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Original Article

Lack of Association of SP110 Gene Polymorphisms with Pulmonary Tuberculosis in Golestan Province, Iran

Semira Kheiri (MSc)
Department of Medical Biotechnology, Faculty of Advanced Medical Technologies, Golestan University of Medical Science, Gorgan, Iran

Zohreh nematollahi(MSc)
Metabolic Disorders Research Center, Golestan University of Medical Science, Gorgan, Iran

Naghmeh Gholipour (PhD)
National Institute of Genetic Engineering and Biotechnology, Tehran, Iran

Jahanbakhsh Asadi (PhD)
Metabolic Disorders Research Center, Golestan University of Medical Science, Gorgan, Iran

Corresponding author: Jahanbakhsh Asadi

Email: Ja_asadi52@yahoo.com

Tel: +989112239011

Address: Golestan University of Medical Science, Gorgan, Iran

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ABSTRACT

Background and Objectives: *Mycobacterium tuberculosis* is the causative agent of pulmonary tuberculosis, a main public health problem that results in 1.5 million deaths annually. A number of epidemiological studies suggested that host genetic factors could play a main role in susceptibility to tuberculosis infection.

SP110 is an interferon-induced nuclear body protein with vital roles in apoptosis, cell cycling and immunity. *SP110* gene has been suggested to be a suitable candidate for limiting TB infections. Thus, we investigated the possible association between *SP110* gene polymorphisms and susceptibility to tuberculosis in the Golestan Province, Iran.

Methods: We investigated the frequency of rs1135791 polymorphism of the *SP110* gene among 100 pulmonary tuberculosis patients and 100 healthy individuals who were referred to the health centers in the Golestan Province (Iran) between 2014 and 2015. Frequency of genotypes was evaluated using amplification refractory mutation system-polymerase chain reaction.

Results: The frequency distribution of TT, TC and CC genotypes among the patients was 65%, 31% and 4%, respectively. In the control group, the frequency distribution of TT, TC and CC genotypes was 56%, 46% and 7%, respectively. There was no significant difference in the frequency of rs1135791 between the patients with pulmonary tuberculosis and the healthy controls (P=0.42).

Conclusion: Based on the results, the SP110 rs1135791 variant is not a genetic risk factor for development of pulmonary tuberculosis in Golestan Province, Iran.

Keywords: rs1135791T, Pulmonary tuberculosis, Golestan Province.

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INTRODUCTION

Tuberculosis (TB) remains a major global health problem and is the second leading cause of death from infectious diseases (1). Two-third of affected individuals are with latent TB infection, but the remaining one-third can spread active TB infections, which ultimately results in 1.5 million deaths annually (2). Family-based studies have provided abundant evidence on the role of genetics in TB susceptibility, and encouraged further host genetic studies to reveal mechanism of TB susceptibility and pathogenesis (3).

Various TB studies on animal models have identified a number of suitable candidate genes for genetic association studies in humans (4). Recently, the intracellular pathogen resistance-1 (*IPR1*) gene located on the super-susceptibility to tuberculosis 1 (sst1) locus has been shown to contribute fundamentally to innate immunity against *Mycobacterium tuberculosis* infection in a murine model (5). *SP110* (51 037 bp on 2q37.1; MIM 604457) is the human homologue of *IPR1* and the role of its variants has been implicated in a number of diseases including TB (7). Results of the studies on *SP110* gene in the individuals with TB have been contradictory. Two single nucleotide polymorphisms (SNPs) of this gene (rs3948464 and rs2114592) were found to be associated with TB in the West African population (8), whereas rs1427294 and rs1135791 have been reported to be significantly associated with TB in India (9) and China (10), respectively. However, similar studies in other populations have not found such association (7). In addition, a meta-analysis of five SNPs could not find a consistent association with TB (11).

Based on the heterogeneity of findings and the lack of enough studies on the Asian population, this study aimed to evaluate the relationship between variation in the *SP110* gene and TB in Golestan Province, Iran.

MATERIAL AND METHODS

The samples were selected from individuals who were referred to the health centers in the Golestan Province (Iran) between 2014 and 2015. This case-control study was performed on 100 pulmonary TB patients (57 males, mean age: 20 years) and 100 healthy controls (50 males, mean age: 25 years). Diagnosis was made based on the standard criteria (radiological findings, positive acid-fast bacilli sputum smear and culture, and response to anti-TB drugs). All study procedures were approved by the Institutional Review Board of Golestan University of Medical Science. Written informed consent was obtained from all participants.

Genomic DNA was isolated from the whole blood samples using commercial DNA isolation kits (Pajouhan Co., Iran). DNA concentration was adjusted to 100 ng/µl by adding deionized water. DNA purity was evaluated by Picodrop. The DNA samples were stored at -20 ^{o}C until analysis.

For genotyping of rs1135791, we used amplification refractory mutation system-polymerase chain (ARMS-PCR) with a forward and two reverse primers specific for the wild type and mutant variants. Two PCR reactions were performed for each sample with a normal ARMS amplification primer and the mutant ARMS primer, and the presence or absence of a PCR product indicated the presence or absence of the target allele.

ARMS-PCR primers correspond to wild-type sequence were as follows: forward consensus primer (5' GGGTGAATTCACAGAGGATGGGG3'); wild type reverse primer (5' TTCAGCAGCTCTCCTAGGGTCA3'). For amplification of the mutant, the wild-type reverse primer was replaced with a mutant reverse primer (5'TTCAGCAGCTCTCCTAGGGTCG3'). All primers were purchased from SinaClon BioSciences, Iran.

The PCR solution (25 µl) contained 100 ng of genomic DNA, 10× PCR buffer, 10 pmol of each primer, 10 nmol of dNTP, 1.5 mmol Mg^{2+} and 1U Taq polymerase.

The cycling conditions were as follows: initial denaturation at 95 °C for 5 minutes, 30 cycles of denaturation at 95 °C for 30 seconds, annealing at 62 °C for 30 seconds, extension at 72 °C for 20 seconds and final extension at 72 °C for 5 minutes. Later, 10 µl from the PCR products was pipetted onto 2.5% agarose gel containing 0.1 Ag/ml of ethidium bromide (Gibco BRL) for gel electrophoresis and visualization of the amplified fragments.

Statistical analysis was performed using MedCalc software (Version 12.1.4.0). P-values less than 0.05 were considered statistically significant.

RESULTS

The patients and healthy controls were separated on the basis of three possible genotypes and the presence or absence of 195 bp PCR products. In the gel electrophoresis, the samples with the TT genotype had a single band for the primer R1 (reverse oligonucleotide primer for amplification of allele T). Samples with the heterozygous genotype (TC) had two bands for primers 1R and 2R. The samples with the CC genotype had a single band for primer 2R (reverse oligonucleotide primer for amplification of allele C). We were able to detect both alleles (T and C) among the patients and control participants.

Odds ratio (OR) was calculated to assess the association between the alleles and TB infection. There was no significant difference in the frequency distribution of the alleles between the patients and the controls (Table 1). Among 100 patients, the frequency distribution of TT, TC and CC genotypes was 65%, 31% and 4%, respectively. In the control group, the frequency distribution of TT, TC and CC genotypes was 57%, 36% and 7%, respectively.

Figure 1. Detection of the rs1135791 mutation by ARMS-PCR. L: 50 bp marker, A1 and A2: normal homozygote, B1, B2, D1, and D2: heterozygous, C1 and C2: mutant homozygote

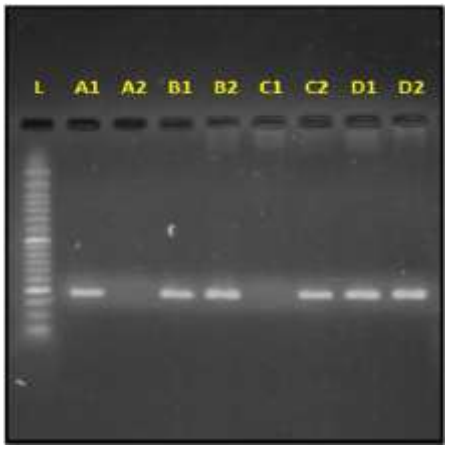

Table 1- Allele frequency distribution of the *SP110* SNPs

Gene	Alleles	Patients(%)	Controls (%)	OR	95% CI	P	X^2
SP110	T	161 (80.5%)	150 (75%)	1.0	Reference	0.2293	1.445
SP110	C	39 (19.5%)	50 (25%)	1.376	0.857-2.211		

DISCUSSION

TB caused by *M. tuberculosis* is a common and deadly infectious disease that primarily affects the lungs but can also spread to other organs, such as the kidneys and the brain. TB is more prevalent in families with a previous history of the disease, and various studies have investigated the role of host genetics in TB susceptibility. Animal studies have shown that the absence of a particular gene can lead to development of the severe form of TB. Several genetic factors have been shown to be involved in the pathogenesis of TB. Hence, studying the genetic basis of TB in different populations can provide valuable information that could be useful for treatment and prevention of the disease. One of the candidate genes for susceptibility to TB is *SP110*, which is located on human chromosome 2q37.1. The gene is a member of the Sp100/Sp140 family and functions as a transcriptional activator and a nuclear hormone receptor co-activator (6). The main cytokine involved in response to viral infections is IFN (12) which itself induces SP110, indicating the role of SP110 in

the IFN response (13). According to previous reports, controlling SP110 could be effective in controlling intracellular infections such as TB. A study on the West African population was the first to report the association of *SP110* SNPs with pulmonary TB (8). However, results of our study and some previous studies in Indonesia, South and West Africa, Russia and India could not demonstrate a relationship between *SP110* SNPs and susceptibility to TB (14-17). However, a study on the Chinese population found an association between rs11556887 and rs1135791 and TB susceptibility (10). As mentioned earlier, we could not find a direct relationship between *SP110* SNPs and TB among Iranians living in the Golestan Province. Additional studies on the genetic basis of TB in different populations could contribute to the improvement of current prevention and treatment methods.

CONCLUSION

Based on the results, the SP110 rs1135791 variant is not a genetic risk factor for development of pulmonary TB in the Golestan Province, Iran.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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