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Short Communication

The IL-17F sequence variant is associated with susceptibility to tuberculosis

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ABSTRACT

The interleukin (IL)-17 gene plays a key role in host defence against infections from microbes, including *Mycobacterium tuberculosis*. Genetic factors contribute to host defence. However, whether genetic variation in IL-17 is associated with altered susceptibility to tuberculosis is unknown. A total of 596 pulmonary tuberculosis (PTB) patients, 176 extra-pulmonary tuberculosis (EPTB) patients, and 622 control patients from a Chinese Han population were recruited. Two single-nucleotide polymorphisms (SNPs) in IL-17F (rs1889570 and rs763780) and one SNP in IL-17A (rs2275913) were genotyped using the SNaPshot technique. Of the three SNPs in the IL-17 gene tested, there was an increased frequency of the rs1889570 G allele and the rs763780 C allele in the PTB patients and an increased frequency of the rs763780 C allele in the EPTB patients compared with the control patients. There were also significant differences in the distribution of the rs763780 genotype between the PTB and EPTB patients and the controls. The patients who had the CT/TT genotype of the rs763780 SNP were more susceptible to tuberculosis, compared to the CC genotype. There was no significant difference observed between the IL-17 SNPs when the PTB and EPTB patients were compared. Genetic variation in IL-17F is associated with altered susceptibility to tuberculosis and may provide valuable information in the development of tuberculosis.

1. Introduction

Tuberculosis (TB) caused by *Mycobacterium tuberculosis* is one of the three major infectious disease killers, with an estimated 8–9 million new cases and 2–3 million deaths from TB annually (Corbett et al., 2003). Despite the high infectivity of *M. tuberculosis*, only 5–10% of infected individuals develop active disease with clinical symptoms (Flynn, 2004). In addition, the manifestation of active disease in patients varies substantially, from mild symptoms to severe or fatal TB, with the latter often characterised by large cavitary lung lesions, miliary TB, or meningitis (Lewinsohn et al., 2004). These observations indicate that protective immunity against *M. tuberculosis* infection plays an important role in disease development (Flynn, 2004). Th17 cells, a new subset of Th (T helper) cells, produce cytokines from the interleukin (IL) 17 family, namely, IL-17A and IL-17F (Lloyd and Hessel, 2010; Steinman, 2007). An increasing amount of evidence has shown that Th17 cells are the major IL-17-producing cells and participate in protective immunity against *M. tuberculosis* (Chen et al., 2009; Khader et al., 2007; Paidipally et al., 2009). Khader et al. (2007) reported an indirect role for the Th17 response in protective immunity in *M. tuberculosis*-infected mouse models. *M. tuberculosis* infection can induce the human Th17 response, and a reduced *M. tuberculosis* antigen-specific Th17 response, which may be due to suppressing Th1 cytokines, has been observed in patients with active disease compared to healthy donors (Scriba et al., 2008). Th17 cells play a central role not only in the development of autoimmune and inflammatory diseases but also in protection against intracellular pathogens (Pitta et al., 2009).

Interest in IL-17 has been growing because of its significance as a marker of Th17. This cytokine has been well characterised in vitro and in vivo and has a central role in protective immunity against *M. tuberculosis*. Upon intratracheal delivery of BCG, IL-17 mRNA was detected in the lungs of infected animals as early as one day after infection (Umemura et al., 2007). IL-17-deficient mice showed delayed granuloma formation, suggesting that IL-17 is essential for initial granuloma organisation, most likely by promoting early neutrophil recruitment. Moreover, IL-17-deficient mice are unable to control *M. tuberculosis* following a high-dose infection (Okamoto Yoshida et al., 2010). Up-regulation of IL-17 expression in T lymphocytes has been correlated with active TB (Dheda et al., 2008). IL-17 expression in lymphocytes from patients with active TB correlates with the severity of the disease (Jurado et al., 2012). Serum IL-17 levels after two months of anti-TB treatment were significantly lower in non-survivors than survivors; serum IL-17 levels may be biomarkers that predict long-term mortality (Chen et al., 2011). Thus, IL-17 may act to enhance the protective immune response against *M. tuberculosis*, with important implications for the therapy of patients with TB.

Abbreviations: IL-17, interleukin (IL)-17; *M. tuberculosis*, *Mycobacterium tuberculosis*; PTB, pulmonary tuberculosis; EPTB, extra-pulmonary tuberculosis; SNPs, single-nucleotide polymorphisms; TB, tuberculosis; Th cell, T helper cell; Th17 cells, Interleukin-17-producing T helper cells; SAP, shrimp alkaline phosphatase; ExoI, ExonucleaseI; HWE, Hardy–Weinberg equilibrium; BMI, body mass index; OR, odds ratios; CI, confidence intervals; PPD, Purified Protein Derivative.

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Epidemiological studies have demonstrated a strong genetic component in the risk of developing TB. Human genetic variation is an important determinant of the outcome of *M. tuberculosis* infection. Association studies have identified various host genetic factors that affect susceptibility to TB, including genes encoding HLA-DRB1 (Lombard et al., 2006), a vitamin D receptor (Roth et al., 2004), SLC11A1 (Taype et al., 2006), and interleukin-10 (IL-10) (Oral et al., 2006). Our group previously demonstrated that specific polymorphisms have an effect on the risk of developing TB in the Chinese population (Liang et al., 2011). Based on experimental findings and gene expression studies, IL-17 is a good positional candidate gene for TB. However, there are no data on whether polymorphisms in the IL-17 gene have an influence on the development of TB in Chinese and other populations. Therefore, we conducted a case–control study in a Chinese population to evaluate correlations between polymorphisms in the IL-17 gene and the risk of developing TB.

2. Materials and methods

2.1. Study population

The studied population comprised 1394 subjects, including 772 patients who were admitted with TB to the Shanghai Pulmonary Hospital affiliated with Tongji University School of Medicine and 622 healthy subjects without TB (control group) (Table 1). The TB patients were divided into the pulmonary tuberculosis (PTB) group (n = 596) and the extra pulmonary tuberculosis (EPTB) group (n = 176) according to clinical case definitions recommended by the World Health Organization (Global Tuberculosis Control, 2002). The PTB patients were diagnosed based on radiographic and clinical presentation, positive smear and culture for *M. tuberculosis*. The EPTB patients presented with signs of TB in organs other than the lungs, such as lymph nodes, abdomen, genitourinary tract, skin, joints and bones, meninges, etc. The diagnosis of EPTB was based on discovering *M. tuberculosis* in cultures of other specimens besides respiratory secretions (Vasankari et al., 2010), fine needle aspiration cytology, biochemical analyses of cerebrospinal/pleural/ascitic fluid, histopathological examination or strong clinical evidence consistent with active extra-pulmonary tuberculosis, followed by a clinician's decision to treat with a full course of anti-tuberculosis chemotherapy. All patients were HIV-negative, and none presented with other infectious diseases or with immunosuppressive conditions. The healthy control subjects were blood donors who were clinically normal and willing to participate in the study. The control subjects were older than 18 years and did not suffer from an inflammatory autoimmune disease or possess a history of chronic infectious disease. They also had no evidence of TB in their medical histories or prior chest radiographies and tested negative for PPD.

This study was approved by the Ethics Committee of the Shanghai Pulmonary Hospital affiliated with Tongji University School of Medicine. Informed consent was obtained from all TB patients and the control subjects participating in this study. All of the patients and control subjects resided in the same region and shared a similar ethnic background.

Table 1
Demographic characteristics of TB patients and controls.

Group Studied	N	Male (%)	Age(years) (mean ± SD)	BMI(kg/m^2) mean ± SD
PTB patients	596	53.2	43.7 ± 12.3	20.1 ± 2.8
EPTB patients	176	56.7	39.5 ± 10.8	19.6 ± 3.2
Tuberculosis of larynx	6	60.1	40.1 ± 11.3	18.3 ± 3.5
Tuberculosis of intestine	8	45.1	40.5 ± 12.4	19.3 ± 2.9
Tuberculous meningitis	29	56.5	31.4 ± 9.2	20.3 ± 2.8
Spondylocace	17	60.5	38.7 ± 11.3	18.8 ± 3.1
Tuberculosis of pericardium	10	62.3	30.5 ± 9.2	21.3 ± 2.8
Tuberculosis of crewels	20	57.8	43.5 ± 10.1	19.1 ± 2.5
Tuberculosis of uropoiesis	11	43.4	39.3 ± 8.9	18.9 ± 3.2
Tuberculous pleurisy	68	61.2	35.4 ± 7.8	19.6 ± 3.3
Bone tuberculosis	7	51.3	42.3 ± 12.3	19.7 ± 3.4
Controls	622	48.9	40.2 ± 8.1	24.0 ± 2.9

2.2. DNA isolation and genotyping

Collected peripheral blood samples were stored at −20 °C until use. The DNA was extracted from EDTA-anticoagulated peripheral blood leukocytes using the QIAamp DNA Blood Mini Kit (QIAamp Genomic DNA Isolation Mini Kit; Qiagen, Shanghai, China) according to the manufacturer's protocol. Polymorphisms were genotyped using the SNaPshot kit provided commercially by Applied Biosystems, which was a rapid and robust assay used to simultaneously genotype SNPs by single-nucleotide primer extension (minisequencing). Following these steps, the population genetic data were obtained. This method was proven to be valid for genotyping [Lian et al., 2010]. The primer sequences are shown in Table 2.

A multiple polymerase chain reaction (PCR) was used to amplify all of the fragments in a total reaction volume of 10 μl containing 1× HotStart Taq buffer, 3.0 mM Mg^{2+}, 0.3 mM NTP, 0.1 μM of each primer, 1 U HotStart Taq polymerase (Qiagen) and 1 μl template DNA. The target DNA sequence was denatured at 95 °C for 2 min, followed by 11 cycles of denaturation at 94 °C for 20 s, annealing at 62 °C decreased by 0.5 °C per cycle, and extension at 72 °C for 1.5 min; 24 cycles of denaturation at 94 °C for 20 s, annealing at 59 °C for 30 s, and extension at 72 °C for 1.5 min; and a final extension at 72 °C for 2 min.

After the multiple amplification was completed, we purified the PCR products using shrimp alkaline phosphatase (SAP) and ExonucleaseI (ExoI). The mixture, including 1 U SAP, 1 U Exo I and 10 μl PCR products, was incubated at 37 °C for 60 min and subsequently incubated at 75 °C for 15 min. Then, the purified PCR products were used as templates for the minisequencing reaction with the commercially available SNaPshot Kit. To detect the polymorphisms, we used minisequencing extension primers rs1889570SF: TTTTTTTTTTTTTTTCACACCTTTTGTC TTGGAGCTG, rs763780SR: TTTTTTTTTTTTTTTTGGATATGCACCTCTTAC TGCACA and rs2275913SF: TTTTTTTTTTTTTTTTTTTTCCTTCCCATTTTC CTTCAGAAG. The SNaPshot Extension Reaction mixture included 5 μl SNaPshotmix, 2 μl purified PCR product, 2 μl ultrapure water and 2 μl extension primer mix (0.8 μM for each extension primer). The cycling protocol was 96 °C for 1 min; 28 cycles of 96 °C for 10 s, 52 °C for 5 s, and 60 °C for 30 s. To purify the extension products, we added 1 U SAP to the extension products, incubated them at 37 °C for 60 min and then incubated them at 75 °C for 15 min. One microliter of purified extension product was then mixed with 9 μl HiDi Formamide and 0.5 μl Liz120 (Applied Biosystems) size standard and denatured at 95 °C for 5 min. Thereafter, we loaded the mixture on the ABI 3730xl DNA Analyzer (Applied Biosystems).

2.3. Statistical analysis

The genotypic and allelic distributions of the patients and normal controls were compared using the SHEsis software (Shi and He, 2005) and the χ^2 test. The Hardy–Weinberg equilibrium (HWE) was

Table 2
The PCR primers of IL-17 SNPs.

SNP	Forward primer (5′–3′)	Reverse primer (5′–3′)
rs763780	TGTAGGAACTTGGGCTGCATCA	TGTTGCAGAGCACTGGGTAAGG
rs1889570	GAGCAATAAAGGTGAAAAAGACAGTCTT	GAGGGGAGGACCCTTCCTGAAT
rs2275913	TCAAGGTACATGACACCAGAAGACC	TAAAATTTCCGCCCCCAATGAG