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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.

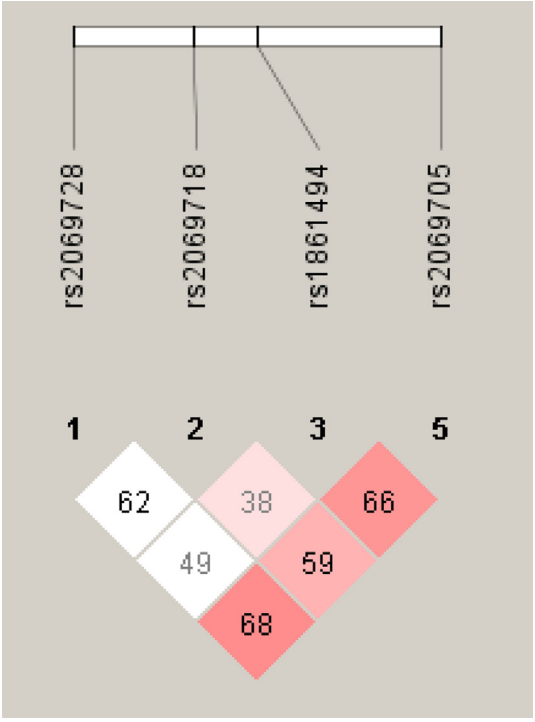

Figure 1. Linkage disequilibrium plot in D′ demonstrating adjacent strength between SNP pairs in the IFN-γ gene. D′ values are multiplied by 100, e.g., 38 in the square at the bottom implies a D′ value of 0.38. Squares without a number have a value of 100, equal to a D′ value of 1. When two SNPs are a complete link, the D′ value is 1. IFN-γ = interferon gamma; SNP = single nucleotide polymorphisms.

Results

In this study, 200 case patients diagnosed with TB and 202 control individuals without a history of TB infection were enrolled. There were significant differences in sex and age between groups. Male sex was more prevalent in the case group (Table 1). Older control individuals were enrolled to reduce the effect of exposure time and aging. The average age of the control group was higher than the case group as expected (Table 1).

Six SNPs in the IFN-γ genomic region were genotyped by TaqMan SNP genotyping assays. None of the genotype distributions of all of the study participants of the tested SNPs deviated from the Hardy-Weinberg equilibrium (HWE). The LD of the polymorphic tSNPs of the IFN-γ gene is shown in Fig. 1. No haploblock was identified at the IFN-γ gene. The SNPs rs1861494 (+2109; also known as IVS2 + 284) and rs2069718 (intron 3) showed different genotype frequencies between TB patients and non-TB individuals. The SNP rs2430561 showed marginally significant association to TB. The SNP rs2069710 (−155 bp; promoter region) was monomorphic in our study participants (Table 2).

The OR analysis showed that the C carrier (CT + TT) of rs1861494, TT homozygous of rs2069718, and AA homozygous of rs2430561 were risk genotypes for susceptibility to TB (Table 3). The genetic effects of the above three SNPs on the susceptibility to TB were further adjusted for the influence of age and sex by logistic regression. As shown in Table 3, the strength of associations is not apparently diluted by adjusting for age and sex.

Discussion

In the current study, we investigated the association between IFN-γ polymorphisms and TB in Han Taiwanese. Two

Table 2 Genotyping frequencies of tSNPs in the TB and non-TB groups and results of the χ2 test

SNP ID	Location	Genotype	Genotype counts		*p*
			TB	Non-TB	
rs2069728	3' near gene	AA	3	4	0.739
		AG	37	32	
		GG	160	166	
rs2069718	Intron 3	CC	9	12	<0.001
		CT	61	110	
		TT	130	80	
rs1861494	Intron 3 (IVS2 + 284)	GG	8	39	<0.001
		AG	97	76	
		AA	95	87	
rs2430561	Intron 1 (+874)	AA	13	4	0.067
		AT	56	54	
		TT	131	144	
rs2069710	Promoter (−155)		No polymorphism	No polymorphism	
rs2069705	Promoter (−1616)	CC	104	106	0.935
		CT	77	75	
		TT	19	21	

tSNPs = tag single-nucleotide polymorphisms; TB = tuberculosis.

Table 3 Odds ratio analysis of IFN-γ SNPs: rs1861494, rs2069718, and rs2430561

SNP ID	Genotype	Genotype counts		OR (95% CI)	Adjusted OR (95% CI)[a]
		Non-TB	TB		
rs1861494	GG (ref.)	39	8	5.74 (2.61, 12.64)	7.02 (2.62, 18.81)
	AA + AG	163	192		
rs2069718	CC + CT (ref.)	122	70	2.83 (1.89, 4.25)	3.47 (2.15, 5.60)
	TT	80	130		
rs2430561	TT + AT (ref.)	198	187	3.44 (1.10, 10.74)	3.85 (1.43, 15.53)
	AA	4	13		

[a] Adjusted for age and sex by logistic regression.
CI = confidence interval; IFN-γ = interferon gamma; OR = odds ratio; SNPs = single-nucleotide polymorphisms; TB = tuberculosis. ref. = reference genotype.

IFN-γ polymorphisms in intron 3, rs1861494 and rs2069718, as well as rs2430561 in intron 1 were associated with TB. The results of this study indicated that polymorphisms of IFN-γ gene may confer the risk of TB in Han Taiwanese.

SNP rs1861494, also known as IVS2 + 284, has been reported to be associated with asthma.[15] The wild-type allele (A) has a higher binding affinity than the polymorphic allele (G) for binding to putative nuclear factor(s).[15] Similar the aforementioned asthma study, G allele has a negative association in asthma and TB. SNP rs2069718 has been reported to be associated with systemic lupus erythematosus.[16] These two SNPs showed different minor allele frequencies (MAFs; 0.33 in rs1861494, 0.26 in rs2069718) and were not in the same haploblock in our study population, although they were all in intron 3 of the IFN-γ gene. This observation may indicate that more than one causative polymorphism in the IFN-γ gene is involved in susceptibility to *M. tuberculosis* infection.

Although none of the genotype distributions of all study participants of the tested SNPs deviated from the HWE, disequilibrium was shown in rs1861494 and rs2069718 in the case and/or control groups. In the control and case groups the HWE test was $p = 0.004$ and $p = 0.006$, respectively, for rs1861494. In rs2069718, the HWE test was $p = 0.001$ in the control group and not significant in the case group. The genotyping was replicated by sequencing, and the results were consistent with genotyping. The deviation from the HWE may be due to sampling bias, though the two selected SNPs obtained significant results in control groups, which was uncommon. In 1949, the pioneering geneticist JBS Haldane recognized that infectious diseases have been the main agent of natural selection during the past 5000 years.[17] Our results may suppose that the control (non-TB) group is the result of such a selection in the Han Taiwanese population. The genotype of rs1861494 in the case (TB) group also deviated from the HWE. This disequilibrium may be due to "artifact selection" derived from the patient inclusion criteria.

The +874 polymorphism of the IFN-γ gene is associated with TB in the Han Chinese population in Hong Kong,[13] and another two SNPs in the IFN-γ gene have been revealed to be strongly associated with TB in the Han Taiwanese population by genotyping the tSNPs. Further investigations on the genetic role of associated SNPs in other Han populations living in different geographic regions and other Asian populations are warranted.

Conflicts of interest

The authors declare that they have no financial or non-financial conflicts of interest related to the subject matter or materials discussed in the manuscript.

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