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ORIGINAL ARTICLE

CD209 promoter −336 A/G (rs4804803) polymorphism is associated with susceptibility to pulmonary tuberculosis in Zahedan, southeast Iran

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Introduction: The association between −336 A/G polymorphism of *CD209* and susceptibility to/protection from tuberculosis is inconsistent.
Aim: The present study aimed at evaluating the possible association between *CD209* rs4804803 (−336 A/G) gene polymorphism and pulmonary tuberculosis (PTB) in a sample of Iranian population.
Materials and methods: This case–control study was performed on 156 PTB patients and 154 healthy individuals. Tetra-amplification refractory mutation system-polymerase chain reaction was used to detect the polymorphisms.
Results: Our findings revealed that the *CD209* rs4804803 increased the risk of PTB in codominant [odds ratio (OR) = 5.16, 95% confidence interval (CI) = 1.60–16.59, $p = 0.006$, GG vs. AA], dominant (OR = 1.69, 95% CI = 1.07–2.66, $p = 0.024$, AG + GG vs. AA), and recessive (OR = 4.20, 95% CI = 1.34–13.16, $p = 0.014$, GG vs. AA + AG) tested inheritance models. Furthermore, the

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rs4804803 G allele increased the risk of PTB (OR = 1.58, 95% CI = 1.12–2.23, $p = 0.011$) as compared to the A allele.
Conclusion: Our data suggest that *CD209* rs4804803 polymorphism increased the risk of PTB in a sample of Iranian population.

Introduction

Tuberculosis (TB) is a global public health problem and remains a major cause of death worldwide, especially in Asia and Africa. One-third of the world's population is infected with *Mycobacterium tuberculosis*. However, only approximately 5–10% of those infected develop the active disease in their lifetime.[1] TB is a highly complex disease, and the reason why some infected individuals develop active disease, while others do not is not yet understood completely. Although pathogens and environmental factors are supposed to contribute to TB, increasing evidence suggests that host genetic factors play a significant role in TB susceptibility.[2–4] Genetic studies on TB have shown that the genetic polymorphisms, potentially involved in the immune response to TB, may lead to susceptibility to or protection from TB. The *DC-SIGN* gene is located on chromosome 19p13.2–3. DC-SIGN might be a key part of the host immunity to TB and one of the candidate genes for susceptibility to TB. DC-SIGN is mainly expressed on dendritic cells (DCs) and alveolar macrophages. DCs are professional antigen presenting cells playing a crucial role in connecting innate and adaptive immunity.[5] DC-SIGN acts as a major receptor for *M. tuberculosis* in human DCs and plays an important first-line role in host defense against pathogens, by internalization and presentation of the bacterium.[6] In mature DCs, DC-SIGN promotes the activation and proliferation of resting T cells and increases primary immune responses.[7]

Although, possible genetic associations between TB and −366 A/G polymorphism of *CD209* have been investigated in previous studies, the findings are contradictory.[8–12] Genetic risk factors for TB may differ among different populations. Subsequently, previously reported genetic associations in other populations should be investigated repeatedly to determine the associations of the genetic risk in each population. Although, the associations between *CD209* −336A/G polymorphism and the risk of TB have been studied, the findings are controversial.[9,10,13–15] To the best of our knowledge, there is no report regarding the impact of this polymorphism on susceptibility to TB in Iranian population. Therefore, the present study was conducted to find out the possible association between −366 A/G polymorphism of *CD209* and the risk of/protection from pulmonary tuberculosis (PTB) in a sample of Iranian population.

Materials and methods

This case–control study was conducted in 156 patients with PTB and 154 healthy individuals, in the Research Center for Infectious Diseases and Tropical Medicine, Bou-Ali Hospital, Zahedan, Iran. The local ethics committee of the Zahedan University of Medical Sciences approved the project, and written informed consent was obtained from all participants. All control individuals were from the same geographical origin and living in the same region as the patients with PTB (Zahedan, southeast Iran).

The diagnosis of PTB was based on clinical symptoms, radiological evidence, and bacteriological investigations such as sputum acid-fast bacillus smear positivity, culture, and response to anti-TB chemotherapy, as described previously.[16,17] Whole-blood samples were collected in sodium-ethylenediaminetetraacetic acid (Na-EDTA) tubes from all participants and genomic DNA was extracted as described previously.[18]

The *CD209* genomic sequence (NT_077812.2) was obtained from the National Center for Biotechnology Information (NCBI). The rs4804803 polymorphism was searched, and primers for tetra-primer amplification refractory mutation system-polymerase chain reaction (T-ARMS-PCR) were designed (Table 1). This method is a rapid and simple technique for detection of single-nucleotide polymorphism.[19–21] In T-ARMS-PCR method, four primers (two external primers and two allele-specific internal primers) were used. Product sizes were 197 bp for the G allele, 292 bp for the A allele, and 442 bp for the control band.

PCR was performed using a PCR premix (AccuPower PCR PreMix, Bioneer, Daejeon, Korea). Into a 0.2-mL PCR tube containing the AccuPower PCR PreMix, 1 μL template DNA (~100 ng/μL), 1 μL of each primer (10 μM), and 15 μL DNase-free water were added. PCR was performed under the following conditions: 95°C for 5 minutes; 95°C for 30 seconds, 61°C for 25 seconds, 72°C for 30 seconds, 30 cycles; and 72°C for 10 minutes. The PCR products were electrophoresed on 2% agarose gels and photographed (Fig. 1). We regenotyped random samples to verify the accuracy of genotyping. No genotyping mistake was found.

Statistical analysis

The statistical analysis of the data was performed using the SPSS 18.0 software (SSPS Inc., Chicago, IL, USA). Demographics and biochemical parameters between the

Table 1 Primers used for the detection of single-nucleotide polymorphism of DC-SIGN rs4804803 polymorphism

Primers	Sequence (5′ to 3′)
Forward inner (G allele)	AGGAACTGGGGGTGCTACCTGACC
Reverse inner (A allele)	ACCCCCTCCACTAGGGCAAGGTTA
Forward outer	AAACTTGCAGTGCCTCCTCAGTTTCC
Reverse outer	AGATGGGCCGGATCTTTCAAGAATTT