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ASSOCIATION STUDY OF THE *FTO* GENE POLYMORPHISMS WITH THE RISK OF PULMONARY TUBERCULOSIS IN A SAMPLE OF IRANIAN POPULATION

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Aim: The aim of the present study was to find out the impact of fat mass and obesity-associated (FTO) gene on risk of pulmonary tuberculosis (PTB) in a sample of Iranian population. *Methods:* This case-control study was carried out on a total of 354 subjects including 185 PTB patients and 169 healthy subjects. Genotyping of *FTO* rs9939609 and rs8050136 variants was done using polymerase chain reaction-restriction fragment length polymorphism method. *Results: FTO* rs9939609 variant showed no statistically significant difference in allele and genotype frequencies between PTB patients and controls. The rs8050136 polymorphism marginally increased the risk of PTB in dominant (OR = 1.53, 95% CI = 1.00–2.33, $p = 0.055$, CA+AA vs. CC) inheritance model tested, where rs8050136 A allele significantly increased the risk of PTB (OR = 1.42, 95% CI = 1.02–1.97, $p = 0.045$) compared with C allele. *Conclusion:* The finding of the present study showed an association between *FTO* rs9939609 variant and risk of PTB. Further studies with larger sample sizes and different ethnicities are necessary to confirm the findings.

Keywords: tuberculosis, FTO, polymorphism

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Introduction

Tuberculosis (TB) is a main health concern, affecting nearly one-third of the world's population [1, 2]. According to WHO report on global TB control, approximately 8.6 million new cases occurred in 2012 [3]. It has been proposed that 5%–10% of infected cases go on to develop active TB [3], which implies that host genetics may influence the risk of the disease. Multiple lines of evidence support a role for genetics in the development of pulmonary tuberculosis (PTB) [4–9] in a sample of Iranian population.

The *FTO* gene is located on chromosome 16 (16q12.2). This gene encodes a protein that plays a role in regulation of food intake [10]. Obesity influence patterns of oxidative stress and altered circulating levels of inflammatory cytokines, which impair immune function, modify leukocyte counts, and affect cell-mediated immune responses [11–13]. There is growing evidence showing that the impairment of the immune function leads to an increased susceptibility of the host to a number of various pathogens, such as *Mycobacterium tuberculosis*, influenza, coxsackievirus, *Helicobacter pylori*, and encephalomyocarditis virus [14–16].

FTO is the strongest known genetic susceptibility locus for obesity. The genetic variants of *FTO* gene were found to be significantly associated with obesity in diverse populations [17–21].

To the best of our knowledge, there is only one report regarding the impact of *FTO* gene polymorphisms on tuberculosis risk [22]. Thus, the present study aimed to examine the possible associations between polymorphisms of *FTO* rs9939609 and rs8050136 variants and susceptibility to PTB in a sample of Iranian population.

Materials and Methods

Patients

This case-control study included 185 confirmed PTB patients and 169 unrelated population-based healthy subjects. The cases were selected from PTB patients admitted to a university-affiliated hospital (Bou-Ali Hospital, Zahedan, referral center for TB). The enrolment procedures have been described previously [23]. Briefly, PTB diagnosis was done using clinical symptoms, chest radiography, presence of acid-fast bacilli on a sputum smear, culturing *M. tuberculosis* organisms, and response to antituberculosis chemotherapy. The project was approved by the local ethics committee of the Zahedan University of Medical

Sciences, and informed consent was taken from all subjects participated in the study. Blood samples were collected in ethylenediaminetetraacetic acid-containing tubes from patients and healthy controls, and genomic DNA was extracted using salting out method as described previously [24].

Genotyping

Genotyping of *FTO* rs9939609 and rs8050136 polymorphisms was performed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The set of primer sequences used in this method are shown in Table I. In each 0.2-mL PCR reaction tube, 1 μL of genomic DNA (~100 ng/mL), 1 μL of each primer (10 μM), 10 μL of 2× Prime Taq Premix (GeNet Bio, Korea), and 7 μL of ddH_2O were added.

Amplification was done with an initial denaturation step at 95 °C for 5min, followed by 30 cycles of 30 s at 95 °C, 30 s at 60 °C, and 30 s at 72 °C with a final step at 72 °C for 10 min. For rs9939609 and rs8050136 genotyping, 10 μL of PCR products were digested with *DdeI* and *MseI* restriction enzymes (Fermentas, Vilnius, Lithuania), respectively. Then, the fragments were separated by electrophoresis in 2% agarose gels. For rs9939609 variant, the A allele was digested into 173- and 27-bp fragments, whereas the T allele was undigested (200-bp fragment) (Figure 1). Regarding rs8050136 variant, the A allele was digested into 182- and 24-bp fragments, whereas the C allele was undigested (206-bp fragment) (Figure 2).

Statistical analysis

Statistical analysis was performed using the SPSS 20.0 package software. The differences between the variables were evaluated by χ^2-test or independent sample *t*-test according to the data. The associations between genotypes and PTB were estimated by computing the odds ratio (OR) and 95% confidence intervals (95% CI) from logistic regression analyses. A *p*-value of <0.05 was considered to be statistically significant.

Table I. The primers used for the detection of *FTO* gene polymorphisms using PCR-RFLP method

Polymorphisms	Sequence (5′->3′)	Restriction enzyme	Product size (bp)
rs9939609 T>A	F: TAGGTTCCTTGCGACTGCTGTGAACTT	*DdeI*	A allele: 173, 27
	R: AGCCTCTCTACCATCTTATGTCCAAACA		T allele: 200
rs8050136 C>A	F: ATGCCAGTTGCCCACTGTGGCATT	*MseI*	A allele: 182, 24
	R: GCAAAATTTCACACACCAAGATGGTCATG		C allele: 206

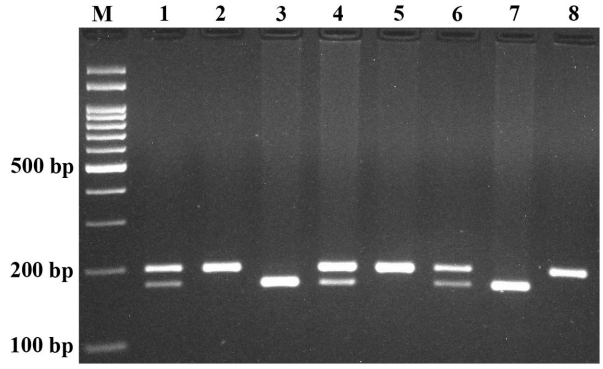

Figure 1. Electrophoresis pattern of PCR-RFLP for the detection of *FTO* rs9939609 polymorphism. The A allele was digested by *DdeI* restriction enzyme into 173- and 27-bp fragments, whereas the T allele was undigested (200-bp fragment). M: DNA marker; lanes 1, 4, and 6: TA; lanes 2, 5, and 8: TT; lanes 3 and 7: AA

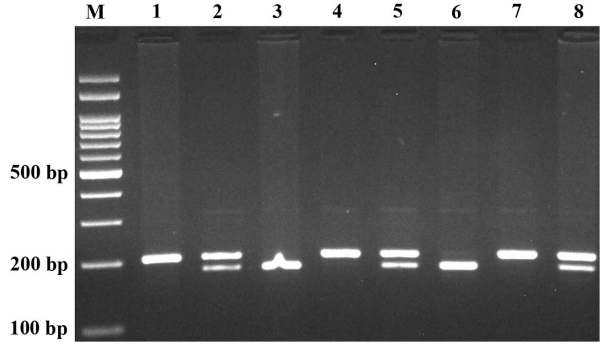

Figure 2. Electrophoresis pattern of PCR-RFLP for the detection of *FTO* rs8050136 polymorphism. The A allele was digested by *MseI* restriction enzyme into 182- and 24-bp fragments, whereas the C allele was undigested (206-bp fragment). M: DNA marker; lanes 1, 4, and 7: CC; lanes 2, 5, and 8: CA; lanes 3 and 6: AA

Results

A total of 354 subjects including 185 PTB patients (72 males, 113 females, mean age: 50.0 ± 19.6 years) and 169 unrelated healthy subjects (75 males, 94 females, mean age: 47.9 ± 15.0 years) were evaluated. There was no statistically significant difference found between cases and controls regarding sex and age ($p > 0.05$). Genotypes and allele frequencies of *FTO* gene polymorphisms are shown in Table II. Allelic (A vs. T) and genotypic comparisons of codominant

Table II. Genotype and allelic frequencies of *FTO* rs9939609 T>A and rs8050136 C>A variant in PTB patients and control subjects

FTO polymorphism	Case *n* (%)	Control *n* (%)	OR (95% CI)	*p*
rs9939609 T>A				
Codominant				
TT	74 (40.0)	74 (43.8)	1.00	–
TA	95 (51.4)	86 (50.9)	1.11 (0.72–1.71)	0.659
AA	16 (8.6)	9 (5.3)	1.78 (0.74–4.28)	0.279
Dominant				
TT	74 (40.0)	74 (43.8)	1.00	–
TA+AA	111 (60.0)	95 (56.2)	1.17 (0.76–1.78)	0.518
Recessive				
TT+TA	169 (91.4)	160 (94.7)	1.00	
AA	16 (8.6)	9 (5.3)	1.68 (0.72–3.92)	0.299
Allele				
T	243 (65.7)	234 (69.2)	1.00	–
A	127 (34.3)	104 (30.8)	1.18 (0.86–1.61)	0.354
rs8050136 C>A				
Codominant				
CC	81 (43.8)	92 (54.5)	1.00	–
CA	90 (48.6)	70 (41.4)	1.46 (0.95–2.25)	0.100
AA	14 (7.6)	7 (4.1)	2.27 (0.87–5.90)	0.107
Dominant				
CC	81 (43.8)	92 (54.5)	1.00	–
CA+AA	104 (56.2)	77 (45.5)	1.53 (1.00–2.33)	0.055
Recessive				
CC+CA	171 (92.4)	162 (95.9)	1.00	–
AA	14 (7.6)	7 (4.1)	1.89 (0.74–4.81)	0.186
Allele				
C	252 (68.1)	254 (75.1)	1.00	–
A	118 (31.9)	84 (24.9)	1.42 (1.02–1.97)	0.045

(TA vs. TT and AA vs. TT), dominant (TA+AA vs. TT), and recessive (AA vs. TA+TT) genetic inheritance models showed no significant association between rs9939609 T>A polymorphism and risk of PTB. Regarding rs8050136 C>A polymorphism, this variant marginally increased the risk of PTB in dominant (OR = 1.53, 95% CI = 1.00–2.33, $p = 0.055$, CA+AA vs. CC) inheritance model tested, where rs8050136 A allele significantly increased the risk of PTB (OR = 1.42, 95% CI = 1.02–1.97, $p = 0.045$) compared with C allele.

The interaction of two polymorphisms on risk of PTB is shown in Table III. The results revealed that AA/AA genotype marginally increased the risk of PTB compared with rs9939609 TT/rs8050136 CC genotype.

Haplotype frequencies of *FTO* gene polymorphisms in PTB patients and controls are shown in Table IV. The results revealed that AA haplotype marginally associated with risk of PTB compared with rs9939609T/rs8050136C.

Table III. Interaction of *FTO* rs9939609 T>A and rs8050136 C>A polymorphisms with PTB risk

rs9939609 T>A	rs8050136 C>A	PTB *n* (%)	Controls *n* (%)	OR (95% CI)	*p*
TT	CC	68 (36.8)	72 (42.6)	1.00	–
TA	CA	83 (44.9)	64 (37.9)	1.42 (0.89–2.26)	0.158
TA	CC	12 (6.5)	20 (11.8)	0.64 (2.89–1.40)	0.326
AA	AA	14 (7.6)	5 (3.0)	2.96 (1.01–8.68)	0.051
TT	CA	6 (3.2)	2 (1.2)	3.18 (0.62–16.29)	0.275
AA	CA	2 (1.1)	4 (2.4)	0.52 (0.09–2.99)	0.683
TA	AA	0 (0.0)	2 (1.2)	–	–

Table IV. Haplotype association of *FTO* rs9939609 T>A and rs8050136 C>A variants with PTB risk

rs9939609 T>A	rs8050136 C>A	PTB (Frequency)	Control (Frequency)	OR (95% CI)	*p*
T	C	0.6397	0.6794	1.00	–
A	A	0.3019	0.2356	1.39 (0.96–2.02)	0.078
A	C	0.0414	0.0721	0.64 (0.32–1.28)	0.210
T	A	0.0170	0.0129	1.48 (0.40–5.41)	0.560

Discussion

Obesity is identified to affect cell-mediated immune responses. Recent studies have shown that genetic polymorphisms in the *FTO* gene are associated with human obesity. In the present study, we examined the possible association between *FTO* gene polymorphisms and the risk of PTB in a sample of Iranian population. Our findings revealed that rs9939609 variant was not associated with the risk of PTB, whereas the rs8050136 C>A variant marginally increased the risk of PTB in dominant inheritance model. The rs8050136 A allele significantly increased the risk of PTB compared with C allele. In contrast to our findings, Feng et al. [22] have found a significant association between *FTO* rs9939609 polymorphism and risk of TB. The AA genotype significantly increased the risk of TB compared with TT genotype (OR = 3.77, 95% CI = 2.26–6.28, $p < 0.001$). Furthermore, the A allele increased the risk of TB (OR = 1.26, 95% CI = 1.08–1.48, $p = 0.004$). But, they did not find an association between rs8050136 variant and risk of TB.

It has been proposed that obesity is a risk factor for cancer. Several studies have shown that *FTO* gene polymorphisms may be significantly associated with risk of various cancers [25, 26].

The effect of *FTO* gene variant on the risk of PTB may not merely be attributed to its role in the risk of obesity. The alteration of human immune response related to this genetic variant may play a role.

The present study has several limitations. First, we have no data regarding body mass index prior to the diagnosis of tuberculosis, and we could not determine the effect of *FTO* variants on obesity. Second, we only selected two variants in the *FTO* gene. Third, we did not determine the gene environmental interactions. Finally, we had no data regarding BCG vaccination of cases and controls.

In summary, our findings revealed an association between rs8050136 variant of *FTO* gene and risk of PTB in a sample of Iranian population. The possible mechanisms that could explain this association remains to be clear. Further studies with larger sample sizes and different ethnicities are necessary to confirm these findings.

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Conflict of Interest

The authors declare no conflict of interest.

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