



R620W functional polymorphism of protein tyrosine phosphatase non-receptor type 22 is not associated with pulmonary tuberculosis in Zahedan, southeast Iran

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ABSTRACT. The protein tyrosine phosphatase non-receptor type 22 (PTPN22) gene, which encodes an intracellular lymphoid-specific phosphatase, is considered an important regulator of T-cell activation. We investigated a possible association between the PTPN22 C1858T (R620W) polymorphism and pulmonary tuberculosis in an Iranian population. Single nucleotide polymorphisms of PTPN22 C1858T (rs2476601) were genotyped in 172 pulmonary tuberculosis cases and 204 normal subjects from Zahedan, Iran. Frequencies of genotypes CC,

CT and TT of the PTPN22 C1858T polymorphism were 98.3, 1.7 and 0% in the pulmonary tuberculosis patients, and 96.1, 3.9 and 0% in the control group, respectively ($P = 0.239$). The frequency of the minor (T) allele was 0.8% in pulmonary tuberculosis patients and 2.0% in controls. Significant differences were not observed in genotype or allele frequencies of PTPN22 C1858T in the comparison between pulmonary tuberculosis patients and healthy subjects in our Iranian population sample.

Key words: PTPN22; Tuberculosis; Polymorphism

INTRODUCTION

Pulmonary tuberculosis (PTB) is still a major health problem worldwide, and it remains the main cause of morbidity and mortality (Naderi et al., 2009, 2010). It has been known that over one-third of the world's population is infected with *Mycobacterium tuberculosis*, of whom only 10% develop the clinical disease (Porter and McAdam, 1994). Persuasive evidence from twin studies indicates that host genetic factors are significant risk factors for development of tuberculosis (Comstock, 1978). The findings of numerous diverse tuberculosis (TB) susceptibility genes in genome-wide screens supported the possibility of multigenic predisposition to TB (Bellamy et al., 2000; Cervino et al., 2002). The lung is the main target of infection with *M. tuberculosis*. It is well-known that in most subjects infected with *M. tuberculosis*, host immunity responses effectively control the infection (North and Jung, 2004).

The PTPN22 gene is located on chromosome 1p13.3-p13.1 and encodes intracellular lymphoid tyrosine phosphatase (LYP) (Cohen et al., 1999). LYP, which is expressed only in cells of hematopoietic origin, dephosphorylates kinases such as Lck, Fyn, and Zap-70, all of them recognized to be important in T-cell signaling (Mustelin et al., 1999). It has been documented that dephosphorylation of these substrates by LYP negatively modulates T-cell activation (Hermiston et al., 2002; Mustelin et al., 2002; Veillette et al., 2002). In addition, LYP binds to the adaptor molecule Grb2 (growth factor receptor-bound protein 2), and this interaction is thought to play a negative regulatory function in T-cell signaling (Hill et al., 2002). Moreover, LYP binds to the C-terminus of Src tyrosine kinase (Csk) and downregulates the activation of T cells. Csk is a key suppressor of kinases that mediate T-cell activation (Cohen et al., 1999). It has been reported that the PTPN22 C1858T (R620W) polymorphism placed at the P1 motif does not bind Csk, preventing formation of the complex and, consequently, suppression of T-cell activation (Bottini et al., 2004).

Several studies have found a significant association between a functional missense PTPN22 C1858T (R620W) polymorphism and vulnerability to numerous autoimmune diseases such as type 1 diabetes, rheumatoid arthritis and systemic lupus erythematosus (Gomez et al., 2005a; Chelala et al., 2007; Lee et al., 2007; Douroudis et al., 2008).

To the best of our knowledge, the data regarding PTPN22 C1858T polymorphism in PTB are scant (Gomez et al., 2005b; Lamsyah et al., 2009), and there is no report in our country. Thus, the aim of the present study was to find out the association between PTPN22 C1858T (R620W) polymorphism and PTB in an Iranian population.

MATERIAL AND METHODS

This case-control study was performed from July 2008 to April 2010 in the Research Center for Infectious Diseases and Tropical Medicine, Bou-Ali Hospital, Zahedan, Iran. The project was approved by the Ethics Committee of Zahedan University of Medical Sciences, and informed consent was obtained from all patients and healthy subjects. The study consisted of 172 PTB (64 males, 108 females; mean age 50.3 ± 20.3) and 204 healthy subjects (94 males, 110 females; mean age 47.5 ± 14.7). The diagnosis of PTB was based on clinical, radiological, sputum acid fast bacillus (AFB) smear positivity, culture and response to antituberculosis chemotherapy, as previously described (Naderi et al., 2009; Hashemi et al., 2011b).

Two milliliters of venous blood was drawn from each subject, and genomic DNA was extracted from peripheral blood as previously described (Hashemi et al., 2010a).

Tetra primer ARMS-PCR is a simple and rapid method for detection of SNPs (Hashemi et al., 2010a,b, 2011a). Tetra primer ARMS-PCR was designed for the detection of PTPN22 R620W (rs2476601) polymorphism. We used two external primers (forward outer primer: 5'-CTCACACATCAGCTTCCCAAAGTG-3', reverse outer primer: 5'-CAACTT TACTGATAATGTTGCTTCAACGGA-3') and two internal primers (forward inner primer: 5'-CAACCACAATAAATGATTCAGGTGTACG-3', reverse inner primer: 5'-ATCCCCCCTC CACTTCCTGGAT-3'). Product sizes were 213 bp for the C allele and 151 bp for the T allele, while the product size of the two outer primers was 314 bp as illustrated schematically in Figure 1.

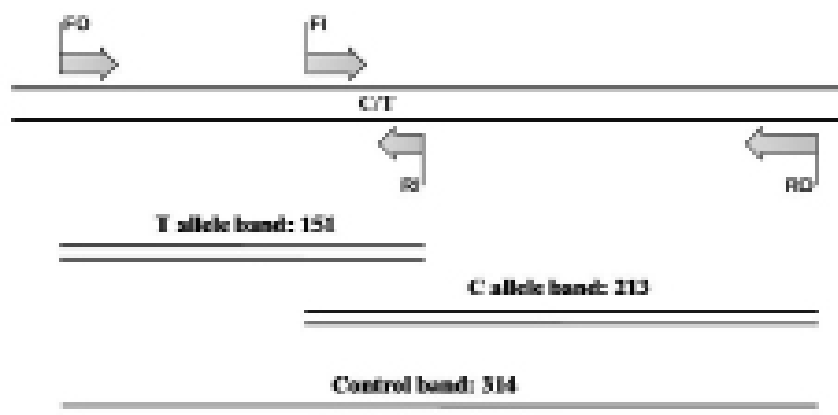


Figure 1. Schematic diagram of the tetra ARMS assay for determination of the PTPN22 C1858T polymorphism. Two forward and two reverse primers are used to generate three potential PCR products. Primers FO and RO give a 314-bp product, which is used for control of DNA quality and quantity. Primers FO and RI amplify the T allele, generating a 151-bp product, and primers FI and RO generate a 213-bp product for the C allele.

PCR was performed using commercially available PCR premix (AccuPower PCR PreMix, BIONEER, Daejeon, Korea) according to the manufacturer recommended protocol. A 0.2-mL PCR tube was used, containing the AccuPower PCR PreMix, 1 μ L template DNA (~ 100 ng/ μ L), 1 μ L of each primer (10 μ M) and 15 μ L DNase-free water. The total volume for PCR was 20 μ L.

PCR cycling conditions were 5 min at 95°C followed by 30 cycles of 30 s at 95°C, 30 s at 64°C, and 30 s at 72°C, and 10 min at 72°C (Corbett Research, Australia). The PCR products were electrophoresed on 3% agarose gels and photographed (Figure 2). To ensure genotyping quality, we regenotyped the samples at random to verify the initial results.

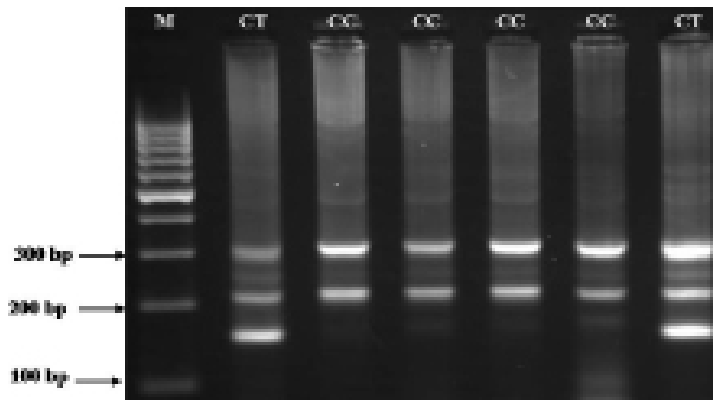


Figure 2. Results of tetra ARMS-PCR of the PTPN22 C1858T polymorphism. Lane M = DNA marker.

The statistical analysis of the data was performed using the SPSS 17.0 software. Genotypes and alleles were compared between the groups using the Fisher exact test.

RESULTS

We determined the genotypic frequency of 1858 PTPN22 C/T polymorphism in PTB and control subjects (Table 1). Genotype frequencies of both patients and healthy controls satisfied the Hardy-Weinberg equilibrium. The CC genotype was observed in 98.3% (169/172) patients, whereas 1.7% (3/172) were heterozygous (CT) and none of the patients had a TT genotype. In the control group, the frequencies of genotypes were 96.1% (196/204) for CC, 3.9% (8/204) for CT and 0% (0/204) for TT (Table 1). No homozygous TT genotype was found among PTB patients or healthy subjects. There were no significant differences between case and control groups regarding PTPN22 C/T polymorphism ($P = 0.239$).

Table 1. Genotype frequencies of the PTPN22 C1858T polymorphism in pulmonary tuberculosis (PTB) patients and healthy subjects.

Genotype	PTB	Healthy subjects
CC	98.3% (169/172)	96.1% (196/204)
CT	1.7% (3/172)	3.9% (8/204)
TT	0.0% (0/172)	0.0% (0/204)
Fisher exact test, $P = 0.239$		

The distribution of PTPN22 C/T allele frequencies in PTB and normal individuals is shown in Table 2. No significant difference was observed between PTB and control subjects regarding allele frequencies (Fisher exact test, $P = 0.241$).

Table 2. Allele frequencies of PTPN22 C1858T in pulmonary tuberculosis (PTB) patients and healthy subjects.

Allele	PTB	Healthy subjects
C	99.1% (341/344)	98.0% (400/408)
T	0.9% (3/344)	2.0% (8/408)

Fisher exact test, P = 0.241

DISCUSSION

In the present 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 Iranian population. We did not detect individuals carrying the TT genotype among patients and controls. To date, there are only two reports regarding the association between PTPN22 polymorphisms and PTB. In contrast to our findings, Lamsyah et al. (2009) observed statistically significant differences in the PTPN22 C1858T genotypic and allelic frequencies between PTB and controls in a Moroccan population. The frequency of the minor allele (T allele) was found to be 0.41% in PTB and 3.2% in normal subjects (Lamsyah et al., 2009). In another study, Gomez et al. (2005b) found statistically significant differences in the PTPN22 C1858T genotypic and allelic frequencies between PTB and controls in a Columbian population. The frequency of the T allele was found to be 1.3% in PTB and 4.3% in normal subjects. The distribution of T allele frequencies in our study is in accordance with these findings (Gomez et al., 2005b; Lamsyah et al., 2009). The finding of no homozygous TT individuals among PTB patients or healthy controls in our study is in line with the findings of Lamsyah et al. (2009) and Gomez et al. (2005b) in a Moroccan and Colombian population, respectively. These studies suggest a potential protective role of the T allele in TB. Our results are in disagreement with these findings and this discrepancy may be due to the different ethnicity of subjects participating in the studies.

It has been reported that the PTPN22 C1858T polymorphism is not involved in vulnerability to *Brucella melitensis*, an intracellular pathogen, which gives rise to human brucellosis (Bravo et al., 2009). Several studies have found a significant association between the PTPN22 C1858T (R620W) polymorphism and vulnerability to various autoimmune diseases (Gomez et al., 2005a; Chelala et al., 2007; Lee et al., 2007; Douroudis et al., 2008).

In the majority of infected subjects, active natural and acquired immune responses successfully control *M. tuberculosis*. The exact reasons as to why only a number of the individuals infected with *M. tuberculosis* develop clinical disease remain unknown. There is some evidence that suggests that host genetic factors may be important risk factors for the development of tuberculosis (Guide and Holland, 2002; Hashemi et al., 2011b; Qu et al., 2011).

Variation in allele frequency of the PTPN22 C1858T (R620W) has been reported among different ethnic populations. The highest frequency of the minor allele (T allele) has been observed in Scandinavian countries [12.3% in Sweden (Reddy et al., 2005) and 15.5% in Finland (Seldin et al., 2005)]. Minor allele frequencies in Western Europe have ranged from 7 to 10% [Spain, 7-7.4% (Orozco et al., 2005); France, 9.2% (Wipff et al., 2006); Germany, 10% (Jagiello et al., 2005); UK, 10.3% (Hinks et al., 2006), and Italy, 2.1% (Bottini et al., 2004)]. The C1858T SNP is considerably less polymorphic in non-Caucasian populations such as Colombian (4.3%) and Moroccan populations (3.22%) (Gomez et al., 2005b; Lamsyah et al., 2009). Actually, this polymorphism has not been found in African or Asian populations

(Begovich et al., 2004; Ban et al., 2005; Mori et al., 2005; Chabchoub et al., 2006; Ikegami et al., 2007; Chabchoub et al., 2009). The frequency of the PTPN22-1858T allele in a Chinese population was found to be 1.43% (Zhang et al., 2008). To the best of our knowledge, for the first time, we found a low frequency of the minor allele (T allele frequency of 0.8% in PTB and 2.0% in normal individuals) in a sample of Iranians, which shows that PTPN22 is less polymorphic in our population. There are few data regarding PTPN22 polymorphisms and tuberculosis. Considering the small sample size of studies, a larger sample size is required to obtain the sufficient statistical power.

In conclusion, we found that the PTPN22 C1858T (R620W) is not involved in the susceptibility to PTB in Iranian patients.

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