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Short Communication

Protein tyrosine phosphatase nonreceptor type 22 (*PTPN22*) gene polymorphism in pulmonary tuberculosis in the Indian population

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ABSTRACT

A variant of the protein tyrosine phosphatase nonreceptor type 22 (*PTPN22*) gene is known to be associated with susceptibility to autoimmune diseases and bacterial infections as it acts as an important regulator of T-cell activation. The objective of this study was to evaluate whether *PTPN22*-C1858T polymorphism is associated with the resistance to pulmonary tuberculosis (PTB). Single-nucleotide polymorphism of *PTPN22*-C1858T (rs2476601) was genotyped in 124 patients with PTB and 130 healthy controls from India using restriction fragment length polymorphism and direct sequencing of the amplified DNA. The frequencies of genotypes CC, CT, and TT were 100%, 0%, and 0%, respectively, in PTB; and 99.2%, 0.8% and 0%, respectively, in healthy control individuals. These values did not differ significantly between the patients and controls. The mutant allele C1858T was found to be a rare allele in Indian population.

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Introduction

Tuberculosis (TB), an infectious disease caused by *Mycobacterium tuberculosis* (*Mtb*), is a major global health problem. The number of TB cases reported worldwide is on the rise partly due to development of multidrug-resistant *Mtb*. TB is also the leading cause of death from infectious diseases, especially in Asia and Africa [1]. According to the World Health Organization *Global Tuberculosis Report 2015*, TB ranked alongside human immunodeficiency virus as a leading cause of death. In 2014, there were an estimated 9.6 million new TB cases, of which 1.5 million deaths were recorded, indicating the high prevalence of this disease. Globally, India has the largest number of TB cases—23% of the global total followed by

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Indonesia (10%) and China (10%) [2]. In 2014, more than 2 million TB cases were reported from India [2].

The World Health Organization estimated that one-third of the global population is infected with *Mtb*, but only 5–10% among them develop the clinically evident disease [3]. This indicates that along with the infective agent other factors also play a role in developing clinically evident disease. Variability in TB susceptibility was demonstrated in Germany, wherein accidental injection of live *Mtb* instead of Bacillus Calmette–Guérin vaccine caused some infants to become seriously ill, whereas others showed no symptoms at all [4]. This finding indicates a clear genetic predisposition as one of the host factors influencing risk factors for the development of TB [5–7].

Access to genetic data using various studies is an important aspect of identifying new genetic associations through genome-wide association studies [8]. Genome-wide association studies have been successful in identifying genetic variants that contribute to complex human traits in diverse populations [9]. Several genes are reported to have been associated with TB pathogenesis such as protein tyrosine phosphatase nonreceptor type 22 (*PTPN22*), *TLR2*, vitamin D receptor (*VDR*), and cytokine genes (interleukin-10 [IL-10], IL-6, interferon-γ, tumor necrosis factor-α, and tumor growth factor-β1) [10].

Protein tyrosine phosphatases are involved in maintaining the T cells in the resting stage and are also responsible for bringing back the activated T cells to the resting phenotype in the absence and presence of antigen, respectively [11,12]. In this study, we focused on Lyp encoded by *PTPN22*. Lyp, a cytosolic phosphatase, is mainly expressed in hematopoietic cells [13,14]. By dephosphorylating many proteins and enzymes in early T-cell receptor signal cascade, Lyp acts as a negative regulator of T-cell receptor signaling [15,16] and is involved in immune and inflammatory responses; additionally, its levels are increased in cells that participate in the immune response against *Mtb* [17]. Previous studies have identified the physiological association between a missense C1858T (R620W) mutation in *PTPN22* and autoimmune diseases such as rheumatoid arthritis [15,18,19], type 1 diabetes [20–23], systemic lupus erythematosus [24], and Graves' disease [25,26]. Vang et al. [16] have reported that the missense allele (C1858T allele) is a gain-of-function variant. It has also been reported that the C1858T allele of *PTPN22* is associated with a higher incidence of bacterial pulmonary infections in patients with chronic mucocutaneous candidiasis [27]. However, the C1858T allele has been shown to play a protective role in pulmonary TB in various populations [28–31]. Interestingly, it has also been reported that the C1858T allele of *PTPN22* is not associated with TB in an Iranian population [32]. Studies in Indian population have evaluated the association between the C1858T allele of *PTPN22* and various diseases such as type 1 diabetes [33,34], sporadic idiopathic hypoparathyroidism [35], and rheumatic heart disease [36], but no such association studies were so far performed for TB patients. The differences in the results concerning the association of the C1858T allele of *PTPN22* with different diseases in various populations necessitated the present investigation in Indian population. This study aims to investigate the association between the single-nucleotide polymorphism (SNP) C1858T allele of *PTPN22* and pulmonary TB in a sample of Indian population.

Materials and methods

Study subjects

Whole-blood samples (2 mL) were collected from 124 pulmonary tuberculosis (PTB) patients at Jayalakshmi Memorial Chest Hospital, Kadapa, Andhra Pradesh, India (60 male patients and 64 female patients; mean age: 45.36 ± 5.92 years). Acid-fast bacilli in sputum samples, chest X-ray examination, and Mantoux and fine-needle aspiration cytology tests were performed to confirm the disease, although the patients were already undergoing treatment. We have randomly selected 130 age- and sex-matched unrelated healthy controls (60 males and 70 females; mean age: 40.25 ± 7.62 years). Individuals recruited to the health control group had no previous history of TB. All patients gave written informed consent. The Ethics Committee of the institute approved this study.

Genomic DNA extraction from whole blood

Genomic DNA was extracted from EDTA-treated frozen whole blood by a rapid nonenzymatic method [37]. Genotyping of *PTPN22*-1858C/T SNP (rs2476601) was performed by restriction fragment length polymorphism in the amplified DNA. A fragment of the *PTPN22* gene was amplified using specific primers (sense and antisense primers were 5′-GATAATGTTGCTT CAACGGAATTTA-3′ and 5′-TCACCAGCTTCCTCAACCACA-3′, respectively). The *Xcm*I site generated by the *PTPN22*-1858C/T transition mutation was used for the detection of SNP. The amplified fragment was then digested with *Xcm*I (New England Biolabs, Beverly, MA, USA) for detecting the SNP. A 10-μL aliquot of the polymerase chain reaction (PCR) product was digested overnight with 10 U of *Xcm*I at 37 °C. After incubation, the restriction digestion mixture was electrophoresed on a 1.2% agarose gel in 1× Tris–acetate–EDTA buffer for 1 h at 125 V. The genotype of all patients and controls obtained using the restriction fragment length polymorphism-PCR assay was verified by direct sequencing using the sense primer.

Statistical analysis

Allele frequencies of *PTPN22* were counted directly. The *p* values were two tailed and the statistical significance was noted at the .05 level. Fisher's exact test and Spearman correlation test were performed using GraphPad Prism 5.0 (GraphPad Software, La Jolla, CA, USA).

Results

The presence of restriction endonuclease site for *Xcm*I (CCANNNNNNNNNTGG) in the PCR-amplified product indicates the presence of mutant allele in *PTPN22*. In samples from heterozygous subjects with mutant T allele, the 215-bp products were digested into 170- and 45-bp fragments. Patients with 1858C alleles did not have any restriction site

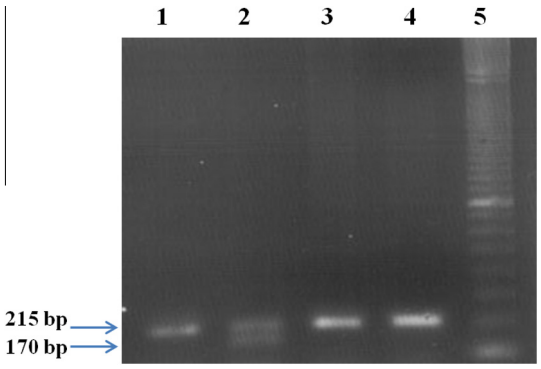

Fig. 1 – *Xcm*I digestion patterns of the *PTPN22*-C1858T polymorphism. A 215-bp fragment of the *PTPN22* gene region was amplified by polymerase chain reaction (PCR) using genomic DNA as template. The PCR product was digested with *Xcm*I restriction endonuclease and then separated on a 1.2% agarose gel and stained with ethidium bromide and visualized using ultraviolet light. Lanes 1 and 3 = undigested amplified product of *PTPN22*. Lane 2 = *PTPN22*-amplified product digested with *Xcm*I (heterozygous) showing 215-bp (upper arrow) and 170-bp (lower arrow) fragments. Lane 4 = *PTPN22*-amplified product digested with *Xcm*I (homozygous). Lane 5 = 100-bp DNA ladder. bp = base pair.

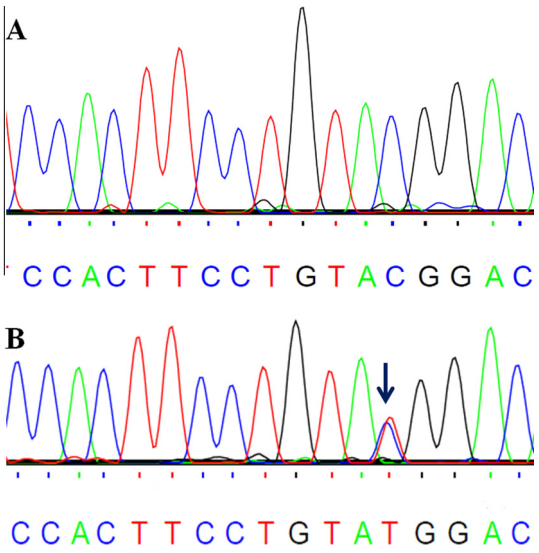

Fig. 2 – DNA sequencing for part of the *PTPN22* gene. (A) Genotype CC. (B) Genotype CT. The arrow in panel B indicates the position of the single-nucleotide polymorphism where the overlapping of two peaks (heterozygous) can be observed.

for *Xcm*I and showed intact 215-bp product (Fig. 1). Restriction digestion of the *PTPN22*-amplified product with *Xcm*I revealed that only one healthy individual was a heterozygous mutant for the C1858T allele (Lane 2) and none of the patients carried the missense allele (Fig. 1).

The frequency of occurrence of the mutant genotype (CT or TT) among PTB patients was 0% (0/124) and there was one heterozygous (CT) mutant (1/130 = 0.8%) in the healthy controls ($p > .99$; Table 1). The frequency of the mutant allele was found to be 0% in the patient group and 0.38% in the healthy control group (Table 1). Furthermore, the mutation was confirmed by direct sequencing of the PCR-amplified product. For the heterozygous genotype with the mutant T allele, two peaks overlapped each other in a chromatogram (Fig. 2), which indicated the presence of both C and T alleles.

Table 1 – Genotype and allele frequencies of the *PTPN22* of the C1858T polymorphism in pulmonary tuberculosis.

Genotype	Patients (pulmonary tuberculosis)	Healthy controls
CC	100% (124/124)	99.2% (129/130)
CT	0% (0/124)	0.8% (1/130)
TT	0% (0/124)	0% (0/130)
Fishers exact test, $p > .99$		
Alleles		
C	100% (248/248)	99.62% (259/260)
T	0% (0/248)	0.38% (1/260)

Discussion

In this study, we found no association between the genotypic and allelic frequencies of the *PTPN22*-C1858T gene polymorphism and susceptibility to PTB in a sample of the Indian population. We have detected only one heterozygous mutant (CT) in healthy control samples and no individuals with homozygous mutant (TT) were detected among patients and controls. The low mutant allele frequency of *PTPN22* was also reported in another study in a different disease in the Indian population [35].

Some of the earlier studies reported a significant association between the T allele and PTB [28,29]. Similar to previous studies on Moroccan population [29], Colombian population [28], and Iranian population [32], a sample of the Indian population also showed the absence of TT genotype in TB cases. The aforementioned studies [28,29] suggest a potential protective role of the T allele in TB. However, in contrast to the previous studies, the distribution of T allele in this study was very low. It has been reported that the *PTPN22*-C1858T polymorphism is not involved in susceptibility to *Brucella melitensis*, an intracellular pathogen, which causes human brucellosis [38]. The observations suggest that *PTPN22*-C1858T confers resistance to TB while also increasing susceptibility to Gram-positive bacteria. In this present study, the mutant allele C1858T was identified as a rare allele at a frequency of 0.0019 (1/508) and as such it cannot be used as a marker for PTB in Indian population.

In conclusion, our study results found that *PTPN22*-C1858T is not associated with the susceptibility to PTB in Indian population.

Conflicts of interest

None of the authors has competing interests.

Acknowledgments

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