Letter to the Editor

Toll-like Receptor 1 Polymorphisms Increased the Risk of Pulmonary Tuberculosis in an Iranian Population Sample*

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A case-control study was carried out that involved 203 individuals diagnosed with pulmonary tuberculosis (PTB) and 203 healthy subjects. Genotyping of *TLR1* rs5743551 and rs5743618 polymorphisms was done using polymerase chain reaction-restriction fragments length polymorphism assay. We found that *TLR1* rs5743551 variant affected the risk of PTB in the codominant (*OR*=3.28, 95% *CI*=1.98-5.45, *P*<0.0001, GA *vs*. GG; *OR*=1.86, 95% *CI*=1.05-3.28, *P*=0.033, AA *vs*. GG) and dominant (*OR*=2.69, 95% *CI*=1.67-4.34, *P*<0.0001, GA+AA *vs*. GG) inheritance models tested. The A allele was associated with a higher risk of PTB than the G allele (*OR*=1.33, 95% *CI*=1.01-1.75, *P*=0.049). The TG genotype of the rs5743618 variant significantly increased the risk of PTB compared to the risk associated with the TT genotype (*OR*=3.29, 95% *CI*=1.82-5.97, *P*<0.0001). The G allele was associated with a higher risk of PTB than the T allele (*OR*=3.00, 95% *CI*=1.69-5.31, *P*=0.0001). Our findings revealed that *TLR1* rs5743551 and rs5743618 polymorphisms affected the risk of PTB in an Iranian population sample. Additional studies with larger sample sizes and involving subjects of different ethnicities are required to validate our present findings.

Tuberculosis (TB) is a major health problem worldwide. According to the World Health Organization (WHO) report on global control of TB, 10.4 million new TB cases and 1.4 million TB deaths occurred in 2015 worldwide[1]. It has been estimated that one-third of the global population is infected with TB, however only 5%-10% of infected individuals develop active TB. This suggests that there are differences in the susceptibility to TB or in the resistance to disease development among individuals.

Toll-like receptors (TLRs) play a key role in the recognition of infectious agents and initial immune response by detecting their specific conserved molecules. TLR2, 4, 5, and 6 are expressed on the surface of certain innate immune cells, where they bind the components of extracellular pathogens[2]. TLR3, 7, 8, and 9 are located intracellularly, in the endosomal compartments of innate immune cells, where they detect nucleic acids of intracellular pathogens. The engagement of TLRs by microbial constituents triggers the induction of inflammatory reactions, antimicrobial host defense, and events important for adaptive immune responses. TLR1 and TLR2 play an important role in host defense against mycobacteria, particularly by mediating the response to mycobacterial triacylated lipopeptides. Ligation of TLRs activates an intracellular signaling cascade that involves several adaptor proteins, such as MyD88 and toll-interleukin 1 receptor domain-containing adaptor protein, and activates macrophages and dendritic cells[2]. Several studies investigated the impact of *TLR1* variants on pulmonary tuberculosis (PTB) susceptibility in diverse populations, but the results were inconclusive[3-9]. Thus, the present study aimed to examine possible associations between polymorphisms of the *TLR1* gene and susceptibility to PTB in a sample of Iranian population.

This case-control study involved 203 PTB patients and 203 age- and sex-matched healthy individuals. The diagnosis of PTB patients and enrolment procedure were described previously[10]. Briefly, the cases were selected from PTB patients admitted to the University-affiliated hospital Bou-Ali Hospital (Zahedan, a referral center for TB in southeast Iran). All control subjects and PTB patients had the same ethnic origin and were living in the same region (Zahedan, southeast Iran). Cases and

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controls had similar age and sex ratios (P=0.698 and 0.267, respectively). Informed consent was taken from all subjects, and the project was approved by the Ethics Committee of the Zahedan University of Medical Sciences.

Genomic DNA was extracted from whole blood samples by salting out method. Genotyping of *TLR1* polymorphisms rs5743551 A/G and rs5743618 was performed using the polymerase chain reaction (PCR)-restriction fragment length polymorphism method. The sequences of the forward and reverse primers for genotyping the rs5743551 variant were 5'-ATATTTTTACTGCCCTGAATCCAA-3' and 5'-GGCCA ACTTCCCTAAACTAAGAAT-3', respectively. For PCR, 1 μL of genomic DNA (~100 ng/mL), 1 μL of each primer (10 μmol/L), 10 μL of 2× Prime Taq Premix (Genet Bio, Daejeon, Korea), and 7 μL ddH_2O were added into each 0.2 mL PCR reaction tube. The amplification was done with an initial 5 min denaturation step at 95 °C, followed by 30 cycles of 30 s at 95 °C, 30 s at 58 °C, and 30 s at 72 °C with a final 10-min long step at 72 °C. PCR products (10 μL) were digested with HindIII restriction enzyme (Fermentas) and separated by electrophoresis in 2% agarose gels. The G allele remained undigested (454-bp), whereas the A allele was digested into 146- and 308-bp fragments (Figure 1).

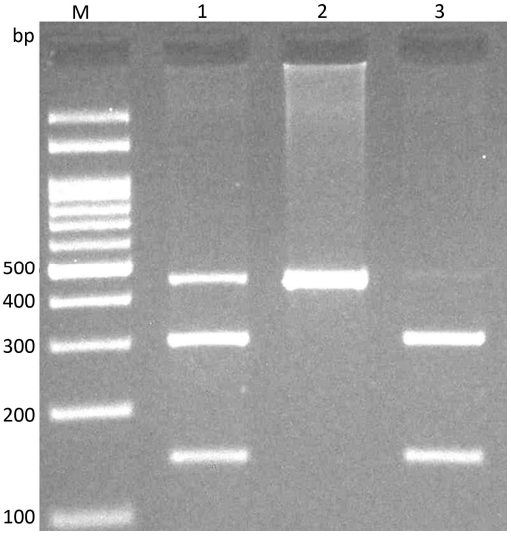

Figure 1. Detection of the *TLR1* rs5743551A/G polymorphism by electrophoresis of the polymerase chain reaction-restriction fragments length polymorphism assay products. The A allele is digested by HindIII restriction enzyme into 308- and 146-bp products, whereas the G allele remains undigested (454 bp). M: DNA marker; lane 1: GA; lane 2: GG; lane 3: AA.

For genotyping the rs5743618 variant, the forward and reverse primers were 3'-CCCAGAA AGAATCGTGCCCA-3' and 5'-TGGATGTGGCAGCTTT AGCA-3', respectively. The PCR cycling conditions for rs5743618 were as follows: an initial 6-min denaturation step at 95 °C was followed by 35 cycles of 40 s at 95 °C, 40 s at 63 °C, and 40 s at 72 °C with a final extension at 72 °C for 10 min. PCR products (10 μL) were digested with PstI restriction enzyme (Fermentas). The T allele remained undigested (500-bp), while the G allele was digested into 149- and 351-bp fragments (Figure 2).

Statistical analysis was done using SPSS 20.0 software. Data were analyzed by the χ^2-test or Student's t-test for independent samples, as appropriate. The associations between genotypes and PTB were computed by calculating the odds ratio (*OR*) and 95% confidence intervals (95% *CI*) from logistic regression analyses. Differences were considered significant if P<0.05.

A total of 406 subjects, including 203 confirmed PTB patients (78 males, 125 females; age 49.83±20.23 years) and 203 unrelated healthy subjects

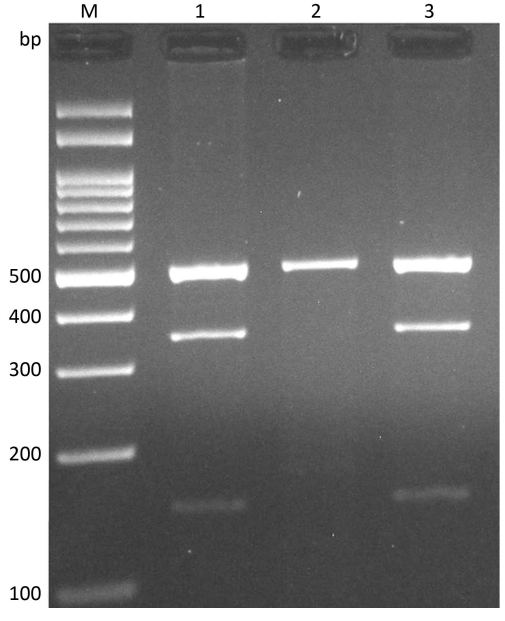

Figure 2. Detection of the *TLR1* rs5743618 polymorphism by electrophoresis of the polymerase chain reaction-restriction fragments length polymorphism assay products. The G allele is digested by PstI restriction enzyme into 351- and 149-bp products, whereas the T allele remains undigested (500 bp). M: DNA marker; lanes 1 and 3: TG; lane 2: TT.

(89 males, 114 females; age 49.15±14.97 years), have been genotyped. There were no statistically significant differences between the groups in the sex ratio and age (*P*>0.05 for both comparisons). Genotypes and allele frequencies of the *TLR1* polymorphisms are shown in Table 1. We found that the rs5743551 polymorphism affected the risk of PTB in codominant (*OR*=3.28, 95% *CI*=1.98-5.45, *P*<0.0001, GA *vs*. GG; *OR*=1.86, 95% *CI*=1.05-3.28, *P*=0.033, AA *vs*. GG) and dominant (*OR*=2.69, 95% *CI*=1.67-4.34, *P*<0.0001, GA *vs*. GG) inheritance models tested. The A allele was associated with the higher risk of PTB than the G allele (*OR*=1.33, 95% *CI*=1.01-1.75, *P*=0.049).

Our findings showed that the TG genotype of rs5743618 significantly increased the risk of PTB compared to the risk associated with the TT genotype (*OR*=3.29, 95% *CI*=1.82-5.97, *P*<0.0001). Furthermore, the G allele was associated with the higher risk of PTB than the T allele (*OR*=3.00, 95% *CI*=1.69-5.31, *P*=0.0001).

TLRs are major sensors of mycobacterial infection and play a key role in the initiation and coordination of the antimycobacterial innate immune response. TLR1 is an essential regulator of the immune response against mycobacterial lipopeptides, in particular, against triacylated lipopeptide. Lipopeptides, which are present in various microbes, stimulate immune responses through TLR1/2 or TLR2/6 heterodimers[2]. In the present study, we investigated a possible association between *TLR1* rs5743551 A/G and rs5743618 G/T polymorphisms and the risk of PTB in a sample of Iranian population. We revealed that both *TLR1* polymorphisms significantly affected the risk of PTB in our population. Qi et al.[3] have found that the GT genotype and G allele of the *TLR1* rs5743618 variant was associated with increased risk of TB in Han Chinese children. In addition, they reported that the GT genotype of the *TLR1* rs5743618 polymorphism correlated with a reduced immune response to TB infection. Ocejo-Vinyals et al.[4] have found that the G allele and GG genotype of the *TLR1* rs57436180 variant increased the risk of PTB susceptibility in a cohort of individuals in the north of Spain.

Sinha et al.[5] investigated the impact of the *TLR1* 743 A>G and 1805 T>G (rs57436180) variants on TB in Indian population. They found that the frequencies

Table 1. Genotypic and Allelic Frequencies of *TLR1* Variants in PTB Patients and Controls

TLR1 Polymorphisms	Case *n* (%)	Control *n* (%)	*OR* (95% *CI*)	*P*
rs5743551 G>A				
Codominant				
GG	32 (15.8)	68 (33.5)	1	-
GA	122 (60.1)	79 (38.9)	3.28 (1.98-5.45)	<0.0001
AA	49 (24.1)	56 (27.6)	1.86 (1.05-3.28)	0.033
Dominant				
GG	32 (15.8)	68 (33.5)	1	
GA+AA	171 (84.2)	135 (66.5)	2.69 (1.67-4.34)	<0.0001
Recessive				
GG+GA	154 (75.9)	147 (74.2)	1	
AA	49 (24.1)	56 (27.6)	0.83 (0.54-1.30)	0.496
Allele				
G	186 (45.8)	215 (53.0)	1	-
A	220 (54.2)	191 (47.0)	1.33 (1.01-1.75)	0.049
rs5743618 T>G				
TT	156 (76.8)	186 (91.6)	1	-
TG	47 (23.2)	17 (8.4)	3.29 (1.82-5.97)	<0.0001
GG	0 (0.0)	0 (0.0)	-	-
Allele				
T	359 (88.4)	389 (95.8)	1	-
G	47 (11.6)	17 (4.2)	3.00 (1.69-5.31)	0.0001

of heterozygous genotypes (743 AG) in TB cases and healthy controls were significantly different (0.47 *vs*. 0.61, respectively; *P*=0.02). In contrast, no significant difference in the frequency of the *TLR1* 1805 T>G polymorphism was observed between cases and controls in that study. Serum level of IL6 in healthy controls with the *TLR1* GG genotype was found to be significantly higher than that in healthy controls with AA (*P*=0.035) and AG (*P*=0.005) genotypes.

Wu et al.[6] have found no significant differences in the genotype or allele frequencies of the *TLR1* rs5743618 (1805 T>G) variant between PTB patients and individuals with latent TB as well as between PTB patients and healthy controls. Dittrich et al.[7] reported that the rs4833095 (Asn248Ser) variant of *TLR1* is associated with protection against TB in an Indian population and with an increased immune response to MTB (*Mycobacterium tuberculosis*) lysate *in vitro*. Ma et al.[8] did not observe an association between the *TLR1* rs5743618 (1805 T>G) variant and PTB risk.

A meta-analysis performed by Zhang et al.[9] indicated that the rs5743618 variant of *TLR1* was not associated with TB overall, but a subgroup analysis showed that this variant was significantly associated with an increased risk of TB in Africans and American Hispanics.

The contradictory results concerning the impact of *TLR1* variants on PTB in different populations may be due to population genetic factors and even geographical differences in MTB strains.

In summary, we revealed that *TLR1* variants are associated with the susceptibility to PTB in an Iranian population sample. Further studies with larger sample sizes and involving subjects of different ethnicities are warranted to confirm these findings.

Conflict of interest The authors declare no conflicts of interest.

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