood asthma [16]. Hayashi et al. conducted a study in Japanese population, and reported that IL-17 rs2275913 and rs3748067 polymorphisms could influence the susceptibility to the development of ulcerative colitis [17]. For the association between IL-17 gene polymorphisms and risk of TB, three studies reported their association, but the results are inconsistent [6, 13, 14]. Ocejo-Vinyals et al. conducted a study in a Spanish population, and reported that IL-17A rs2275913 was associated with susceptibility of pulmonary tuberculosis [13]. Peng et al. conducted a study in Chinese population, and found that T allele of the IL-17 rs763780 is associated with increased risk of TB, and no significant association between rs2275913 polymorphisms and risk of TB [14]. However, Tiwari et al. did not find IL-17 gene polymorphisms are not associated with development of TB [6]. In our study, we found that CC genotype of rs763780 gene polymorphism was associated with increased risk of TB. The discrepancies of these results may be caused by differences in ethnicities, study design, and sample size as well as by chance.

There are several limitations in this study. First, we only selected cases and controls in one place, which may limit the representation of other populations. The IL-17 rs3748067 and rs763780 were not in line with Hardy-Weinberg in the controls. These indicate that selection bias may be existed in this study. Second, the small sample size of this study could limit the statistical power to find the association between IL-17 gene polymorphisms and risk of TB. Third, the development of TB could be influence by other genetic factors. Therefore, further large sample study is greatly needed to confirm the association between IL-17 gene polymorphisms and development of TB.

In conclusion, we suggest that CC genotype of IL-17 rs763780 gene polymorphism was associated with increased risk of TB, and rs763780 play a critical role in the etiology of TB. These findings could be helpful in identifying individuals at increased risk for developing TB, and further large sample studies are greatly needed to confirm these associations.

Disclosure of conflict of interest

None.

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References

[1] Lawn SD, Zumla AI. Tuberculosis. Lancet 2011; 378: 57-72.

[2] Cooke GS, Hill AV. Genetics of susceptibility to human infectious disease. Nat Rev Genet 2001; 2: 967-77.

[3] Bellamy R. Susceptibility to mycobacterial infections: the importance of host genetics. Genes Immun 2003; 4: 4-11.

[4] Abhimanyu, Mangangcha IR, Jha P, Arora K, Mukerji M, Banavaliker JN; Indian Genome Variation Consortium, Brahmachari V, Bose M. Differential serum cytokine levels are associated with cytokine gene polymorphisms in north Indians with active pulmonary tuberculosis. Infect Genet Evol 2011; 11: 1015-22.

[5] Feng FM, Liu XX, Sun YH, Zhang P, Sun SF, Zhang B, Wang XT, Lu LJ. Independent and

joint effects of the IL-6 and IL-10 gene polymorphisms in pulmonary tuberculosis among the Chinese Han population. Genet Mol Res 2014; 13: 7766-72.

[6] Tiwari U, Ramachandran VG, Das S, Kumar S. Interleukin-3 and interleukin-17 do not play a dynamic role in the immunopathogenesis of osteoarticular tuberculosis. Indian J Tuberc 2014; 61: 142-7.

[7] Selvaraj P, Alagarasu K, Harishankar M, Vidyarani M, Nisha Rajeswari D, Narayanan PR. Cytokine gene polymorphisms and cytokine levels in pulmonary tuberculosis. Cytokine 2008; 43: 26-33.

[8] Harrington LE, Hatton RD, Mangan PR, Turner H, Murphy TL, Murphy KM, Weaver CT. Interleukin 17-producing CD4 + effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. Nat Immunol 2005; 6: 1123-32.

[9] Chen X, Zhang M, Zhu X, Deng Q, Liu H, Larmonier N, Graner MW, Zhou B. Engagement of Toll-like receptor 2 on CD4(+) T cells facilitates local immune responses in patients with tuberculous pleurisy. J Infect Dis 2009; 200: 399-408.

[10] Khader SA, Bell GK, Pearl JE, Fountain JJ, Rangel-Moreno J, Cilley GE, Shen F, Eaton SM, Gaffen SL, Swain SL, Locksley RM, Haynes L, Randall TD, Cooper AM. IL-23 and IL-17 in the establishment of protective pulmonary CD4+ T cell responses after vaccination and during Mycobacterium tuberculosis challenge. Nat Immunol 2007; 8: 369-77.

[11] Paidipally P, Periasamy S, Barnes PF, Dhiman R, Indramohan M, Griffith DE, Cosman D, Vankayalapati R. NKG2D-dependent IL-17 production by human T cells in response to an intracellular pathogen. J Immunol 2009; 183: 1940-5.

[12] Pitta MG, Romano A, Cabantous S, Henri S, Hammad A, Kouriba B, Argiro L, el Kheir M, Bucheton B, Mary C, El-Safi SH, Dessein A. IL-17 and IL-22 are associated with protection against human kala azar caused by Leishmania donovani. J Clin Invest 2009; 119: 2379-87.

[13] Ocejo-Vinyals JG, de Mateo EP, Hoz MÁ, Arroyo JL, Agüero R, Ausín F, Fariñas MC. The IL-17 G-152A single nucleotide polymorphism is associated with pulmonary tuberculosis in northern Spain. Cytokine 2013; 64: 58-61.

[14] Peng R, Yue J, Han M, Zhao Y, Liu L, Liang L. The IL-17F sequence variant is associated with susceptibility to tuberculosis. Gene 2013; 515: 229-32.

[15] Jin EH, Choi EY, Yang JY, Chung HT, Yang YS. Significant association between IL-17F promoter region polymorphism and susceptibility to asthma in a Korean population. Int Arch Allergy Immunol 2011; 155: 106-10.

[16] Chen J, Deng Y, Zhao J, Luo Z, Peng W, Yang J, Ren L, Wang L, Fu Z, Yang X, Liu E. The polymorphism of IL-17 G-152A was associated with childhood asthma and bacterial colonization of the hypopharynx in bronchiolitis. J Clin Immunol 2010; 30: 539-45.

[17] Hayashi R, Tahara T, Shiroeda H, Saito T, Nakamura M, Tsutsumi M, Shibata T, Arisawa T. Influence of IL17A polymorphisms (rs2275913 and rs3748067) on the susceptibility to ulcerative colitis. Clin Exp Med 2013; 13: 239-44.

[18] Espinoza JL, Takami A, Onizuka M, Kawase T, Sao H, Akiyama H, Miyamura K, Okamoto S, Inoue M, Ohtake S, Fukuda T, Morishima Y, Kodera Y, Nakao S; Japan Marrow Donor Program. A single nucleotide polymorphism of IL-17 gene in the recipient is associated with acute GVHD after HLA-matched unrelated BMT. Bone Marrow Transplant 2011; 46: 1455-63.