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

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Received June 3, 2016; Accepted September 21, 2016

DOI: 10.3892/br.2016.763

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): 1.42-4.53; P=0.002, and OR=2.26, 95% 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). Individuals defective in the genes for *IFNγ* or *IFNγ* receptor (*IFNγR*) have been indicated to be susceptible for mycobacterial infections including MTB (6). Previously, we showed an association between *IFNγ* and *IFNγR* variants and risk of pulmonary TB (PTB) (7,8).

Interferon-induced transmembrane protein-3 (IFITM3) is a double transmembrane protein that can be upregulated by IFNs and participates in INF-triggered processes, such as homotypic cell adhesion, anti-proliferative activities in tumor pathogenesis, and the innate immune response to virus infections (9-13). The *IFITM3* gene is mapped to an *IFITM* gene cluster on chromosome 11p15.5 (14). In a genome wide scan, Stein *et al* (15) identified that one of the TB-linked loci was located in this chromosome region. To the best of our knowledge, there is only one report regarding the impact of *IFITM* gene polymorphisms on the risk of TB (16). Therefore, the present study aimed to examine the possible associations between polymorphisms of *IFITM3* gene and susceptibility to PTB in a sample of Iranian population.

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 *IFTIM3* rs7478728 and rs3888188 polymorphisms was performed using the polymerase chain reaction (PCR)-restriction fragment length polymorphism

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Key words: tuberculosis, *IFITM3*, polymorphism

Table I. Primer sequences used for the detection of *IFITM3* gene polymorphisms.

Polymorphisms	Sequence (5'→3')	Restriction enzyme	Product size (bp)	Anneling temperature (˚C)
rs7478728 C>T	F: TTAGCCCTCAGCCCCTCTTTCGTC R: CTGTTGACAGGAGAGAAGAAGGTT	*Alw*26I	T allele: 217, 29 C allele: 246	53
rs3888188 T>G	F: CACAGTGAGGGTTATGGGAGAC R: ACTGTTGACAGGAGAGAAGAAGGTT	*Hpy*188I	G allele: 340, 246 T allele: 586	54

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

method. The primer sequences are shown in Table I. In each 0.20 ml PCR reaction tube, 1 μl of genomic DNA (~100 ng/ml), 1 μl of each primer (10 μM), 10 μl of 2X Prime Taq Premix (Genet Bio Inc., Daejeon, Korea), and 7 μl ddH_2O were added.

Amplification was carried out with an initial denaturation step of 5 min at 95˚C followed by 30 cycles of 30 sec at 95˚C, annealing at 53˚C for rs7478728 and 54˚C for rs3888188 for 30 sec and extension at 72˚C for 30 sec. Final extension was performed at 72˚C for 5 min.

For rs7478728, 10 μl of PCR products was digested with *Alw*26I restriction enzyme (Fermentas, Glen Burnie, MD, USA) and then separated by electrophoresis in 2% agarose gels. The C allele was undigested (246-bp), while the T allele was digested and produced 217- and 29-bp fragments (Fig. 1).

For the rs3888188 variant, the PCR products were digested with *Hpy*188I restriction enzyme (Fermentas). The T allele was undigested (586-bp), while the G allele digested and produced 340- and 246-bp fragments (Fig. 2).

To confirm the genotyping quality for each polymorphism, ~20% of random samples were regenotyped and the findings confirmed the preceding genotyping results.

Statistical analysis. Statistical analysis of the data was performed using the SPSS 20.0 software (IBM SPSS, Armonk, NY, USA). The analysis was performed by the χ^2 test or independent sample t-test according to the data. The associations between genotypes and PTB were calculated by computing the odds ratio (OR) and 95% confidence interval (CI) from logistic regression analyses. The Hardy-Weinberg equilibrium (HWE) for cases and controls was calculated by χ^2 test. P<0.05 was considered to indicate a statistically significant difference.

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

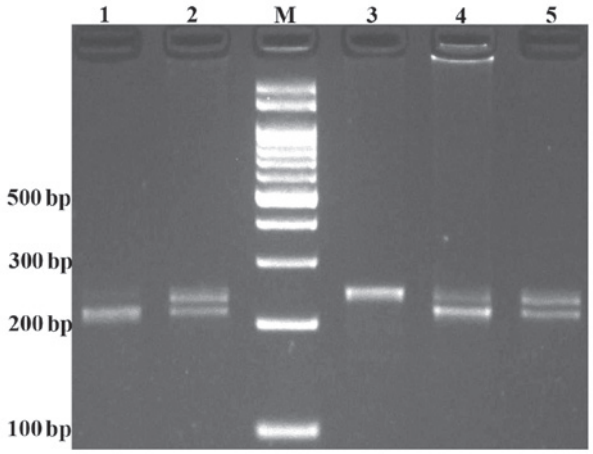

Figure 1. *IFITM3* rs7478728 C>T polymorphism using polymerase chain reaction-restriction fragment length polymorphism methods. The C allele was undigested (246-bp), while the T allele was digested by the *Alw*26I restriction enzyme, and 217- and 29-bp fragments were produced. M, DNA marker; lane 1, TT; lanes 2, 4 and 5, CT; and lane 3, CC.

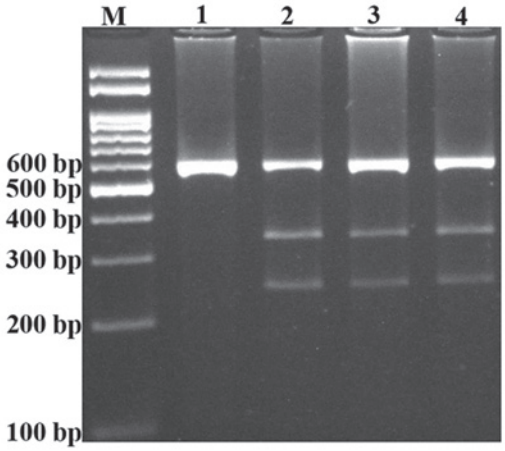

Figure 2. *IFITM3* rs3888188 T>G polymorphism using polymerase chain reaction-restriction fragment length polymorphism methods. The T allele was undigested (586-bp), while the G allele was digested by the *Hpy*188I restriction enzyme and produces 340- and 246-bp fragments. M, DNA marker; lane 1, TT; and lanes 2-4, TG.

Table II. Frequency distribution of *IFITM3* rs7478728 and rs3888188 gene polymorphisms in PTB and controls.

Polymorphisms	Case n (%)	Control n (%)	OR (95% CI)	P-value
rs7478728 C>T				
Codominant				
CC	38 (20.2)	43 (25.4)	1.00	-
CT	141 (75.0)	121 (71.6)	1.32 (0.80-2.17)	0.337
TT	9 (4.8)	5 (3.0)	2.04 (0.63-6.61)	0.362
Dominant				
CC	38 (20.2)	43 (25.4)	1.00	-
CT+TT	150 (79.8)	126 (74.6)	1.35 (0.82-2.21)	0.293
Recessive				
CC+CT	179 (95..2)	164 (97.0)	1.00	
TT	9 (4.8)	5 (3.0)	1.65 (0.54-5.02)	0.538
Allele				
C	217 (57.7)	207 (61.2)	1.00	-
T	159 (42.3)	131 (38.8)	1.16 (0.86-1.56)	0.377
rs3888188 T>G				
TT	139 (73.9)	148 (87.6)	1.00	-
TG	49 (26.1)	21 (12.4)	2.48 (1.42-4.35)	0.002
GG	0 (0.0)	0 (0.0)	-	-
Allele				
T	327 (87.0)	317 (93.8)	1.00	-
G	49 (13.0)	21 (6.2)	2.26 (1.33-3.86)	0.003

IFITM3, interferon-induced transmembrane protein-3; PTB, pulmonary tuberculosis; OR, odds ratio; CI, confidence interval.

Table III. Interaction of *IFITM3* rs7478728 and rs3888188 gene polymorphisms on PTB risk.

rs7478728 C>T	rs3888188 T>G	Case n (%)	Control n (%)	OR (95% CI)	P-value
CC	TT	29 (15.4)	39 (23.1)	1.00	-
CT	TT	104 (55.3)	104 (61.5)	1.34 (0.77-2.34)	0.329
CT	TG	37 (19.7)	17 (10.1)	2.93 (1.38-6.19)	0.006
TT	TT	6 (3.2)	5 (3.0)	1.61 (0.45-5.81)	0.524
CC	TG	9 (4.8)	4 (2.4)	3.03 (0.85-10.80)	0.128
TT	TG	3 (1.6)	0 (0.0)	-	-

IFITM3, interferon-induced transmembrane protein-3; PTB, pulmonary tuberculosis; OR, odds ratio; CI, confidence interval.

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).

The genotype of *IFITM3* rs7478728 variant in cases and controls was not in HWE (χ^2=54.1, P<0.001 and χ^2=43.64, P<0.001, respectively). Regarding the *IFITM3* rs3888188 variant, the genotype in controls (χ^2=0.74, P=0.389) but not in cases (χ^2=4.22, P=0.040) was in HWE.

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.

Acknowledgements

The present study was funded by a dissertation research grant (M.D. thesis of FA no. 7265) from the Zahedan University of Medical Sciences. The authors would like to thank the patients and healthy subjects who willingly participated in the study.

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