Original Article

Lack of Association between rs4331426 Polymorphism in the Chr18q11.2 Locus and Pulmonary Tuberculosis in an Iranian Population*

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Abstract

Objective The effect of rs4331426 polymorphism in the Chr18q11.2 locus on pulmonary tuberculosis (PTB) risk was evaluated.

Methods This case-control study included 208 PTB patients and 204 healthy subjects. Genotyping of the rs4331426 variant was conducted using polymerase chain reaction restriction fragment length polymorphism.

Results The frequencies of genotypes AA, AG, and GG polymorphisms were 83.1%, 15.9%, and 1.0% in the PTB group and 84.3%, 15.2%, and 0.5% in the control group, respectively. The frequency of the minor (G) allele was 8.9% in the PTB group and 8.1% in controls. Neither genotype nor allele frequencies of the rs4331426 variant showed statistically significant differences between PTB and controls. In addition, stratification by sex showed no significant association between the rs4331426 variant and PTB risk in males or females.

Conclusion In conclusion, the results of this study do not support an association between the rs4331426 polymorphism and risk of PTB in an Iranian population.

Key words: Tuberculosis; rs4331426; Polymorphism

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INTRODUCTION

Tuberculosis (TB), a disease caused by *Mycobacterium tuberculosis* infection, remains a public health problem worldwide[1-2]. According to a WHO report on the global control of TB, there were nearly 8.6 million new cases in 2012[3]. It has been estimated that one-third of the global population is infected with TB, whereas 5%-10% of infected cases will develop active TB[3-4], indicating a key role for genetic factors in host immunity. Increasing evidence indicates that host genetic factors play a key role in the susceptibility to TB[5-11].

Numerous genetic polymorphisms associated with TB susceptibility have been identified in genome-wide association studies (GWAS)[12-16]. In contrast to methods that test one or a few genetic regions, GWAS scans the entire genome to identify strong associations between polymorphisms and TB

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susceptibility. Among the variants recognized by GWAS, rs4331426 located in the noncoding region of chromosome 18 (18q11.2) showed the highest association scores for TB in an African population[15]. Wang et al.[17] investigated this variant in a Chinese population and found conflicting results. They found that the rs4331426 G allele was protective, while the G allele was a risk allele in the African population. The findings of other studies[18-19] in Chinese populations did not support an association between the rs4331426 variant and risk/protection of TB. There is no data regarding the effect of this variant on TB risk in Iranian populations. Thus, the present study examined the possible associations between the rs4331426 polymorphism and susceptibility to PTB in an Iranian population.

MATERIALS AND METHODS

This case-control study included 208 PTB patients and 204 age- and sex-matched healthy individuals. The cases were selected from unrelated PTB patients admitted to a university-affiliated hospital (Bou-Ali Hospital, Zahedan, referral center for TB). The enrollment process and study design have been described previously[7,10,20]. Briefly, PTB was diagnosed by clinical symptoms, radiological evidence, and bacteriological investigations such as sputum acid-fast bacillus smear positivity, culture, and response to antituberculosis chemotherapy. All control subjects were unrelated adults selected from the Zahedan population, without recent signs, symptoms, or history of active TB. The project was approved by the local ethics committee of the Zahedan University of Medical Sciences, and informed consent was obtained from all participants. Genomic DNA was extracted from whole blood using the salting out method. DNA was extracted from whole blood samples using the salting out method as described previously[21].

Genotyping of the rs4331426 A>G variant was conducted by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP). The forward and reverse primers were 5′-GGAATAA CATATGCTTGCCCGT-3′ and 5′-TGCACCACCTCTTGTA GATGAG-3′, respectively. PCR was performed using commercially available PCR premix (Bioneer, South Korea) according to the manufacturer's protocol. In each 0.20-mL PCR tube, 1 µL of genomic DNA (~100 ng/mL), 1 µL of each primer (10 µmol/L), and 17 µL ddH_2O were added. The PCR cycling conditions were as follows: initial denaturation for 6 min at 95 °C followed by 35 cycles of 40 s at 95 °C, 58 °C for 30 s, 72 °C for 30 s, and final extension at 72 °C for 10 min. Ten microliters of PCR products were digested with the HhaI restriction enzyme (Fermentas, Vilnius, Lithuania). The A allele was undigested (305 bp), while the G allele was digested and produced fragments of 259 and 46 bp (Figure 1).

Statistical Analysis

Statistical analysis was conducted using the SPSS 20.0 software (SPSS, Inc., Chicago, IL, USA). Data were analyzed by χ^2-test or independent sample *t*-test for data fitting. The associations between genotypes and PTB were estimated by computing the odds ratio (OR) and 95% confidence intervals (95% CI) from unconditional logistic regression analyses. *P*-values <0.05 were considered statistically significant. Sample power was calculated by using STATA 10 software (StataCorp, College Station, TX, USA) and is shown in Table 1.

RESULTS

A total of 412 subjects including 208 confirmed PTB patients (85 males, 123 females; aged 51.16±20.08 years) and 204 unrelated healthy subjects (88 males, 116 females; aged 49.28±15.10 years) were evaluated. There was no statistically significant difference among the groups regarding sex and age (*P*=0.690, *P*=0.284). Genotypes and allele

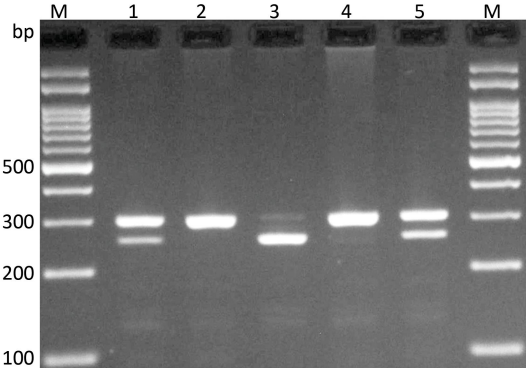

Figure 1. Photograph of rs4331426 A>G polymorphism using polymerase chain reaction restriction fragments length polymorphism (PCR-RFLP). G allele was digested by HhaI restriction enzyme and produced 259- and 46-bp fragments, while the A allele remained undigested (305-bp). M: DNA marker; lanes 1 and 5: AG; lanes 2 and 4: AA; lane 3: GG.

frequencies of the rs4331426 A>G polymorphism are shown in Table 1. The findings showed that neither the genotype nor the allelic frequencies of the rs4331426 variant showed statistically significant differences between the PTB group and controls. This variant was not associated with the risk/protection of PTB.

Moreover, the possible association between the rs4331426 variant and PTB in males and females was evaluated. As shown in Table 2, no significant association between rs4331426 and PTB in males or females was observed.

We estimated the Hardy-Weinberg equilibrium separately for cases and controls. The rs4331426 genotypes in cases and controls were in Hardy-Weinberg equilibrium (χ^2=0.092, *P*=0.761 and χ^2=0.099, *P*=0.752, respectively).

DISCUSSION

In the present study, we investigated the possible association between rs4331426 A>G at the Chr18q11.2 locus and risk of PTB in an Iranian population. Our findings revealed that this variant was not associated with PTB in our population. Stratification by sex revealed no significant association between the rs4331426 variant and PTB risk in males or females.

Table 1. Genotype and Allele Frequencies of rs4331426 A>G Polymorphism in Pulmonary Tuberculosis (PTB) and Healthy Subjects

rs4331426 A>G	PTB *n* (%)	Control *n* (%)	OR (95% CI)	*P*	Study Power (%)
Genotype					
AA	173 (83.1)	172 (84.3)	1	-	3.7
AG	33 (15.9)	31 (15.2)	1.06 (0.62-1.81)	0.892	2.9
GG	2 (1.0)	1 (0.5)	1.99 (0.18-22.15)	0.945	2.6
AG+GG	35 (16.9)	32 (15.7)	1.09 (0.64-1.84)	0.79	3.9
Allele					
A	379 (91.1)	375 (91.9)	1	-	4.8
G	37 (8.9)	33 (8.1)	1.11 (0.68-1.81)	0.709	4.8

Table 2. Genotype and Allelic Frequencies of rs4331426 A>G Polymorphism in Male and Female Pulmonary Tuberculosis (PTB) Patients and healthy Subjects

rs4331426 A>G	PTB *n* (%)	Control *n* (%)	OR (95% CI)	*P*
Male				
AA	65 (76.5)	75 (85.2)	1	-
AG	18 (21.1)	13 (14.8)	1.20 (0.73-3.51)	0.321
GG	2 (2.4)	0 (0.0)	5.76 (0.27-122.30)	0.221
AG+GG	20 (23.5)	13 (14.8)	1.77 (0.82-3.85)	0.176
Allele				
A	148 (87.0)	163 (92.6)	1	-
G	22 (13.0)	13 (7.4)	1.86 (0.91-3.83)	0.108
Female				
AA	108 (87.8)	97 (83.6)	1	-
AG	15 (12.2)	18 (15.5)	0.75 (0.36-1.57)	0.459
GG	0 (0.0)	1 (0.9)	0.30 (0.01-7.44)	0.476
AG+GG	15 (12.0)	19 (16.4)	0.71 (0.34-1.47)	0.709
Allele				
A	231 (93.9)	212 (91.4)	1	-
G	15 (6.1)	20 (8.6)	0.69 (0.34-1.38)	0.297

In a GWAS study, Thye et al.[15] identified a susceptibility locus of rs4331426 on chromosome 18q11.2 that increased the risk of TB (OR=1.19, 95% CI=1.13-1.27, P=6.8×10^{-9}, G allele *vs.* A allele) in an African population. Lee et al.[22] reported that the rs4331426 variant was associated with the susceptibility to TB in a female Han Chinese population. The AG genotype increased the risk of TB (OR=4.34, 95% CI=1.30-14.52, P=0.011) compared to the AA genotype. Ji et al.[18] and Dai et al.[19] found no significant association between the rs4331426 variant and risk of PTB in Chinese populations. However, Wang et al.[17] found that the AG+GG genotype significantly decreased the risk of TB in a Han Chinese population (OR=0.64, 95% CI=0.45-0.93, P=0.014) compared to the AA genotype. Furthermore, the G allele decreased the risk of TB (OR=0.62, 95% CI=0.44-0.87, P=0.006). Chen et al.[23] found no association between the rs4331426 polymorphism and risk of TB in a Han Chinese population.

Recently, a meta-analysis performed by Miao et al.[24] revealed that the rs4331426 variant may not contribute to TB susceptibility in Chinese people. Xue et al.[25] performed a meta-analysis and found that the rs4331426 polymorphism was associated with an increased risk of TB, particularly in an African subgroup. However, their findings revealed no significant association between the rs4331426 variant and risk of TB in an Asian subgroup.

The discrepancies between these studies may be related to genetic and environmental differences between the populations investigated.

In summary, our results do not support an association between rs4331426 A>G in the Chr18q11.2 locus and the risk of PTB in an Iranian population. Further studies of this variant should include larger sample sizes and different ethnicities to determine its effect on TB risk.

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Western Blotting

All authors have no conflict of interests to declare.

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