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ORIGINAL ARTICLE

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

Mohammad Naderi [a], Mohammad Hashemi [b,c,*], Mohsen Taheri [d,e], Hossein Pesarakli [a], Ebrahim Eskandari-Nasab [c], Gholamreza Bahari [c]

[a] *Research Center for Infectious Diseases and Tropical Medicine, Zahedan University of Medical Sciences, Zahedan, Iran*
[b] *Cellular and Molecular Research Center, Zahedan University of Medical Sciences, Zahedan, Iran*
[c] *Department of Clinical Biochemistry, School of Medicine, Zahedan University of Medical Sciences, Zahedan, Iran*
[d] *Research Center of Genetics in Non-contagious Diseases, Zahedan University of Medical Sciences, Zahedan, Iran*
[e] *Department of Genetics, School of Medicine, Zahedan University of Medical Sciences, Zahedan, 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

* Corresponding author. Department of Clinical Biochemistry, School of Medicine, Zahedan University of Medical Sciences, Zahedan 98167-43463, Iran.
E-mail addresses: mhd.hashemi@gmail.com, hashemim@zaums.ac.ir (M. Hashemi).

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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

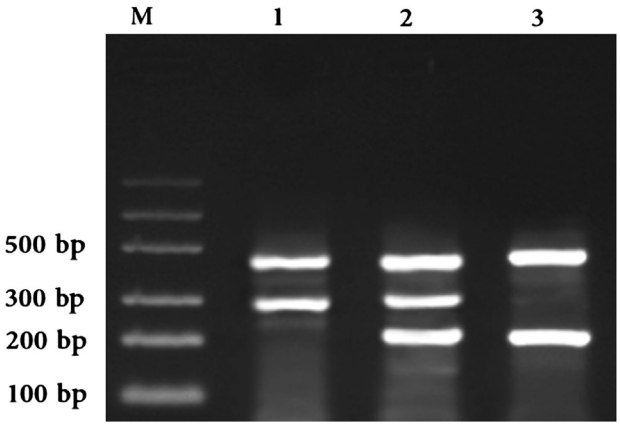

Figure 1. Electrophoresis pattern of T-ARMS-PCR for detection of −366 A/G (rs4804803) polymorphism of *DC-SIGN*. Lane 1 = GG; Lane 2 = AG; Lane 3 = AA; M = DNA marker; T-ARMS-PCR = tetra-amplification refractory mutation system-polymerase chain reaction.

groups were analyzed by independent sample *t* tests for continuous data and χ^2 test for categorical data. The associations between genotypes and PTB were estimated by computing the odds ratio (OR) and 95% confidence interval (95% CI) from logistic regression analyses. A *p* value of less than 0.05 was considered statistically significant.

Results

A total of 156 PTB patients (58 males and 98 females; aged 48.1 ± 15.6 years) and 154 healthy individuals (67 males and 87 females; aged 49.8 ± 20.7 years) were enrolled in the study. There was no significant difference between the groups regarding age and sex ($p = 0.403$ and $p = 0.297$, respectively). The genotype and allele frequencies of *CD209* rs4804803 polymorphism in PTB patients and control individuals are shown in Table 2. A significant difference was found between PTB patients and controls regarding *CD209* rs4804803 polymorphism ($\chi^2 = 9.21$, df = 2, $p = 0.010$).

Our findings showed that *CD209* rs4804803 polymorphism increased the risk of PTB in codominant (OR = 5.16, 95% 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 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.

Discussion

In the present study, we examined the impact of rs4804803 *DC-SIGN* polymorphism on the risk of PTB in a sample of Iranian population. Our findings showed that GG genotype as well as the G allele increased the risk of PTB in our population. In agreement with our finding, Barreiro et al[8] reported that −336 A decreased the risk of developing TB in a cohort of individuals of South African colored origin. By contrast, Vannberg et al[10] observed that −336 G exhibited significant protection against pulmonary TB in sub-Saharan Africa. In contrast to our finding, Olesen et al,[11] in the north African city of Tunis, and Ben-Ali et al,[9] in the west African city of Guinea-Bissau, found no association between −336 A/G polymorphism and TB. Furthermore, Zheng et al[12] have found that −336A/G (rs4804803) polymorphism of DC-SIGN is not associated with susceptibility to TB in Chinese population. Kobayashi et al[22] have found no association between this variant and the risk of TB in Indonesian population. Gomez et al[23] reported that −336 A/G variant did not increase the risk of TB in non-African individuals from northwestern Colombia,.

During preparation of this manuscript, two meta-analyses were published. A meta-analysis of 10 studies (2598 TB patients and 2614 control individuals) performed by Miao et al[13] found no association between *CD209* −336A/G polymorphism and the risk of TB (OR = 1.02, 95% CI = 0.90−1.15, for G vs. A allele). Another meta-analysis was carried out by Chang et al.,[15] based on the data extracted from 14 studies (3610 cases and 3539 controls). They observed that *CD209* promoter −336A/G

Table 2 Frequency distribution of DC-SIGN rs4804803 gene polymorphisms in PTB and healthy individuals (control)

Genotypes	PTB *n* (%)	Controls *n* (%)	OR (95% CI)	*p*	OR (95% CI) [a]	*p*
Codominant						
AA	61 (39.1)	79 (51.3)	1.00	—	1.00	—
AG	80 (51.3)	71 (46.1)	1.46 (0.92−2.32)	0.109	1.49 (0.94−2.39)	0.090
GG	15 (9.6)	4 (2.6)	4.86 (1.53−15.37)	0.007	5.16 (1.60−16.59)	0.006
Dominant						
AA	61 (39.1)	79 (51.3)	1.00	—	1.00	—
AG + GG	95 (60.9)	75 (48.7)	1.64 (1.06−2.58)	0.031	1.69 (1.07−2.66)	0.024
Recessive						
AA + AG	141 (91.4)	150 (97.4)	1.00	—	1.00	—
GG	15 (9.6)	4 (2.6)	3.99 (1.29−12.31)	0.016	4.20 (1.34−13.16)	0.014
Alleles						
A	202 (46.7)	229 (74.4)	1.00	—		
G	110 (53.3)	79 (25.6)	1.58 (1.12−2.23)	0.011		

[a] Adjusted for sex and age.
CI = confidence interval; OR = odds ratio; PTB = pulmonary tuberculosis.

polymorphism was not associated with TB susceptibility in all genetic models (OR = 1.04, 95% CI = 0.91−1.19 for G vs. A; OR = 1.13, 95% CI = 0.84−1.53 for GG vs. AA; OR = 1.04, 95% CI = 0.87−1.24 for GG + AG vs. AA; and OR = 1.11, 95% CI = 0.88−1.39 for GG vs. AG + AA). Analyzing the association between *CD209* −336A/G polymorphism and the risk of TB in different ethnicities, they found that the GG genotype increased the risk of TB in Asians.[15] Our result is in agreement with this finding.

Ogarkov et al[24] have found a correlation between infection with Beijing genotype and human *CD209* −336A/G polymorphism. The frequency of −336G allele was significantly lower among the patients affected with Beijing strains than among those infected with non-Beijing strains. In another study, they investigated a possible combination of *M. tuberculosis* lineage and human host allele/genotype correlating with adverse outcome of the disease.[14] They proposed that although carriers of the *CD209* −336A allele are more sensitive to infection with a Beijing strain, a combination of human *CD209* −336G allele and *M. tuberculosis* Beijing genotype leads more frequently to lethal outcomes in pulmonary TB male patients in Caucasian population.[14]

It has been proposed that −336A/G variant in the *CD209* (DC-SIGN) gene promoter region regulates promoter activity by affecting a Sp1-like binding site.[12]

Although the data indicated a discrepancy, both studies suggested an important role of −366 A/G polymorphism of *CD209* in the development of TB. There is no clear explanation for the different findings in different studies. Ethnic, genetic, and/or environmental factors may interact in various ways to either decrease or increase the susceptibility to TB in different regions.[25]

This is the first report regarding the association between −366 A/G polymorphism of C*D209* and PTB in Iranian population.

In conclusion, our results indicate that *CD209* rs4804803 polymorphism increased the risk of PTB in a sample of Iranian population. Larger studies are needed to validate our findings.

Conflicts of interest

All authors declare no conflicts of interest.

Acknowledgments

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References

1. Barry 3rd CE, Boshoff HI, Dartois V, et al. The spectrum of latent tuberculosis: rethinking the biology and intervention strategies. *Nat Rev Microbiol* 2009;**7**:845−55.
2. Stein CM. Genetic epidemiology of tuberculosis susceptibility: impact of study design. *PLOS Pathog* 2011;**7**:e1001189.
3. de Wit E, van der Merwe L, van Helden PD, Hoal EG. Gene−gene interaction between tuberculosis candidate genes in a South African population. *Mamm Genome* 2011;**22**:100−10.
4. Hashemi M, Sharifi-Mood B, Nezamdoost M, et al. Functional polymorphism of interferon-gamma (IFN-gamma) gene +874T/A polymorphism is associated with pulmonary tuberculosis in Zahedan, Southeast Iran. *Prague Med Rep* 2011;**112**:38−43.
5. Pathak SK, Skold AE, Mohanram V, Persson C, Johansson U, Spetz AL. Activated apoptotic cells induce dendritic cell maturation via engagement of Toll-like receptor 4 (TLR4), DC-SIGN and beta-2 integrins. *J Biol Chem* 2012;**287**:13731−42.
6. Tailleux L, Schwartz O, Herrmann JL, et al. DC-SIGN is the major *Mycobacterium tuberculosis* receptor on human dendritic cells. *J Exp Med* 2003;**197**:121−7.
7. Geijtenbeek TB, Torensma R, van Vliet SJ, et al. Identification of DC-SIGN, a novel dendritic cell-specific ICAM-3 receptor that supports primary immune responses. *Cell* 2000;**100**:575−85.
8. Barreiro LB, Neyrolles O, Babb CL, et al. Promoter variation in the DC-SIGN-encoding gene CD209 is associated with tuberculosis. *PLOS Med* 2006;**3**:e20.
9. Ben-Ali M, Barreiro LB, Chabbou A, et al. Promoter and neck region length variation of DC-SIGN is not associated with susceptibility to tuberculosis in Tunisian patients. *Hum Immunol* 2007;**68**:908−12.
10. Vannberg FO, Chapman SJ, Khor CC, et al. CD209 genetic polymorphism and tuberculosis disease. *PLOS One* 2008;**3**.e1388.
11. Olesen R, Wejse C, Velez DR, et al. DC-SIGN (CD209), pentraxin 3 and vitamin D receptor gene variants associate with pulmonary tuberculosis risk in West Africans. *Genes Immun* 2007;**8**:456−67.
12. Zheng R, Zhou Y, Qin L, et al. Relationship between polymorphism of DC-SIGN (CD209) gene and the susceptibility to pulmonary tuberculosis in an eastern Chinese population. *Hum Immunol* 2011;**72**:183−6.
13. Miao R, Li J, Sun Z, Li C, Xu F. Association between the CD209 promoter -336A/G polymorphism and susceptibility to tuberculosis: a meta-analysis. *Respirology* 2012;**17**:847−53.
14. Ogarkov O, Mokrousov I, Sinkov V, Zhdanova S, Antipina S, Savilov E. 'Lethal' combination of *Mycobacterium tuberculosis* Beijing genotype and human CD209 -336G allele in Russian male population. *Infect Genet Evol* 2012;**12**:732−6.
15. Chang K, Deng S, Lu W, et al. Association between CD209 -336A/G and -871A/G polymorphisms and susceptibility of tuberculosis: A Meta-Analysis. *PLOS One* 2012;**7**:e41519.
16. Naderi M, Hashemi M, Kouhpayeh H, Ahmadi R. The status of serum procalcitonin in pulmonary tuberculosis and non-tuberculosis pulmonary disease. *J Pak Med Assoc* 2009;**59**:647−8.
17. Naderi M, Hashemi M, Mehdizadeh A, et al. Serum adenosine deaminase activity and total antioxidant capacity of plasma in pulmonary tuberculosis and non-tuberculosis pulmonary disease. *Turk J Med Sci* 2010;**40**:701−6.
18. Hashemi M, Moazeni-Roodi AK, Fazaeli A, et al. Lack of association between paraoxonase-1 Q192R polymorphism and rheumatoid arthritis in southeast Iran. *Genet Mol Res* 2010;**9**:333−9.
19. Hashemi M, Moazeni-Roodi A, Bahari A, Taheri M. A tetra-primer amplification refractory mutation system-polymerase chain reaction for the detection of rs8099917 IL28B genotype. *Nucleosides Nucleotides Nucleic Acids* 2012;**31**:55−60.
20. Hashemi M, Hoseini H, Yaghmaei P, et al. Association of polymorphisms in glutamate-cysteine ligase catalytic subunit and microsomal triglyceride transfer protein genes with nonalcoholic fatty liver disease. *DNA Cell Biol* 2011;**30**:569−75.
21. Hahsemi M, Rezaei H, Eskandari Nasab E, Kaykhaei MA, Zakeri Z, Taheri M. Association between chemerin

rs17173608 and vaspin rs2236242 gene polymorphisms and the metabolic syndrome, a preliminary report. *Gene* 2012; **510**:113—7.
22. Kobayashi K, Yuliwulandari R, Yanai H, et al. Association of CD209 polymorphisms with tuberculosis in an Indonesian population. *Hum Immunol* 2011;**72**:741—5.
23. Gomez LM, Anaya JM, Sierra-Filardi E, Cadena J, Corbi A, Martin J. Analysis of DC-SIGN (CD209) functional variants in patients with tuberculosis. *Hum Immunol* 2006;**67**:808—11.
24. Ogarkov OB, Medvedeva TV, Nekipelov OM, Antipina SL, Men'shikov ML. [Study of DC-SIGN gene polymorphism in patients infected with *Mycobacterium tuberculosis* strains of different genotypes in the Irkutsk Region]. *Probl Tuberk Bolezn Legk* 2007:37—42. [In Russian].
25. Fares A. Racial differences in susceptibility to infection by *Mycobacterium tuberculosis*. *Ann Trop Med Public Health* 2012;**5**:307—12.

Web references

26. National Center for Biotechnology Information. The *CD209* genomic sequence (NT_077812.2). Available at: http://www.ncbi.nlm.org [accessed 10.11.2011].