MAJOR ARTICLE

Common Polymorphisms in the *PKP3-SIGIRR-TMEM16J* Gene Region Are Associated With Susceptibility to Tuberculosis

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(See the editorial commentary by Wilkinson, on pages 525–7.)

Background. Tuberculosis has been associated with genetic variation in host immunity. We hypothesized that single-nucleotide polymorphisms (SNPs) in *SIGIRR*, a negative regulator of Toll-like receptor/IL-1R signaling, are associated with susceptibility to tuberculosis.

Methods. We used a case-population study design in Vietnam with cases that had either tuberculous meningitis or pulmonary tuberculosis. We genotyped 6 SNPs in the *SIGIRR* gene region (including the adjacent genes *PKP3* and *TMEM16J*) in a discovery cohort of 352 patients with tuberculosis and 382 controls. Significant associations were genotyped in a validation cohort (339 patients with tuberculosis, 376 controls).

Results. Three SNPs (rs10902158, rs7105848, rs7111432) were associated with tuberculosis in discovery and validation cohorts. The polymorphisms were associated with both tuberculous meningitis and pulmonary tuberculosis and were strongest with a recessive genetic model (odds ratios, 1.5–1.6; $P = .0006–.001$). Coinheritance of these polymorphisms with previously identified risk alleles in Toll-like receptor 2 and TIRAP was associated with an additive risk of tuberculosis susceptibility.

Conclusions. These results demonstrate a strong association of SNPs in the *PKP3-SIGIRR-TMEM16J* gene region and tuberculosis in discovery and validation cohorts. To our knowledge, these are the first associations of polymorphisms in this region with any disease.

Mycobacterium tuberculosis infects one-third of the world's population, causes active disease in 9 million new cases per year, and leads to almost 2 million deaths annually [1]. Infection with *M. tuberculosis* may result in several outcomes including immediate bacillary multiplication (primary progressive tuberculosis) with or without dissemination, dormant infection (latency), or development of active tuberculosis at a time point remote from the original infection (reactivation). The mechanisms responsible for different tuberculosis outcomes are poorly understood.

Several lines of evidence support the influence of host genetics on susceptibility to tuberculosis including twin-based, observational, candidate gene association, linkage, and genomewide association studies [2–6]. The direct study of tuberculosis susceptibility in humans allows for unique insights not possible in animal models, such as the dissection of tuberculosis phenotypes [3]. We and others have identified associations between common polymorphisms in innate immunity genes and susceptibility to tuberculosis and clinical phenotypes [7–10]. Much of this work has focused on pattern recognition receptors and their associated adaptor proteins [6–11]. Among the most important and best studied pattern recognition receptors are Toll-like receptors (TLRs), a family of transmembrane

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proteins with extracellular leucine-rich repeats, that initiate the host immune response to a variety of pathogens including mycobacteria [2, 6, 12, 13]. Several TLRs, including TLR1/2/4/6/8/9 mediate recognition of mycobacteria and have gene variants that are associated with susceptibility to tuberculosis [6, 8, 9, 14–16]. Although much progress has been made in understanding activation of TLR pathways, mechanisms underlying inhibition and regulation of these pathways are less well understood [17]. Among the identified negative regulators of TLR-pathways are Toll-interacting protein, suppressor of cytokine signaling 1, a variant of myeloid differentiation (MyD88s), and a single immunoglobulin interleukin (IL) 1 receptor (SIGIRR, also known as Toll IL-1 receptor 8) [17, 18]. The role of TLR pathway negative regulators in tuberculosis infection and disease has not been well studied.

M. tuberculosis pathogenesis involves activation of multiple proinflammatory and antiinflammatory signaling pathways in macrophages and dendritic cells (DCs) that subsequently activate the adaptive immune response [12, 19–22]. SIGIRR is a member of the IL-1R family that inhibits MyD88-dependent TLR and IL-1R signaling [23, 24]. The mechanism for this negative-regulatory effect may involve binding of IRAK1 and TRAF-6 preventing signal propagation [25]. *SIGIRR* is conserved among vertebrates [26]. Studies of SIGIRR-deficient mice suggest an important role for SIGIRR in the negative regulation of innate immune pathways and the subsequent effects on adaptive immune responses [27, 28]. There have been no genetic studies of *SIGIRR* to assess its possible role in human disease.

Because SIGIRR has a negative regulatory effect on TLR pathways, we hypothesized that polymorphisms in the *SIGIRR* gene region are associated with susceptibility to tuberculosis disease and clinical subtypes. We report the association of *SIGIRR* gene region single-nucleotide polymorphisms (SNPs) with tuberculosis. To our knowledge, this is the first reported association of a *SIGIRR* gene region SNP with any disease.

METHODS

Human Subjects

All subjects were recruited from study sites in Ho Chi Minh City, Vietnam. Study subjects with tuberculous meningitis were recruited from 2 hospitals: Pham Ngoc Thach (PNT) Hospital for Tuberculosis and the Hospital for Tropical Diseases that serve the local community and act as tertiary referral centers. Adults (≥15 years of age) were admitted to these centers from 1997 through 2008 with clinical meningitis (nuchal rigidity, abnormal cerebrospinal fluid [CSF] parameters), negative human immunodeficiency virus (HIV) test results, and a positive Ziehl–Neelsen stain for acid-fast bacilli and/or *M. tuberculosis* culture from CSF ("definite tuberculous meningitis"). All tuberculous meningitis cases in the "discovery" cohort were definite tuberculous meningitis. In addition to definite tuberculous meningitis, the validation cohort included subjects with "probable tuberculous meningitis," defined as clinical meningitis plus ≥1 of the following: chest radiograph consistent with active tuberculosis, acid-fast bacilli found in any specimen other than CSF, and clinical evidence of other extrapulmonary tuberculosis [29]. Severity of tuberculous meningitis at presentation was assessed using the British Medical Research Council tuberculous meningitis grade [30].

Discovery cohort subjects with pulmonary tuberculosis were recruited in 2003–2004 from a network of district tuberculosis control units. These subjects were outpatients (≥15 years of age), had no history of tuberculosis treatment, no evidence of miliary or extrapulmonary tuberculosis, negative HIV test results, and sputum smear positive for acid-fast bacilli or *M. tuberculosis* cultured from sputum. Subjects with pulmonary tuberculosis enrolled for the validation cohort were recruited from the PNT hospital from 2006–2008 and differed from the discovery cohort in that both outpatients and inpatients were included, with the majority being outpatients.

Control subjects were enrolled at Hung Vuong Hospital where umbilical cord blood was collected from newborns. All subjects were unrelated and >99% were of the Vietnamese Kinh ethnicity. Written, informed consent was obtained from patients or their relatives if the patient could not provide consent. All protocols were approved by human subject review committees at the Hospital for Tropical Diseases, PNT hospital, Health Services of Ho Chi Minh City, Hung Vuong Hospital, Oxford Tropical Research Ethics Committee, and the University of Washington.

SNP Selection and Assessment of Linkage Disequilibrium

We identified haplotype-tagging SNPs from the Han Chinese population using International HapMap Project data from the Genome Variation Server (SeattleSNPs Program for Genomic Applications, http://gvs-p.gs.washington.edu/GVS/). We searched a region on chromosome 11p15, 50 kilobase upstream and downstream of the *SIGIRR* gene (which spans 11.7 kilobase over 10 exons) for tagged SNPs using an R^2 cutoff of .8 for linkage disequilibrium and a minor allele frequency cutoff of .1. We selected 6 SNPs in this region that were located in *SIGIRR* and 2 adjacent genes: *PKP3* and *TMEM16J*. Two of the SNPs were intronic to *SIGIRR* (rs7396562 and rs4074794), 1 was intronic to *TMEM16J* (rs7111432), 2 were intronic to *PKP3* (rs10902158 and rs7105848), and 1 was a synonymous coding SNP in *PKP3* (rs11748) (Figure 1).

For SNPs located outside the *SIGIRR* gene that showed association with our tuberculosis outcomes, we used data from the 1000 Genomes Project to assess linkage disequilibrium with polymorphisms in the *SIGIRR* gene (http://browser.1000genomes.org/index.html). We accessed genotyping results that included combined data from the Han Chinese and Japanese populations [31].

Genomic Techniques

Genomic DNA was prepared using the QIAamp DNA blood kit (Qiagen) from peripheral blood samples. Genotyping in the

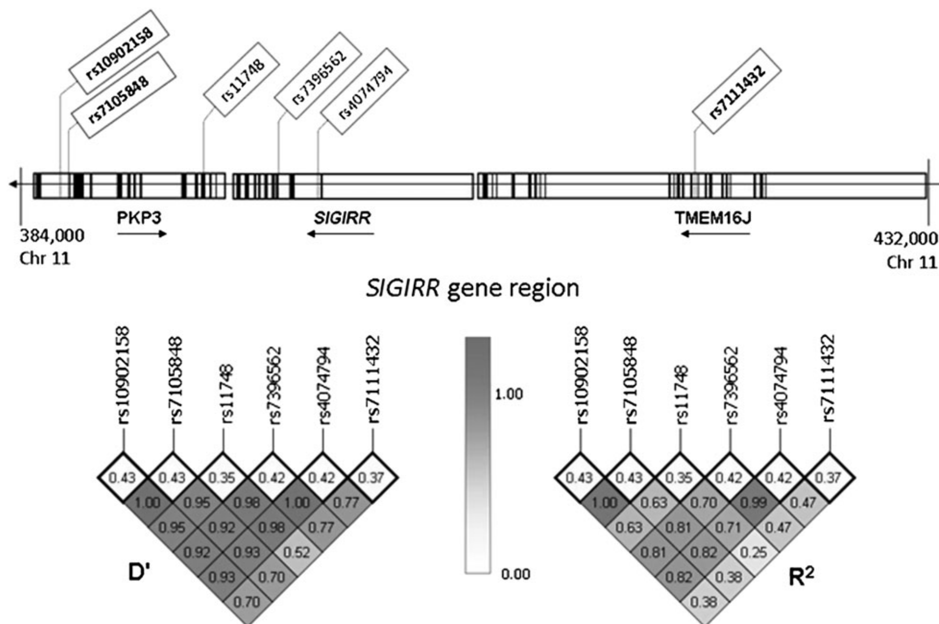

Figure 1. The genomic positions of 6 single-nucleotide polymorphisms (SNPs) in the *SIGIRR* gene region. Exons are shown as black rectangles. D' and R^2 values were calculated from control subjects in the discovery cohort. Values are shown numerically and by shading, based on the legend in the middle. The minor allele frequency is shown in the bolded box immediately below each corresponding SNP. SNPs for which significant associations were found in both cohorts are in bold type.

subjects was performed using a chip-based matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) Mass Array technique (Sequenom), as described elsewhere [32]. Cluster plots were visually inspected to ensure accurate genotyping calls. The call rate for each SNP exceeded 94% in the discovery cohort. All candidate SNPs were in Hardy-Weinberg equilibrium ($P > .05$) among control subjects according to a χ^2 goodness-of-fit test and were further evaluated for association with tuberculosis.

Statistical Methods

We examined associations between allelic and genotypic frequencies in our discovery cohort and the outcome of "any tuberculosis" using Stata 11 software (StataCorp) and the user-written package "genassoc" [33]. SNPs were assessed for association with tuberculosis in the discovery cohort using genotypic models. Those SNPs that had a significant association ($P < .05$) were genotyped in the validation cohort. If findings were confirmed in the validation cohort ($P < .05$), then analysis of the combined cohorts for those SNPs was performed. In the combined cohort, SNPs were investigated under additional genetic models (dominant, recessive, heterozygous advantage, and additive) and for association with the clinical subtypes of tuberculosis. For example, in the recessive model, carriers of allele 0 (00 and 01 genotypes) were compared with homozygous subjects for allele 1 (11 genotype). The Pearson χ^2 test and Student's *t* test were used to assess categorical and continuous clinical variables, respectively. To compare nested logistic regression models, variables were manually deleted and effects on the model assessed using partial likelihood ratio tests [34]. Interactions were assessed by means of the *P* value associated with the cross-product term between the risk genotypes of interest. Measures of linkage disequilibrium were assessed using the "pwld" command in Stata. Haplotypes were constructed with an expectation–maximization algorithm implemented using the "hapipf" function in Stata.

Six candidate polymorphisms were genotyped in our study. Because we used a 2-stage study design to validate findings from the discovery cohort in a validation cohort, we report uncorrected *P* values throughout the manuscript. We tested for population stratification in the discovery cohort using 24 unlinked SNPs that are randomly distributed throughout the genome and not known to be in linkage disequilibrium with any known gene; the null hypothesis is that the allele frequencies at each marker loci are the same in case and control groups [35–37].

RESULTS

Association of *SIGIRR* Region SNPs With Tuberculosis

We used a case-population study design to examine whether polymorphisms in the *SIGIRR* gene region were associated with susceptibility to tuberculosis. We examined 352 tuberculosis case patients (182 pulmonary tuberculosis, 170 tuberculous meningitis) and 382 controls in our discovery cohort and 339 tuberculosis case patients (212 pulmonary tuberculosis,

127 tuberculous meningitis) and 376 controls in our validation cohort (Table 1). Although study subjects self-identified their ancestry as Vietnamese Kinh, we formally tested for levels of background genetic differences between cases and controls by genotyping the entire discovery cohort for a panel of 24 independent SNP markers [35, 36]. The mean χ^2 statistic for the 24 SNPs comparing allelic frequencies between cases and controls was 1.6 ($P = .20$), suggesting that no significant population stratification was present in our discovery cohort (Supplemental Table 1).

We first examined whether 6 SNPs were associated with all forms of tuberculosis in our discovery cohort (Table 2). When we evaluated the SNPs with a genotypic model, 5 SNPs were identified as significantly associated with tuberculosis: rs10902158 ($P = .02$), rs7105848 ($P = .03$), rs11748 ($P = .04$), rs7396562 ($P = .02$), and rs7111432 ($P = .0003$). We used a second cohort to validate SNPs associated with tuberculosis in the discovery cohort. In a genotypic model, 3 of the 5 SNPs were significant: rs10902158 ($P = .0001$), rs7105848 ($P = .006$), and rs7111432 ($P = .007$). There was evidence of mild to moderate linkage disequilibrium in the combined cohort between rs7111432 and rs7105848 ($D' = .69$; $R^2 = .35$), and rs7111432 and rs10902158 ($D' = .70$; $R^2 = .36$) (Figure 1). There was complete linkage disequilibrium between rs7105848 and rs10902158 in the control subjects ($D' = 1.0$; $R^2 = 1.0$); however, this was not true in case patients ($R^2 = .93$). Together, these results suggested that 3 polymorphisms in the *SIGIRR* region were strongly associated with susceptibility to tuberculosis in discovery and validation cohorts.

Table 1. Clinical Characteristics of Discovery and Validation Cohorts

Characteristics	Discovery Cohort (n = 734)	Validation Cohort (n = 715)
Case patients, No. (%)		
Pulmonary tuberculosis	182 (25)	212 (30)
Tuberculous meningitis	170 (23)	127 (18)
Definite	170	54
Probable	0	73
Controls, No. (%)	382 (52)	376 (50)
Age, median ± SD, y		
Case patients	35 ± 16.4	35 ± 14.9
Pulmonary tuberculosis	36.5 ± 16.7	35 ± 12.7
Tuberculous meningitis	33 ± 16.0	36 ± 17.6
Male sex, No. (%)		
Case patients	199 (57)	229 (68)
Pulmonary tuberculosis	114 (63)	162 (76)
Tuberculous meningitis	85 (50)	67 (53)
Controls	207 (54)	172 (46)
Tuberculous meningitis grade, No. (%)[a]		
1	42 (25)	29 (23)
2	81 (48)	28 (22)
3	42 (25)	9 (7)
Not recorded	5 (3)	61 (48)

Abbreviation: SD, standard deviation.

[a] British Medical Research Council grade; there was a significant difference in grades between cohorts ($P < .001$).

Analysis of rs10902158, rs7105848, and rs7111432 in the Combined Cohort

For the 3 significant polymorphisms, we examined these associations further under different genetic models and for different clinical phenotypes in a combined cohort of the discovery and validation sample sets. For SNP rs10902158, the P value for the genotypic model was .00008 for the combined sample sets (Table 3). Testing the association with tuberculosis under different genetic models, we found the strongest association was consistent with a recessive model (odds ratio [OR], 1.53; 95% confidence interval [CI], 1.19–1.97; $P = .0006$) (Table 3 and data not shown). Similarly, polymorphisms rs7105848 and rs7111432 were strongly associated with tuberculosis in a recessive model (ORs, 1.52 [95% CI, 1.18–1.96, $P = .0007$] and 1.61 [95% CI, 1.20–2.17; $P = .001$], respectively).

Association of *SIGIRR* Gene Region SNPs With Various Phenotypes of Tuberculosis

We next performed several secondary analyses to further examine the association of these 3 *SIGIRR* region polymorphisms and susceptibility to tuberculosis. We examined whether these associations were specific for tuberculous meningitis or pulmonary tuberculosis. All 3 SNPs (rs10902158, rs7105848, rs7111432) were associated with both pulmonary and meningeal tuberculosis to a similar degree (Table 3). We constructed haplotypes of the SNPs that were significantly associated with tuberculosis to analyze whether there were additive associations among the different alleles. Because rs10902158 and rs7105848 are in near complete linkage disequilibrium, we included only rs10902158 in addition to rs7111432 in this analysis. We found 2 haplotypes (01 and 10) that were associated with all types of tuberculosis combined (ORs, 1.38 [95%, CI, 1.02–1.88] and 1.38 [1.10–1.71], respectively) (Table 4). We found 1 haplotype (10) associated with pulmonary tuberculosis and 2 haplotypes (01 and 10) associated with tuberculous meningitis (Table 4). These results suggest that the haplotype analyses did not reveal stronger associations than those found in single polymorphism analyses.

We evaluated whether misclassification of subjects with tuberculous meningitis may have affected our outcomes. For tuberculous meningitis cases, the discovery cohort exclusively contained those with definite tuberculous meningitis, whereas the validation cohort had subjects with both definite and probable tuberculous meningitis (Table 1). We examined associations between the 3 validated SNPs and tuberculous meningitis in subjects with definite tuberculous meningitis only. In this

Table 2. ***SIGIRR* Gene Region Single-Nucleotide Polymorphisms (SNPs) and Genotype Frequencies in Control and Tuberculosis Groups**

SNP and Polymorphism Base Pair	Group	Allele Frequency, No. (%)		Subjects With Genotype, No. (%)			Genotypic Comparison	
		0	1	00	01	11	χ^2 (2 *df*)	*P*
rs10902158	Discovery control	434 (0.568)	330 (0.432)	127 (0.332)	180 (0.471)	75 (0.196)		
A/G 386308	Discovery cases	348 (0.509)	336 (0.491)	103 (0.301)	142 (0.415)	97 (0.284)	7.616	.02
	Validation control	406 (0.544)	340 (0.456)	111 (0.298)	184 (0.493)	78 (0.209)		
	Validation cases	360 (0.556)	288 (0.444)	126 (0.389)	108 (0.333)	90 (0.278)	18.234	.0001
rs7105848	Discovery control	432 (0.568)	328 (0.432)	127 (0.334)	178 (0.468)	75 (0.197)		
T/C 386546	Discovery cases	344 (0.510)	330 (0.490)	102 (0.303)	140 (0.415)	95 (0.282)	7.070	.03
	Validation control	409 (0.547)	339 (0.453)	111 (0.298)	183 (0.492)	78 (0.210)		
	Validation cases	354 (0.535)	308 (0.465)	115 (0.347)	124 (0.375)	92 (0.278)	10.206	.006
rs11748	Discovery control	496 (0.653)	264 (0.347)	170 (0.447)	156 (0.411)	54 (0.142)		
393980	Discovery cases	386 (0.621)	236 (0.379)	140 (0.450)	106 (0.341)	65 (0.209)	6.638	.04
	Validation control	473 (0.650)	255 (0.350)	154 (0.423)	165 (0.453)	45 (0.124)		
	Validation cases	395 (0.637)	225 (0.363)	132 (0.426)	131 (0.423)	47 (0.152)	1.323	.52
rs7396562 C/A	Discovery control	442 (0.580)	320 (0.420)	135 (0.354)	172 (0.451)	74 (0.194)		
A/G 398352	Discovery cases	341 (0.536)	295 (0.464)	111 (0.349)	119 (0.374)	88 (0.277)	7.588	.02
	Validation control	394 (0.576)	290 (0.424)	120 (0.351)	154 (0.450)	68 (0.199)		
	Validation cases	357 (0.572)	267 (0.428)	118 (0.378)	121 (0.388)	73 (0.234)	2.784	.25
rs4074794	Discovery control	439 (0.581)	317 (0.419)	135 (0.357)	169 (0.447)	74 (0.196)		
G/A 399815	Discovery cases	349 (0.535)	303 (0.465)	111 (0.340)	127 (0.390)	88 (0.270)	5.701	.06
rs7111432	Discovery control	481 (0.635)	277 (0.365)	150 (0.396)	181 (0.478)	48 (0.127)		
G/A 419659	Discovery cases	406 (0.613)	256 (0.387)	146 (0.441)	114 (0.344)	71 (0.215)	16.547	.0003
	Validation control	466 (0.626)	278(0.374)	144 (0.387)	178 (0.478)	50 (0.134)		
	Validation cases	414 (0.645)	228 (0.355)	149 (0.464)	116 (0.361)	56 (0.174)	9.800	.007

SNPs are arranged in the order that they are located on the chromosome. SNPs are listed by reference SNP ID and followed by major/minor alleles and chromosomal location in base pairs. 0, common allele; 1, allele with minor frequency.

analysis, rs7111432 remained significantly associated with tuberculous meningitis in a recessive model (OR, 1.71; 95% CI, 1.12–2.57; $P = .008$), whereas rs7105848 and rs10902158 were no longer associated with tuberculous meningitis (ORs, 1.11 [95% CI, .75–1.62] and 1.28 [.88–1.85], respectively). The exclusion of subjects with probable tuberculous meningitis did not significantly change the associations between the 3 validated SNPs and any tuberculosis. We found no association between the validated SNPs and survival or neurologic outcome in subjects with tuberculous meningitis. There were no associations between the 3 SNPs and *M. tuberculosis* lineage (Beijing vs non-Beijing) (data not shown).

Coinheritance of *SIGIRR* Gene Region SNPs and Known Tuberculosis Risk Alleles

SIGIRR is a negative regulator of TLR signaling and may directly interact with TLRs through its TIR domain. We previously found Toll-interleukin 1 receptor domain containing adaptor protein (TIRAP) SNP C558T and TLR2 SNP C597T to be associated with susceptibility to tuberculosis [7, 8]. We examined whether coinheritance of *SIGIRR* variants with other tuberculosis risk genotypes was associated with additive or synergistic risks of susceptibility to tuberculosis. We first evaluated nested logistic regression models that included either rs7111432 alone or with rs10902158. Using a likelihood ratio test, we found nosignificant difference between the models ($P = .24$) and included only rs7111432 for subsequent coinheritance model analyses. We coded rs7111432 using a recessive genetic model (00/01 vs 11), in conjunction with the other risk SNPs that were categorized according to the genetic model associated with risk in the original publications (TIRAP C558T—additive, TLR2 C597T—recessive). For all combinations, we observed an additive increase in risk for tuberculosis. Coinheritance of the risk genotype (11) at rs7111432 with 2 copies of the risk allele at TIRAP C558T (11) had an OR for tuberculosis of 7.3 (95% CI, 2.7–20.2) compared with an individual without either risk genotype (Table 5). Coinheritance of the risk alleles at rs7111432 (11) and TLR2 SNP C597T (11) had an increased OR for all tuberculosis of 4.0 (95% CI, 1.9–8.5). We do not present a model with all 3 genotypes, because none of the subjects had all of the susceptibility genotypes. The cross-product interaction term was not significant for either of these coinheritance models. Together, these results suggest that coinheritance of *SIGIRR* polymorphisms with TLR2 and TIRAP risk alleles is associated with an additive rather than synergistic risk of susceptibility to tuberculosis.

Table 3. Associations of rs7105848 and rs711432 in Combined Cohorts With All Tuberculosis, Pulmonary Tuberculosis, and Tuberculous Meningitis

		Allele Frequency, No. (%)		Subjects With Genotype, No. (%)			Genotypic Model		Recessive Model	
SNP	Group	0	1	00	01	11	χ^2	*P*	OR (95% CI)	*P*
rs10902158	Control	840 (0.556)	670 (0.444)	238 (0.315)	364 (0.482)	153 (0.203)			1	
A/G	All tuberculosis	709 (0.531)	625 (0.469)	229 (0.343)	251 (0.376)	187 (0.280)	18.963	.00008	1.53 (1.19–1.97)	.0006
	Pulmonary tuberculosis	408 (0.540)	348 (0.460)	133 (0.352)	142 (0.376)	103 (0.272)	12.861	.002	1.47 (1.09–1.98)	.008
	Tuberculous meningitis	301 (0.521)	277 (0.479)	96 (0.332)	109 (0.377)	84 (0.291)	12.400	.002	1.61 (1.17–2.22)	.002
rs7105848	Control	837 (0.557)	667 (0.443)	238 (0.316)	361 (0.480)	153 (0.203)			1	
T/C	All tuberculosis	698 (0.522)	638 (0.478)	217 (0.325)	264 (0.395)	187 (0.280)	14.505	.0007	1.52 (1.18–1.96)	.0007
	Pulmonary tuberculosis	394 (0.516)	370 (0.484)	123 (0.322)	148 (0.387)	111 (0.291)	13.124	.001	1.60 (1.19–2.15)	0.001
	Tuberculous meningitis	304 (0.531)	268 (0.469)	94 (0.329)	116 (0.406)	76 (0.266)	6.238	.04	1.42 (1.02–1.97)	.03
rs7111432	Control	947 (0.630)	555 (0.370)	294 (0.391)	359 (0.478)	98 (0.130)			1	
G/A	All tuberculosis	820 (0.629)	484 (0.371)	295 (0.452)	230 (0.353)	127 (0.195)	25.132	3×10^{-6}	1.61 (1.20–2.17)	.001
	Pulmonary tuberculosis	490 (0.662)	250 (0.338)	189 (0.511)	112 (0.303)	69 (0.186)	31.544	1×10^{-7}	1.52 (1.07–2.17)	.01
	Tuberculous meningitis	330 (0.585)	234 (0.415)	106 (0.376)	118 (0.418)	58 (0.206)	9.379	.009	1.73 (1.18–2.50)	.003

SNPs are listed by reference SNP ID and followed by major/minor alleles. For calculations of OR, each group was compared with the control group. 0, common allele; 1, allele with minor frequency. ''All tuberculosis'' includes both pulmonary and meningeal tuberculosis.

Abbreviations: CI, confidence interval; OR, odds ratio; SNP, single-nucleotide polymorphism.

Linkage Disequilibrium Between Validated and *SIGIRR* Polymorphisms

After we originally hypothesized that polymorphisms in *SIGIRR* are associated with tuberculosis susceptibility, we next evaluated linkage disequilibrium between our 3 validated SNPs (located in genes adjacent to *SIGIRR*) and *SIGIRR* polymorphisms. Using data from the 1000 Genomes Project (HCB/JPT populations), we identified 7 SNPs in the *SIGIRR* gene (Supplemental Figure 1). For these 7 SNPs, we found values for D' that ranged from .85 to .96 for rs10902158/rs7105848 and .70 to .89 for rs7111432.

Table 4. *SIGIRR* Gene Region Haplotype Frequencies in Control and Tuberculosis Groups

	Haplotype (rs10902158 [A/G] and rs7111432 [G/A])			
Group	AG–00	AA–01	GG–10	GA–11
Control	748.5 (0.258)	95.1 (0.033)	208.1 (0.072)	464.2 (0.160)
All tuberculosis	628.4 (0.217)	110.2 (0.380)	239.8 (0.083)	403.6 (0.139)
All tuberculosis vs control				
OR (95% CI)	1	1.38 (1.02–1.88)	1.38 (1.10–1.71)	1.04 (.87–1.24)
P	…	.03	.003	.66
Pulmonary tuberculosis	380.0 (0.165)	48.0 (0.021)	140.4 (0.061)	219.6 (0.095)
Pulmonary tuberculosis vs control				
OR (95% CI)	1	1.0 (.67–1.46)	1.33 (1.03–1.71)	0.93 (.76–1.15)
P	…	.98	.02	.51
Tuberculous meningitis	248.4 (0.118)	62.2 (0.029)	99.6 (0.047)	183.8 (0.087)
Tuberculous meningitis vs control				
OR (95% CI)	1	1.97 (1.36–2.84)	1.45 (1.09–1.93)	1.20 (.95–1.51)
P	…	.0001	.008	.11

Data are estimated haplotype number (frequency) unless otherwise indicated. Haplotypes represent alleles composed of 2 *SIGIRR* gene region single-nucleotide polymorphisms: rs10902158 (A/G) and rs7111432(G/A). ORs were calculated in reference to the 00 haplotype. ''All tuberculosis'' includes both pulmonary and meningeal tuberculosis. 0, common allele; 1, allele with minor frequency.

Abbreviations: CI, confidence interval; OR, odds ratio.

Table 5. Coinheritance of *SIGIRR* Single-Nucleotide Polymorphism (SNP) rs7111432 and Select SNPs Associated With Susceptibility to Tuberculosis

rs7111432 genotype	TIRAP C558T	TLR2 C597T	All Tuberculosis (n = 314)	Controls (n = 387)	OR (95% CI)	P
00/01	00	…	0.678	0.819	Reference	
00/01	01	…	0.100	0.047	2.01 (1.26–3.19)	.003
00/01	11	…	0.013	0.005	4.03 (1.59–10.17)	.003
11	01	…	0.022	0.008	3.66 (1.97–6.78)	.000
11	11	…	0.003	0.003	7.34 (2.66–20.23)	.000
00/01	…	00/01	0.711[a]	0.830[b]	Reference	
00/01	…	11	0.08[a]	0.043[b]	2.22 (1.21–4.07)	.01
11	…	00/01	0.19[a]	0.122[b]	1.82 (1.21–2.74)	.004
11	…	11	0.019[a]	0.005[b]	4.04 (1.93–8.45)	.000

SNPs are listed by reference SNP ID. For rs7111432, genotype 11 is associated with susceptibility to tuberculosis. For calculations of ORs, each group was compared with the reference group. 0, common allele; 1, allele with minor frequency. ''All tuberculosis'' includes both pulmonary and meningeal tuberculosis.

Abbreviations: CI, confidence interval; OR, odds ratio; SNP, single-nucleotide polymorphism.

[a] For these data, n = 311.

[b] For these data, n = 376.

Values for R^2 were .47–.78 and .46–.53 for rs10902158/rs7105848 and rs7111432, respectively. These results suggest that the 3 tuberculosis-associated polymorphisms are in linkage disequilibrium with SNPs within the *SIGIRR* gene.

DISCUSSION

The primary finding of our study is that 3 *SIGIRR* gene region SNPs (rs10902158, rs7111432, rs7105848) were associated with susceptibility to tuberculosis in independent cohorts of Vietnamese subjects. To our knowledge, these are the first associations of *SIGIRR* gene region SNPs with susceptibility to any infectious disease.

Although the 3 SNPs are located in genes adjacent to *SIGIRR* (rs10902158 and rs7105848 in *PKP3* and rs7111432 in *TMEM16J*), *SIGIRR* remains the most promising gene for a functional association with tuberculosis disease. The 3 SNPs are in moderate to strong linkage disequilibrium with polymorphisms in the *SIGIRR* gene and *SIGIRR* is known to regulate immune responses. In addition, *PKP3* and *TMEM16J* are not known to play a role in immunity. The mechanism of how *SIGIRR* polymorphisms may regulate susceptibility to tuberculosis is unknown. The most direct effect is probably through inhibition of TLR-IL-1R signaling in innate immune cells [17, 23, 26]. Early reports suggested that SIGIRR is highly expressed in monocytes and immature DCs, but not on macrophages[23, 38]. However, a recent study found that SIGIRR is abundantly expressed intracellularly in macrophage colony-stimulating factor– differentiated macrophages and DCs [39]. In an aerosolized *M. tuberculosis* in vivo murine infection model [27], *Sigirr*$^{-/-}$ mice had increased mortality in comparison to wild-type mice. Interestingly, this mortality difference, which was associated with hepatic necrosis, was not accompanied by a difference in bacterial loads. The *Sigirr*$^{-/-}$mice had evidence of a hyperinflammatory response with greater cellular recruitment of macrophages and neutrophils and significantly higher levels of tumor necrosis factor α and IL-1β at 4 weeks after infection [27]. There was no difference in interferon-γ production by lung CD4 and CD8 cells. In addition, survival among SIGIRR-deficient mice was improved by administration of neutralizing antibodies to tumor necrosis factor α and IL-1β. Together, these studies suggest that SIGIRR regulates the immune response to *M. tuberculosis* through a direct inhibitory effect on innate immune cells.

In addition to the regulation of innate immune responses by SIGIRR, studies also indicate that SIGIRR modulates adaptive immunity [27, 28, 40]. SIGIRR-deficient mice infected with *Candida albicans* were susceptible to mucosal and disseminated disease and had increased mortality compared to wild-type mice [40]. These poor outcomes were associated with increased frequency of interferon-γ and IL-17 producing T cells in the lymph nodes of *Sigirr*$^{-/-}$ mice and accompanied by higher levels of *C. albicans*–induced IL-1, IL-12p70, and IL-23 in *Sigirr*$^{-/-}$ DCs in comparison with wild-type mice. These data suggest that SIGIRR modulates T-cell polarization through regulation of DC function. Interestingly, SIGIRR may also directly regulate T-cell function. Bulek and colleagues [28] polarized naive T cells from SIGIRR-deficient and wild-type mice into T-helper (Th) 1 and Th2 cells and found significantly higher levels of SIGIRR expression in Th2 cells. Furthermore, in vivo models using IL-33 stimulation (a promoter of Th2 responses) and ovalbumin-induced asthma showed significantly higher serum and lung tissue levels of Th2 cytokines (IL-4, IL-5, and IL-13) in SIGIRR-deficient mice. These data are intriguing, because Th2 cytokines have been shown to inhibit autophagy of *M. tuberculosis* and promote alternative activation of macrophages [41, 42]. These studies of the effect of

SIGIRR on T-cell polarization and cytokine profiles suggest additional mechanisms for an impact on tuberculosis susceptibility.

A potential limitation of our study and case-population studies in general is the misclassification of controls, because some of the cord-blood subjects may develop tuberculosis during their lifetime. The use of cord-blood controls is a common design method in gene association studies and reflects adult background population genotype frequencies [7, 43, 44]. Although the use of cord-blood samples may lead to a modest loss of power, the misclassification of controls underestimates the genetic risk of polymorphisms. Misclassification also may have been introduced through the inclusion of patients with "probable" tuberculous meningitis in our cohort as the definition did not require microbiologic confirmation of *M. tuberculosis* in the CSF. In sensitivity analyses, we found that the association of rs7111432 with tuberculous meningitis persisted after excluding subjects with probable tuberculous meningitis. A second limitation relates to multiple comparisons. Because of our prespecified study design using discovery and validation cohorts, we accepted a *P* value of less than .05 as significant. However, if we had used a conservative Bonferroni correction to establish a significant *P* value in the discovery cohort (.008), we would still have selected SNP rs7111432 for validation.

A third limitation is that the association findings may not be due to polymorphisms within *SIGIRR*. Our aim was to select haplotype-tagging SNPs in the *SIGIRR* gene due to its association with the immune response to pathogens. However, the SNPs chosen for genotyping based on linkage disequilibrium patterns were not all within *SIGIRR*. The tuberculosis-associated SNPs identified in our study are from intronic regions outside the *SIGIRR* gene: rs711432 is intronic to *TMEM16J*, and rs7105848 and rs10902158 are intronic to *PKP3* (plakophilin 3). *PKP3* is upstream of *SIGIRR* and codes for a protein that binds with desmosomes as part of epithelial architecture [45]. *TMEM16J*, located downstream of *SIGIRR,* has an unknown function [46]. Neither *PKP3* nor *TMEM16J* have a reported role in infectious disease pathogenesis. Based on what little is known, we would not expect these genes to be functionally associated with susceptibility to tuberculosis. We hypothesize that the associated SNPs are in linkage disequilibrium with a functionally important variant in the *SIGIRR* gene or promoter region. This would be consistent with the results of our haplotype and logistic regression analyses and will be examined with future fine-mapping studies. Finally, candidate gene association studies are subject to confounding due to population substructure [47]. However, our study population was the Vietnamese Kinh, a highly homogenous population in Southeast Asia, and we found no evidence of population stratification in the discovery cohort using control SNPs [36, 48].

In summary, we found an association between SNPs in the *SIGIRR* gene region and the development of both pulmonary tuberculosis and tuberculous meningitis in discovery and validation cohorts of Vietnamese adults. These findings suggest that negative regulators of inflammation may be important in the control of *M. tuberculosis*.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://www.oxfordjournals.org/our_journals/jid/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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