Original Article

Association of the *TLR1* variant rs5743557 with susceptibility to tuberculosis

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Background: Toll-like receptor 1 (TLR1) and TLR6 play important roles in the innate immune response against *Mycobacterium tuberculosis* (M.TB) via interactions with TIR domain-containing adaptor protein (TIRAP) and myeloid differentiation primary response 88 (MYD88). The aim of this study was to investigate the relationship of *TLR1*, *TLR6*, *MYD88* and *TIRAP* polymorphisms with susceptibility to latent tuberculosis infection (LTBI) and tuberculosis (TB).

Methods: In total, 204 uninfected healthy controls (HC), 201 individuals with LTBI and 209 TB patients were enrolled. Two interferon-γ release assays were used to differentiate individuals with LTBI from uninfected controls. TagSNPs of the four genes were genotyped by the SNPscan™ Kit. The Haploview 4.2 and SHEsis software packages were combined to perform linkage disequilibrium (LD) and haplotype analyses. Multifactor dimensionality reduction (MDR) software was used to investigate gene-gene interaction. The Stata 12.0 software was used to perform meta-analysis of the relationship between rs5743557 and TB susceptibility.

Results: The AA genotype of rs5743557 was associated with reduced TB risk (P=0.006) and the AA/GA genotypes of *TLR1* rs5743604 were associated with increased TB risk (P=0.017) when the LTBI group was compared with the TB group. The frequency of *TLR1* haplotype rs4833095-rs5743604 CG was significantly higher in the LTBI group than in the TB group (P=0.019877). However, only the relationship between rs5743557 and TB susceptibility remained significant after 1000-fold permutation testing (P=0.023). The meta-analysis suggested that rs5743557_A was associated with decreased TB risk in the Chinese adult population (P<0.001, OR 0.80, 95% CI: 0.72–0.88). No significant gene-gene interactions were found.

Conclusions: The results of our study suggest that the tagSNP rs5743557 of *TLR1* is associated with the risk of TB.

Keywords: Myeloid differentiation factor 88; Toll-like receptor 1 (TLR1); Toll-like receptor 6 (TLR6); TIR domain-containing adaptor protein (TIRAP); tuberculosis (TB)

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Introduction

According to the 2016 Global Tuberculosis Report, there were an estimated 10.4 million new tuberculosis (TB) cases and 1.8 million TB deaths worldwide in 2015 (1). Although one third of the worldwide population has been infected with *Mycobacterium tuberculosis* (M.TB) (2), only approximately 5–10% of those infected develop clinical TB in their lifetime (3,4). In China, the TB infection rate was as high as 31.4% and there were 420 million individuals with latent tuberculosis infection (LTBI), as described in the 2000 National Epidemiological Sampling Survey of Tuberculosis (5).

Toll-like receptor 1 (TLR1) and Toll-like receptor 6 (TLR6) are members of the TLR2 subfamily (6) and play important roles in the innate immune response against M.TB by interacting with myeloid differentiation primary response 88 (MYD88) and TIR domain-containing adaptor protein (TIRAP). Variants in four genes (*TLR1*, *TLR6*, *MYD88* and *TIRAP*) were reported to be associated with TB risk and to affect the function of their respective genes. The A allele of rs4833095 in *TLR1* was shown to decrease susceptibility to TB in the Indian population, to increase the TNF response to M.TB lysates in mononuclear cells and to increase NF-κB expression in the HEK cell line (7). The T allele of rs5743810 in *TLR6* was shown to decrease NF-κB signaling activity when HEK293 cells were stimulated by the di-acylated lipopeptide PAM2 and by M.TB lysates (8). The AG genotype of rs6853 in *MYD88* was associated with reduced risk of pulmonary tuberculosis (PTB) and the allele A increased production of TNF-α, IFN-γ and NO (9). Heterozygosity for rs8177374 (S180L) in *TIRAP* was associated with reduced TB susceptibility (9) and the mutation reduced the affinity of TIRAP protein for the interferon-γ receptor and influenced responses to TB (10).

Results from numerous genetic association studies focused on TB risk have been inconsistent. Possible reasons for this lack of reproducibility include different phenotype definitions for TB cases and controls, differences in allele distribution and linkage disequilibrium (LD) among ethnic groups, varieties of M.TB strains, as well as complex gene-gene and gene-environment interactions (11,12).

Moreover, a meta-analysis of 16 published studies identified a 380 gene meta-signature which was uniquely expressed in active TB patients in nine or more datasets (13). In addition, a study revealed that Toll-like receptor-associated genes including *MYD88* and *TLR6* were differentially expressed in TB and LTBI patients (14). Therefore, we hypothesized that genetic and immunologic status are different between LTBI and TB groups and the Toll-like receptor related genes are involved in the pathogenesis of active TB. Furthermore, separating uninfected healthy controls from LTBI individuals might help to reduce discrepancies and further our understanding of the genetic determinants in TB and LTBI susceptibility.

A review for U.S. Preventive Services Task Force demonstrated that the T-SPOT.TB and QuantiFERON TB-GIT tests had higher sensitivity and specificity than the tuberculin skin test (TST) for identifying TB infection (15). Despite the difference in sensitivity between the two interferon-γ release assays (IGRAs), QuantiFERON TB-GIT assay and T-SPOT.TB, they are both widely used to recognize LTBI. Compared with the TST, IGRAs could differentiate TB infection from most nontuberculous mycobacterial (NTM) infection. In addition, Bacille Calmette-Guerin vaccination was common in China and therefore we utilized both IGRAs in this study to discriminate individuals with LTBI from uninfected controls in order to explore the relationship of tagSNPs of four genes (*TLR1*, *TLR6*, *MYD88* and *TIRAP*) with susceptibility to LTBI and TB. In addition, a meta-analysis of the relationship between rs5743557 and TB risk was performed based on recently published reports.

Methods

Study population

Our study recruited 614 unrelated Han volunteers, including 209 TB patients, 201 LTBI subjects and 204 uninfected healthy controls (HC) from 2014 June to 2015 December in the West China Hospital, Chengdu, Sichuan. The confirmed active TB patients were identified based on syndromes, signs, computed tomography (CT) image, detection of acid-fast bacilli (AFB) and TB-DNA in samples such as sputum, biopsy tissues or bronchoalveolar lavage fluid and culture results as described in the diagnosis for PTB in China (16). In addition to culture, the finding of TB-DNA offers strong evidence to confirm TB infection instead of NTM infection. According to the Centers for Disease Control, patients with both positive Nucleic acid amplification test (NAAT) and AFB smear results, or with two or more specimens yielding positive NAAT results can be presumed to have TB (17). Moreover, patients with typical CT imaging and positive TB-DNA results can be diagnosed as confirmed TB in China (16).

Both LTBI and HC participants were chosen from close contacts of bacteria-confirmed PTB patients. All LTBI and HC had neither TB history nor signs indicative of TB according to their chest X-ray results and symptoms, and participants diagnosed with TB during one-year follow up were excluded. LTBI was defined as a state of persistent immune response to M.TB antigens without evidence of clinically active TB (18). Provided that the negative and positive controls were appropriate, the IGRA result was considered positive if the IFN-γ value was >14 pg/mL after subtracting the value of the negative control in the QuantiFERON TB-GIT test (Wantai Biological Pharmaceutical Co., Beijing, China), or if the number of spots was ≥16 in the T-SPOT.TB test. Participants with positive IGRA results were identified as LTBI and participants with negative IGRA results were included as HC. Individuals with HIV infection, primary immune deficiency, autoimmune diseases, diabetes mellitus, malignant tumors, or being treated with immunosuppressive medications were excluded.

All study protocols were approved by the Ethics Committee of the West China Hospital of Sichuan University in China, as shown in File No. 131, approved in 2013. A 3 mL venous blood sample was obtained from each volunteer after acquiring informed consent. The study outcomes will never affect the future management of the patients.

SNP selection and genotyping

Thirteen tagSNPs were selected as previously described (19), including four SNPs in *TLR1* (rs4833095, rs5743557, rs5743596 and rs5743604), four in *TLR6* (rs1039559, rs3775073, rs5743808 and rs5743827), two in *MYD88* (rs6853, rs7744) and three in *TIRAP* (rs595209, rs8177375 and rs3802813). Genomic DNA was extracted from whole blood using AxyPrep genomic DNA Mini kits (Axygen, USA). All SNPs were genotyped by custom-by-design 2x48-Plex SNPscan™ Kit (Cat#:G0104, Genesky Biotechnologies Inc., Shanghai, China). In addition, 5% duplicate samples were tested to evaluate genotyping repeatability and quality.

Meta-analysis of association between rs5743557 and TB susceptibility

The research strategy was the same as in our previously published report (20). Only case-control studies focusing on association between rs5743557 and TB susceptibility were included. After evaluating the Newcastle Ottawa Scale (NOS) of each included study, information including first author, publication year, population, sample size of the cases and controls, HIV status, Hardy-Weinberg equilibrium (HWE), allele frequency of cases and controls, OR and 95%CI was abstracted. Studies were excluded if the genotype distribution deviated from HWE or the NOS scores were <4.

Statistical analysis

The chi-squared test, non-paramateric test and binary logistic regression were performed using the Statistical Package for Social Sciences version 17.0 software (SPSS Inc., Chicago, IL, USA) for analysis of demographic characteristics and association under three genetic models (dominant, recessive and additive). It is well known that the Bonferroni correction is too conservative as it fails to take correlation among SNPs into account, which might lead to a high false negative rate. Therefore, we applied the permutation test instead, which is considered to be the gold standard of multiple testing correction in genome-wide association studies (21), to adjust the results in our study. Both the HWE and permutation test were calculated using the Plink 1.90 software (Shaun Purcell, Christopher Chang, www.cog-genomics.org/plink/1.9/) (22). Haploview 4.2 and the online software SHEsis were combined to perform LD and haplotype analysis, as well as permutation correction of the haplotype results (23,24). Nonparametric multifactor dimensionality reduction (MDR) was used to investigate gene-gene interactions. Statistical power was calculated with the PS: Power and Simple Size Calculation version 3.1.2 software (Dupont WD, Plummer WD, Nashville, Tennessee of USA, http://biostat.mc.vanderbilt.edu/). A significant P value was considered as a two-sided $P<0.05$. The Stata 12.0 software was used to accomplish the meta-analysis. We used a fixed effects model when the heterogeneity results showed $P>0.1$ and I^2 <50%, otherwise a random effects model was applied. In addition, subgroup analysis was performed.

Results

Meta-analysis results of relationship between rs5743557 and TB risk

As shown in the flow diagram (*Figure S1*), only three reports including 2,179 TB patients and 2,049 healthy controls were enrolled (20,25,26). These reports contained

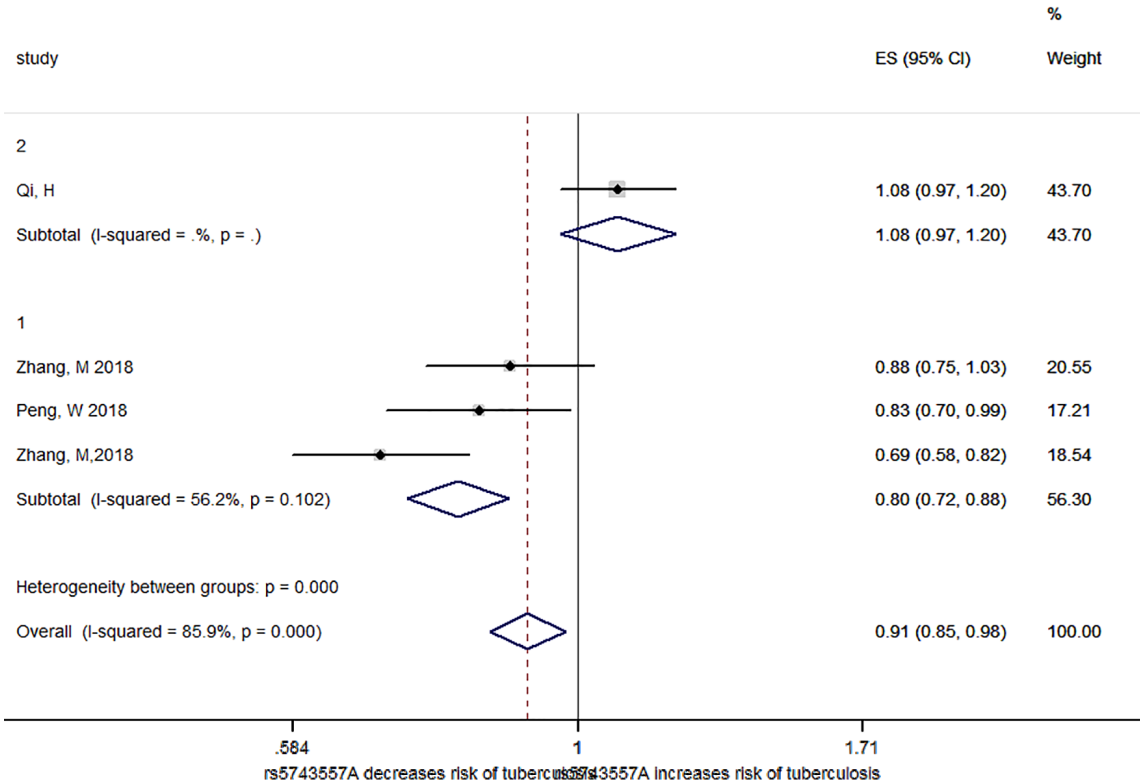

Figure 1 Forest plot for the subgroup analysis of the association between rs5743557 and susceptibility to tuberculosis by age. 1 represents adults, 2 represents children. Each study cohort is represented by the name of the first author and publication year. The point estimate of the OR and 95% CI for each comparison are shown; the pooled OR and 95%CIs were derived from random-effects models. The I2 and P values are the heterogeneity test results. OR, odds ratio; CI, confidence interval.

of four studies, two of Chinese Han adults, one of Chinese Tibetan adults and one of Chinese Han children (*Table S1*). As demonstrated in *Figure 1* (20,25,26), there was high degree of heterogeneity among the included studies (P<0.001 and I^2=85.9%). After stratifying by age, no significant heterogeneity was observed (P=0.102), and rs5743557_A was associated with reduced TB risk and the odds ratio was 0.80 in the Chinese adult group (P<0.001, 95% CI: 0.72–0.88). As shown in *Figure 2*, when the Chinese adult population was grouped by Han and Tibetan nationality, no significant heterogeneity was observed (P=0.626, I^2=0%)and the relationship between rs574355_ A and TB risk in Chinese Han adults was still present (P=0.01, OR 0.86, 95% CI: 0.76–0.96).

Demographic characteristics

The demographic characteristics of all participants are summarized in *Table 1*. The LTBI group was older than the HC (P=0.027) and TB (P<0.001) groups. No significant difference was found in gender distribution. All uninfected controls were either negative for the QuantiFERON TB-GIT assay (n=180) or for the T-SPOT.TB test (n=24). All LTBI individuals were either positive for the QuantiFERON TB-GIT assay (n=180) or for the T-SPOT. TB test (n=21). As shown in *Table 1*, 51 (24.4%) active TB patients were culture positive, 64 (30.6%) had AFB positive biopsy results, 134 (64.1%) were positive for TB-DNA detection and 176 (84.2%) patients were found to be AFB positive in specimens. In addition, 142 (67.9%) active TB patients were either positive for TB culture or TB-DNA detection. According to a meta-analysis, the prevalence of NTM infections among suspected TB patients was 6.3% (5.4–7.4%) in mainland China and was 6.2% (4.6–8.2%) in the South-west region of China (27). Thus, NTM likely contributed little to our study results. Among all TB patients, 103 cases had satisfactory IGRAs with 90 positive for the QuantiFERON TB-GIT assay and two positive for the T-SPOT.TB test. The remaining 11 cases were negative

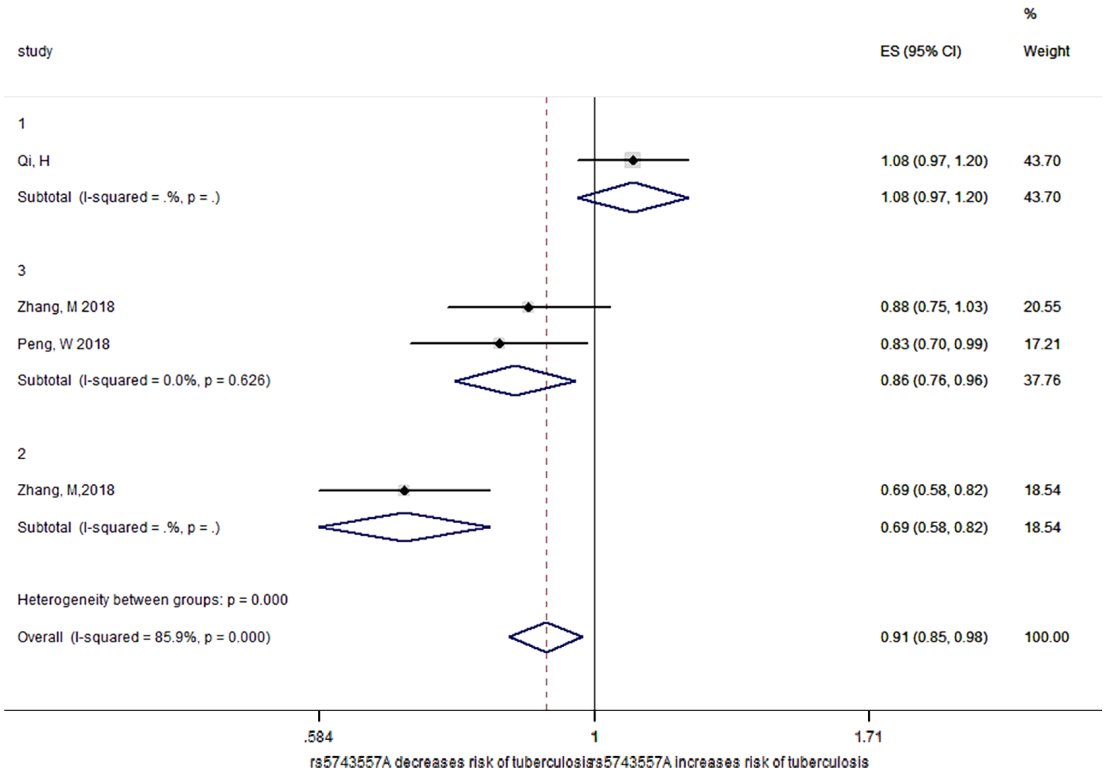

Figure 2 Forest plot for the subgroup analysis of the association between rs5743557 and susceptibility to tuberculosis by nationality. 1 represents Chinese Han children, 2 represents Chinese Tibetan adults, 3 represents Chinese Han adults. Each study cohort is represented by the name of the first author and publication year. The point estimate of the OR and 95% CI for each comparison are shown; the pooled OR and 95%CIs were derived from random-effects models. The I2 and P values are the heterogeneity test results. OR, odds ratio; CI, confidence interval.

Table 1 Demographic characteristics of the study populations

Characteristic	HC, n=204, n (%)	LTBI, n=201, n (%)	TB, n=209, n (%)	HC *vs.* LTBI, P value	LTBI *vs.* TB, P value
Age (mean ± IQR), year	HC *vs.* LTBI: 47.39±22.5; LTBI *vs.* TB: 43.84±29.5			0.027	<0.001
Gender					
Male	93 (46.0)	95 (47.0)	107 (51.0)		
Female	111 (54.0)	106 (53.0)	102 (49.0)	0.735	0.426
TB culture positive, n (%)			51 (24.4)		
TB-DNA positive, n (%)			134 (64.1)		
Biopsy with positive acid-fast bacilli, n (%)			64 (30.6)		
Acid-fast bacilli positive, n (%)			176 (84.2)		
TB culture or TB-DNA positive, n (%)			142 (67.9)		
QuantiFERON TB-GIT assay/T-SPOT.TB positive, n (%)		180 (90.0)/21(10.0)	90 (87.4)/2 (2.0)		
QuantiFERON TB-GIT assay/T-SPOT.TB negative, n (%)	180 (88.2)/24 (11.8)		11 (10.7)/0 (0)		

HC, healthy controls; LTBI, latent tuberculosis infection; TB, tuberculosis; IQR, interquartile range

for the QuantiFERON TB-GIT assay and the negative rate (10.7%) was comparable to that from a previous large epidemiological study (28).

LD structure of all tagSNPs

As shown in *Table S2*, the genotype distributions of rs4833095, rs5743557 and rs1039559 were not in HWE in the HC group. The genotype call rate of all SNPs was 99.79% and the consistency of the 5% repeat genotyping was 99.81%. Displayed in *Figure 3A*,*B*, two SNP pairs (rs4833095-rs5743604 and rs5743596-rs5743557) of *TLR1* were in strong LD with $r^2 \geq 0.65$ and D' approaching to 1. LD between SNP pairs of *TLR6* and *TIRAP* were low ($r^2 < 0.36$), and the two tagSNPs of *MYD88* were not in LD at all ($r^2 = 0$).

Gene-gene interactions between four genes using MDR analysis

The MDR model was applied to screen for interactions among the thirteen SNPs in the four genes on LTBI and TB risk. Gene-gene interactions results of the MDR analysis are shown in *Table S3*. Between the HC and LTBI groups, rs4833095 of *TLR1* formed the best model with 53.14% testing balanced accuracy and 10/10 cross-validation consistency and there was no gene-gene interaction. Between the LTBI and TB groups, rs5743604 of *TLR1*, rs1039559 of *TLR6* and rs7744 of *MYD88* formed the best model with 53.48% testing balanced accuracy and 7/10 cross-validation consistency. However, after 1000-fold permutation testing, no significant gene-gene interaction was found.

Associations between SNPs and LTBI or TB susceptibility

To investigate the relationship between the tagSNPs and susceptibility to LTBI and TB, we compared the HC group with the LTBI group and the LTBI group with TB group. As shown in *Table 2*, when comparing the HC group and LTBI group, TT /TC genotypes of rs4833095 and AA/GA genotypes of rs5743604 in *TLR1* were associated with reduced susceptibility to TB infection (P=0.007 and P=0.034), while genotype AA of rs5743557 in *TLR1*was associated with increased risk of TB infection (P=0.012). In addition, when comparing the LTBI group and TB group, the AA genotype of both rs5743596 and rs5743557 was associated with decreased TB risk (P=0.036 and P=0.006), while the AA/GA genotypes of rs5743604 were associated with increased susceptibility to TB (P=0.017). None of the tagSNPs of the *TLR6*, *MYD88* and *TIRAP* genes were associated with susceptibility to LTBI or TB (results shown in *Table S4*). However, only the relationship between rs5743557 and TB susceptibility remained significant after 1000-fold permutation (P=0.023).

After calculating the power for all tagSNPs, rs5743557 and rs5743604 had power approaching 80% when comparing the TB group with the LTBI group, and rs4833095 had similar power when comparing the HC group with the LTBI group (*Table S2*). Considering that rs4833095 deviated from HWE in the HC group, only results of rs5743557 and rs5743604 between TB group and the LTBI group should be taken into consideration.

Associations between haplotypes and LTBI or TB susceptibility

The rs4833095-rs5743604 CG haplotype of *TLR1* was significantly higher in the LTBI group than in the TB group (P=0.019877). However, this result was no longer significant association after 1000-fold permutation testing (P=0.191, shown in *Table 3*). For the *TLR1* gene, no significant haplotype associations were observed when the HC group was compared with the LTBI group.

Discussion

Although rs4833095 of *TLR1* (7), rs5743810 of *TLR6* (8), rs6853 of *MYD88* (9) and rs8177374 of *TIRAP* (10) have been found to influence the host response to M.TB, we focused this study on the relationship between tagSNPs of these four genes and two stages of TB progression. Our study revealed that the AA genotype of rs5743557 was associated with reduced risk of TB compared with the LTBI group after permutation correction. The meta-analysis suggested rs5743557_A was associated with decreased TB susceptibility in the Chinese adult population.

It is well known that TLR1 is able to recognize triacyl lipoproteins of mycobacteria as a heterodimer with TLR2, as shown by the defective response to triacylated lipopeptides in TLR1-deficient mice (29). rs5743557, located 595 bp upstream of the *TLR1* gene, might influence the promoter activity of this gene. rs5743557_A was reported to be significantly associated with decreased TB risk in Chinese Tibetans, while this relationship was only observed in Chinese Han women and no significant association was seen in Chinese Han children (20,25,26).

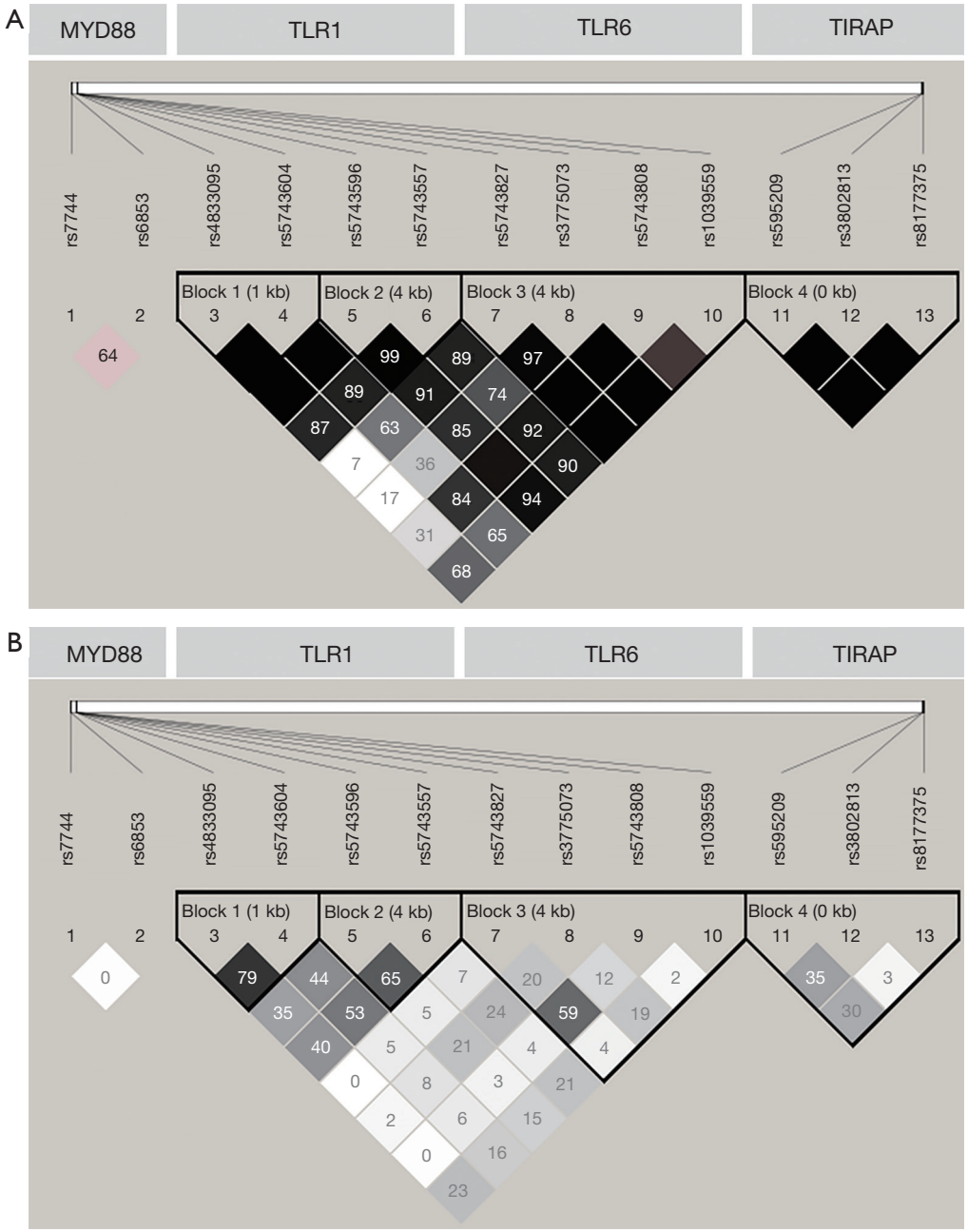

Figure 3 Linkage disequilibrium (LD) of all tagSNPs of four genes. (A). Linkage disequilibrium (LD) of all tagSNPs of four genes displayed in form of D'. Each colored cell is related to the strength of LD between the corresponding two markers. The number in each cell represents the LD parameter D' (×100), an empty cell indicates D' (×100) =100. rs7744 and rs6853 are located in MYD88; rs4833095, rs5743604, rs5743596, and rs5743557 are located in TLR1; rs5743827, rs3775073, rs5743808 and rs1039559 are located in TLR6; rs595209, rs3802813 and rs8177375 are located in TIRAP. (B) LD of all tagSNPs of four genes displayed in form of r2 (×100). Each colored cell is related to the strength of LD between the corresponding two markers. The number in each cell represents the LD parameter r2 (×100). All SNPs are displayed as in (A).

Table 2 Associations between four tagSNPs of TLR1 and the risk of LTBI and TB

SNPs	Genetic models	Alleles/ genotypes	HC, n (%)	LTBI, n (%)	TB, n (%)	HC *vs.* LTBI			LTBI *vs.* TB		
						P[a]	OR (95% CI)[a]	P*	P[a]	OR (95% CI)[a]	P*
rs4833095	Dominant	TT + TC	148 (72.6)	119 (59.5)	145 (69.4)	0.007	0.56 (0.37–0.86)	0.065	0.074	1.48 (0.96–2.28)	0.343
		CC	56 (27.5)	81 (40.5)	64 (30.6)						
	Recessive	TT	32 (15.7)	33 (16.5)	42 (20.1)	0.882	1.04 (0.61–1.78)	1	0.386	1.27 (0.74–2.15)	0.972
		TC + CC	172 (84.4)	167 (83.5)	167 (79.9)						
	Additive	CC/TC/TT				0.074	0.77 (0.58–1.03)	0.520	0.095	1.28 (0.96–1.71)	0.445
rs5743557	Dominant	AA + GA	140 (68.6)	138 (69.0)	140 (67.0)	0.837	1.05 (0.68–1.61)	1	0.475	0.85 (0.55–1.32)	1
		GG	64 (31.4)	62 (31.0)	69 (33.0)						
	Recessive	AA	26 (12.7)	44 (22.0)	24 (11.5)	0.012	1.99 (1.17–3.39)	0.111	0.006	0.45 (0.26–0.8)	0.023
		GA + GG	178 (87.3)	156 (78.0)	185 (88.5)						
	Additive	GG/GA/AA				0.120	1.26 (0.94–1.69)	0.807	0.045	0.73 (0.54–0.99)	0.485
rs5743596	Dominant	AA + GA	116 (56.8)	110 (55.0)	109 (52.2)	0.813	0.95 (0.64–1.42)	1	0.544	0.88 (0.58–1.33)	1
		GG	88 (43.1)	90 (45.0)	100 (47.8)						
	Recessive	AA	18 (8.8)	26 (13.0)	14 (6.7)	0.144	1.61 (0.85–3.06)	0.842	0.036	0.47 (0.23–0.95)	0.207
		GA + GG	186 (91.1)	174 (87.0)	195 (93.3)						
	Additive	GG/GA/AA				0.605	1.08 (0.8–1.46)	1	0.153	0.79 (0.58–1.09)	0.783
rs5743604	Dominant	AA + GA	158 (77.5)	136 (68.0)	167 (79.9)	0.034	0.62 (0.4–0.97)	0.300	0.017	1.78 (1.11–2.85)	0.056
		GG	46 (22.5)	64 (32.0)	42 (20.1)						
	Recessive	AA	43 (21.1)	41 (20.5)	52 (24.9)	0.784	0.93 (0.58–1.52)	1	0.273	1.32 (0.81–2.16)	0.922
		GA + GG	161 (78.9)	159 (79.5)	157 (75.1)						
	Additive	GG/GA/AA				0.126	0.8 (0.6–1.065)	0.783	0.031	1.4 (1.03–1.87)	0.166

[a], adjusting for age and gender when analyzing genotype models in a binary logistic regression analysis model; *, adjusting for multiple comparisons by 1,000-fold permutation testing. HC, uninfected healthy controls; LTBI, latent tuberculosis infection; TB, tuberculosis; OR, odds ratio; CI, confidence interval.

Table 3 Haplotype analysis of four tagSNPs of TLR1 in the comparison of LTBI *vs.* TB

Haplotype	TB (freq)	LTBI (freq)	Chi2	Pearson's P	Odds ratio (95% CI)	P*
rs4833095-rs5743604						
CA	32.00 (0.077)	25.00 (0.062)	0.623	0.429922	1.244 (0.723–2.139)	1
CG	199.00 (0.476)	223.00 (0.557)	5.426	0.019877	0.721 (0.548–0.950)	0.191
TA	187.00 (0.447)	152.00 (0.380)	3.823	0.050583	1.321 (0.999–1.746)	0.396
Global result	400	418	5.445	0.066		
rs5743557-rs5743596						
AA	123.00 (0.294)	134.87 (0.337)	1.819	0.177447	0.816 (0.607–1.097)	0.874
AG	41.00 (0.098)	47.13 (0.118)	0.854	0.355397	0.812 (0.521–1.264)	1
GG	254.00 (0.608)	216.87 (0.542)	3.418	0.064502	1.300 (0.984–1.717)	0.475
Global result	400	418	3.454	0.178		

*, P value after 1,000-fold permutation testing using the Haploview software. LTBI, latent tuberculosis infection; TB, tuberculosis; OR, odds ratio; CI, confidence interval.

After meta-analysis in our paper, rs5743557_A showed a relationship with decreased TB susceptibility in the Chinese adult population. Our study confirmed the meta-analysis results considering the association seen in the comparison of TB with LTBI. More information about rs5743557 and TB risk in other populations is needed to further confirm our results. As is well known, the immune system is quite different between children and adults, and children are more likely to develop active TB after infection (30,31). Thus, separating adults from children is necessary to better understand the mechanisms underlying TB pathogenesis in each group.

rs5743595 of *TLR1*, which was in strong LD with rs5743557 in the Chinese Han Beijing population, was reported to augment expression of *TLR1* mRNA, TLR1 proteins and specific cytokines as determined by RT-PCR, flow cytometry and ELISA (32). However, whether rs5743557 participated in the regulation of *TLR1* function is unclear. Instead, many reports have observed functional roles of rs4833095, which is a non-synonymous polymorphism and the amino acid change was reported to alter TLR1 folding or function (33). rs4833095 was also associated with lack of surface expression and impaired function of TLR1 (34). However, no association between rs4833095 and TB was observed in most studies and after meta-analysis (20). In consideration of the lack of relationship between rs4833095 and susceptibility to TB, the functional effects of rs4833095 might affect the risk of other diseases. Thus, special attention should be focused on rs5743557 or other linked polymorphisms, which may be the key variants in the relationship between *TLR1* and TB susceptibility.

In a similar way to TLR1, TLR6 is known to recognize lipopeptides possessing a diacylglycerol group in the form of TLR6/TLR2 heterodimers (35), leading to activation of MYD88 dependent pathways. TIRAP, together with MYD88, is involved in TLR1, TLR2, and TLR4 signaling pathways and activates downstream NF-κB, leading to induction of cytokine secretion and the proinflammatory response. One study demonstrated that *TLR6*-deficient mice showed an impaired response to diacylated lipopeptides (29). *MYD88* null mice were reported to exhibit increased susceptibility to virulent M.TB infection and rapidly succumb to TB (36). *TIRAP*-deficient mice also showed impaired MYD88-dependent pathways involving TLR2 and TLR4 (37).

rs5743808 of *TLR6* was reported to associate with increased TB risk in the African American population (33). rs6853 of *MYD88* was shown to have a relationship with TB risk in an Indian population and revealed functional effects (9). No other tagSNP of *TLR6*, *MYD88* and *TIRAP* has been reported to significantly associate with TB risk in previous studies. Although a study suggested *MYD88* and *TLR6* were uniquely expressed in TB patients compared with LTBI subjects (14), the lack of significant results of the two genes in our study does not provide any confirmatory evidence to support a pathogenic role of these genes in TB. Nevertheless, in view of the important roles played by TLR6, MYD88 and TIRAP in the immune response

against M.TB and the limited genetic epidemiologic information about them, further studies with larger sample size are required to verify their definitive relationship with LTBI and TB susceptibility.

Considering the large population of LTBI, early recognition and targeted treatment of high-risk LTBI patients will make a great contribution to reaching the End TB Strategy goal by the year 2035. Studies focused on finding a measureable way to predict the risk of TB infection and active TB from LTBI progression has recently begun. Histone deacetylase related genes were differentially expressed in the genome-wide transcriptional profiles between uninfected healthy controls and LTBI individuals (38). One study identified a 16 gene expression signature for predicting the risk of LTBI progression into active TB with a sensitivity of 66.1% and a specificity of 80.6% (39). Another study has shown that the TNFα-only T-cell phenotype was observed more frequently in recently acquired LTBI compared with remotely acquired LTBI (40). While great efforts have been made in predicting TB infection and progression, a meta-analysis revealed a 380 gene meta-signature with only five genes involved in all included studies (13). Thus, searching for genes and transcript markers to predict TB progression is still an active area of research.

Our study was designed to expand the knowledge of the relationship between four innate immunity genes and the risk of LTBI and TB, however, only one significant genetic association was identified. The main weaknesses of our study were the limited sample size and a lack of a replication study to verify our findings. However, our study also has some advantages. This is the first report to comprehensively investigate association between polymorphisms of four genes (*TLR1*, *TLR6*, *MYD88* and *TIRAP*) and susceptibility to two stages of TB progression, namely LTBI and active TB. In addition, the enrolled Han participants were mostly from Sichuan Province, Southwestern China, and thus had minimal geographical and ethnic heterogeneity.

In conclusion, our study found a significant association between rs5743557 of *TLR1* and risk of TB in the Chinese adult population. Validation of this association and experiments to determine the underlying mechanism are required. In addition, a larger study sample is still needed to investigate association between the other three critical genes (*TLR6*, *MYD88* and *TIRAP*) and susceptibility to LTBI and TB. This study provides important clues to elucidate the relationship of innate immunity to the development of TB.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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