Association study of polymorphisms in interferon-γ receptor genes with the risk of pulmonary tuberculosis

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Abstract. Tuberculosis (TB) is an infectious disease caused by mycobacterium, which most commonly affects the lungs. The adaptive immune response in *Mycobacterium tuberculosis* is predominantly mediated by the interferon-γ (IFN-γ) signaling pathway, which is regulated by IFN-γ receptors (IFNGR). IFN-γ activates the transcription of a number of genes that are important in immune responses, thus the appropriate function of IFNGR appears to be important in host defense against mycobacteria. In the present study, 22 genetic variants in *IFNGR1* and *IFNGR2* were genotyped in 673 patients and 592 normal controls to investigate the association between *IFNGR1* and *IFNGR2* polymorphisms and the risk of TB. Statistical analyses revealed that four genetic variants in *IFNGR1*, *rs9376269*, *rs9376268*, *rs9376267* and *rs56251346* were marginally associated with the risk of TB (P=0.02-0.04), while other single nucleotide polymorphisms in *IFNGR1* and *IFNGR2* did not exhibit any associations. However, the significance of the four genetic variants *rs9376269*, *rs9376268*, *rs9376267* and *rs56251346* was eliminated following a multiple testing correction of the data (P>0.05). The present results revealed that certain genetic variants in IFNGR genes may be associated with TB development, which may be useful preliminary data for future investigation.

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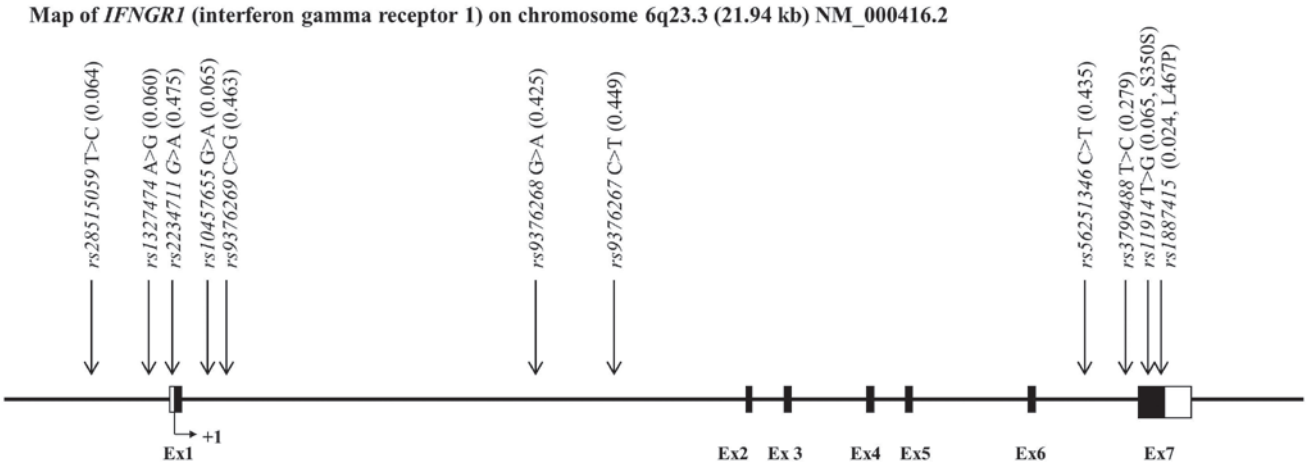
Introduction

Tuberculosis (TB) is an infectious disease commonly caused by mycobacteria (1). TB is considered to be an acute global health problem with ~9 million novel TB cases and 1.4 million fatalities each year (2). TB commonly originates in the lungs, but is able to spread to other parts of the body, leading to extra-pulmonary diseases (3). Among the patients infected with TB, ~10% progress to active TB during their lifespan and the remaining individuals remain asymptomatic (4). The immune responses of TB patients are mainly regulated by T helper 1 cells, which secrete interferon-γ (IFN-γ) (5). IFN-γ mediated immune responses activate macrophages, which induce the secretion of other cytokines, including interleukin (IL)-1, IL-12 and tumor necrosis factor (TNF)-α (6). Previously, genome-wide association studies have revealed that genetic variation in genes involved in immune responses, including IL-1, IL-12 and TNF-α, is associated with the risk of TB (7-9).

The IFN-γ-induced signaling pathway is activated by interacting with its receptor composed of two subunits, IFN-γ receptor (IFNGR) 1 and 2, which encode the ligand-biding chain (α-chain) and the non-ligand binding chain, respectively. IFNGR is involved in a positive feedback loop of IFN-γ expression (10). Genetic variation in cytokine-associated genes, including *IFNGR1* and *IFNGR2*, have previously been found to be important in other viral/host-mediated immune responses in TB (11-16). Among the genetic variants in *IFNGR1*, the

A



B Haplotype of *IFNGR1*

Hap.	*rs28515059*	*rs1327474*	*rs2234711*	*rs10457655*	*rs9376269*	*rs9376268*	*rs9376267*	*rs56251346*	*rs3799488*	*rs11914*	Freq.
ht1	C	A	A	G	C	G	C	C	T	T	0.407
ht2	C	A	G	G	G	A	T	T	C	T	0.276
ht3	C	A	G	G	G	A	T	T	T	T	0.145
ht4	T	A	G	A	C	G	C	C	T	G	0.062
ht5	C	G	A	G	C	G	C	C	T	T	0.060

C LDs among *IFNGR1* polymorphisms

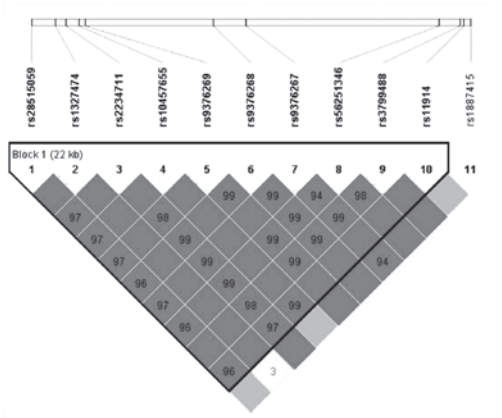


Figure 1. Schematic physical map of *IFNGR1*. (A) Polymorphisms of *IFNGR1*. Black blocks indicate coding exons; white blocks indicate 5'- and 3'-untranslated regions. First base of translation site is denoted as nucleotide +1. (B) Haplotypes of *IFNGR1* in a Korean population. (C) LDs among *IFNGR1* polymorphisms. IFNGR, interferon-γ receptors; LD, linkage disequilibrium; ht, haplotype.

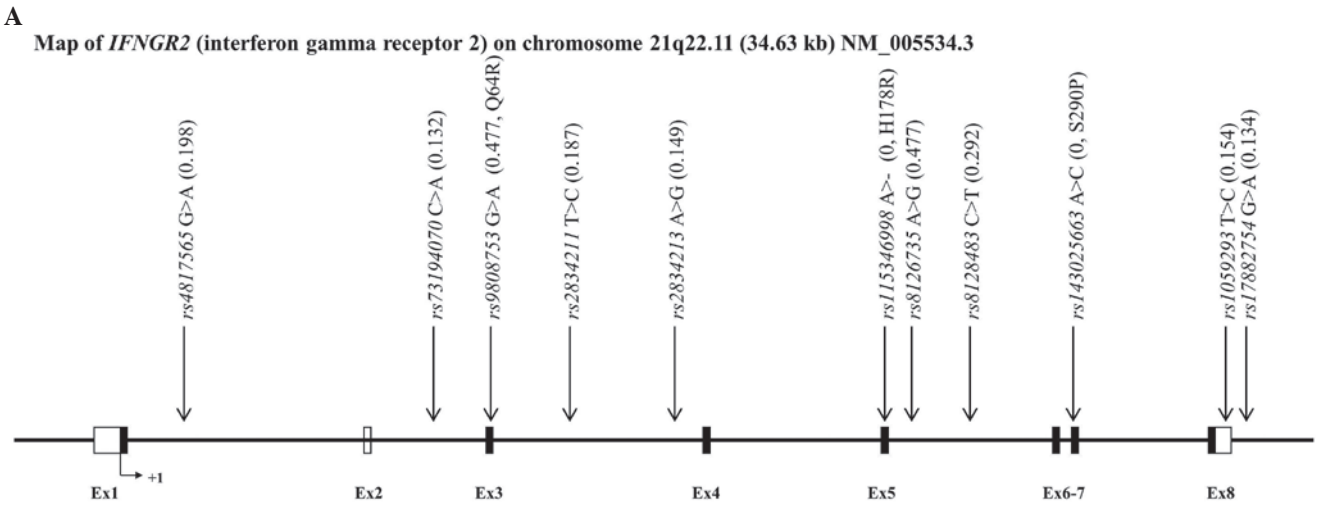
single nucleotide polymorphism (SNP) *rs2234711* has been revealed to be a major marker of disease protection. In a recent Chinese study, patients with *rs2234711* had a significantly lower prevalence of TB [odds ratio (OR)=0.82, P<0.001] (17). However, to date, an association between the risk for TB and genetic variation in the *IFNGR1* and *IFNGR2* genes had not been demonstrated in a Korean population. In the present study, the association of polymorphisms in the *IFNGR1* and *IFNGR2* genes with the risk of TB in the Korean population was investigated.

Patients and methods

Patients. A total of 673 patients with clinical manifestation of pulmonary TB (mean age, 45.81 years; range, 16-92 years, 388 males and 285 females) were recruited from Soonchunhyang University Bucheon Hospital (Bucheon, Republic of Korea). Polymerase chain reaction was used to assess all sputum acid-fast bacillus culture-positive samples to distinguish *Mycobacterium tuberculosis* (MTB) from non-tuberculous mycobacteria (NTM). The diagnosis of pulmonary TB was confirmed by the isolation of MTB from the sputum or bronchoalveolar lavage fluid. Patients with an NTM infection were excluded from the present study. Patients with TB who had a family history of the disease were also excluded from the study to eliminate the additional risk factors of added exposure to TB. A total of 592 healthy controls (mean age, 50.22 years; range, 9-87 years, 277 males and 315 females) were simultaneously recruited from a randomly sampled population who had attended the clinic for routine health checkups in the same regional area. Only patients above the age of 40 years were included in the normal control group to exclude the possibility of TB infection among young individuals who may subsequently develop the condition. Individuals with other medical diseases/conditions, including human immunodeficiency virus, hepatitis, diabetes, alcoholism, autoimmune diseases and cancer were also excluded from the present study.

The ethnicity of all patients and controls was Korean. Written informed consent was obtained from all patients prior to the start of the experiment. The experimental protocol was approved by the Institutional Review Board of Soonchunhyang University Bucheon Hospital (IRB no. schbc-biobank-2012-001).

SNP genotyping. Candidate SNPs of the *IFNGR1* and *IFNGR2* genes were selected from Japanese and



B

Haplotype of *IFNGR2*

Hap.	rs73194070	rs9808753	rs2834211	rs2834213	rs8126735	rs8128483	rs1059293	rs17882754	Freq.
ht1	C	G	T	A	A	C	T	G	0.515
ht2	C	A	C	A	G	C	T	G	0.185
ht3	C	A	T	G	G	T	C	G	0.148
ht4	A	A	T	A	G	T	T	A	0.128

C

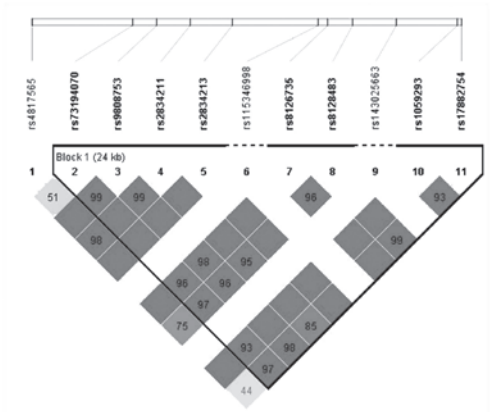


Figure 2. Schematic physical map of *IFNGR2*. (A) Polymorphisms of *IFNGR2*. Black blocks indicate coding exons; white blocks indicate 5'- and 3'-untranslated regions. First base of translation site is denoted as nucleotide +1. (B) Haplotypes of *IFNGR2* in a Korean population. (C) LDs among *IFNGR2* polymorphisms. IFNGR, interferon-γ receptors; LD, linkage disequilibrium; Ex, exon; ht, haplotype.

Han Chinese data from the 1,000 Genomes database (http://browser.1000genomes.org/index.html) based on the allele frequency and linkage disequilibrium (LD) status in the Asian population. Additional SNPs which had been previously investigated were also selected (14). A total of 11 SNPs of the *IFNGR1* gene and 11 SNPs of the *IFNGR2* gene were selected based on the following criteria: Minor allele frequency (MAF; >5%) and LD (r^2>0.98). A total of 22 polymorphisms were genotyped in 673 TB patients and 592 normal controls using a TaqMan assay on the ABI prism 7900HT sequence detection system (Applied Biosystems, Foster City, CA, USA) (18). Quality control of the genotyping was performed in 10% of the samples by duplicate checking (rate of concordance in duplicates, >99.5%). Selected SNPs and probe information on the polymorphisms is shown in Table I.

Statistical analysis. The level of LD was obtained using Haploview version 4.2 software (Broad Institute, Cambridge, MA, USA; http://www.broadinstitute.org/mpg/haploview), with examination of Lewontin's D' (|D'|) and the LD coefficient r^2 between all pairs of bi-allelic loci (19). Haplotypes were estimated using PHASE version 2.1 software (Stephen Laboratory, University of Chicago, Chicago, IL, USA) (20). A comparison of genotype distributions between TB patients and healthy controls was performed using a logistic regression model adjusted for age (continuous value) and gender (male=0, female=1) as co-variates using SAS software, version 9.3 (SAS Inc., Cary, NC, USA). The effective number of independent marker loci was calculated for multiple testing corrections using SNPSpD (http://genepli.qimr.edu.au/general/daleN/SNPSpD/), a program based on the spectral decomposition of matrices of pair-wise LD between SNPs (21). The total sum of independent marker loci in the gene was calculated as 7.7553 for *IFNGR1* and 9.3328 for *IFNGR2*, and this value was applied to correct for multiple testing.

Results

Genotyping and haplotype analysis of IFNGR1 and IFNGR2. In the present study, a total of 22 polymorphisms (11 in *IFNGR1* and 11 in *IFNGR2*) were selected, based on their MAF, location and LD status, and genotyped in 673 TB cases and 592 healthy controls. Detailed information regarding polymorphisms, including allele, amino acid change, position, MAF, heterozygosity and P-values for the Hardy-Weinberg equilibrium are shown in Table II. LDs among SNPs were

Table I. Probe information for IFNGR1 and IFNGR2.

Gene	Loci	Assay on demand ID or probe sequence
IFNGR1	*rs28515059*	C__63095558_10
	rs1327474	C___2523634_10
	rs2234711	C__11693991_10
	rs10457655	C__30506149_10
	rs9376269	C__30272193_20
	rs9376268	C__30470198_10
	rs9376267	C__30182293_10
	rs56251346	TGTTTACAAAGTGGGCACATC[a]
		ATTGGAAACATTTCCCCATC[b]
		CATTACTTGC[c]
		CATTATTTGC[d]
	rs3799488	C__25647358_10
	rs11914	C___7578627_10
	rs1887415	C__11693851_30
IFNGR2	*rs4817565*	GACATTGCCACAACATCCAG[a]
		GAGCCTGGCCTCACTTTTTA[b]
		ACCTGTCCAT[c]
		ACCTATCCAT[d]
	rs73194070	ACTGTGAGGGAGCATTGACC[a]
		CCGAAGGCAGACAGGTAAAG[b]
		ACCACCCCCC[c]
		ACCACACCCC[d]
	rs9808753	C___2443413_1_
	rs2834211	C__16072862_10
	rs2834213	C___2443417_10
	rs115346998	AGAAGGCTCCCTCATCATCA[a]
		TCTTGCCTGTTGGATTCCTC[b]
		TGTCCATTAC[c]
		TGTCCGTTAC[d]
	rs8126735	TGAAGCATCTCCAGTGCCTA[a]
		GAGCCAAACACAAAGGAAGC[b]
		TTATAATGGT[c]
		TTATGATGGT[d]
	rs8128483	GAAGAGGCACATGGAGGAAA[a]
		CCTGGCAGACAACAGTTCAC[b]
		TCATCGCTCC[c]
		TCATTGCTCC[d]
	rs143025663	GTTTCACACTCCACCAAGCA[a]
		GCTGCAGTGAGCAGAGATTG[b]
		TTACAGATAG[c]
		TTACCGATAG[d]
	rs1059293	C___2443435_10
	rs17882754	TCATGGGAACTCAGCAAACA[a]
		CTCAAGTGATCCACCCACCT[b]
		CAGGGCCTAG[c]
		CAGGACCTAG[d]

IFNGR, interferon-γ receptor; [a]forward; [b]reverse; [c]labeled with VIC fluorophore; [d]labeled with 6-carboxyfluorescein.

Table II. Allele information of *IFNGR1* and *-2* polymorphisms in Korean patients (n=1265).

Gene	SNP	Allele	Position	AA Change	Genotype			MAF	Heterozygosity	HWE		
					C/C	C/R	R/R			TB	NC	Total
IFNGR1	*rs28515059*	C>T	5' flanking		1,106	151	5	0.064	0.119	0.803	0.728	0.949
	rs1327474	A>G	5' flanking		1,117	145	3	0.060	0.112	0.374	0.859	0.451
	rs2234711	G>A	5' UTR		345	632	282	0.475	0.499	0.744	0.987	0.818
	rs10457655	G>A	Intron1		1,101	154	5	0.065	0.122	0.841	0.669	0.876
	rs9376269	C>G	Intron1		357	643	264	0.463	0.497	0.5	0.049	0.415
	rs9376268	G>A	Intron1		411	633	221	0.425	0.489	0.939	0.157	0.396
	rs9376267	C>T	Intron1		377	636	248	0.449	0.495	0.415	0.054	0.491
	rs56251346	C>T	Intron6		395	640	230	0.435	0.491	0.904	0.136	0.296
	rs3799488	T>C	Intron6		650	522	92	0.279	0.403	0.239	0.901	0.358
	rs11914	T>G	Exon7	S350S	1,105	155	5	0.065	0.122	0.837	0.647	0.861
	rs1887415	T>C	Exon7	L467P	1,206	58	1	0.024	0.046	0.609	0.451	0.726
IFNGR2	*rs4817565*	G>A	Intron1		813	402	50	0.198	0.318	0.488	0.424	0.972
	rs73194070	C>A	Intron2		946	303	16	0.132	0.230	0.472	0.145	0.130
	rs9808753	G>A	Exon3	Q64R	341	642	282	0.477	0.499	0.645	0.682	0.540
	rs2834211	T>C	Intron3		836	384	45	0.187	0.305	0.669	0.553	0.912
	rs2834213	A>G	Intron3		917	315	30	0.149	0.253	0.754	0.726	0.634
	rs115346998	A>-	Exon5	H178R	1,265	–	–	–	–	–	–	–
	rs8126735	A>G	Intron5		339	645	281	0.477	0.499	0.402	0.798	0.436
	rs8128483	C>T	Intron5		641	509	115	0.292	0.414	0.664	0.351	0.336
	rs143025663	A>C	Exon7	Q290P	1,264	1	0	0.000	0.001	0.985	-	0.989
	rs1059293	T>C	3' UTR		911	319	35	0.154	0.260	0.294	0.654	0.271
	rs17882754	G>A	3' flanking		942	1,248	17	0.134	0.233	0.607	0.124	0.158

C/C, C/R, and R/R refer to the common homozygote, heterozygote and minor homozygote, respectively. MAF, minor allele frequency; HWE, Hardy-Weinberg equilibrium; TB, tuberculosis; NC, normal control; AA, amino acid; IFNGR, interferon-γ receptor; UTR, untranslated region.

Table III. Logistic analysis of *IFNGR1* polymorphisms.

				MAF		Codominant			Dominant			Recessive		
Loci	Allele	Position	AA change	TB	NC	OR (95% CI)	P	p^{corr}	OR (95% CI)	P	p^{corr}	OR (95% CI)	P	p^{corr}
rs28515059	C>T	5' flanking		0.062	0.065	0.94 (0.68-1.30)	0.72	1	0.93 (0.66-1.31)	0.67	1	1.25 (0.21-7.61)	0.81	1
rs1327474	A>G	5' flanking		0.058	0.062	0.93 (0.66-1.30)	0.65	1	0.94 (0.66-1.33)	0.71	1	0.47 (0.04-5.51)	0.55	1
rs2234711	G>A	5' UTR		0.464	0.487	0.90 (0.77-1.06)	0.21	1	0.89 (0.69-1.15)	0.37	1	0.85 (0.65-1.11)	0.24	1
rs10457655	G>A	Intron1		0.063	0.067	0.93 (0.67-1.28)	0.66	1	0.92 (0.65-1.28)	0.61	1	1.26 (0.21-7.64)	0.80	1
rs9376269	C>G	Intron1		0.476	0.448	1.13 (0.97-1.33)	0.13	0.97	1.03 (0.80-1.32)	0.80	1	1.40 (1.06-1.85)	**0.02**	0.15
rs9376268	G>A	Intron1		0.442	0.405	1.18 (1.00-1.38)	**0.05**	0.40	1.14 (0.90-1.45)	0.27	1	1.40 (1.04-1.88)	**0.03**	0.22
rs9376267	C>T	Intron1		0.461	0.435	1.13 (0.96-1.32)	0.15	1	1.02 (0.80-1.30)	0.88	1	1.40 (1.05-1.87)	**0.02**	0.16
rs56251346	C>T	Intron6		0.452	0.415	1.19 (1.01-1.40)	**0.04**	0.29	1.17 (0.92-1.48)	0.22	1	1.40 (1.04-1.88)	**0.03**	0.19
rs3799488	T>C	Intron6		0.295	0.262	1.19 (0.99-1.43)	0.06	0.46	1.27 (1.02-1.60)	**0.04**	0.28	1.12 (0.72-1.73)	0.62	1
rs11914	T>G	Exon7	S350S	0.063	0.068	0.93 (0.67-1.28)	0.64	1	0.91 (0.65-1.28)	0.59	1	1.26 (0.21-7.68)	0.80	1
rs1887415	T>C	Exon7	L467P	0.019	0.029	0.67 (0.40-1.12)	0.13	0.98	0.68 (0.40-1.15)	0.15	1	–	–	–
ht1				0.399	0.416	0.92 (0.78-1.08)	0.33	1	0.95 (0.75-1.21)	0.69	1	0.82 (0.60-1.10)	0.19	1
ht2				0.291	0.259	1.19 (0.99-1.43)	0.06	0.40	1.26 (1.01-1.58)	**0.04**	0.31	1.16 (0.74-1.80)	0.52	1
ht3				0.146	0.144	1.03 (0.82-1.30)	0.78	1	1.00 (0.77-1.28)	0.97	1	1.65 (0.70-3.92)	0.25	1
ht4				0.061	0.064	0.94 (0.68-1.30)	0.71	1	0.93 (0.66-1.30)	0.66	1	1.26 (0.21-7.68)	0.80	1
ht5				0.058	0.062	0.93 (0.66-1.30)	0.65	1	0.94 (0.66-1.33)	0.71	1	0.47 (0.04-5.51)	0.55	1

Effective number of independent marker loci in *IFNGR1* were calculated to correct for multiple testing using SNPSpD (http://genepi.qimr.edu.au/general/daleN/SNPSpD/). The number of independent marker loci in *IFNGR1* was calculated as 7.7553. Bold P-values indicate statistical significance. TB, tuberculosis; MAF, minor allele frequency; OR, odds ratio; CI, confidence interval; IFNGR, interferon-γ receptor; NC, normal control, UTR, untranslated region; ht, haplotype.

Table IV. Logistic analysis of *IFNGR2* polymorphisms.

				MAF		Codominant			Dominant			Recessive		
Loci	Allele	Position	AA change	TB	NC	OR (95% CI)	P	p^{corr}	OR (95% CI)	P	p^{corr}	OR (95% CI)	P	p^{corr}
rs4817565	G>A	Intron1		0.200	0.197	1.02 (0.84-1.24)	0.84	1	1.06 (0.84-1.34)	0.62	1	0.83 (0.47-1.47)	0.52	1
rs73194070	C>A	Intron2		0.134	0.130	1.06 (0.83-1.35)	0.63	1	1.05 (0.81-1.35)	0.74	1	1.44 (0.51-4.03)	0.49	1
rs9808753	G>A	Exon3	Q64R	0.481	0.471	1.03 (0.88-1.21)	0.72	1	1.04 (0.81-1.34)	0.77	1	1.04 (0.79-1.36)	0.78	1
rs2834211	T>C	Intron3		0.184	0.192	0.95 (0.78-1.17)	0.64	1	0.97 (0.77-1.23)	0.82	1	0.78 (0.43-1.44)	0.43	1
rs2834213	A>G	Intron3		0.154	0.142	1.06 (0.85-1.32)	0.61	1	1.07 (0.83-1.37)	0.61	1	1.09 (0.52-2.30)	0.82	1
rs115346998	A>-	Exon5	H178R	–	–	–	–	–	–	–	–	–	–	–
rs8126735	A>G	Intron5		0.484	0.470	1.05 (0.89-1.23)	0.57	1	1.10 (0.85-1.41)	0.48	1	1.03 (0.79-1.35)	0.84	1
rs8128483	C>T	Intron5		0.300	0.283	1.07 (0.90-1.27)	0.45	1	1.11 (0.89-1.39)	0.37	1	1.03 (0.70-1.53)	0.87	1
rs143025663	A>C	Exon7	Q290P	0.001	–	–	–	–	–	–	–	–	–	–
rs1059293	T>C	3' UTR		0.160	0.146	1.07 (0.86-1.33)	0.53	1	1.07 (0.83-1.38)	0.59	1	1.21 (0.60-2.42)	0.60	1
rs17882754	G>A	3' flanking		0.137	0.132	1.06 (0.84-1.34)	0.64	1	1.03 (0.80-1.34)	0.80	1	1.66 (0.60-4.57)	0.33	1
ht1				0.490	0.478	1.04 (0.89-1.22)	0.65	1	1.07 (0.83-1.38)	0.59	1	1.03 (0.79-1.34)	0.84	1
ht2				0.181	0.189	0.96 (0.78-1.18)	0.68	1	0.97 (0.76-1.22)	0.77	1	0.86 (0.47-1.60)	0.64	1
ht3				0.154	0.141	1.07 (0.86-1.33)	0.56	1	1.08 (0.84-1.39)	0.56	1	1.09 (0.52-2.29)	0.82	1
ht4				0.131	0.126	1.07 (0.84-1.36)	0.60	1	1.06 (0.82-1.38)	0.66	1	1.33 (0.47-3.82)	0.59	1

The effective number of independent marker loci in IFNGR2 was calculated to correct for multiple testing using SNPSpD (http://genepi.qimr.edu.au/general/daleN/SNPSpD/). The number of independent marker loci in *IFNGR2* was calculated as 9.3328. Bold P-values indicate statistical significance. TB, tuberculosis; MAF, minor allele frequency; OR, odds ratio; CI, confidence interval; NC, normal control; AA, amino acid; IFNGR, interferon-γ receptors; ht, haplotype; UTR, untranslated region.

obtained by calculating |D'| and r^2 values. Among the investigated polymorphisms, ten polymorphisms in *IFNGR1* and eight in *IFGNR2* were used for LD block construction of each gene. The genetic variants *rs1887415*, *rs115346998* and *rs143025663* were excluded from LD block construction due to its low frequency (MAF<5%). As a result, one LD block was constructed in *IFNGR1* that contained five major haplotypes (ht), which exhibited a MAF>5% (Fig. 1). Among the *IFNGR1* haplotypes, *IFNGR1*_ht4 and *IFNGR1*_ht5 exhibited equivalence with *rs28515059* and *rs1327474*, respectively, and those haplotypes were excluded from the further analysis. In the case of *IFNGR2*, one LD block was constructed and it contained four major haplotypes, which exhibited a MAF>5% (Fig. 2).

Correlation analyses of SNPs in IFNGR1 and IFNGR2 with TB. The case-control analysis of the correlation between *IFNGR1* or *IFNGR2* polymorphisms and the risk of TB was conducted (Tables III and IV). The correlation analysis revealed that the two SNPs in *IFNGR1*, *rs9376268* and *rs56251346*, induced an increased risk for TB under a co-dominant model (OR=1.18 and 1.19; P=0.05 and 0.04, respectively). The two SNPs exhibited similar genetic effects with a higher level of significance under a recessive model (OR=1.40; P=0.03 for the two SNPs). Along with *rs9376268* and *rs56251346*, two SNPs in intron 1, *rs9376269* and *rs9376267*, also induced an increased risk for TB under a recessive model (OR=1.40; P=0.02 for the two SNPs). However, the level of significance was not retained following the correction for multiple testing in all analysis models (P>0.05). Polymorphisms in the coding region, *rs11914* (S350S) and *rs1887415* (L467P), were not associated with an increased risk for TB. In the haplotype analysis, *IFNGR1*_ht2 exhibited a marginal association with the risk for TB under a dominant model (P=0.04), although its association was eradicated following the correction for multiple testing. However, no genetic polymorphisms and haplotypes in *IFNGR2* exhibited significant correlations with the risk of developing TB.

Discussion

In previous studies, genetic variations in the genes involved in the IFN-γ signaling pathway have been associated with the risk of developing several mycobacterial diseases, particularly TB (13-15). Defects in the proper functioning of IFN-γ-meditated immune responses is a major cause of disease susceptibility (22). IFN-γ activates transcription of a large number of cytokines, including those secreted by macrophages, including IL-12 and TNF-α, which have roles in immune responses, thus the appropriate function of the IFNGR appears to be important in host defense against mycobacteria (23).

In the present study, a logistic analysis was conducted to identify a possible significant association between genetic variants in the IFNGR genes and TB in a Korean population. Previous studies have revealed a correlation of the *IFNGR1* polymorphisms *rs2234711*, *rs1327474* and *rs11914*, with TB (Table V) (13,14,17,24,25). Studies in African populations have revealed that the prevalence of TB was lower in African populations with the minor alleles of *rs11914* (S350S) and *rs2234711*, suggesting a protective effect (OR=0.66; P=0.022

Table V. Comparison of previous studies on *IFNGR1*-TB association.

			Studied allele				
Reference	Study population	Study patients (cases/control, n)	*rs1327474* P-value (OR)	*rs2234711* P-value (OR)	*rs11914* P-value (OR)	*rs9376268* P-value (OR)	*rs56251346* P-value (OR)
Awomoyi *et al* (2004) (24)	Gambian	320/320	0.34 (1.19)	0.5 (1.01)[a]	0.23 (1.41)	–	–
Cooke *et al* (2006) (25)	African	682/619	–	**0.041** (0.75)	–	–	–
He *et al* (2010) (14)	Chinese	222/188	NS	NS	–	–	–
de Wit *et al* (2011) (13)	African	505/318	–	–	**0.022** (0.66)	–	–
Lu J *et al* (2014) (17)	Chinese	1434/1412	–	**<0.001** (0.82)	–	–	–
Present study (2014)[b]	Korean	673/592	0.65 (0.93)	0.21 (0.90)	0.64 (0.93)	**0.05** (1.18)	**0.04** (1.19)

Polymorphisms that were commonly investigated (*rs1327474*, *rs2234711*, *rs11914*) and exhibited significant results in the present study (*rs9376268*, *rs56251346*) are listed. OR, odds ratio; NS, not significant; –, not performed. [a]Minor allele is reversed compared with the present study. [b]Presented values are derived from co-dominant model of logistic analysis. Bold P-values indicate statistical significance. IFNGR, interferon-γ receptors; TB, tuberculosis.

Table VI. Comparison of genetic distribution in ethnic groups of polymorphisms in *IFNGR1* and *IFNGR2*.

			MAF				Fisher's exact test					
Gene	Loci	Allele	KOR	AS	AF	CA	KR vs. AS	KR vs. AF	KR vs. CA	AS vs. AF	AS vs. CA	AF vs. CA
IFNGR1	*rs28515059*	C>T	0.064	0.075	0.049	0.172	0.3703	0.0984	<.0001	0.0277	<.0001	<.0001
	rs1327474	A>G	0.060	0.054	0.045	0.38	0.6130	0.3154	0.4094	0.2682	0.3146	0.1509
	rs2234711	G>A	0.475	0.497	0.49	0.443	0.9348	0.0458	<.0001	0.2491	<.0001	<.0001
	rs10457655	G>A	0.065	0.078	0.293	0.178	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
	rs9376269	C>G	0.463	0.419	0.11	0.262	0.1787	<.0001	<.0001	<.0001	<.0001	<.0001
	rs9376268	G>A	0.425	0.39	0.059	0.265	<.0001	<.0001	<.0001	<.0001	0.0021	<.0001
	rs9376267	C>T	0.449	0.409	0.12	0.265	0.2480	<.0001	<.0001	<.0001	<.0001	<.0001
	rs56251346	C>T	0.435	0.401	0.059	0.265	0.1354	<.0001	<.0001	<.0001	<.0001	<.0001
	rs3799488	T>C	0.279	0.253	0.01	0.128	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
	rs11914	T>G	0.065	0.073	0.059	0.169	<.0001	<.0001	<.0001	0.0874	<.0001	<.0001
	rs1887415	T>C	0.024	0.016	0.02	–	<.0001	<.0001	–	0.7987	–	–
IFNGR2	*rs4817565*	G>A	0.198	0.223	0.03	0.112	0.0077	<.0001	0.0002	<.0001	0.0006	<.0001
	rs73194070	C>A	0.132	0.134	0.083	0.213	0.2535	0.0019	<.0001	0.0663	0.0217	<.0001
	rs9808753	G>A	0.477	0.465	0.222	0.112	0.1713	<.0001	<.0001	<.0001	<.0001	0.0003
	rs2834211	T>C	0.187	0.204	0.018	0.112	0.0324	<.0001	0.0044	<.0001	<.0001	<.0001
	rs2834213	A>G	0.149	0.177	0.033	0.243	0.3102	<.0001	<.0001	<.0001	0.0901	<.0001
	rs115346998	A>G	–	0.005	–	–	–	–	–	–	–	–
	rs8126735	A>G	0.477	0.46	0.242	0.087	0.2313	<.0001	<.0001	<.0001	<.0001	<.0001
	rs8128483	C>T	0.292	0.336	0.364	0.194	0.2078	0.0022	0.0001	0.4482	<.0001	<.0001
	rs143025663	A>C	0.000	0.005	–	–	0.0449	–	–	–	–	–
	rs1059293	T>C	0.154	0.199	0.171	0.454	0.0826	0.4749	<.0001	0.4726	<.0001	<.0001
	rs17882754	G>A	0.134	0.163	0.012	0.109	–	–	–	–	–	–

Selected SNPs are identical to those in Table III. Minor allele frequency and P-values were calculated based on information from the 1,000 Genomes database. African populations included ASW (African ancestry in Southwest USA), LWK (Luhya in Webuye, Kenya), MKK (Maasai in Kinyawa, Kenya) and YRI (Yoruban in Ibadan, Nigeria). Asians populations included CHB (Han Chinese in Beijing, China), CHD (Chinese in Metropolitan Denver, CO, USA), JPT (Japanese in Tokyo, Japan) and Korean individuals from the present study. Caucasians populations included CEU (Utah residents with Northern and Western European ancestry from the CEPH collection) and TSI (Tuscany in Italy). MAF, minor allele frequency; KOR, Patients in the prersent study; AS, Asian; AF, African; CA, Caucasian. IFNGR, interferon-γ receptor.

and OR=0.75; P=0.041, respectively) (13,25). The protective effect of *rs2234711* on TB prevalence has also been observed in a Chinese population (OR=0.82, P<0.001) (17). In another Chinese study, *rs7749300,* which revealed a marked LD with *rs2234711* and *rs1327474*, were significantly associated with the risk of TB (OR=3.96; P=0.0003, from haplotype analysis of three SNPs) (14). However, *rs7749300* was not investigated in the present study due to perfect LD with *rs2234711* in the 1,000 Genomes database. However, previously demonstrated genetic effects were not replicated in the present study, which may be due to differences in the genetic diversity among the populations. In the case of *IFNGR2*, two polymorphisms (*rs2834213* and *1059293*) exhibited a protective effect against the risk of developing TB (OR=0.69-0.70; P=0.0073-0.0088) (26); however, these findings were not replicated in the present study.

In order to investigate whether the present results were due to ethnic differences or not, the genetic composition of IFNGR genes were compared between ethnicities. Frequency analysis and Fisher's exact test were additionally conducted among the four groups, which included a Korean population from the present study, as well as African, Asian and Caucasian populations from the 1,000 Genomes database (Table VI). As a result, the SNP *rs11914* exhibited a significant difference in allelic distribution between Korean and African individuals. Genetic compositions of *rs11914* in the Japanese and Chinese populations also differed from that of Korean individuals. Along with the *rs11914* SNP, other investigated SNPs, including *rs10457655*, *rs9376269*, *rs9376268*, *rs9376267*, *rs56251346* and *rs3799488*, have demonstrated a wide degree of frequency variance depending on the populations (P<0.05).

Previous studies have demonstrated that dysfunction in the IFN-γ pathway caused by genetic variation may contribute to a further impairment in cellular immune function in IFN-γ-mediated diseases, which may increase the susceptibility to disease. A specific promoter polymorphism, *rs1327474*, and one coding region polymorphism, *rs11914* (S350S), were found to be significantly associated with the risk of arthritis in a European population (27). Other SNPs, *rs3799488* and *rs10457655*, exhibited associations with the risk of rectal cancer prevalence and risk of atopic dermatitis, respectively, in a Caucasian population (28,29).

Of note, functional analysis of *IFNGR1* identified that the non-synonymous SNP *rs1887415* (L467P) does not functionally differ from the wild-type receptors (30). In addition, *IFNGR1* L467P has been reported to be associated with the high immunoprotein levels against diseases (31,32). Previous studies of rs1887415 may be a plausible explanation for the protective effect against TB (OR=0.63) since *IFNGR1* interacts with the IFN-γ immune responses that induce secretion of other cytokines. The association analyses demonstrated that genetic variants in the ligand-binding chain of IFNGR (*IFNGR1*) affect the IFN-γ pathway, although genetic variants in the signal-transducing chain of IFNGR (IFNGR2), including three non-synonymous SNPs (Q64R, H178R, Q290P), do not affect the IFN-γ pathway.

In conclusion, a correlation analysis between polymorphisms in IFNGR genes and the risk of TB revealed that four SNPs, *rs9376269*, *rs9376268*, *rs9376267* and *rs56251346*, were marginally associated with the development of TB. The present study was the first to report, to the best of our knowledge, the importance of *IFNGR1* and *IFNGR2* as genetic factors in mycobacterial infectious disease, which may prove useful for identifying the etiology of TB in a Korean population.

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