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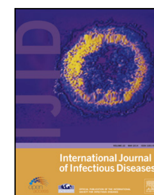

An association study of NRAMP1, VDR, MBL and their interaction with the susceptibility to tuberculosis in a Chinese population

Linlin Wu [a], Haijun Deng [b,1], Yihui Zheng [b,1], Mikael Mansjö [c], Xubin Zheng [a], Yi Hu [a,*], Biao Xu [a]

[a] Department of Epidemiology, School of Public Health, Fudan University, 138 Yi Xue Yuan Rd, Shanghai 200032, China; Key Laboratory of Public Health Safety (Fudan University), Ministry of Education, Shanghai, China
[b] Disease Control and Prevention Center, Putuo District, Shanghai 200062, China
[c] The Public Health Agency of Sweden (former Swedish Institute for Communicable Disease Control), Solna, Sweden

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SUMMARY

Objectives: To investigate natural-resistance-associated macrophage protein 1 (NRAMP1), mannose-binding lectin (MBL), vitamin D receptor (VDR) gene polymorphisms and their interaction with susceptibility to pulmonary tuberculosis (PTB) in a Chinese population.
Methods: A case-control study was conducted in PTB (n=151), age- and sex- matched healthy controls (HCs) (n=453). Genetic polymorphisms of NRAMP1 (INT4, D543NA and 3′UTR), MBL (HL, PQ, XY and AB) and VDR (FokI and Taq) were analyzed by using PCR-restriction fragment length polymorphism (RFLP) and PCR- single- strand conformation polymorphism (SSCP) techniques. Multifactor dimensionality reduction (MDR) analysis was carried out to assess the effects of the interaction between SNPs.
Results: The distribution of NRAMP1- 3′UTR (TGTG/del), MBL- HL (H/L) and FokI (F/f) were significantly different between PTB patients and HCs ($p<0.05$). HPYA (OR: 1.88; 95% CI: 1.22-2.91), LPXA (OR: 3.17; 95% CI: 1.69- 5.96), LQYA (OR: 3.52; 95%CI: 1.50-8.23) and LPYB (OR: 12.37; 95%CI: 3.75- 40.85) of MBL were risk haplotypes for PTB. The TGTG- H- f (OR: 1.70; 95%CI: 1.10-2.62) and del- H-f (OR: 3.48; 95% CI: 1.45-8.37) of 3′UTR- HL- FokI were also high-risk haplotypes associated with tuberculosis.
Conclusions: Our study suggests that genotypes of many polymorphic genes are associated with TB, it is necessary to further explore the mechanism of genotypes and gene-gene interaction in susceptibility to tuberculosis.
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1. Introduction

Tuberculosis (TB) remains a global health problem with 8.8 million new cases and 1.1 million deaths each year.[1] Although it is estimated that about one third of the world's population have been infected with Mycobacterium tuberculosis (*M.TB*), only 10% of those infected develop an active TB disease during their life time.[2] This suggests that individual differences may act upon the susceptibility to tuberculosis. Polymorphisms in natural-resistance-associated macrophage protein 1 (NRAMP1), vitamin D receptor (VDR) and mannose- binding lectin (MBL) genes have been found to be associated with TB in different ethnic groups. However, the effect of genetic variation remain to be inconclusive.

NRAMP1 has been shown to be a critical element in the regulation of intracellular membrane vesicle trafficking of macrophages.[3] The potential roles of NRMAP1 polymorphisms (3′UTR, INT4 and D543N) in the development of pulmonary tuberculosis (PTB) have been investigated in various racial groups, but the results have been inconsistent. In a West Africans and Gambian population,[4] three NRAMP1 polymorphisms (3′UTR, INT4 and D543N) were significantly associated with TB, while no association was found between NRAMP1 polymorphisms and the disease in Taiwanese,[5] Thai,[6] Moroccans[7] and Brazilians.[8]

MBL is a calcium– dependent plasma collagenous lectin which plays an important role in innate immune defense against infectious agents.[9] The results of previous studies investigating the influence of MBL gene variants on susceptibility to TB and its

* Corresponding author. Tel.: +86 21 54237677; fax: +86 21 54237710.
E-mail address: yhu@fudan.edu.cn (Y. Hu).
[1] Contributed equally as first authors

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progression are controversial.[10–13] Several studies have reported MBL deficiency associated with conferring protection against TB, whereas others have reported susceptibility related with TB. There are few studies on the relationship between MBL gene polymorphisms and the development of TB in the Chinese Han population and it remains unclear whether MBL deficiency status will lead to the increased susceptibility to TB infection.

In comparison with the NRAMP1 gene, fewer populations have been studied in relation to the VDR gene. Previous studies have not reached a consensus regarding the association between the VDR gene variants and the risk of developing TB. In Gambian[14] and London population,[15] a significant interaction between FokI and TaqI genotypes of the VDR gene and susceptibility to PTB was observed, while another study found that there was no association between the VDR gene and PTB in Cambodians.[2]

The discrepant results observed so far are supposed to be partially due to diverse ethnic backgrounds. Gene-gene interaction analysis is a new approach to elucidate susceptibility to complex diseases; until recently, few studies investigating gene-gene interactions in relation to infectious diseases such as TB have been performed. Additionally, considering the minor allele frequency more than 5% in the Chinese population (Hapmap Date Rel 28 Phase II+III), the present study aimed to investigate 9 polymorphisms in NRAMP, MBL and VDR genes as well their gene-gene interaction in relation to the susceptibility to TB. The identification of host genes and the analysis of gene-gene interaction may provide a theoretical basis for exploring the high incidence of TB in Han ethnic population and establish theoretical foundation for the mechanism of ethnic variation in susceptibility to TB. This may also help to better understanding of the pathogenesis of TB and development of prophylactic or treatment strategies.

2. Materials and methods

2.1. Study design and subjects

A 1:3 case- control study was conducted in 151 PTB patients and 453 healthy controls (HCs). All subjects agreed to take part in the study. The patients included in this study were newly diagnosed PTB patients registered in the Putuo district from January 2013 to August 2013. The PTB cases were selected according to the national diagnostic criteria of China, with positive sputum smear and/or culture and significant symptoms of typical PTB, chest radiography consistent with active disease. The HC group composed of healthy individuals who matched in age and gender. None of the controls showed any clinical manifestations of PTB at the time of blood sample collection, and all were confirmed to be not PTB by X-ray examination. Subjects, who were HIV positive and known to present any autoimmune, any chronic inflammatory, or any other disease conditions, were excluded from the study. The study was approved by the Ethics Committee of Fudan University, and informed consents were obtained from all subjects before blood sampling and questionnaire investigation.

2.2. Determination of sample size

The sample size was determined by the following factors: the known prevalence of NRAMP, VDR, MBL genetic polymorphisms in the Han population, α and β errors, and the expected difference in the prevalence of TLR genetic polymorphisms. We used a value of at least 10% for the prevalence of polymorphisms, an α error of 0.05, a β error of 0.2, a 10% expected difference, and match ratio of 3:1. The minimum sample size was estimated to be 100 for the PTB group and 300 for the HC group.

2.3. DNA extraction

Blood samples were collected from 151 PTB patients and 453 HCs. Venous blood (5.0 ml) with anticoagulant was used for DNA extraction and detection of gene polymorphism. Genomic DNA was extracted by the salting-out technique[16] from 5 ml of EDTA- anti-coagulated blood samples, suspended in sterile water and stored at -4 °C until use. DNA concentration was determined by spectrofluorometry (Spectra Max Gemini; Molecular Devices, Sunnyvale, CA) using the PicoGreen assay (Molecular Probes, Eugene, OR).

2.4. Genotyping of NRAMP1 and VDR Genes

Our study was performed to determine the distribution of gene polymorphisms by using Polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP). For the NRAMP1 gene, the INT4, 3′ UTR and D543N polymorphism sites were investigated. For the VDR gene, the TaqI and FokI polymorphism sites were detected.

PCR-RFLP analysis was used to identify the polymorphisms of NRAMP1 and VDR genes. The primers used for amplification of NRAMP1 and VDR gene polymorphisms were as previously reported[17] and listed in Table 1. PCR amplifications were performed using purified DNA in 25 ul reaction volumes. Thermocycling parameters were as follows: 95 °C for 5 min and 35 cycles of 94 °C for 45 s, 58 °C for 45 s and 72 °C for 45 s, with a final extension at 72 °C for 10 min. PCR products of INT4, D543N and 3′UTR were then digested by Apa I, Ava II, Fok I restriction endonuclease respectively, and the polymorphisms of VDR gene were genotyped by FokI and TaqI. Digestion products were separated by electrophoresis on a 2.5% agarose gel, stained with ethidium bromide, and visualized under ultraviolet light. The genotypes were defined according to generated fragment patterns. To ensure the validity of result, we also did the DNA sequencing on the 5% representative isolates to check the fragment generated from RFLP.

2.5. Mutations on HL, AB, XY and PQ loci mutation of MBL gene

Polymerase chain reaction (PCR) and single-strand conformation polymorphism (SSCP) technique were used to study the types and frequencies of gene mutations in HL, AB, XY and PQ loci of MBL gene. PCR amplifications were performed using purified DNA in 25 ul reaction volumes. Thermocycling parameters were as follows: 94 °C for 4 min and 35 cycles of 94 °C for 30 s, 59 °C for 60 s

Table 1
The primer sequences of NRAMP1 and VDR genes

Locus	Primer
NRAMP1 -INT4	Forward primer: 5′-CCT GCC TCC TCA CAG CTT CT-3′ Reverse primer: 5′-TGTGCTATCAGTTGAGCCTG-3′
NRAMP1 -3‘UTR	Forward primer: 5′-GCA TCT CCCC CAA TTC ATG CT-3′ Reverse primer: 5′ - AAC TGT CCC ACT CTA TCC TG -3′
NRAMP1 -D534N	Forward primer: 5′-GCATCTCCCCAATTCATGGT-3′ Reverse primer: 5′-AACTGTCCCACTCTATCCTG-3′
VDR-TaqI	Forward primer: 5′-AGCTGGCCCTGGCACTGACTCTGCTCT-3′ Reverse primer: 5′-ATGGAAACACCTTGCTTCTTCTCCCTC-3′
VDR-FokI	Forward primer: 5′-CAGAGCATGGACAGGGAGC-3′ Reverse primer: 5′-AGGAGAGGCAGCGGTACTG-3′
MBL-AB	Forward primer: 5′-AGTCGACCCAGATTGTAGGACAGAG-3′ Reverse primer: 5′- AGGATCCAGGCAGTTTCCTCTGGAAGG-3′
MBL-HL	Forward primer: 5′-GCTTACCCAGGCAAGCCTGTG-3′ Reverse primer: 5′-ACTTACCCAGGCAAGCCTGTC-3′
MBL-PQ	Forward primer: 5′-CTCAGTTAATGAACACATATTTACCG-3′ Reverse primer: 5′-CTCAGTTAATGAACACATATTTACCA-3′
MBL-XY	Forward primer: 5′-GGTCCCATTTGTTCTCACTCCCACC-3′ Reverse primer: 3′-GAAAGCATGTTTATAGTCTTCCAGC-3′

and 72 °C for 45 s, with a final extension at 72 °C for 5 min. PCR amplification products were analyzed on a polyacrylamide gel to detect alterations in PCR-amplified products. The primer sequences were previously reported[10,12,18] and listed in Table 1.

2.6. Statistical analysis

Data were managed and analyzed using SPSS program, Windows version 16.0 (SPSS Inc., Chicago, IL, USA). Hardy-Weinberg equilibrium was analyzed using SNP stats online software (Institut CataA d' oncologia). For each polymorphism, allele and genotype frequencies in differences in different groups were examined by the chi-squared test or Fisher's exact test when appropriate. Odds ratios (OR) and 95% confidence intervals (CIs) were calculated to quantify the degree of association between the polymorphisms and tuberculosis. Multifactor dimensionality reduction (MDR) analysis was also carried out to assess the interaction between SNPs using MDR software. A two- tailed $P<0.05$ was considered statistically significant.

3. Results

3.1. Genotypes and alleles distribution of NRAMP1, MBL and VDR genes in two groups

The genotype distribution of NRAMP1, MBL and VDR polymorphisms in all subjects did not deviate from the Hardy-Weinberg equilibrium ($p> 0.05$). The frequencies of 3′UTR TGTG+/+, TGTG+/del, del/del genotypes, TGTG+ and del alleles were 64%, 33%, 3%, 81% and 19% in PTB patients, whereas the frequencies in HCs were 80%, 19%, 1%, 89% and 11% respectively. At the NRAMP1- 3′ UTR polymorphic site, the frequency of TGTG +/del genotype was higher in cases compared to HCs ($p<0.001$; OR, 2.083; 95%CI, 1.380- 3.144). A significant difference was observed in the alleles, the frequency of TGTG del allele was higher in cases compared to HCs. However, PTB patients and HCs had very similar distribution of alleles and genotype frequencies in the INT4 and D543N genes. (Table 2)

In PTB patients, the H/H, H/L, L/L genotype frequencies of the MBL gene were 30%, 42% and 27%, while these were 22%, 55% and 23% in healthy individuals respectively. Significant difference was observed between the two groups in the frequency of HL genotype of the MBL gene (p=0.008; OR, 0.608; 95% CI, 0.419- 0.882). There were no significant differences in the PQ, XY, AB genotypes and alleles of the MBL gene between cases and controls.

The genotype frequencies for VDR-FF, VDR-Ff and VDR-ff of FokI polymorphisms were 38%, 46%, 16% in PTB patients and 50%, 40%, 10% in controls. VDR-FF were significantly overrepresented in the HC group (p= 0.010; OR, 0.609; 95% CI, 0.418- 0.888). The F and f alleles of FokI were 22% and 78% in cases, while being 70% and 30% in the HC group and there was statistically significant difference between the two groups (p= 0.004; OR, 1.478; 95% CI, 1.134- 1.950). The frequencies of T, t alleles in TaqI gene were 96% and 4% in PTB patients to compare with 89% and 11% in HCs. A significant difference between the two groups was observed for the T and t alleles of the TaqI gene (p=0.001; OR, 2.758; 95% CI, 1.524- 4.992). (Table 2)

3.2. Association of gene polymorphisms with susceptibility to tuberculosis

According to the value of Akaike information criterion (AIC) and Bayesian information criterion (BIC), the optimal models included co-dominant, dominant, recessive, over-dominant and additive index models. The optimal models of D543N and TaqI were not identified. The result also showed that INT4, PQ, XY and AB were not associated with tuberculosis under these genetic models. 3′UTR related to the occurrence of tuberculosis in the co-dominant (TGTG/del.vs.TGTG/TGTG: OR, 2.14; 95%CI: 1.41-3.24), dominant (del/del.vs.del/TGTG: OR, 2.18; 95%CI: 1.46-3.27), overdominant (TGTG/del.vs.del/del: OR, 2.08; 95%CI, 1.38-3.14) and additive index (OR, 2.02; 95%CI, 1.40-2.92) models, while no association between 3′UTR and tuberculosis was observed in recessive model ($p>0.05$). There was association between HL and tuberculosis in the co-dominant (H/L.vs.H/H: OR, 0.57; 95%CI, 0.36-0.88), dominant (L/L.vs.H/L: OR, 0.65; 95%CI, 0.43-0.99), over-dominant (H/L.vs.L/L: OR, 0.61; 95%CI, 0.42-0.88) models. FokI gene of VDR was associated with tuberculosis in the co-dominant (F/f.vs.F/F: OR, 2.07; 95%CI, 1.17-3.67; F/f.vs.F/F: OR, 1.53; 95%CI, 1.03-2.29), dominant (F/f-f/f.vs.F/F: OR, 1.64; 95%CI, 1.13-2.39), and additive index (OR, 1.46; 95%CI, 1.12-1.91) models. (Table 3)

3.3. The association between the combined haplotypes of different SNPs of MBL and tuberculosis

We then further studied the relationship between the multi-loci haplotypes of MBL and tuberculosis, because there was obviously linkage disequilibrium between every two SNPs of MBL. The results showed that there were 15 haplotypes with frequencies from 34.3% to 0.1%. Five haplotypes had frequencies lower than 1% and were thus defined as rare haplotypes. With the highest frequency of the haplotype as control, the risk haplotypes for TB were HPYA (OR, 1.88; 95%CI, 1.22-2.91), LPXA (OR, 3.17; 95%CI, 1.69-5.96), LQYA (OR, 3.52; 95%CI, 1.50-8.23) and LPYB (OR, 12.37; 95%CI, 3.75-40.85). No significant associated were detected between other haplotypes and tuberculosis. (Table 4)

3.4. The association between the combined haplotypes of multi-locus and the susceptibility to PTB

Based on the analysis of loci, we found that the 3′UTR of the NRAMP1 gene, the HL of the MBL gene and the FokI of the VDR gene were associated with the occurrence of tuberculosis. Therefore, we analyzed the association between the haplotypes and tuberculosis. The three loci could be combined into 8 haplotypes and the frequencies of these haplotypes varied from 30.6% to 1.7%. The results showed that the TGTG-H-f (OR:1.70,95%CI, 1.10-2.62) and del-H-f (OR: 3.48; 95%CI, 1.45-8.37) were higher risk haplotypes for tuberculosis. Other haplotypes were not associated with tuberculosis. (Table 5)

4. Discussion

Tuberculosis is a major public health threat. Although it is estimated that one third of the world's population has been infected with the bacillus which may cause TB, a relatively small proportion of the people (10%) infected with bacillus will progress to active TB disease.[2] It is suggested that the susceptibility to TB is multifactorial disease, influenced by many factors including HIV infection, environmental and host genetic factors.[19–22] Recently, various studies have been reporting that host genetic factors may play an important role in the susceptibility to TB. In our study, we investigated the association between the NRAMP1, MBL and VDR gene polymorphisms and their single and interaction in relation to the susceptibility to PTB and analyzed gene-gene interaction in Shanghai city.

The polymorphisms in the regions of the NRAMP1 gene had been studied in relation to tuberculosis in many populations.[23,24] Our study reported that there were associations of 3′UTR variants with susceptibility to TB. Similar to our finding, a significant association of 3′UTR of NARMP1 variants with susceptibility to TB was detected in Chinese Han[25] and in Korean[26] populations,

Table 2
Genotype and allele distribution of NRAMP1, MBL and VDR genes in two groups

	Genotype and allele	Cases N(%)	Controls N(%)	P value	OR(95%CI)
NRAMP1-INT4	Genotypes				
	C/C	6 (4.0)	14 (3.1)	0.599	1.298(0.490~3.439)
	G/C	45 (30.0)	166 (36.6)	0.127	0.734(0.493~1.092)
	G/G	100 (66.0)	273 (60.3)	0.192	1.293(0.879~1.902)
	Alleles				
	C	57 (18.9)	194 (21.4)	0.346	0.854(0.614~1.187)
	G	245 (81.1)	712 (78.6)	0.346	1.171(0.843~1.627)
NRAMP1-D543N	Genotypes				
	G/A	15 (9.9)	33 (7.3)	0.297	1.404(0.740~2.663)
	G/G	136 (90.1)	420 (92.7)	0.297	0.712(0.376~1.351)
	Alleles				
	G	287 (95.0)	873 (96.4)	0.308	0.723(0.387~1.351)
	A	15 (50.0)	33 (3.6)	0.308	1.383(0.740~2.582)
NRAMP1-3′UTR	Genotypes				
	TGTG+/+	97 (64.2)	361 (79.7)	0.000*	0.458(0.306~0.686)
	TGTG+/del	50 (33.1)	87 (19.2)	0.000*	2.083(1.380~3.144)
	TGTGdel/del	4 (2.6)	5 (1.1)	0.175	2.438(0.646~9.199)
	Alleles				
	TGTG+	244 (80.8)	809 (89.3)	0.000*	0.504(0.354~0.720)
	TGTGdel	58 (19.2)	97 (10.7)	0.000*	1.983(1.389~2.829)
MBL-HL	Genotypes				
	H/H	46 (30.5)	101 (22.3)	0.043*	1.527(1.012~2.303)
	H/L	64 (42.4)	248 (54.7)	0.008*	0.608(0.419~0.882)
	L/L	41(27.1)	104 (33.0)	0.296	1.251(0.822~1.904)
	Alleles				
	H	156 (51.7)	450 (49.7)	0.550	1.083(0.834~1.405)
	L	146 (48.3)	456 (50.3)	0.550	0.924(0.712~1.199)
MBL-PQ	Genotypes				
	P/P	124 (82.1)	364 (80.4)	0.633	1.123(0.697~1.808)
	P/Q	26 (17.2)	87 (19.2)	0.588	0.875(0.540~1.418)
	Q/Q	1 (0.7)	2 (0.4)	0.738	1.503(0.135~16.697)
	Alleles				
	P	274 (90.7)	815 (90.0)	0.696	1.093(0.700~1.705)
	Q	28 (9.3)	91 (10.0)	0.696	0.915(0.586~1.428)
MBL-XY	Genotypes				
	X/X	7 (4.6)	15 (3.3)	0.425	1.419(0.568~3.550)
	X/Y	47 (31.1)	120 (26.5)	0.270	1.254(0.838~1.876)
	Y/Y	97 (64.2)	318 (70.2)	0.171	0.763(0.517~1.125)
	Alleles				
	X	61 (20.2)	150 (16.6)	0.149	1.276(0.916~1.776)
	Y	241 (79.8)	756 (83.4)	0.149	0.784(0.563~1.092)
MBL-AB	Genotypes				
	A/A	112 (74.2)	348 (76.8)	0.508	0.866(0.567~1.325)
	A/B	37 (24.5)	97 (21.4)	0.429	1.191(0.772~1.838)
	B/B	2 (1.3)	8 (1.8)	0.713	0.747(0.157~3.555)
	Alleles				
	A	261 (86.4)	793 (87.5)	0.618	0.907(0.618~1.331)
	B	41 (13.6)	113 (12.5)	0.618	1.102(0.751~1.618)
VDR-FokI	Genotypes				
	FF	57 (37.7)	226 (49.9)	0.010*	0.609(0.418~0.888)
	Ff	70 (46.4)	181 (40.0)	0.167	1.299(0.896~1.882)
	ff	24 (15.9)	46 (10.1)	0.056	1.672(0.982~2.847)
	Alleles				
	F	184 (60.9)	633 (69.9)	0.004*	0.673(0.513~0.882)
	f	118 (39.1)	273 (30.1)	0.004*	1.487(1.134~1.950)
VDR-Taq	Genotypes				
	TT	138 (91.4)	403 (89.0)	0.398	1.317(0.694~2.498)
	Tt	13 (8.6)	50 (11.0)	0.398	0.759(0.400~1.440)
	Alleles				
	T	289 (95.7)	806 (89.0)	0.001*	2.758(1.524~4.992)
	t	13 (4.3)	100 (11.0)	0.001*	0.363(0.200~0.656)

Note: Genotype frequencies were compared between the patients and control subjects by χ^2 test or Fisher's exact test when appropriate. Allele frequencies were compared between the patients and control subjects by use of a χ^2 test for 2-by-2 contingency tables. N: the sample size. OR: odd ratio; CI: confidence interval.
* $p<0.05$.

respectively. By contrast, there were no associations between the 3′UTR variant and TB in Thai,[6] Moroccans,[7] Danes,[27] and Brazilians.[8] We found no association between allele variants at locus INT4, D543N of NRAMP1 in patients versus HCs and this finding did not confirm a previous investigate in West Africans.[4] Also, there are ethnic variations in the allelic frequency distribution for the investigated polymorphism markers. Notably, the candidate susceptible genotype, 3′UTR TGTG+/del heterozygote, was very rare in Caucasians,[4,28] but was present in a high proportion of Asian population groups.[5,27,29,30] From Hapmap, we found that the allele frequencies of D543N were different, such as 86% in Chinese Han population, 85.1% in Russians, 99.1% in European and 94.2% in India. Other co-variables such as socio-economic factors, nutritional status, and interactions between

Table 3
The association between the NRAMP1 gene polymorphism with susceptibility to tuberculosis under different genetic models

Locus	model	Genotype	Cases (%)	Controls(%)	OR (95%CI)	p	AIC	BIC
INT4	Codominant	G/G	273 (60.3)	100 (66.2)	1	0.29	682.8	696.0
		G/C	166 (36.6)	45 (29.8)	0.74 (0.50-1.11)			
		C/C	14 (3.1)	6 (4.0)	1.17 (0.44-3.13)			
	Dominant	G/G	273 (60.3)	100 (66.2)	1	0.19	681.6	690.4
		G/C-C/C	180 (39.7)	51 (33.8)	0.77 (0.53-1.14)			
	Recessive	G/G-G/C	439 (96.9)	145 (96.0)	1	0.61	683.0	691.8
		C/C	14 (3.1)	6 (4.0)	1.30 (0.49-3.44)			
	Overdominant	G/G-C/C	287 (63.4)	106 (70.2)	1	0.12	680.9	689.7
		G/C	166 (36.6)	45 (29.8)	0.73 (0.49-1.09)			
	Additive index	–	–	–	0.85 (0.60-1.19)	0.33	682.3	691.1
D543N		G/G	420 (92.7)	136 (90.1)	1	0.31	682.3	691.1
		G/A	33 (7.3)	15 (9.9)	1.40 (0.74-2.66)			
3′UTR	Codominant	TGTG/TGTG	361 (79.7)	97 (64.2)	1	<0.01*	671.1	684.3
		TGTG/del	87 (19.2)	50 (33.1)	2.14 (1.41-3.24)			
		del/del	5 (1.1)	4 (2.6)	2.98 (0.78-11.30)			
	Dominant	TGTG/TGTG	361 (79.7)	97 (64.2)	1	<0.01*	669.3	678.1
		TGTG/del-del/del	92 (20.3)	54 (35.8)	2.18 (1.46-3.27)			
	Recessive	TGTG/TGTG-TGTG/del	448 (98.9)	147 (97.3)	1	0.20	681.7	690.5
		del/del	5 (1.1)	4 (2.6)	2.44 (0.65-9.20)			
	Overdominant	TGTG/TGTG-del/del	366 (80.8)	101 (66.9)	1	<0.01*	671.5	680.3
		TGTG/del	87 (19.2)	50 (33.1)	2.08 (1.38-3.14)			
	Additive index	–	–	–	2.02 (1.40-2.92)	<0.01*	669.4	678.2
H/L	Codominant	H/H	101 (22.3)	46 (30.5)	1	0.03*	678.0	691.3
		H/L	248 (54.8)	64 (42.4)	0.57 (0.36-0.88)			
		L/L	104 (23)	41 (27.1)	0.87 (0.52-1.43)			
	Dominant	H/H	101 (22.3)	46 (30.5)	1	0.046*	679.3	688.1
		H/L-L/L	352 (77.7)	105 (69.5)	0.65 (0.43-0.99)*			
	Recessive	H/H-H/L	349 (77.0)	110 (72.8)	1	0.30	682.2	691.0
		L/L	104 (23)	41 (27.1)	1.25 (0.82-1.90)			
	Overdominant	H/H-L/L	205 (45.2)	87 (57.6)	1	0.01*	676.4	685.2
		H/L	248 (54.8)	64 (42.4)	0.61 (0.42-0.88)*			
	Additive index	–	–	–	0.92 (0.71-1.20)	0.54	682.9	691.7
P/Q	Codominant	P/P	364 (80.3)	124 (82.1)	1	0.82	684.9	698.1
		P/Q	87 (19.2)	26 (17.2)	0.88 (0.54-1.42)			
		Q/Q	2 (0.4)	1 (0.7)	1.47 (0.13-16.33)			
	Dominant	P/P	364 (80.3)	124 (82.1)	1	0.63	683.1	691.9
		P/Q-Q/Q	89 (19.6)	27 (17.9)	0.89 (0.55-1.43)			
	Recessive	P/P-P/Q	451 (99.6)	150 (99.3)	1	0.75	683.2	692
		Q/Q	2 (0.4)	1 (0.7)	1.50 (0.14-16.70)			
	Overdominant	P/P-Q/Q	366 (80.8)	125 (82.8)	1	0.59	683.0	691.8
		P/Q	87 (19.2)	26 (17.2)	0.88 (0.54-1.42)			
	Additive index	–	–	–	0.91 (0.58-1.44)	0.69	683.1	691.9
X/Y	Codominant	Y/Y	318 (70.2)	97 (64.2)	1	0.37	683.3	696.5
		X/Y	120 (26.5)	47 (31.1)	1.28 (0.85-1.93)			
		X/X	15 (3.3)	7 (4.6)	1.53 (0.61-3.86)			
	Dominant	Y/Y	318 (70.2)	97 (64.2)	1	0.17	681.5	690.3
		X/Y-X/X	135 (29.8)	54 (35.8)	1.31 (0.89-1.93)			
	Recessive	Y/Y-X/Y	438 (96.7)	144 (95.4)	1	0.46	682.8	691.6
		X/X	15 (3.3)	7 (4.6)	1.42 (0.57-3.55)			
	Overdominant	Y/Y-X/X	333 (73.5)	104 (68.9)	1	0.27	682.1	690.9
		X/Y	120 (26.5)	47 (31.1)	1.25 (0.84-1.88)			
	Additive index	–	–	–	1.26 (0.91-1.75)	0.16	681.3	690.2
A/B	Codominant	A/A	348 (76.8)	112 (74.2)	1	0.70	684.6	697.8
		A/B	97 (21.4)	37 (24.5)	1.19 (0.77-1.83)			
		B/B	8 (1.8)	2 (1.3)	0.78 (0.16-3.71)			
	Dominant	A/A	348 (76.8)	112 (74.2)	1	0.51	682.9	691.7
		A/B-B/B	105 (23.2)	39 (25.8)	1.15 (0.75-1.76)			
	Recessive	A/A-A/B	445 (98.2)	149 (98.7)	1	0.71	683.2	692.0
		B/B	8 (1.8)	2 (1.3)	0.75 (0.16-3.56)			
	Overdominant	A/A-B/B	356 (78.6)	114 (75.5)	1	0.43	682.7	691.5
		A/B	97 (21.4)	37 (24.5)	1.19 (0.77-1.84)			
	Additive index	–	–	–	1.10 (0.75-1.62)	0.62	683.1	691.9
FokI	Codominant	F/F	226 (49.9)	57 (37.8)	1	0.02	677.5	
		F/f	181 (40)	70 (46.4)	1.53 (1.03-2.29)			
		f/f	46 (10.2)	24 (15.9)	2.07 (1.17-3.67)			
	Dominant	F/F	226 (49.9)	57 (37.8)	1	0.01*	676.5	
		F/f-f/f	227 (50.1)	94 (62.2)	1.64 (1.13-2.39)			
	Recessive	F/F-F/f	407 (89.8)	127 (84.1)	1	0.06	679.9	
		f/f	46 (10.2)	24 (15.9)	1.67 (0.98-2.85)			
	Overdominant	F/F-f/f	272 (60)	81 (53.6)	1	0.17	681.4	
		F/f	181 (40)	70 (46.4)	1.30 (0.90-1.88)			
	Additive index	–	–	–	1.46 (1.12-1.91)	0.01*	675.6	
Taq		T/T	403 (89)	138 (91.4)	1	0.39	682.6	

Note: N: the sample size; OR: odds ratio; 95% CI: 95% confidence interval; AIC: Akaike's information criterion; BIC: Bayesian information criterion;
* $p<0.05$.

Table 4
The association between the combined haplotypes of different SNPs of MBL and susceptibility to TB

	HL	PQ	XY	AB	Controls Freq(%)	Cases Freq(%)	OR(95%CI)	P
1	L	P	Y	A	37.09	25.39	1	–
2	H	P	Y	A	29.27	34.03	1.88 (1.22 - 2.91)*	0.01
3	H	P	Y	B	8.67	6.40	1.01 (0.53 - 1.92)	0.99
4	H	P	X	A	5.29	10.01	1.92 (0.91 - 4.07)	0.09
5	L	P	X	A	7.05	8.19	3.17 (1.69 - 5.96)*	0.00
6	L	Q	Y	A	3.59	7.56	3.52 (1.50 - 8.23)*	0.00
7	H	Q	Y	A	3.57	0	–	–
8	L	P	Y	B	0.91	5.96	12.37 (3.75 - 40.85)*	0.00
9	L	Q	X	A	1.27	1.25	0.47 (0.09 - 2.33)	0.35
10	H	P	X	B	1.87	0.76	1.07 (0.15 - 7.94)	0.94
11	H	Q	Y	B	0.44	0.46	0.45 (0.04 - 5.70)	0.54
12	L	Q	Y	B	0.10	0		
13	L	Q	X	B	0.32	NA		
14	H	Q	X	B	0.15	NA		
15	H	Q	X	A	0.60	NA		

Note: OR: odds ratio; 95% CI: 95% confidence interval; Freq: frequency;
* $p<0.05$.

genes may not be excluded. Therefore, this needed to have in-depth study on the function of these genes.

Our study revealed that the HL genotype of the MBL gene was found to be a protective factor by exhibiting a significantly higher proportion in HC group compared to PTB group, which is different from the previous study reported in other Chinese areas.[10] The conflicting result could be due to the influences of the different study design as well as the modified factors. Meanwhile, the promoters PQ and XY were not associated with the development of TB. This is consistent with another study in China.[10] We also indicated that the frequencies of AB genotype and A/B allele were not significantly different between PTB patients and HCs. Similar to our study, a meta-analysis demonstrated no association between AB/AA genotypes and PTB infection. However, there are a number of studies reporting higher B allele frequency among TB patients, suggesting a risk of the B allele in TB infection.[4,31] In contrast, other groups have presented evidence supporting an association between MBL genetic variants in the structural region and protection from TB infection.[11,32,33] Therefore, the question of whether the mutant alleles are advantageous or disadvantageous in TB infection deserves further investigation in other populations. The analysis of haplotypes showed that LPAB and LPXA conferred susceptibility to tuberculosis. This is similar with other studies in different populations.[10,34] Meanwhile, the previous functional study suggested the activity of luciferase in HYP haplotype was relatively highest, followed by LYP and LYQ haplotypes, while the activity of LXP haplotype was lowest.[35] Our study indicated that XB haplotype was super dominant in the case group. As demonstrated in the previous study, XB haplotype increased the susceptibility to TB by causing the low levels of serum MBL and the deficiency of MBL.[36] This may explain why the Chinese were more likely to develop tuberculosis.

Table 5
The association between multi-locus combined haplotypes and tuberculosis

3UTR	LH	FokI	Cases Freq(%)	Controls Freq(%)	OR (95%CI)	P
TGTG	L	F	0.2874	0.3099	1.09 (0.73-1.63)	0.683
TGTG	H	F	0.2206	0.3223	1.70 (1.10-2.62)*	0.014
TGTG	H	f	0.1806	0.1249	0.66 (0.40-1.09)	0.103
TGTG	L	f	0.1194	0.1358	1.17 (0.67-2.05)	0.579
del	L	F	0.0606	0.0396	0.65 (0.29-1.49)	0.306
del	H	F	0.0748	0.0226	3.48 (1.45-8.37)*	0.003
del	H	f	0.0407	0.0269	1.52 (0.56-4.13)	0.412
del	L	f	0.0160	0.0180	1.13 (0.30-4.31)	1.000

Note: OR: odds ratio; 95% CI: 95% confidence interval; Freq: frequency; *$p<0.05$

Vitamin D metabolism can lead to the activation of macrophages and subsequently restrict the intracellular growth of *M.TB*.[37,38] Therefore, the polymorphism in the VDR gene may be involved in genetic susceptibility to TB. In our study, we found that the frequencies of FokI- Ff and ff genotypes were higher in the case group than in the control group, indicating that the Ff (OR, 1.53, 95% CI: 1.03- 2.29) and ff (OR, 2.07, 95% CI: 1.17- 3.67) genotype frequencies were associated with an increased risk of developing TB. No significant differences of TaqI- Tt and tt genotype frequencies were observed between TB patients and HCs. The similar studies also showed that FokI may be associated with TB, while TaqI is not involved in the TB development in Chinese Han and Kazak populations.[14] The most plausible explanation for the association between FokI genotype and the disease is that the FokI polymorphism site defines a C/T transition at the first of two potential translation initiation sites in exon 2 and the VDR gene with the FF genotype demonstrated an increased transcription rate. The T allele of TaqI gene was considered a susceptible allele and t was the resistant allele. From Hapmap databases, the frequencies of T allele in TaqI gene were 97.1% in Chinese, 75.0% in African, 89.7% in Japanese and 56.2% in European populations. This may be one of the reasons for the TB high prevalence in the Han population. The relation between VDR gene polymorphism and susceptibility to TB has been studied in different populations. Polymorphisms of the VDR gene have been associated with TB resistance in Gambia, and data showed a significantly lower frequency of TaqI-tt genotype among TB patients.[14] Among Gujarati Asians in West London,[15] a significant interaction between vitamin D status and FokI and TaqI genotypes was also observed. In Tuvinians from Tuva Republic and Russians from Tomsk city, no association between Fok I genotype and PTB was found.[39] A study in Cambodia[2] and Tanzania[40] found no association between VDR gene and PTB. The association may be caused by genetic variation, interaction of gene- gene or gene- environment. Further study of the interaction of gene- gene and gene-environment in different ethnic populations is needed. This will be helpful for the prediction of high risk population, and lay a foundation for the development of appropriate prevention strategies. Therefore, our study also analyzed the association between multi-loci haplotypes and tuberculosis. We found that TGTG-H-f (OR:1.66,95%CI:1.03-2.53) and del-H-f (OR: 4.82; 95%CI: 1.94-11.95) were higher risk haplotypes for tuberculosis. Other haplotypes were not associated with tuberculosis. Based on these observations, the present study demonstrated a complex interaction between the host and pathogen influenced by environmental factors. Meanwhile, the following in-depth functional study will be

necessary to better understand the mechanism of tuberculosis development as well as to provide a new molecular target therapy approach for the development of vaccines and anti-TB drugs.

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