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TIRAP rs8177374 gene polymorphism increased the risk of pulmonary tuberculosis in Zahedan, southeast Iran

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ABSTRACT

Objective: To evaluate the possible association between Toll-interleukin 1 receptor (TIR) domain containing adaptor protein (TIRAP; also known as MAL) *rs1893352* and *rs8177374 (S180L)* gene polymorphisms and pulmonary tuberculosis (PTB) in a sample of Iranian population. **Methods:** This case-control study was performed on 174 PTB and 177 healthy subjects. Tetra amplification refractory mutation system-polymerase chain reaction (T-ARMS-PCR) was used to detect the polymorphisms. **Results:** Our finding showed that neither the overall *Chi*-square comparison of PTB and control subjects nor the logistic regression analysis indicated any association between rs1893352 polymorphism and PTB. Regarding rs8177374 polymorphism, the CT genotype as well as CT+TT increased the risk of PTB in comparison with CC genotype (*OR*=4.73, 95% *CI*=2.65-8.45, *P*<0.0001 and *OR*=6.47, 95% *CI*=3.68-11.38, *P*<0.0001, respectively). The rs8177374 T allele increased the risk of PTB in comparison with C allele (*OR*=4.21, 95% *CI*=2.43-7.26, *P*<0.0001). **Conclusions:** Our finding indicates that TIRAP rs8177374 polymorphism is associated with PTB in a sample of Iranian population.

1. Introduction

Tuberculosis, caused by *Mycobacterium tuberculosis (M. tuberculosis)*, is a global public health problem worldwide especially in Asia and Africa[1]. Approximately one-third of the world's population is currently infected with *M. tuberculosis*. In the majority of infected people, the immune response is able to sufficiently control the infection, but only 5%-10% of infected individuals will develop the disease[2]. According to the World Health Organization (WHO), in 2011, there were an estimated 8.7 million new cases of TB and 1.4 million died[3]. The precise reasons why only some of the individuals exposed to *M. tuberculosis* (Mtb) develop disease and others eradicate or limit the disease remain unknown. Toll-like receptors (TLRs) are type I transmemberane receptors that recognize evolutionary conserved pathogen-associated molecular patterns (PAMPs) and activate cells of the innate immune system. These PAMPs include bacterial, viral, and parasitic products, as well as endogenous ligands[4]. Ligation of TLRs by PAMPs leads to intracellular signaling in which several adaptor proteins are involved, namely the myeloid differentiation primary response protein 88 (MyD88), the My88D adaptor-like protein (Mal), the TIR domain-containing adapter inducing interferon-beta (TRIF) and the TRIF-related adaptor molecule (TRAM)[5-7]. TLRs play important roles in both recognition and innate immune response to *M. tuberculosis*[8]. They expressed on various immune cells including monocytes, macrophages, dendritic cells and epithelial cells of the immune system and recognize a wide variety of molecules, which lead to the transcription of proinflammatory genes through a complex signaling cascade[9]. Recently, we have found a significant

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association betweenTLR2 polymorphism at position 597T/C and susceptibility to PTB in a sample of Iranian population[16].

The TIRAP gene, encodes the human MAL adaptor protein, is located on chromosome 11q24.2 and spans 14.5 kb with 6 exons and encodes a protein of 221 amino acids[11]. Map of the human TIRAP gene is shown schematically in Figure 1. Several studies investigated the association between TIRAP gene polymorphisms and risk of tuberculosis (TB), but the results were controversial[11-14]. In the present study, an attempt was made to find out whether *TIRAP rs1893352* and *rs8177374 (S180L)* gene polymorphisms are associated with susceptibility or resistance to pulmonary tuberculosis in a sample of Iranian population.

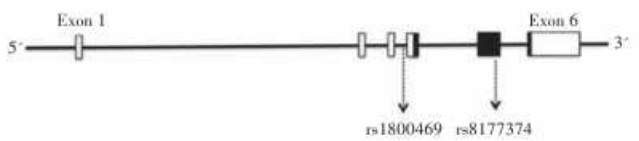

Figure 1. Map of the human *TIRAP* gene.
Exons 1-6 are numbered and represented by black (coding exons) and white boxes (5`and 3`UTRs). The positions of rs1893352 and rs8177374 are designated.

2. Materials and methods

2.1. Patients

This case-control study was performed on 174 patients with PTB and 177 population-based healthy subjects. The diagnosis of PTB was confirmed by meeting either of the following two standards: 1) sputum smear or culturing was positive; 2) sputum smear and culturing was negative but X-ray radiography was abnormal and antituberculosis chemotherapy was effective. Control subjects were selected from the Zahedan population showing no recent signs, symptoms, or history of pulmonary infections[15-17]. All control individuals were unrelated to patients and from the same geographical origin and living in the same region as the patients with PTB (Zahedan, southeast Iran). The project was approved by the local Ethics Committee of the Zahedan University of Medical Sciences, and written informed consent was taken from all participants. Blood samples were collected in Na-EDTA tubes from patients and healthy controls and stored at -20 ℃ until DNA extraction. Genomic DNA extraction from blood samples was carried out as described previously[18].

Table 1
Primers used for polymorphism determination of TIRAP.

Primers		Sequence (5′ -> 3′)
rs1893352	Forward outer	GCGTGTGCCACAGTGAGGAAGACCTGGT
	Reverse outer	CCGACAGCCTTTTCCAGAAGCTGCGCTT
	Forward inner (T allele)	GAGGGCTGCACCATCCCCCTGCTTTT
	Reverse inner (C allele)	GTGGGTAGGCAGCTCTGCTGAGGCACG
rs8177374	Forward outer	GTGTCTGGCCCTAATCTCATGAGGAAT
	Reverse outer	GCACTACACTCAGGAACACAGCAGAGTC
	Forward inner (G allele)	AAGTGCAGCCTTTGTGATTCTCTCTCTATG
	Reverse inner (A allele)	CATGGAGCAGCCATCCTACAGAGTGT

Table 2
The genotypes and allele distribution of TIRAP/MAL rs1893352 and rs8177374 polymorphisms in cases (pulmonary tuberculosis) and control groups.

Polymorphism	Casen (%)	Controln (%)	OR (95% *CI*)	*P*
rs1893352				
AA	124 (71.3)	140 (79.1)	1.00	-
AG	48 (27.6)	36 (20.3)	1.51 (0.91-2.47)	0.132
GG	2 (1.1)	1 (0.6)	2.25 (0.20-25.22)	0.603
AG+GG	50 (28.7)	37 (20.9)	1.53 (0.94-2.49)	0.108
Allele				
A	296 (85.1)	316 (89.0)	1.00	-
G	52 (14.9)	38 (11.0)	1.42 (0.91-2.22)	0.144
rs8177374				
CC	112 (64.4)	159 (89.8)	1.00	-
CT	60 (34.5)	18 (10.2)	4.73 (2.65-8.45)	<0.0001
TT	2 (1.1)	0 (0.0)	7.09 (0.34-149)	0.173
CT+TT	62 (35.6)	18 (10.2)	6.47 (3.68-11.38)	<0.0001
Allele				
C	284 (81.6)	336 (94.9)	1.00	-
T	64 (18.4)	18 (5.1)	4.21 (2.43-7.26)	<0.0001

2.2. Genotyping

The TIRAP/MAL genomic sequence (NT_033899.8) was obtained from the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov). The polymorphisms were searched and primers were designed for T-ARMS-PCR, which is a simple and rapid method for detection of SNPs[19-22] (Table 1). In this method two external primers (control band) and two inner primers (allele specific primers) are used. For rs8177374 polymorphism the products sizes were: 258-bp for T allele, 300-bp for C allele and 505-bp for two outer primers (control band) (Figure 2). The products sizes for detection of rs1893352 polymorphism were; 172-bp for A allele, 248-bp for G allele and 364-bp for the control band (Figure 3).

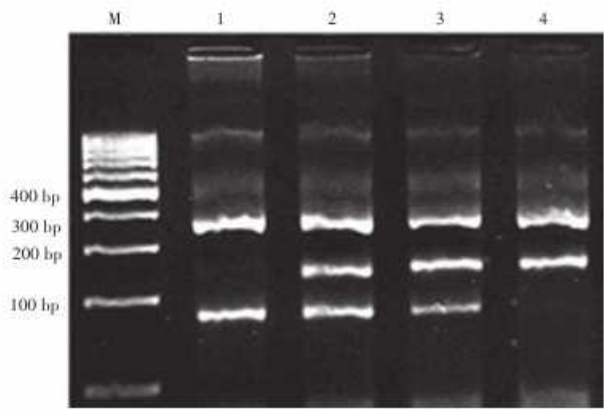

Figure 2. Photograph of the PCR products of the TIRAP/MAL rs1893352 polymorphism.
Product sizes were 172-bp for A allele, 248-bp for G allele, and 364-bp for two outer primers (control band). M: DNA marker; Lane 1: rs1893352 AA; Lanes 2,3: AG; Lane 4: GG.

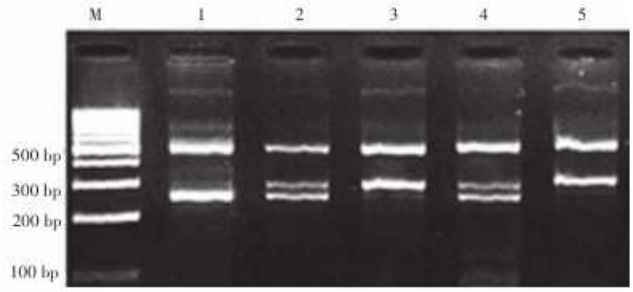

Figure 3. Photograph of the PCR products of TIRAP rs8177374 polymorphism.
The products sizes were: 258-bp for T allele, 300-bp for C allele and 505-bp for two outer primers (control band). M: DNA marker; Lanes 1: rs8177374 TT; Lanes 2,4: TC, Lanes 3, 5: CC.

PCR reactions consisted of a total volume of 20 μL containing 250 μM dNTPs, 0.4 μM of each primer, 2 mM $MgCl_2$, 1 U Taq DNA polymerase and 50 ng genomic DNA. The PCR cycling conditions were 5 min at 95 ℃ followed by 30 cycles of 30 s at 95 ℃, 30 s at 62 ℃ for rs1893352 and 61 ℃ for rs8177374, respectively, and 25 s at 72 ℃ with a final step at 72 ℃ for 10 min to allow for complete extension of all PCR fragments. The PCR products were analyzed by electrophoresis on a 2% agarose gel containing 0.5 μg/mL ethidium bromide and visualized by transillumination with UV light and photograph was taken (Figure 1, 2). To confirm the genotyping results, selected PCR amplified DNA (n=3 respectively, for each genotype) were examined by DNA sequencing and the results were concordant with those determined by T-ARMS-PCR. We regenotyped approximately 20% of the samples to verify the initial results. The check confirmed the previous genotyping results by 100%.

2.3. Statistical analysis

The statistical analysis of the data was performed using the SPSS 18.0 software. Independent sample t-test and χ^2 test were used for compare age and sex between the groups, respectively. The associations between genotypes and PTB were estimated by computing the odds ratio (*OR*) and 95% confidence intervals (95% *CI*) from logistic regression analysis.

3. Results

The study subjects consisted of 174 PTB patients [64 men and 110 women; age: (50.2±20.5) years] and 177 unrelated healthy controls [80 men and 97 women; age: (46.9±15.5) years].

There was no significant difference among the groups concerning sex and age (P>0.05). We compared the genotype frequencies of TIRAP rs8177374 and rs1893352 polymorphisms between the groups. The rs1893352 genotypes in both controls and cases were in HWE (χ^2=0.66, P=0.417 and χ^2=1.26, P=0.260, respectively. Neither the overall χ^2 exact comparison of PTB and control subjects nor the logistic regression analysis indicated any association between this polymorphism and PTB (Table 2).

As shown in Table 1, CT as well as CT+TT genotypes of TIRAP/MAL rs8177374 increased the risk of PTB in comparison with CC genotype (*OR*=4.73, 95% *CI*=2.65-8.45, P<0.0001 and *OR*=6.47, 95% *CI*=3.68-11.38, P<0.0001, respectively). Allele frequencies for C and T were found to be 0.816 and 0.184 in the PTB and 0.949 and 0.051 in the control group, respectively. The rs8177374 T allele increased the risk of PTB in comparison with C allele (*OR*=4.21, 95% *CI*=2.43-7.26, P<0.0001).

The TIRAP rs8177374 genotypes in controls but not in cases were in HWE (χ^2=0.51, P=0.456 and χ^2=3.85, P=0.049,

respectively). The deviation of cases from HWE provides support for an association of TIRAP rs8177374 variant with PTB.

4. Discussion

Tuberculosis is the most common cause of death. It has been proposed that tuberculosis disease development depends on interactions between the pathogen, environment, and the host. Many studies support that the differences in host immune genes is associated with susceptibility or resistance to TB[23-28].

TIRAP is involved in the MyD8-depedent signaling pathways in receptors, which recognizes a wide range of pathogenic bacteria and induces activation of NF-κB and transcription of proinflammatory genes and ultimately activates the mechanisms of host defense and the clearance of the pathogens[29-31].

In the present study, we investigated the impact of *TIRAP/MAL rs8177374* and *rs1893352* gene polymorphisms and susceptibility to PTB in a sample of Iranian population. A significant difference was found between patients with PTB and controls regarding rs8177374 polymorphism. High frequency of heterozygous TIRAP rs8177374 (S180L) in PTB infected individuals point to the role of this genotype as a risk factor for the development of disease. No significant difference was found between the groups concerning rs1893352 polymorphism in our population.

In contrast to our finding, Khor *et al*[11] reported that the heterozygous genotype of rs8177374 (S180L) was associated with reduced risks of PTB in West African and Algerian populations. In agreement with our finding they found no association between rs1893352 variant and TB. Nejentsev *et al*[12] have found no association between S180L variant and susceptibility to PTB in Russian, Ghanaian, and Indonesian populations. A meta-analysis performed by Miao *et al*[32] suggests that TIRAP S180L polymorphism is not associated with susceptibility to TB.

Zhang *et al*[33] investigated polymorphisms of TIRAP in a case-control study of 212 patients with tuberculosis and 215 controls in a Chinese population. They observed that TIRAP G286A (D96N, rs8177400) polymorphism was associated with susceptibility to TB. They found no significant association between rs3802813, rs3802814, rs8177374, rs7932766, rs7932976, rs8177375 polymorphisms and TB. A study carried out in Vietnam population revealed that the TIRAP S180L (rs8177374) polymorphism was not associated with PTB, while rs8177374 TT genotype increased the risk of meningeal tuberculosis[34]. Taype *et al* have found no association between TIRAP S180L variant and TB in Peruvian population[14]. In agreement with our finding, a study conducted by Selvaraj *et al*[13] showed that TIRAP Leu180 allele (rs8177374 T allele) was associated with an increased risk of PTB in South Indian population. They observed a trend towards an increased frequency of CT and TT genotypes in PTB patients (*OR*=1.33, 95% *CI*=0.86-2.04 and *OR*=3.10, 95% *CI*=0.96-10.05, respectively).

The disparity between different studies might be due to the difference in ethnic, genetic and host-pathogen and gene environment interaction in various ways to either decrease or enhance the susceptibility to TB in different regions.

TLRs play important roles in innate immune response to *M. tuberculosis*. Since the TIRAP mediates downstream signaling of TLR2 and TLR4 inducing pro-inflammatory response and a variant of this protein has been reported to diminish TLR2 signaling[30], the genetic variations of TIRAP might alter host response to PTB. It has been reported that a TIRAP Ser180 to leu substitution (rs8177374) has less ability to bind with TLR-2 in comparison with the 180Ser variant and impairs TLR-2-mediated NF-kB signaling, which leads to functional deficiency in TIRAP[11].

In conclusion, our data suggest that TIRAP rs8177374 (S180L) polymorphism increased the risk of PTB in a sample of Iranian population. Larger studies with different ethnicities are required to validate our findings.

Conflict of interest statement

We declare that we have no conflict of interest.

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