Evaluation of interferon-induced transmembrane protein-3 (IFITM3) rs7478728 and rs3888188 polymorphisms and the risk of pulmonary tuberculosis

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Abstract. The current study aimed to examine the possible association between the interferon-induced transmembrane protein-3 (IFITM3) gene polymorphisms and risk of pulmonary tuberculosis (PTB) in a sample population. This case-control study was conducted on 188 PTB patients and 169 healthy subjects. The rs7478728 and rs3888188 variants of IFITM3 were genotyped using polymerase chain reaction-restriction fragment length polymorphism. The findings showed no significant association between rs7478728 polymorphism and risk of PTB. Regarding rs3888188 polymorphism, the TG genotype as well as G allele significantly increased the risk of PTB [odds ratio (OR)=2.48, 95% confidence interval (CI): 1.33-3.86; P=0.003, respectively]. In conclusion, the findings revealed that rs3888188 polymorphism increased the risk of PTB in a sample of Iranian population. Additional investigation with larger sample sizes and different ethnicities are needed to verify our findings.

Introduction

Tuberculosis (TB), caused by Mycobacterium tuberculosis (MTB) infection, is a public health problem globally (1,2). According to the WHO report on the worldwide control of TB, approximately 8.6 million new cases occurred in 2012 (3). Although almost 33% of the population is infected with TB, only 5-10% of infected cases develop active TB (3), which suggests a major role of genetic factors in host immunity. Interferon-γ (IFNγ) is produced and released by host cells in response to the presence of numerous pathogens (4). It plays a key role in macrophage activation during MTB infection (5).

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Key words: tuberculosis, IFITM3, polymorphism

Materials and methods

Patients. This case-control study was performed on 188 PTB patients and 169 age- and gender-matched healthy individuals. The enrollment process and study design are described elsewhere (17-23). Briefly, the cases were chosen from PTB patients admitted to a University-Affiliated Hospital (Bou-Ali Hospital, Zahedan, Iran, referral center for TB) with no clinical symptoms or family history of TB. TB was diagnosed by clinical symptoms, posterior-anterior chest radiography, presence of acid-fast-bacilli on a sputum smear, and culturing MTB organisms from a specimen taken from the patient and response to therapy, as described previously (20-23). The project was approved by the local Ethics Committee of the Zahedan University of Medical Sciences and informed consent was obtained from all subjects. DNA was extracted from whole blood samples using the salting out method (24).

Genotyping. Genotyping of IFITM3 rs7478728 and rs3888188 polymorphisms was performed using the polymerase chain reaction (PCR)-restriction fragment length polymorphism
Table I. Primer sequences used for the detection of IFITM3 gene polymorphisms.

<table>
<thead>
<tr>
<th>Polymorphisms</th>
<th>Sequence (5’→3’)</th>
<th>Restriction enzyme</th>
<th>Product size (bp)</th>
<th>Annealing temperature (˚C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs7478728 C&gt;T</td>
<td>F: TTAGGCCCTAGCCCTCTTTTCT</td>
<td>Alw26I</td>
<td>T allele: 217, 29</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td>R: CTGGTGACAGGAGAGAAGAGT</td>
<td>C allele: 246</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs3888188 T&gt;G</td>
<td>F: CACAGTGAGGTTATGGGAGAC</td>
<td>Hpy188I</td>
<td>G allele: 340, 246</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td>R: ACTGTTGACAGGAGAGAAGAGT</td>
<td>T allele: 586</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

F, forward; R, reverse; IFITM3, interferon-induced transmembrane protein-3.

Results

Patient characteristics. A total of 357 subjects including 188 confirmed PTB patients (73 males, 115 females; ages 50.0±19.5 years) and 169 unrelated healthy subjects (75 males, 94 females; ages 47.9±15.0 years) were assessed. There was no statistically significant difference among the groups regarding gender and age (P>0.05).

Association between the polymorphisms and PTB risk. Genotypes and allele frequencies of the IFITM3 polymorphisms are shown in Table II. Regarding rs7478728 polymorphism, the findings indicated that this variant was not associated with the risk of PTB in codominant (OR=1.32, 95% CI: 0.80-2.17, P=0.337, CT vs. CC; OR=2.04, 95% CI: 0.63-6.61, P=0.362, TT vs. CC), dominant (OR=1.35, 95% CI: 0.82-2.21, P=0.293, CT+TT vs. CC), and recessive (OR=1.65, 95% CI: 0.54-5.02, P=0.538 TT vs. CC+CT) inheritance model tested. The T allele
was not associated with the risk of PTB (OR=1.16, 95% CI: 0.86-1.56, P=0.377) compared to C allele.

Regarding the rs3888188 variant, the results revealed that TG genotype significantly increased the risk of PTB compared to TT genotype (OR=2.48, 95% CI: 1.42-4.35; P=0.002). Similarly, the G allele increased the risk of PTB in comparison with T allele (OR=2.26, 95% CI: 1.33-3.86; P=0.003).

The interaction of the two variants of the IFITM3 gene was analyzed (Table III) and the findings suggested that the CT/TG genotype significantly increased the risk of PTB compared to CC/TT genotype (P=0.006).

Discussion

In the present study, we examined the possible association between IFITM3 rs7478728 and rs3888188 polymorphisms and the risk of PTB in a sample of Iranian population. Our findings did not support an association between rs7478728
variant and risk of PTB in the population studied. However, we found that TG genotype as well as G allele of rs3888188 polymorphism significantly increased the risk of PTB. There is only one study concerning the possible association between IFITM3 variants and risk of TB (16). Shen et al (16) have found that the rs3888188 G allele increased the risk of pediatric TB (OR=1.30, 95% CI: 1.08-1.56; P=0.039). In addition, they found that the rs7478728 T allele was significantly associated with pediatric TB (OR=1.34, 95% CI: 1.07-1.68; P=0.010), but not after Bonferroni correction (P=0.082). Authors of that study also evaluated the effect of rs3888188 (-204 T>G) variant on IFITM3 transcription in vitro and found that the promoter activity of rs3888188 G allele was lower than that of the T allele. Similarly, peripheral-blood mononuclear cells carrying the rs3888188 GG genotype showed a reduced IFITM3 mRNA level compared to cells carrying TT or GT genotype. It was concluded that the rs3888188 variant is a functional promoter polymorphism of IFITM3 that increased the risk of pediatric TB in the Han Chinese population (16). IFITM3 has been recognized as a key component of the IFNγ signaling pathway and down-regulation of IFITM3 via siRNA significantly reduced the antiviral activities of IFNγ by 40-70% (12,13). It is thus a potential candidate gene for TB susceptibility. IFITM proteins are key mediators of the host antiviral response (11-13,25,26). Everitt et al (25) showed that mice lacking IFITM3 gene display fulminant viral pneumonia following infection with a low-pathogenicity influenza virus. Similarly, in an in vitro study, an increase in viral replication was observed in the absence of IFITM3, and re-introduction of IFITM3 limited the replication of the influenza A virus (25).

One of the limitations of the present study is the relatively small sample sizes. There is no clear explanation for deviation from HWE for the IFITM3 rs7478728 variant in our population. The probable reason may be due to genetic drift.

In conclusion, our findings suggest that IFITM3 rs3888188 polymorphism significantly increased the risk of PTB in a sample of Iranian population. Additional studies with larger sample sizes and diverse ethnicities are necessary to confirm these findings.

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References


