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Association of genetic polymorphisms of CISH with the risk of pulmonary tuberculosis in Zahedan, Southeast Iran

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ABSTRACT

Background: In the current study we aimed to find out the impact of cytokine-inducible Src homology 2 domain protein (CISH) gene polymorphisms on the risk of pulmonary tuberculosis (PTB) in a sample of Iranian population.

Materials and methods: Polymorphisms of *CISH* rs2239751, rs414171, and rs6768300 were determined in 200 PTB patients and 200 healthy subjects using T-ARMS-PCR or PCR-RFLP method.

Results: The results showed that rs414171 A>T genotypes significantly decreased the risk of PTB (OR = 0.16, 95% CI = 0.10–0.27, $p < 0.0001$, AT vs AA; OR = 0.31, 95% CI = 0.14–0.68, $p < 0.0001$, TT vs AA; OR = 0.19, 95% CI = 0.12–0.29, $p < 0.0001$, AT+TT vs AA; OR = 0.29, 95%CI = 0.20–0.42, $p < 0.0001$, T vs A). For rs6768300, the findings indicated that this variant decreased the risk of PTB (OR = 0.52, 95% CI = 0.33–0.82, $p = 0.005$, CG vs GG; OR = 0.57, 95% CI = 0.38–0.87, $p = 0.012$, C vs G). No significant association was observed between *CISH* rs2239751 polymorphism and risk/protection of PTB.

Conclusion: Our findings indicated that *CISH* rs414171 and rs6768300 variants might be associated with protection from PTB.

Introduction

Tuberculosis (TB), an infectious disease, is the leading cause of global morbidity and mortality mainly in Asia and Africa.[1,2] Roughly one-third of the world's population is infected with TB; however, approximately 5–15% of those infected individuals will develop active TB during their lifetime, typically within the first 2–5 years after the initial infection. It has been estimated that 9.6 million (5.4 million men, 3.2 million women and 1.0 million children) new cases occurred in 2014 according to the global tuberculosis report from World Health Organization (WHO).[3] Though environmental and social factors also contribute to susceptibility to TB, there is

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considerable evidence that host genetic factors play a key role in susceptibility to the disease.[4–6] In humans, the cytokine-inducible Src homology 2-containing protein is encoded by the *CISH* gene, which is located on the short arm of chromosome 3 (3p21.3).[7] It contains four exons, of which exons 2–4 encode the CISH protein which is an important negative regulator for inflammatory cytokine signaling.[7,8] It has been proposed that the interleukin-2 (IL-2)-mediated immune response is essential for host defense against infectious pathogens and CISH play a key role in controlling IL-2 signaling.[9]

Few studies have investigated the impact of *CISH* polymorphisms on TB and found an association between the variants and the risk of TB.[10–12] To the best of our knowledge, there is no report regarding the possible association between *CISH* gene polymorphisms and the risk of PTB in the Iranian population. Thus, the present study aimed to examine the possible associations between rs2239751, rs414171 and rs6768300 polymorphisms of *CISH* gene and susceptibility to PTB in a sample of the Iranian population.

Materials and methods

Study population

Two hundred patients diagnosed with PTB and 200 healthy subjects were enrolled in this case-control study. The study design and enrollment process have been described previously.[13,14] The cases were selected from PTB patients admitted to a university-affiliated hospital (Bou-Ali Hospital, Zahedan, referral center for TB). Briefly, diagnosis of PTB was based on clinical symptoms, radiological evidence, and bacteriological investigations such as sputum Acid Fast Bacilli (AFB) smear positivity, culture, and response to anti-tuberculosis chemotherapy. All control subjects were unrelated adults selected through the population without signs, symptoms or history of TB and from the same geographical origin, as the patients with PTB.

The local ethics committee of the Zahedan University of Medical Sciences approved the project, and informed consent was obtained from all individual participants included in the study. Genomic DNA was extracted from the whole blood by the salting out method as described previously.[15]

Genotyping

In this study we designed a tetra-primer amplification refractory mutation system polymerase chain reaction (T-ARMS PCR) for detection of CISH rs2239751 and rs414171polymorphism.

For rs2239751 we used two external primers (FO: GGGAAGACTACTTCTCCCTTGCTGTCT, RO: GCTGATGTGGTAGCTGGGTGTATGAATA) and two internal primers (FI (C allele)): GAACAAAGTTTTAGACTGCTGCGCTCTAC, RI (A allele): TTCTAGGTACATGTGTGTGCCCGTTT.

PCR was performed in 25 μl reaction volumes containing 0.4 μM of each primer, 250 μM of each dNTP, 1 U Taq DNA polymerase with 1.5 mM $MgCl_2$, and 50 ng genomic DNA. The PCR cycling conditions was as follows: an initial denaturation step of 5 min at 95 °C followed by 30 cycles of 30 s at 95 °C, annealing at 62 °C for 30 s and extension at 72 °C for 30 s. Final extension was performed at 72 °C for 5 min. The PCR products were separated by electrophoresis in 2% agarose gels, and observed under ultraviolet light. Product sizes were C allele 180-bp, A allele 251-bp, and control 376-bp, as shown in Fig. 1.

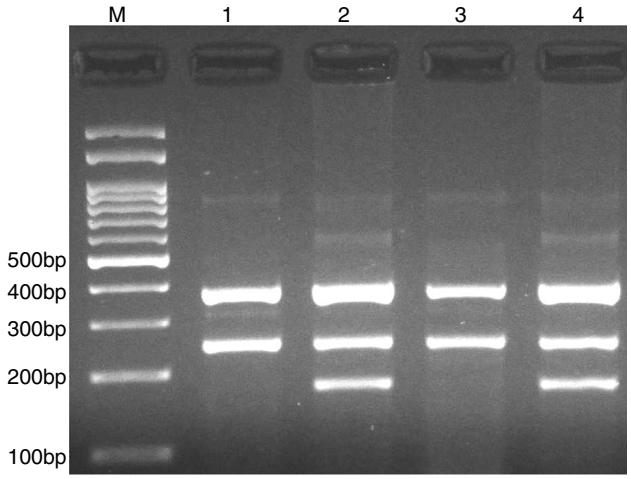

Fig. 1 – Photograph of tetra amplification refractory mutation system-polymerase chain reaction (T-ARMS-PCR) for detection of *CISH* rs2239751 A>C polymorphism. Product sizes were 180-bp for C allele, 251-bp for A allele, and 376-bp for control. M, DNA Marker; Lanes 1 and 3, AA; Lanes 2 and 4, AC.

For rs414171 we used two external primers FO: ACGCCGACAGACCTCCTTGGAGGAG, RO: GAAGCAGCGTCTTCCTAGAACCGCGG, and two internal primers FI (T allele): TGCTATTGGCCCTCCCCGACCACT, RI (A allele): CGCGACGCTGAAGGTGGAGCTGT.

PCR was performed in 25 μl reaction volumes containing 0.4 μM of each primer, 250 μM of each dNTP, 1 U Taq DNA polymerase with 1.5 mM $MgCl_2$, and 50 ng genomic DNA. The PCR cycling conditions was as follows: an initial denaturation step of 5 min at 95 °C followed by 30 cycles of 30 s at 95 °C, annealing at 68 °C for 30 s and extension at 72 °C for 30 s. Final extension was performed at 72 °C for 5 min. The PCR products were separated by electrophoresis in 2% agarose gels, and observed under ultraviolet light. Product sizes were A allele 290-bp, T allele 208-bp and control 452-bp, as shown in Fig. 2.

Detection of *CISH* rs6768300 polymorphism was done by PCR restriction fragment length polymorphism (PCR-RFLP) method. The set of forward and reverse primers were: 5′-GCGAGCTGCTGCCTAATC-3′ and 5′-GCTCGGCTCCACCTTCAG-3′, respectively.

PCR was performed in 25 μl reaction volumes containing 0.4 μM of each primer, 250 μM of each dNTP, 1 U Taq DNA polymerase with 1.5 mM $MgCl_2$, and 50 ng genomic DNA. The PCR cycling conditions was as follows: an initial denaturation step of 5 min at 95 °C followed by 30 cycles of 30 s at 95 °C, annealing at 58 °C for 30 s and extension at 72 °C for 30 s. Final extension was performed at 72 °C for 5 min. PCR products were digested with HhaI restriction enzyme.

The PCR products were separated by electrophoresis in 2% agarose gels, and observed under ultraviolet light. G allele