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ORIGINAL INVESTIGATION

Association of *IFNGR2* gene polymorphisms with pulmonary tuberculosis among the Vietnamese

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Abstract Interferon-γ (IFN-γ) is a key molecule of T helper 1 (Th1)-immune response against tuberculosis (TB), and rare genetic defects of IFN-γ receptors cause disseminated mycobacterial infection. The aim of the present study was to investigate whether genetic polymorphisms found in the Th1-immune response genes play a role in TB. In our study, DNA samples were collected from two series of cases including 832 patients with new smear-positive TB and 506 unrelated individuals with no history of TB in the general population of Hanoi, Vietnam. Alleles of eight microsatellite markers located around Th1-immune response-related genes and single nucleotide polymorphisms near the promising microsatellites were genotyped. A set of polymorphisms within the interferon gamma receptor 2 gene (*IFNGR2*) showed a significant association with protection against TB ($P = 0.00054$). Resistant alleles tend to be less frequently found in younger age at diagnosis ($P = 0.011$). Luciferase assays revealed high transcriptional activity of the promoter segment in linkage disequilibrium with resistant alleles. We conclude that the polymorphisms of *IFNGR2* may confer resistance to the TB development of newly infected individuals. Contribution of the genetic factors to TB appeared to be different depending on age at diagnosis.

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Introduction

Tuberculosis (TB) remains one of the major health problems worldwide (Lopez et al. 2006): According to an estimate, approximately one-third of the world's population is infected with *Mycobacterium tuberculosis* (*M. tuberculosis*), and more than 9 million people develop active TB disease every year. Of these patients, 80% are from 22 high-burden countries including Vietnam (World Health Organization 2010).

Development of TB has been considered to be a two-stage process, infection with *M. tuberculosis* and progression to disease. In total, 5–10% of immunocompetent individuals initially infected with *M. tuberculosis* develop active TB during their lifetime (Frieden et al. 2003). When young vulnerable individuals fail to inhibit growth of the pathogen, they often develop the disease within 2 years of infection. In the remaining individuals, containment of *M. tuberculosis* is successful, though the agent is not

eliminated completely, which leads to life-long latent infection (Russell 2007). When immune levels are impaired after years of infection, reactivation of dormant bacteria leads to disease manifestation, which contributes to the development of elderly TB, though new TB patients affected by re-infection also have to be taken into account (Tufariello et al. 2003). Protective immunity to control the initial infection, orchestrated by immune cells including T cells and macrophages, is influenced by a variety of factors including genetic predisposition (Möller et al. 2010a).

T helper 1 (Th1)-type immune system is crucial to protection against mycobacterial diseases, in which interferon-γ (IFN-γ) has a key role (Lin and Flynn 2010). Although T cell response to mycobacterial infection in human beings is difficult to be addressed experimentally (Cooper 2009), it is known that genetic defects of Th1 molecules can be found in genes such as interferon gamma receptor 1 (*IFNGR1*), interferon gamma receptor 2 (*IFNGR2*), signal transducer and activator of transcription 1, 91 kDa (*STAT1*), interleukin 12B (*IL12B*) and interleukin 12 receptor, beta 1 (*IL12RB1*) cause severe mycobacterial diseases (Zhang et al. 2008). These observations have highlighted IFN-γ/interleukin-12 (IL-12) axis and their polymorphisms have been investigated in mycobacterial infection: association of promoter polymorphism in *IFNGR1* with TB was reported in African populations in independent studies, whereas association of *IFNGR2* with TB has not been published in the literature (Cooke et al. 2006; Stein et al. 2007). Associations with *IL12B* and *IL12RB1* were not consistently shown (Möller et al. 2010b). In the present study, we analyzed genetic polymorphisms of major Th1 cytokine receptors (*IFNGR1*, *IFNGR2*, *IL12RB1* and *IL12RB2*) and signal transduction molecules (*STAT1* and *STAT4*) in Hanoi–Vietnamese and reported a disease association and functional significance of polymorphisms in *IFNGR2*.

Materials and methods

Study population

The patients and control subjects were recruited in Hanoi, Vietnam (Horie et al. 2007). In total, 832 smear-positive pulmonary TB patients without previous episodes of TB (age 41 ± 14.4, males 77.6%) and 506 healthy volunteers without previous and present history of TB (age 37 ± 10.3, males 50.0%) participated in this study. All of them were unrelated Hanoi, Vietnamese. TB patients were all recruited immediately after the diagnosis was made. The TB panel A (n = 277, age 41 ± 13.5, males 73.3%) was collected in 2003–2004, and the second TB panel B (n = 555, age 41 ± 14.8, males 79.8%) was collected in 2007–2009. Pulmonary physicians diagnosed all the patients as new active pulmonary TB and treated them with anti-TB drugs based on the guidelines of the national TB program. Informed consent was obtained from all participants. The study protocol was approved by the ethics committees of the Ministry of Health, Vietnam and the National Center for Global Health and Medicine, Japan. Since 4 patients in panel A were human immunodeficiency virus (HIV) positive by previously described PCR assay (Panteleeff et al. 1999) with minor modifications and 49 patients in panel B were HIV seropositive (Hang et al. 2011), they were excluded from further analysis.

Microsatellite markers

We used eight microsatellite markers (*IFNGR1*-MS1, *IFNGR2*-MS1, *IFNGR2*-MS2, *IL12RB1*-MS1, *IL12RB2*-MS1, *IL12RB2*-MS2, *STAT1*-MS1 and *STAT4*-MS1) located in the major Th1-immune response genes (Tanaka et al. 2005) for screening of genetic polymorphisms associated with active TB. A part of the samples, 98 TB patients from the TB panel A and 200 controls were analyzed as described under (Tanaka et al. 2005).

Single nucleotide polymorphisms (SNP) screening in *IFNGR2* of Vietnamese samples

Forty-eight control samples were subjected to PCR amplifications of promoter and seven exon regions of *IFNGR2* and their sequences were analyzed for polymorphisms. GC content of genomic sequence upstream of the translation initiation codon was high (78.2% of nucleotides −1 to −500), and PCR condition was optimized for GC-rich template. The genomic DNA was extracted from anticoagulated blood with QIAamp DNA midi kit (QIAGEN, Hamburg, Germany). PCR was performed using TaKaRa LA Taq with GC buffer I (TaKaRa, Shiga, Japan) with primers 5′-CTCCCAACAGGCGTCAAACGACATGGTG-3′ and 5′-TGGTCCCTGCTCCACCGCTGCTACTACAAA-3′. PCR cycling condition was 40 cycles of 95°C for 30 s, 67°C for 30 s and 72°C for 2 min. Amplified products (1,607 bp) were purified and sequenced with BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) using 3100 Genetic Analyzer (Applied Biosystems), with primers 5′-AGCTTAATATGTACTTTGGGG-3′ and 5′-CACCCACTCTGAGCACCCGG-3′. This method was also used for the typing of three promoter SNPs, rs8134145, rs8126756 and rs17882748. Sequencing primers that have the allelic variant at their 3′-end 5′-GGAGGGGTGGGGGCTCCAGGGAAA-3′, 5′-GCAGGGCCCGCCTCTTCCCGAGCA-3′ and 5′-GGGCTCCAGGGAAAGCCCGGGGGT-3′ were also designed, and allele-specific sequencing was

performed to directly determine the haplotypes of the three promoter SNPs.

Selection of representative SNPs around *IFNGR2* and genotyping

Representative SNPs around *IFNGR2* were selected from HapMap database (The International HapMap Consortium 2005). SNP genotype data of Han Chinese in Beijing (CHB) encompassing 350 kb from *IL10RB* to *CRYZL1* were analyzed by Haploview 4.2 (Barrett et al. 2005), and 27 representative SNPs were chosen based on the method of block-by-block tags in linkage disequilibrium (LD) blocks determined by confidence interval method (Gabriel et al. 2002). The *IFNGR2* SNPs identified as mentioned above and selected SNPs were genotyped in 273 TB patients of panel A and 506 controls. Genotyping was performed by the Digitag2 assay that has previously been described in another study (Nishida et al. 2007).

Rapid amplification of cDNA end (5′-RACE) of *IFNGR2*

The exact 5′ end of exon 1 was confirmed with FirstChoice RLM-RACE Kit (Ambion, Austin, TX, USA) using total RNA of THP-1 cells (ATCC TIB-202) stimulated with 10 ng/ml of phorbol myristate acetate (Schwende et al. 1996), U937 cells (ATCC CRL-1593.2) and Jurkat cells (ATCC TIB-152).

Luciferase assay

Promoter region of *IFNGR2* (Rhee et al. 1996) consisting of 1,167 bp (position −1,172 to −6 of initiation codon) was amplified by PCR and inserted into *Xho* I and *Bgl* II sites of pGL4.10 vector (Promega, Madison, WI, USA). Three plasmids of the observed haplotypes (CCC, ATC, ATT of rs8134145, rs8126756 and rs17882748) were constructed, and their sequences were confirmed to be devoid of any additional nucleotide difference. Reporter plasmids were mixed with pRL-TK (Promega) and transfected to Jurkat human T-cell leukemia cells with Lipofectamin LTX (Invitrogen, Carlsbad, CA, USA) in triplicate. Cells were harvested after 24 h and luciferase activity was measured using Dual-Luciferase Reporter Assay System (Promega). The transfection experiments were repeated twice with three independent subclones of each plasmid.

Statistical analysis

Disease associations with markers were assessed by Chi-square test or Fisher's exact test, and P values less than 0.05 were considered significant. Statistical analysis was performed using Stata version 10 (StataCorp, College Station, TX, USA). When necessary, P values were subjected to Bonferroni's correction for multiple comparisons. To determine whether genotype frequencies in the populations are compatible with Hardy–Weinberg equilibrium, Hardy–Weinberg exact tests were carried out using the program Arlequin version 3.11 (Excoffier et al. 2007). To assess pairwise LD between polymorphisms, we calculated Lewontin's D' and r square (r^2) for polymorphisms by Haploview version 4.2 (Barrett et al. 2005). TB disease associated with genetic variations was assessed by odds ratios unadjusted or adjusted for sex, age at recruitment and its interaction using logistic models. Tendency of having resistant alleles in the order of age at diagnosis was also tested using a similar logistic model within the TB group. Difference in luciferase activity between the haplotype under consideration and the other haplotypes was assessed by Wilcoxon rank sum/Mann–Whitney U test.

Results

Microsatellite markers

Microsatellite marker *IFNGR2*-MS1 located in 5′-upstream region of *IFNGR2* showed significant association with TB even after Bonferroni's correction (Table 1) and the frequency of *IFNGR2*-MS1-325 allele was significantly lower in TB patients than in controls (Supplementary table 1). *IFNGR2*-MS2, the other microsatellite was located in intron 2 of *IFNGR2* and the frequency of *IFNGR2*-MS2-252 allele was also lower in TB patients than in controls (uncorrected $P = 0.0024$), but not significant after Bonferroni's correction. *IFNGR2*-MS1-325 allele and *IFNGR2*-MS2-252 allele were in LD ($D' = 0.91$, $r^2 = 0.64$).

Screening of genetic polymorphisms in *IFNGR2*

Forty-eight control samples were subjected to PCR amplifications of promoter and seven exons of *IFNGR2* and their sequences were analyzed for possible polymorphisms. In the exonic sequences of *IFNGR2*, a non-synonymous SNP, rs9808753 was found in exon 2, and another SNP, rs1059293 was shown in 3′-untranslated region (UTR) of exon 7, while there were no SNPs in exon–intron boundaries. In the 5′ region up to −850 bp of the translation initiation codon, three SNPs, rs8134145, rs8126756 and rs17882748 were also identified.

Genotyping of selected SNPs around *IFNGR2*

Association of microsatellite markers of *IFNGR2* with TB prompted us to identify relevant SNPs that may show

Table 1 Association results for microsatellite markers

Marker	Locus	No. of alleles (total)	No. of alleles (<5% grouped[a])	*P* value[b] (2 × m)	Minimum *P* value[b] (2 × 2)	*P* value[c] Corrected	*P* value HWE
IFNGR1-MS1	6q23.3	14	7	0.419	0.0549	NS	0.4858
IFNGR2-MS1	21q22.11	8	5	0.016	0.0009	0.036	0.2762
IFNGR2-MS2		6	4	0.013	0.0024	NS	0.0326
IL12RB1-MS1	19p13.1	4	4	0.366	0.1600	NS	0.1606
IL12RB2-MS1	1p31.3-p31.2	12	6	0.155	0.0267	NS	0.7499
IL12RB2-MS2		6	4	0.540	0.2228	NS	0.7289
STAT1-MS1	2q32.2	13	5	0.563	0.3010	NS	0.0254
STAT4-MS1	2q32.2-q32.3	11	5	0.232	0.1046	NS	0.5243

NS not significant, *HWE* Hardy–Weinberg equilibrium

[a] Alleles with frequencies less than 5% were grouped

[b] Fisher's exact test

[c] Bonferroni's correction. 2 × 2 minimum *P* value was multiplied by the numbers of total alleles (40)

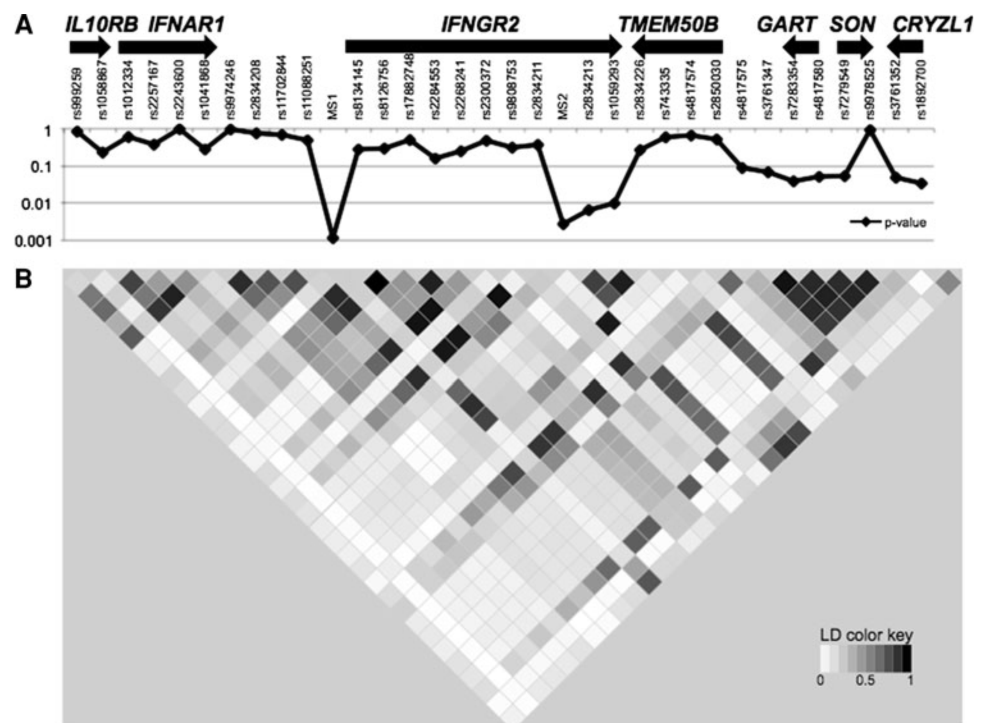

Fig. 1 Association *P* values and pairwise LD of genotyped polymorphisms around *IFNGR2* region. **a** Association results for 32 SNPs in 273 patients (panel A) and 506 controls are shown. *P* values of microsatellite markers in 98 patients and 200 controls are also included. Positions of genes are shown on the top. **b** Pairwise LD (r^2) between 32 SNPs and 2 microsatellite markers determined by the Haploview program is shown. In the calculation of pairwise LD, microsatellite alleles except for one showing the smallest *P* value were grouped, and the microsatellite locus was regarded as having only two alleles

further association. We genotyped 27 SNPs selected around *IFNGR2*, 3 SNPs in the 5′ upstream region, a non-synonymous SNP in exon 2, and a 3′UTR SNP in exon 7 of *IFNGR2* in 273 TB patients (panel A) and 506 controls (Fig. 1, Supplementary table 2). The rs2834213 SNP in intron 2 and the rs1059293 SNP in 3′UTR were associated with TB ($P = 0.0073$, OR 0.69 95% confidence interval [CI] 0.52–0.91; $P = 0.0088$, OR 0.70 95% CI 0.54–0.92). These SNPs were in Hardy–Weinberg equilibrium in the control group. We confirmed that SNPs in other nearby genes were not associated with the disease (Supplementary table 2). As expected, the resistant G allele of rs2834213 and C allele of rs1059293 were both in LD with *IFNGR2*-MS1-325 allele and *IFNGR2*-MS2-252 allele (Supplementary Fig. 1). Particularly, the resistant G allele of rs2834213 in intron 2 was in high LD with *IFNGR2*-MS1-325 allele ($D' = 0.94$, $r^2 = 0.83$), that is located 1.9 kb upstream of the translation initiation codon.

Directly determined haplotypes consisting of three SNPs in the 5′ GC-rich region of *IFNGR2*

In addition to single SNPs associated with the disease, we also characterized a set of SNPs in the 5′ GC-rich region of

the gene, rs8134145, rs8126756 and rs17882748, since these three SNPs are closely located within 300 bp upstream of the transcription start site as discussed below, which may influence *IFNGR2* expression. When we directly determined haplotypes of three 5′ SNPs by allele-specific sequencing in 273 patients and in 506 controls, three common haplotypes (CCC, ATC and ATT) accounted for 99.7% of chromosomes. The haplotype ATC was in high LD with the intron 2 SNP rs2834213 ($D' = 0.97$, $r^2 = 0.82$), and frequencies of the ATC haplotype were significantly lower in patients than in controls ($P = 0.036$, OR 0.76 95% CI 0.58–0.99). Haplotypes carrying SNPs in the entire *IFNGR2* region and their frequencies were estimated in 273 patients and in 506 controls. Consequently, the G allele of the intron 2 SNP rs2834213, the C allele of the 3′UTR SNP rs1059293 and the directly determined haplotype ATC, are uniquely contained in the same haplotype as shown in Supplementary table 3.

Transcription start site (TSS) of *IFNGR2*

In the public database, the aforementioned 5′ SNPs, rs8134145, rs8126756, and rs17882748 are regarded as variants in 5′ UTR, since TSS of the reference cDNA sequence (NM_005534.3) is located at position −648 of the translation initiation codon. However, multiple TSS were actually reported in *IFNGR2,* the positions of which were distributed from the initiation codon to almost 990 bp upstream, presumably due to cell type differences (Rhee et al. 1996). For this reason, we determined the 5′ ends by 5′ RACE in our study. As a result, TSS obtained from all immune cell lines tested were 121 bp upstream of the initiation codon. Thus, the positions of the three SNPs were calculated as −295, −285 and −8 from the TSS, indicating that they are promoter variants in these cell types.

Association results of TB panel B

We selected the intron 2 SNP, rs2834213 as a representative SNP for the disease-resistant polymorphisms and genotyped 503 patients in TB panel B, which were compared with the original control subjects ($N = 506$) in Table 2. The G allele of rs2834213 was significantly associated with TB in panel B ($P = 0.0025$, OR 0.71 95% CI 0.57–0.89). In a logistic model to assess possible confounders, adjusted odds ratios was compared with non-adjusted odds ratios for the G allele, which were hardly affected by sex, age at recruitment and its interaction term, indicating that the *IFNGR2* SNP remained significantly associated with TB in dominant and recessive models respectively ($P = 0.016$ and $P = 0.004$; table not shown).

Furthermore, we set up another logistic model to examine the relationship between having the TB-resistant

Table 2 Association results of rs2834213 A/G SNP

Sample	Allele (frequency)		Genotype (%)			*P* value			OR (95% CI)		
						Allele	Genotype		Allele	Genotype	
	A	G	A/A	A/G	G/G		Dominant	Recessive		Dominant	Recessive
TB panel A	452 (0.837)	88 (0.163)	186 (68.9)	80 (29.6)	4 (1.5)	0.0073	0.047	0.0050	0.69 (0.52–0.91)	0.73 (0.57–0.92)	0.25 (0.08–0.72)
TB panel B	838 (0.833)	168 (0.167)	347 (67.0)	144 (28.6)	12 (2.4)	0.0025	0.015	0.0068	0.71 (0.57–0.89)	0.72 (0.56–0.95)	0.40 (0.20–0.80)
TB combined	1290 (0.834)	256 (0.166)	533 (69.0)	224 (29.0)	16 (2.1)	0.00054	0.0075	0.00048	0.70 (0.57–0.86)	0.73 (0.57–0.92)	0.35 (0.18–0.65)
Controls	786 (0.780)	222 (0.220)	311 (61.7)	164 (32.5)	29 (5.8)						

TB tuberculosis, *OR* odds ratio, *CI* confidence interval

Table 3 Tendency of having G allele (rs2834213) in the order of age strata at the time of diagnosis ($N = 757$)

Age at diagnosis (year)	GA or GG genotype (*n*/*N*)	(%)	Odds ratio per 10-year change* (95% CI)
16–25	35/124	28.2	0.88 (0.79–0.98)
26–35	43/171	25.1	
36–45	53/165	32.1	
46–55	54/171	31.6	
56–65	35/87	40.2	
65–	15/39	38.5	

* In a logistic model, the trend of having the G allele was calculated as odds ratio when the patients are 10-years younger at the time of diagnosis ($P = 0.019$)

G alleles (as binary outcome) and age at diagnosis (as a continuous variable). In patients from panel A and B ($n = 757$), the TB-resistant G allele was less frequently found, as the age at diagnosis was younger ($P = 0.011$). Similarly, in the age-stratified analysis, when the patients are 10 years younger at the time of diagnosis, the odds ratio (OR) for having the G allele was 0.88 (95% CI, 0.79–0.98) and this trend remained significant ($P = 0.019$) (Table 3).

Luciferase assay

We constructed plasmids containing 5′ fragments in which only nucleotide sequences of the three promoter SNPs rs8134145, rs8126756, and rs17882748 are different and measured transcriptional activity of the three promoter segments (CCC, ATC, and ATT) in Jurkat human T-cell leukemia cells. Consequently, the resistant ATC haplotype had significantly higher transcriptional activity than CCC haplotype and ATT haplotype ($P = 0.037$ respectively) by Mann–Whitney U test (Fig. 2).

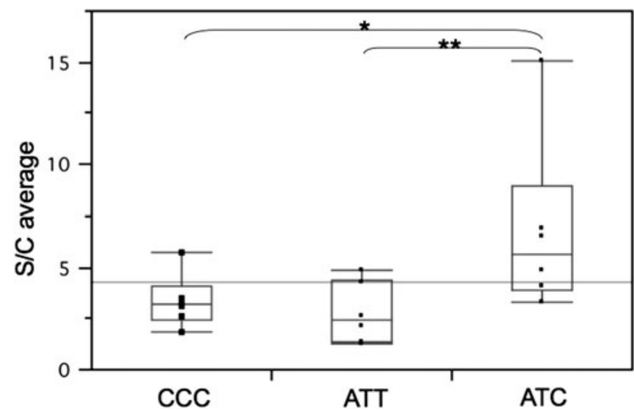

Fig. 2 Dual luciferase reporter assays. The ratios of Firefly luciferase activity (signal S) to Renilla luciferase activity (control C) are displayed using *box* and *whisker plots*. Three subcloned plasmids were prepared, and each subcloned plasmid was tested in triplicate and S/C values were averaged. The experiments were carried out twice independently. As a result, six independent S/C values were obtained for each haplotype. ATC haplotype showed significantly higher transcriptional activity than CCC haplotype and ATT haplotype (*, **$P = 0.037$, respectively) by Mann–Whitney U test. No significant difference was observed between CCC and ATT haplotypes (data not shown)

Discussion

IFN-γ plays a crucial role in host defense against intracellular pathogens mainly through activation of macrophages and regulation of Th1 cell response (Boehm et al. 1997). IL-12 released from dendritic cells and macrophages drives production of IFN-γ via IL-12 receptors, IL12RB1 and IL12RB2, on Th1 cells and subsequent activation of STAT4. In turn, IFN-γ binds to IFN-γ receptors composed of IFNGR1 and IFNGR2 subunits and transduces STAT1 signals to target cells (Bach et al. 1997).

In this study, we first screened eight microsatellite markers within the genes encoding these Th1 cytokine receptors and signal transducers, and demonstrated that the *IFNGR2* marker alleles showed significant association with active TB. SNPs around the gene were analyzed and a strong disease association with the intron 2 SNP rs2834213, the 3′UTR SNP rs1059293 and the 5′ promoter segment characterized by three SNPs was thus demonstrated. Possible influence of population substructure was kept to a minimum, since their ethnicity was Hanoi Vietnamese in which more than 99% were the Kinh people (Hoa et al. 2008).

To our knowledge, this is the first report of *IFNGR2* polymorphisms associated with TB. Intron 2 SNP, rs2834213 was most robustly associated with TB, but its biological importance is currently unclear. Indeed, it was not located near the splice sites (5,582 nucleotides downstream of splice donor site and 877 nucleotides upstream of splice acceptor site). A SNP in 3′UTR of exon 7 rs1059293 was in strong LD with the rs2834213 and also associated with TB, but it was 99 nucleotides upstream of polyadenylation signal. *IFNGR2* did not carry any non-synonymous SNP in high LD with rs2834213.

In an attempt to search functional polymorphism(s) in strong LD with the intron SNP (rs2834213) further, we identified the 300 bp promoter segment containing three SNPs. HapMap database does not have data of the three promoter SNPs, presumably due to high GC content that hinders high throughput genotyping method. The direct haplotyping revealed that it was also associated with the disease as well as the intron 2 SNP. Although we demonstrated that the promoter ATC haplotype showing an inverse disease association has high transcriptional activity in vitro and may confer resistance to TB, we could not

conclude which polymorphism around *IFNGR2* is primarily responsible for the disease until the functional roles of other SNPs showing more robust association are fully studied.

Among previous TB association studies with Th1-related genes, CC genotype at the -56 C/T SNP (rs2234711) of *IFNGR1* was repeatedly associated with TB in African populations (Cooke et al. 2006; Stein et al. 2007). In our study, *IFNGR1*-MS1-158 allele was in strong LD with −56 SNP (Tanaka et al. 2005), but this *IFNGR1* marker allele was not associated with TB. The lack of association is presumably because of insufficient power to detect weak genetic effects. Otherwise, it could be due to population-specific LD, when the true causative variant was not −56 SNP itself.

Experimental data have shown that IFNGR2 is a key regulator for IFN-γ-STAT1 signaling in T cells (Schroder et al. 2004; Regis et al. 2006). During the development of Th1 cells, *IFNGR2* transcription is reduced in the IFN-γ rich condition and this reduction alleviates a potentially harmful anti-proliferative action of IFN-γ-STAT1 signaling. However, IFNGR2 expression is not completely suppressed, because temporary activation of STAT1 is still necessary for Th1 system. *IFNGR2* transcription is thus fine-tuned during the Th1 differentiation process. In the promoter region, the transcriptional activity of the resistant haplotype ATC was higher than the other two common haplotypes in the Jurkat T cell line at baseline levels. Although physiological modulation of *IFNGR2* expression is not easily simulated in a single cell-type model, this segment may have a potential to influence Th1 function through *IFNGR2* regulation.

In this study, another interesting finding is that the resistant allele tend to be less frequently observed in younger patients at the time of diagnosis, a surrogate for age at onset in new patients. This effect was moderate but significant. The allele frequency in older age at diagnosis nearly reached the level of the control population. It is likely that the elderly kept latent infection of *M. tuberculosis* for long years, and the age-associated decline in immune response caused development of active TB, while the younger patients developed active TB soon after initial infection (Tufariello et al. 2003). In intermediate or low burden countries, there are more elderly patients and the effect of the resistant allele of *IFNGR2* may be smaller.

Moreover, in African countries with high rates of TB and HIV co-infection, HIV is the strongest risk factor for TB development (Reid et al. 2006). By contrast, the proportion of HIV-positive TB patients is only 8.8% in the Vietnamese TB panel B and 1.4% in TB panel A, therefore possible effect of the resistant allele on HIV infection could not be determined in this study. In the previous reports, other polymorphisms of *IFNGR2* were associated with liver fibrosis of chronic hepatitis C virus infection and with viremia of hepatitis B virus infection (Nalpas et al. 2010; Huang et al. 2011). Because IFN-γ is a key cytokine for the control of infectious diseases, association of *IFNGR2* polymorphisms with HIV infection needs be clarified.

One limitation in our study is a single control panel of the Vietnamese population. Results of the first case–control set were only partially confirmed because of incomplete independence of the two study sets, though sample size itself was not small. Another limitation is that our control panel may include asymptomatic individuals with latent TB infection, because performing tuberculin testing is not common in Vietnam. Considering two-stage process of infection with the pathogen and progression to disease, we cannot directly specify which stage of TB was more affected by *IFNGR2* in our study population. Future use of interferon gamma release assays to detect latent infection of *M. tuberculosis* in this field might be helpful to arrive at a solution (Pai et al. 2008). Because of the complexity of LD structure and the age-dependent effect as regards these variations, carefully conducted studies should be undertaken to reproduce our results in other populations. Validation studies by re-sequencing are also warranted. In non-Asian populations, however, the LD of rs2834213 does not appear to reach the promoter region of *IFNGR2* (data not shown), indicating that the functional promoter haplotype may not be easily found in disease marker association studies by the conventional tag SNP-based approach in other populations.

We conclude that the polymorphisms of *IFNGR2* may confer resistance to TB in Vietnam. It appeared to be different depending on age at diagnosis. Further functional studies are needed to elucidate the genetic susceptibility to TB, fully considering complicated immune process regarding early or late onset of the disease.

Ethical standards We declare that these experiments comply with the current laws of Japan and Vietnam.

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Conflict of interest The authors declare that they have no conflict of interest.

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