

Lack of an Association between Interleukin-12 Receptor β 1 Polymorphisms and Tuberculosis in Koreans

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Key Words

Tuberculosis · Genetic susceptibility · Interleukin-12 receptor · Single nucleotide polymorphism · Koreans

Abstract

Background: The fact that only 10% of people infected with *Mycobacterium tuberculosis* develop clinical tuberculosis (TB) suggests the presence of genetic factors in the pathogenesis of TB. To date, a number of single nucleotide polymorphisms (SNPs) in several candidate genes have been proposed as genetic risk factors of TB; however, reports are conflicting. **Objectives:** We investigated whether SNPs in the interleukin (IL)-12 receptor β 1 gene are associated with TB in Koreans. **Methods:** One hundred and fifteen patients with bacteriologically or pathologically confirmed TB and 151 healthy anonymous blood donors were enrolled. The genotypes of 5 SNPs on IL-12 receptor β 1 gene, +705A/G (Q214R), +1158T/C (M365T), +1196G/C (G378R), +1637G/A (A525T) and +1664 C/T (P534S), were determined by PCR-RFLP. **Results:** No difference was observed between TB patients and controls in terms of the genotype frequencies of the 5 SNPs of the IL-12 receptor β 1 gene or of their haplotypes. **Conclusions:** In view of the finding that these

SNPs have been reported to be associated with TB in the Japanese and Moroccan populations, our results may reflect racial differences in genetic susceptibility to TB.

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Introduction

Tuberculosis (TB) is the second most common cause of death due to infectious disease in the world. An estimated 8.3 million new cases of TB occurred in the year 2000, and 1.8 million deaths from TB were reported in the same year [1]. Moreover, the incidence of HIV-associated TB continues to increase [2, 3], and TB caused 11% of all adult AIDS deaths in 1997 [1].

The fact that only 10% of people infected with *Mycobacterium tuberculosis* develop clinical TB [4] suggests that genetic factors play a role in the pathogenesis of TB. This hypothesis is further supported by the higher concordance rate of TB in monozygotic twins rather than in dizygotic twins [5]. In this context, several polymorphisms in genes associated with immune defense against *M. tuberculosis* have been tested. Polymorphisms in the following genes: NRAMP1 [6–10], vitamin D receptor [11, 12], interleukin (IL)-1 [13, 14], IL-10 [14] and TNF α [15] have

Table 1. Baseline characteristics of study subjects

Patients	115
Male/female	65/50
Age, years	
Median	36
Range	15–89
Presence of TB history in family ¹	36 (31.3)
Types of TB	
Pulmonary TB including pleuritis	79 (68.7)
Extrapulmonary TB ²	36 (31.3)

Figures in parentheses are percentages.

¹ Family including third-degree relatives.

² Pulmonary TB with other organ involvement was classified as an extrapulmonary TB.

been reported to be genetic factors involved in the development of clinical TB. Unfortunately, these associations have proven to be irreproducible in most cases.

IL-12-dependent interferon- γ production is crucial to the host's defense mechanism against mycobacteria [16, 17]. Moreover, polymorphisms in the coding sequence of the IL-12 receptor gene have been reported to be associated with tuberculosis in the Japanese and Moroccan populations [18, 19], but not with leprosy in Koreans [20]. In this study, we examined whether single nucleotide polymorphisms (SNPs) in IL-12 receptor β 1 gene are associated with TB in Koreans.

Materials and Methods

Patients and Controls

One hundred and fifteen patients with bacteriologically or pathologically confirmed TB were enrolled. Patients with a positive HIV test or being administered immunosuppressive agents were excluded. A control group comprised of 151 healthy anonymous blood donors was evaluated to determine the distribution of alleles and genotypes in a random control population. All enrollees were Korean. This study was approved by our institutional review board and written informed consent was obtained from all subjects, before blood sampling.

Genotyping Using PCR-RFLP

Three milliliters of peripheral blood was drawn from each subject into EDTA-containing tubes. DNA was extracted using a Puregene DNA Isolation Kit (Gentra Systems, Minneapolis, Minn., USA) following the manufacturer's protocol. We typed for 5 previously reported SNPs that cause missense mutations in the coding sequence of the IL-12 receptor β 1 gene in Koreans [20], namely +705A/G (Q214R, NCBI SNP ID:rs 11575934), +1158T/C (M365T, NCBI SNP ID:rs 375947), +1196G/C (G378R, NCBI

SNP ID:rs 401502), +1637G/A (A525T, NCBI SNP ID:rs 11575935) and +1664 C/T (P534S, HGBASE database ID: SNP001745641). For +705A/G genotyping, genomic DNA was amplified using the primer set 5'-ggttaagtgactgtgccaag-3' and 5'-ctcaaacactggcctaag-3'. The PCR fragment obtained was restricted with *Bbv*I (New England BioLabs, Beverly, Mass., US) at 37°C for 10 h. For +1158T/C typing (M365T), 5'-aacaaacgacctgtacc-3' and 5'-caaacctctctggccta-3', and *Hsp*92II (Promega, Madison, Wisc., USA) were used at 37°C. For +1196G/C, 5'-aacaaacgacctgtacc-3' and 5'-agagtgagagccacctgag-3', and *Msp*I (Promega, Madison, Wisc., USA) were used at 37°C. For +1637G/A and +1664 C/T, 5'-ggctgtgtagccagcct-3' and 5'-ggaagcgcagtcagtcag-3' were used and restricted with *Mae*II (Roche, Mannheim, Germany) at 50°C and *Bsr*I (New England BioLabs) at 65°C, respectively. PCR was carried out in PCR buffer (1.5 mM MgCl₂, 10 mM Tris-HCl, pH 9.0, 50 mM KCl, 0.1% Triton[®] X-100), 100 μ M of each dNTP, 25 μ M primers and 1 unit of *Taq* polymerase (Invitrogen, Carlsbad, Calif., USA) in a final volume of 50 μ l. Following an initial denaturation at 95°C for 5 min, samples were subjected to 30 cycles of denaturation at 95°C for 30 s, annealing at 57–60°C (according to the requirements of the primer set) for 30 s and elongation at 72°C for 30 s, followed by a final elongation step at 72°C for 10 min. The reaction products of the PCRs and enzyme restrictions were analyzed on 3% agarose gel (+705A/G, +1158T/C, +1637G/A, +1664 C/T) or 8% polyacrylamide gel (+1196G/C). All patient genotypes showing unrestricted fragments in PCR-RFLP were confirmed by sequencing.

Statistical Analysis

The associations between the various polymorphisms and TB were investigated using the two-tailed χ^2 test. Differences with *p* values of <0.05 were considered significant. Odds ratios and 95% confidence intervals were calculated to assess the relative disease risk conferred by the genotypes. Haplotypes were generated using Haploview (version 2.05), and SPSS (version 11.0) was used for all statistical analyses.

Results

Baseline Clinical Characteristics of the TB Patients

Table 1 shows the baseline characteristics of the 115 TB patients. Median age was 36 years and 65 of the 115 TB patients were male. Thirty-six (31.3%) patients had at least one family member with a history of TB. Seventy-nine (68.7%) patients had pulmonary TB including TB pleuritis and the other 36 (31.3%) had extrapulmonary TB.

Genotype Frequencies of the 5 SNPs in TB Patients and Controls

Of the 5 missense mutations, C1664T alone was not polymorphic (table 2). The genotype distributions of the 4 other SNPs were in Hardy-Weinberg equilibrium. No difference in genotype distributions was observed between patients and controls. The most common haplo-

type in both groups was A-T-G (705-1158-1196), and the proportions of this haplotype in the two groups were similar (69.6% in TB patients vs. 63.9% in controls, $p = 0.94$) (table 3).

Discussion

This study denies the proposed association between polymorphisms in IL-12 receptor $\beta 1$ gene and TB [18, 19] in the Korean population. We genotyped 5 SNPs that have been alleged to cause missense mutations, +705A/G (Q214R), +1158T/C (M365T), +1196G/C (G378R), +1637G/A (A525T) and +1664 C/T (P534S), in the IL-12 receptor $\beta 1$ gene using PCR-RFLP in TB patients and healthy controls, and found no association between these SNPs and TB in Koreans.

IL-12 promotes cell-mediated immunity to intracellular pathogens by inducing type-1 helper T cell responses and interferon- γ production by binding to high-affinity $\beta 1/\beta 2$ heterodimeric IL-12 receptor complexes on T cells and natural killer cells [21]. Moreover, mutations causing IL-12 receptor $\beta 1$ deficiencies have been reported to cause severe forms of disseminated infection by salmonella and mycobacteria [22–24]. The report that a missense mutation in IL-12 receptor $\beta 1$ caused abdominal TB in a child [25] stimulated researchers to establish a relationship between SNPs in the IL-12 receptor $\beta 1$ gene and TB. Although SNPs in the 3' UTR of the IL-12 receptor $\beta 1$ gene were reported not to be associated with TB [26], Akahoshi et al. [18] reported that SNPs in the coding sequence of the IL-12 receptor $\beta 1$ gene are associated with TB in the Japanese and Moroccan populations [19]. However, we failed to confirm this association in the Korean population.

In TB susceptibility association studies, conflicting results are not uncommon. For example, missense mutations of D543N in the NRMAP1 gene were proposed to be a genetic risk factor for TB in Gambia [6], and this was later confirmed in the Japanese [7]. However, this association was denied in New Guinea [8] and Taiwanese [10] populations. In addition, the association between the *TaqI* polymorphism in vitamin D binding receptor and TB proposed in the Gambian population [11] was not reproduced in Gujarati Hindus [12] or in the Cambodian people [27].

Lohmueller et al. [28] argued that a sizable fraction of reported associations are supported by strong evidence of replication and that observed inconsistencies are usually due to false-negative results produced by underpowered

Table 2. Allele frequencies of the 5 SNPs in TB patients and controls

	TB (n = 115)	Control (n = 151)	OR (95% CI)
A705G			
A	160 (69.6)	194 (64.2)	1.27 (0.88–1.83)
G	70 (30.4)	108 (35.8)	1
$p = 0.22$			
T1158C			
T	161 (70.0)	198 (65.6)	1.22 (0.84–1.77)
C	69 (30.0)	104 (34.4)	1
$p = 0.30$			
G1196C			
G	161 (70.0)	198 (65.6)	1.22 (0.84–1.77)
C	69 (30.0)	104 (34.4)	1
$p = 0.30$			
G1637A			
G	223 (97.0)	291 (96.3)	1.20 (0.45–3.15)
A	7 (3.0)	11 (3.7)	1
$p = 0.81$			
C1664T			
C	230 (100)	302 (100)	n.a.
T	0	0	n.a.
$p = 1.00$			

Figures in parentheses are percentages. OR = Odds ratio; CI = confidence interval; n.a. = nonapplicable.

Table 3. Haplotype frequencies in TB patients and controls

Haplotype	TB (n = 230)	Control (n = 302)
A-T-G	160 (69.6)	193 (63.9)
G-C-C	69 (30.0)	102 (33.8)
Others	1 (0.4)	7 (2.3)

Figures in parentheses are percentages.
 $p = 0.94$.

studies. However, the present study fulfills the suggested strategies for genetic association studies [29] and involves 115 TB patients and 151 controls, which is comparable to the 98 patients and 197 controls in the study of Akahoshi et al. [18]. Furthermore, two large-scale association studies involving more than 300 TB patients [11, 27] suggest a possible ethnicity-specific association among different racial groups as well. Thus, a failure to replicate associations between polymorphisms of IL-12 receptor $\beta 1$ and TB in Korean population might be understood in

this context. A meta-analysis of the various results available should elucidate the association between the IL-12 receptor β 1 polymorphism and TB.

In conclusion, we found no association between the polymorphisms of the IL-12 receptor β 1 gene and TB in Koreans. Given that an association was previously reported in the Japanese and Moroccan populations, these results may reflect racial differences with respect to TB susceptibility. A large-scale association study is needed to

more precisely elucidate the true association between polymorphisms of IL-12 receptor β 1 and TB in both populations.

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