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ORIGINAL ARTICLE

Interferon gamma polymorphisms associated with susceptibility to tuberculosis in a Han Taiwanese population

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Background: Polymorphisms of the interferon gamma (IFN-γ) gene are associated with the risk of tuberculosis (TB) in different populations. However, the genetic susceptibility to TB in Han Chinese living in Taiwan is still unknown. The purpose of this study is to evaluate whether the polymorphisms of the IFN-γ gene are associated with TB in Han Taiwanese.
Methods: A total of 200 TB patients and 202 age-matched non-TB individuals were enrolled. Five tag single nucleotide polymorphisms (tSNPs) and rs2430561 (+874) of IFN-γ were selected from a public database. The genotypes were determined using polymerase chain reaction assays.
Results: Three IFN-γ polymorphisms in intron 3, rs1861494 and rs2069718, and rs2430561 in interon 1 were strongly associated with TB. The C carrier (CT+TT) of rs1861494, TT homozygous of rs2069718, and AA homozygous of rs2430561 were risk genotypes for susceptibility to TB.
Conclusion: The IFN-γ polymorphisms, rs1861494, rs2069718, and rs2430561, may confer the risk of TB in Han Taiwanese.

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Introduction

Tuberculosis (TB) remains a major worldwide health concern and is characterized as one of three epidemics by the World Health Organization.[1] In 2006, more than 1.5 million people died of TB, an estimated 9.1 million new cases appeared, and the number of total TB cases worldwide reached about 14 million.[2] In Taiwan, TB is a major disease with an annual incidence of about 16,000 confirmed cases. The proportion of ethnic populations on the island is about 2% native aborigines and 98% Han Chinese (Council of Indigenous Peoples, Executive Yuan Taiwan, 2007). Previous studies in Taiwan have demonstrated a fivefold higher incidence of TB among aborigines compared to Han Chinese.[3] In addition, polymorphisms of the *NRAMP1* gene appear to be associated with susceptibility to TB among aborigines, but not among the Han Chinese population.[3] The genetic susceptibility to TB in Han Chinese living in Taiwan is still unknown.

Interferon gamma (IFN-γ) is a key T helper (Th) type 1 cytokine produced primarily by natural killer cells and T cells. Its production plays a pivotal role in macrophage activation for controlling *Mycobacterium tuberculosis* infection.[4] Mice with a disrupted IFN-γ gene, when challenged with *M. tuberculosis*, fail to produce reactive nitrogen intermediates that restrict the growth of the bacilli.[5] Humans with an inherited complete or partial IFN-γ receptor deficiency are highly susceptible to infection by atypical mycobacteria.[6] There is a single-nucleotide polymorphism (SNP) +874 (A/T; rs2430561) located at the 50-end of a CA repeat at the first intron of human IFN-γ. The +874 T allele is linked to the 12 CA repeats, whereas the A allele is linked to the non-12 CA repeats.[7] The specific sequence of the T allele provides a binding site for the transcription factor nuclear factor-kB (NF-kB). As NF-kB induces IFN-γ expression, this T allele correlates with high IFN-γ expression, whereas the A allele correlates with low expression.[7] Apart from +874 (A/T), two potentially functional polymorphisms have also been reported at the promoter −179 (G/T)[8] and 30-untranslated region +4766 (C/T).[9] Several studies have suggested that a more common polymorphism at position +874 is associated with the risk of TB in different populations.[10–13]

In addition to the +874 and potentially functional SNPs mentioned above, we proposed other SNPs in IFN-γ should be unrevealed to associate with TB infection. In this study, the association between tag SNPs (tSNPs) of IFN-γ and tuberculosis in Han Taiwanese was investigated. The results indicated that polymorphisms of IFN-γ, not reported previously, confer genetic susceptibility to tuberculosis in this population.

Materials and methods

Study population

A total of 200 patients who were treated for active TB at the General Taoyuan Hospital (Taoyuan, Taiwan) between 2007 and 2008 were surveyed consecutively. The inclusion criteria were as follows: adult patients newly diagnosed with active TB, having evident lesions of TB by simple X-ray, computed tomography, and positive results of sputum smears and cultures for mycobacteria. In the control group, 200 volunteer individuals without active TB or a history of TB were enrolled.

Written informed consent was obtained from each patient and volunteer enrolled in this study. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Ethics Committee of Taoyuan General Hospital, Taoyuan, Taiwan.

DNA extraction and genotyping of the SNPs

Genomic DNA was extracted from oral swabs collected from the 200 TB patients and 202 non-TB participants using a QIAamp DNA Mini Kit (QIAGEN, Valencia, CA, USA) according to the manufacturer's instructions. The extracted genomic DNA was analyzed using agarose gel electrophoresis and quantitatively determined by spectrophotometry, and stored at −80 °C until use.

The tSNPs of the IFN-γ genomic region, upstream 1500 base pairs and downstream 1500 base pairs, were selected according to the SeattleSNPs website (http://pga.mbt.washington.edu/education.html). The SeattleSNPs database showed eight polymorphisms (MAF $\geq$ 0) in our target region. According to Han-Chinese Beijing (HCB) data, five tSNPs were selected (minimum $R^2 = 0.8$) from the eight polymorphisms. All SNP genotyping was performed using TaqMan SNP Genotyping Assays (ABI: Applied Biosystems Inc. Foster City, CA, USA). The SNP rs2430561, also known as +874, was also selected. The primers and probes of the selected SNPs were from an ABI assay on demand (AOD) kit. Reactions were carried out according to the manufacturer's protocol (TaqMan SNP Genotyping Assays, protocol, Part Number 4332856 Rev. C). The probe fluorescence signal detection was performed using an ABI Prism 7900 Real-Time PCR System.

Statistical analysis

The quality of the genotype data were evaluated by Hardy-Weinberg equilibrium (HWE) proportion tests. Intermarker linkage disequilibrium (LD) measures, r^2 and D′, were estimated and haplotype blocks were defined using the Haploview program.[14] The association analyses were tested by the χ^2 test. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated from contingency tables. SNP(s) showing significant association ($p \leq 0.05$) in the tests were further evaluated using logistic regressions adjusted for age and sex in the OR analysis. All statistical analyses were performed using SPSS version 17.0 (SPSS Inc., Chicago, IL, USA).

Table 1 Demographic data of the study participants

	TB	Non-TB	*p*
Age (mean ± SD)	55.95 ± 18.455	65.01 ± 10.520	<0.001
Sex (*n*)	Male: 137 Female: 63	Male: 100 Female: 102	<0.001

SD = standard deviation; TB = tuberculosis.