PRINT: ISSN 0972-3757 ONLINE: 2456-6360
Int J Hum Genet, 9(3-4): 239-243 (2009)
DOI: 10.31901/24566330.2009/09.03-4.04

CD209 Promoter Single Nucleotide Polymorphism -336A/G and the Risk of Susceptibility to Tuberculosis Disease in the Moroccan Population

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KEYWORDS *CD209*. Genetic Variation. Single Nucleotide Polymorphism. Tuberculosis

ABSTRACT Tuberculosis (TB) remains a major cause of morbidity and mortality worldwide. *Mycobacterium tuberculosis* is the causal agent of this infection and its major receptor is CD209 which is expressed on human Dendritic Cells (DC). This interaction could influence bacterial persistence and immunity response. The aim of this study was to evaluate the functional polymorphism -336G/A SNP in TB susceptibility in the Moroccan population. We performed a case-control study within a cohort that included 122 pulmonary TB patients and 151 healthy controls. All subjects were genotyped by TaqMan SNP genotyping assays. No significant difference was observed in allele or genotype frequencies of -336G/A *CD209* between TB patients and healthy controls. Further studies are needed to confirm our finding including a large sample size.

INTRODUCTION

Tuberculosis (TB) is a major world cause of morbidity and mortality. Every year, some 8.8 million people develop active TB, with almost 1.6 million deaths in 2005, despite the availability of antibiotics (WHO 2007). The majority of cases arise in developing countries and the high incidence rate per capita is observed, in particular, in sub-Saharan Africa, including 195 000 patients infected with HIV (WHO 2007). Only 5 to 10% of immunocompetent humans are susceptible to TB and over 85% of them develop the disease exclusively in the lungs. The mechanisms underlying resistance against TB remain largely unknown. The important role of genetics in the development of TB is supported by twin studies in humans which report significant differences in the development of TB between monozygotic and dizygotic twins (Jepson et al. 2001; Comstock 1978). The genetic factors that contribute to susceptibility and development of pulmonary TB most likely engage an interaction between multiple alleles located on different genes and chromosomes (Hill 2006).

Among genes that have been reported strongly associated to TB susceptibility disease is *CD209*, located on chromosome 19p13.3 (Barreiro et al. 2006). This gene encodes for a molecule namely Dendritic Cell-Specific ICAM3-Grabbing Non-integrin (DCSIGN), a C-type lectin, which is expressed on subsets of dendritic cells (DCs) and alveolar macrophages (Tailleux et al. 2003a; Tailleux et al. 2005). Several ligands are reported to bind DC-SIGN as ICAM-2, ICAM-3 (intercellular adhesion molecule) etc (Geijtenbeek et al. 2000a; Geijtenbeek et al. 2000b). In addition, DC-SIGN has been shown to recognize others ligands that are expressed on surface of variety of pathogens including Cytomegalovirus (Halary et al. 2002), human and simian immunodeficiency viruses (Pohlmann et al. 2001), *Leishmania amastigotes* (Colmenares et al. 2002), *Aspergillus fumigatus*, (Serrano-Gomez et al. 2004) and *Heliobacter pylori* (Bergman et al. 2004). DC-SIGN has been reported to act as major *Mycobacterium tuberculosis* (Mtb) receptor on human dendritic cells (Tailleux et al. 2005). In deed, the authors of this study have been demonstrated that Mtb enters human monocyte-derived DCs after binding to DC-SIGN through interaction with

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mannosylated lipoarabomannan (ManLAM). DC-SIGN seems also to modulate cytokine production by influencing Th1/Th2 (T cell helper) balance (Geijtenbeek et al. 2003; Tailleux et al. 2003b). The interaction between dendritic cells and Mtb is very important for increasing a protective anti-mycobacterial immune response and for determining the outcome of infection (Henderson et al. 1997; Fortsch et al. 2000; Van Kooyk et al. 2003).

Although the role demonstrated by various studies concerning DC-SIGN molecule in the pathogenesis of TB disease there is few reports focusing in the genetic variation of *CD209* gene. In fact, a statistical significant association has been reported between *CD209* promoter single nucleotide polymorphism (SNP) 336A/G (rs4804803) and TB (Barreiro et al. 2006; Vannberg et al. 2008; Olesen et al. 2007). The aim of this study was to evaluate, for the first time in Moroccan population, the potential role of *CD209* gene polymorphism in the development of TB disease.

MATERIALS AND METHODS

Subjects: Venous blood samples were collected from 122 pulmonary TB patients **(**PTB**),** newly diagnosed, included 99 males and 23 females. The mean age (± Standard Deviation, SD) was 33.69 ± 13.18 years, ranging from 16 to 73 years. All patients were evaluated by microbiological diagnosis (microscopic examination and culture of sputum samples), medical history, physical examination and chest x-ray (Table 1). We selected only patients with both smear and culture positives. Nevertheless, none of the patients studied had extrapulmonary tuberculosis and none had other obvious infections such as hepatitis or human immunodeficiency virus or had serious medical illness. Additionally, a sample of 151 volunteers unrelated healthy controls matched by sex and age. The patients and the controls belonged to the same ethnic origin, socio-economic status. This study was approved by the local ethics committee (Direction d'Epidémiologie et de Lutte contre les Maladies Infectieuses) and informed written consent was obtained from each subjects of this study.

CD209 -336 Genotyping: Total genomic DNA was isolated from peripheral blood of all TB patients and healthy controls using QIAamp DNA Mini kit (Qiagen GmbH, Hilden, Germany). All samples were genotyped by TaqMan SNP Genotyping assays (Applied Biosystems, Foster City, CA, USA). Briefly, a promoter region containing -336 SNP G/A (rs4804803) was amplified using the subsequent primers: The primer sequences were 5'-GGACAGTGCTT CCAGGAACT-3' (sense) and 5'- TGTGTTA CACCCCCTCCACTAG-3' (antisense) and the TaqMan minor groove binder probe sequences were 5'-TACCTGCCTACCCTTG-3' and 5'-CTGCCCACCCTTG-3'; the probes were labeled with the fluorescent dyes VIC and fluorescein-Aminohexyl Amidite (FAM), respectively. The polymerase chain reactions (PCR) was carried out in total volume of 5µl and it was performed on an Applied Biosystems 7500 Fast Real Time PCR System. PCR reaction conditions and the measuring of allele-specific fluorescence protocol were same to those previously described (Gómez et al. 2006).

Statistical Analysis: Initially, each population (patients and controls) was tested for conformity to Hardy-Weinberg equilibrium. Allelic and genotypic frequencies were determined by direct counting and were compared between PTB patient and healthy control groups. Univariate analysis was performed with the chi-square test and Fisher's exact test. P value less than 0.05 was considered significant. The odds ratios (OR) and 95% confidence intervals (CI) were calculated to evaluate the risk between genotype or allele and TB. All statistical analysis was performed using Epi Info™ software Version 3.4 (Centers for Disease Control, Atlanta, GA, USA, October 17, 2007).

RESULTS

A total of 273 subjects (122 PTB patients and 151 healthy controls) were analyzed for A336G *CD209* gene variation. The distribution of the observed genotypes and alleles frequencies of *CD209* A336G SNP in PTB patients and unrelated healthy controls are given in Table2.

Both patients and healthy controls were in Hardy–Weinberg equilibrium with no significant chi-squared values for the observed and expected genotype frequencies of *CD209* gene tested polymorphisms. Our results show no significant difference in allelic or genotypic frequencies of -336G/A *CD209* between PTB patients and healthy controls (Table 2). But, the frequency of

Table 1. Distribution of tuberculosis patients according to their clinical and demographic characteristics.

	PTB patients N (%) 122	
Age (years)		
Mean (± SD)	33.69 (± 13.18)	
Gender		
Male	99	
Female	23	
Clinical Feature		
Haemoptysis	(42/122)	34.42%
Expectoration	(109/122)	88.52%
Fever	(117/122)	95.90%
Cough	(121/122)	99.19%
Abnormality revealed by chest x-ray examination.	(122/122)	100%

GA genotype was observed higher in healthy controls than PTB, (40.40% vs 30.33 %, respectively). In contrast, the AA homozygous genotype was observed more frequent in PTB patients than in healthy controls (62.29 % vs 50.99 %, respectively).

In deed, no significant association was found between -336G/A *CD209* variant neither with clinical features nor with demographic parameters (age and sex).

DISCUSSION

In the present report, we found no significant difference in allelic and genotypic frequencies between PTB patients and healthy controls (P>0.05). To our knowledge, this is the first study to analyze the *CD209* promoter polymorphism in a Moroccan population. Independent validations of association in different populations are essential in the study of the genetic susceptibility of complex diseases. In fact**,** Barreiro et al. (2006) has been reported a significant association between -336A allele and protection against TB in South African Cape Coloureds. In deed, in this study, the authors found a positive association between -336G variant and TB disease, suggesting a potential role to increase the risk of developing TB. Similarly data has recently been reported in Indian population when -366G/G genotype was positively associated with susceptibility to TB (Selvaraj et al. 2009). In contrast, opposite data has been observed in a large sample of individuals from sub-Saharan Africa, when significant protection against pulmonary TB was shown with -336G (Vannberg et al. 2008). the same authors has been also observed a significant decrease number of this variant allele in patients with cavitory tuberculosis disease than those without cavities (p = 0.000031) (Vannberg et al. 2008. Furthermore, it has been reported that this rare allele is associated with lower *CD209* expression in vitro than the ancestral -336A variant (Sakuntabhai et al. 2005). Theses findings observed in different population underly the presence of divergent mechanisms that are involved in the pathogenesis of TB disease.

The divergent results between our study and those reported in African sub-Saharan population could be due in one hand to the difference in the genetic background and in the other hand to the weak sample size of our study (122 vs 2176) (Vannberg et al. 2008). Furthermore, we reported that the -336G allelic frequencies differ widely in populations from Caucasians and African population (respectively, the means value observed were around 19.45% vs 42%), due most likely of geographically determined selection pressures (Boily-Larouche et al. 2007). In contrast, allelic frequency of -336G allele observed in our population was similar to that reported in Tunisian population (Ben-Ali et al. 2007) (respectively, 25.81% vs 25.9%). The -336G allelic frequencies observed in North African population (Moroccan and Tunisian) were most closely to those observed in Caucasians than in sub-Saharan

Table 2. *CD209* genotypes and allelic distributions in patients with pulmonary tuberculosis and in healthy controls.

	PTB Patients N= 122 (%)	*Healthy controls N=151 (%)*	*P value*	*OR (95% CI)*
GG	9 (7.38)	13 (8.61)	0.71	0.85 (0.32 - 2.21)
GA	37 (30.33)	61 (40.40)	0.08	0.64 (0.38 - 1.10)
AA	76 (62.29)	77 (50.99)	0.06	1.59 (0.95 - 2.66)
AA+GA	113 (92.62)	138 (91.39)	0.71	1.18 (0.45 – 3.13)
G	55 (22.54)	87 (28.81)	0.096	0.72 (0.48 - 1.08)
A	189 (77.46)	215 (71.19)	0.096	1.39 (0.92 - 2.09)

Abbreviations: CI= confidence interval; OR = odds ratio.

African population. This issue might have a clinical outcome in term of susceptibility, not only tuberculosis but also for other infectious diseases. The absence of a significant association in our study between TB and healthy controls corroborates with that reported on Tunisian population (Ben-Ali et al. 2007). The two populations, Tunisian and Moroccan, are in close geographic proximity to each other and sharing similarly history; this particularity could explain the same result reported here. Likewise, in our previous study, no significant association was found in the distribution of *CD209* -336 G/A alleles or genotypes in Colombian TB patients as compared with controls (Gómez et al. 2006).

Finally, the lack of association reported in the present study between *CD209* G/A SNP and PTB should be confirmed in a large population sample. Indeed, further studies are needed to explore other *CD209* functional polymorphisms.

ACKNOWLEDGEMENT

We thank all patients and healthy controls for their participation in this study. We are thankful also to all clinicians and nursing staff at different prefectures (Tanger, Larache Rabat, Salé, Skhirate-Temara, Casablanca, Settat, Safi) for their contributions in clinical examinations. This study was supported in part by grants SAF06-00398 and CTS1880, Spain.

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