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Original Article

Genetic polymorphisms of CCL1 rs2072069 G/A and TLR2 rs3804099 T/C in pulmonary or meningeal tuberculosis patients

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Abstract: CCL1, one of the members of the CC chemokine family, is an inflammatory mediator that stimulates the migration of human monocytes. CCL1 expression is induced by *Mycobacterium tuberculosis* and TLR ligands in macrophage. TLR2 plays critical role in host immune response against *M. tuberculosis* infection by regulating the macrophage activation and cytokine secretion. *M. tuberculosis* causes different clinical forms of tuberculosis (TB) disease. Single-nucleotide polymorphisms (SNPs) in the CCL1 gene and TLR2 gene may be associated with the development of different clinical forms of TB, depending on the different immune mechanisms. This study was to evaluate the possible association between CCL1 rs2072069 G/A or/and TLR2 rs3804099 T/C (T597C) polymorphisms and pulmonary tuberculosis (PTB) or/and tuberculous meningitis (TBM) in a sample of the Chinese adult population. A case-control study was designed to compare the allele frequency and genotype distribution between control (n=386) and TB (n=341) who had either PTB (n=230) or TBM (n=111). The genotype typing was performed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique. TLR2 variant genotype 597CC was associated with susceptibility to PTB rather than to TBM. In the male PTB subgroup, 597CC genotype was identified in a higher rate, compared with male control subgroup. This study demonstrates that T597C polymorphism of TLR2 is a risk factor for susceptibility to PTB rather than to TBM in a sample of Chinese adult population. Patient gender may affect the outcome of *M. tuberculosis* infection. TLR2 gene may influence the development of PTB and TBM by different immune mechanisms.

Keywords: CCL1, TLR2, polymorphism, tuberculosis (TB), pulmonary tuberculosis (PTB), tuberculous meningitis (TBM)

Introduction

Tuberculosis (TB), caused by *Mycobacterium tuberculosis*, remains one of the world's deadliest communicable diseases. In 2013, an estimated 9.0 million people developed TB and 1.5 million died from the disease [1]. According to the 2014 global tuberculosis report, India and China alone accounted for 24% and 11% of the estimated 9 million people who developed TB in 2013, respectively. TB ranks as the second leading cause of death from an infectious disease worldwide, after the human immunodeficiency virus (HIV) [1]. The most common manifestation in adults of *M. tuberculosis* infection is pulmonary tuberculosis (PTB), which accounts for 80% of all forms of TB disease [2]. Tuberculous meningitis (TBM) develops in around 1% of all active TB cases, which is the most devastating form of *M. tuberculosis* infection with greater morbidity and mortality. Many of the survivors with TBM are left with chronic neurological sequelae [2, 3]. TBM results from the haematogenous dissemination of *M. tuberculosis* from the lung [4]. It remains unknown why most TB-infected persons remain disease-free and why some people with disseminated TB develop TBM and central nervous infection, while most people have localized disease in the lungs only. There are many known factors affecting TB development, such as environmental or bacterial factors, human immunodeficien-

Table 1. Studied gene polymorphisms of CCL-1 and TLR2 with their locations, primer sequences, sizes of the amplicons, and annealing temperature

Reference SNP no	Gene	Enzyme	Nucleotide composition (5′→3′)	Expected size of PCR product (bp)	Annealing temperature (°C)
rs2072069	CCL-1	*MvaI*	CCTCCTACTTCCTGTCCT CGTACTCCAGCTTGATTT	280	58
rs3804099	TLR2	*TaiI*	TTTAT CGTCTTCCTG GTTC CAAATCAGTATCTCGCAGTT	361	60

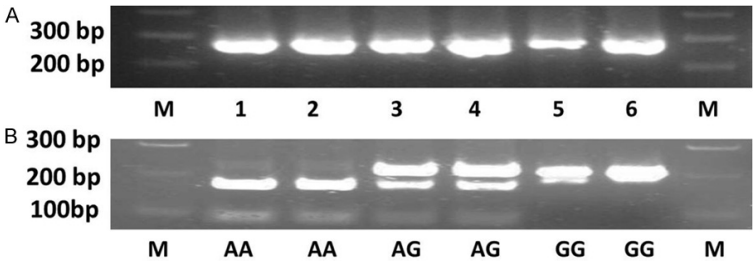

Figure 1. A. PCR products were resolved by 3% agarose gel electrophoresis before digestion by restriction endonuclease. B. PCR-RFLP performed to genotype CCL-1 rs2072069 polymorphism. M=DNA size marker.

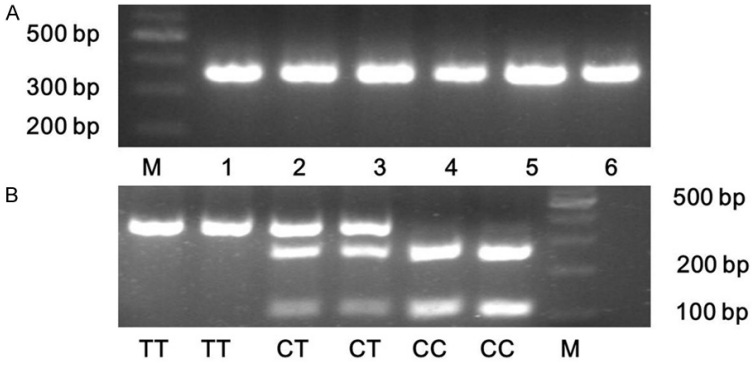

Figure 2. A. PCR products were resolved by 3% agarose gel electrophoresis before digestion by restriction endonuclease. B. PCR-RFLP performed to genotype TLR2 rs3804099 polymorphism. M=DNA size marker.

cy virus infection, gender and age. The occurrence of TB at different rates among particular races, ethnicities, and families indicates a genetic predisposition to TB susceptibility. Furthermore, many studies have indicated that host genetic factors are important determinants of susceptibility or resistance to TB disease [5]. A large body of evidence suggests that the effective immune system plays a critical role in avoiding *M. tuberculosis* dissemination as well as in the pathogenesis of the different clinical forms of TB development [6].

Macrophages act as the first line of immune defense for *M. tuberculosis* and provide a major habitat for *M. tuberculosis* to reside in the host. Macrophages mediate the host innate immune response to *M. tuberculosis* through pathogen recognition and activation of an inflammatory response. Macrophages also function as antigen presenting cells to trigger adaptive immunity. Successful stimulation of innate and adaptive immune responses results in containment of *M. tuberculosis* replication. In contrast, failure to contain *M. tuberculosis* replication is associated with different clinical forms of TB development [2, 7]. Macrophages also express Toll-like receptors (TLRs) that recognize conserved pathogen-associated-molecular patterns (PAMPs) on *M. tuberculosis* to mediate the production of cytokines [8]. TLRs act as key receptors in the innate response to *M. tuberculosis* and represent crucial triggers for adaptive immune response [9, 10]. Among these receptors, TLR2 seems to play a vital role in recognition of *M. tuberculosis* structural components of the cell membrane [11]. TLR2 knockout mice revealed defects in granuloma formation, which demonstrates TLR2 plays a vital role in host innate immune response and in containment of *M. tuberculosis* replication [12, 13]. The mycobacterial TLR2 agonists include arabinose-capped lipoarabinomannan (AraLAM), mannosylated lipoarabinomannan (Man-LAM), 19-kDa lipoprotein antigen of *M. tuberculosis*, mycobacterial glycolipid, phosphatidylinositol dimannoside (PIM), lipomannan (LM) [14]. Polymorphisms of TLR2 show susceptibility to tuberculosis, representing significance of TLR2 in immune

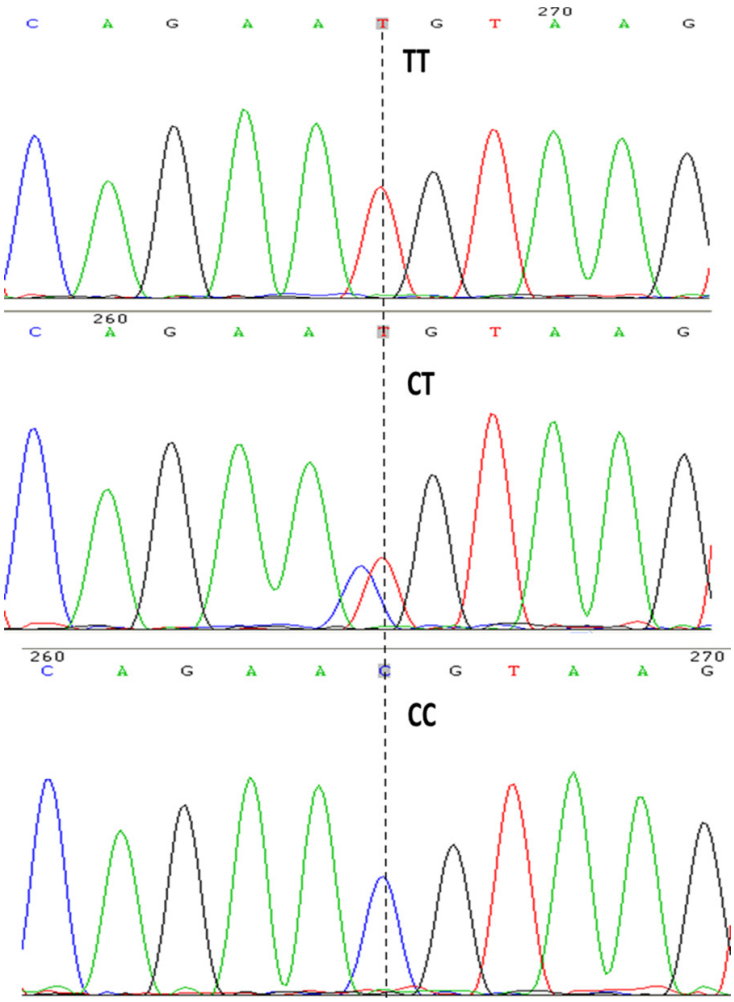

Figure 3. Obtained phenogrames from the randomly chosen individuals by direct sequencing performed to genotype TLR2 rs3804099 polymorphism.

response to *M. tuberculosis* [15, 16]. Chemokines associated with susceptibility to *M. tuberculosis* infection include IL-8 (CXCL8), monocyte chemoattractant protein 1 (MCP-1, CCL2), CCL5, and CXCL10 (IP-10) [17].

It has been shown that polymorphism rs2072069 of CCL1 gene was associated with susceptibility to TBM rather than to PTB in Vietnamese population, which is the first identification of CCL1 as a gene involved in host susceptibility to TB disease [2]. NTT Thuong et al have found that TLR2 T597C polymorphism was associated with TBM rather than PTB and the association increased with the severity of neurologic symptoms of TBM [18]. A significant association between TLR2 T597C polymorphism and PTB has been found in a sample of Iranian population [19]. However, the inconsistent results regarding TLR2 T597C polymorphism in the two ethnic groups imply genetic heterogeneity or other possibilities. No study regarding TBM and CCL1 gene or TLR2 gene polymorphisms has been reported in the Chinese adult population. Therefore, this study was to investigate the association of CCL1 rs2072069 G/A or/and TLR2 T597C polymorphisms and PTB or/and TBM.

Materials and methods

TB and control subjects

Informed consent has been obtained from all participants or their guardian involved in this study. The research has been approved by the Ethics Committee of the Second Hospital of Hebei Medical University. The subjects with TBM were enrolled from Department of Neurology in the Second Hospital of Hebei Medical University. The diagnosis of TBM patients was based on clinical meningitis in addition to having a modified positive Ziehl-Neelsen stain for acid-fast bacill from the cerebrospinal fluid [20]. The adults consecutively admitted to the department of neurology in this hospital due to TBM from January 2014 to September 2014 were recruited. For these 111 subjects with TBM in the study, all of them had a positive stain for acid fast bacilli. Inclusion criteria included acid-fast bacilli seen through modified Ziehl-Neelsen stain from the CFS, negative HIV test results, no history of autoimmune disease, primary immunodeficiency, treatment with immunosuppressive therapy and other clinical forms of TB except miliary pulmonary TB.

Totally 230 patients diagnosed with active PTB were enrolled between January 2014 and September 2014 from Hebei Provincial Chest Hospital and Hospital of Infectious Disease of Shijiazhuang, Hebei province. All of them were bacteriologically identified. None of the patients

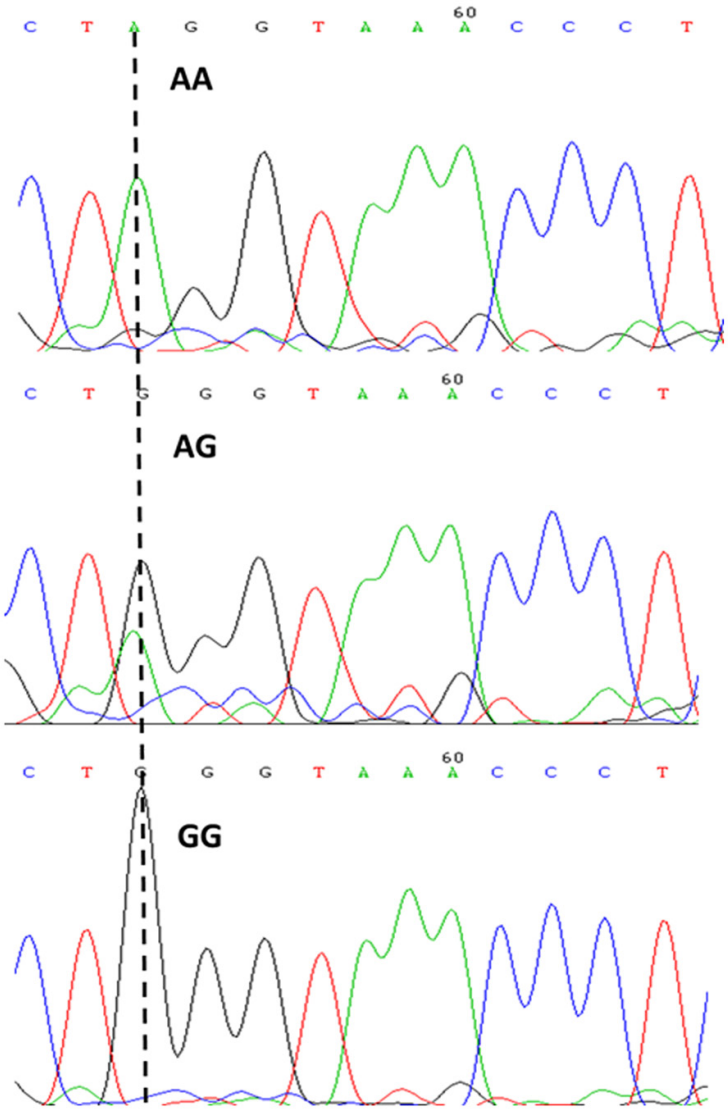

Figure 4. Obtained phenograms from the randomly chosen individuals by direct sequencing performed to genotype CCL1 rs2072069 polymorphism.

had miliary or extra-pulmonary TB, HIV infection, history of autoimmune disease, or treatment with immunosuppressive therapy.

Control subjects were from in-patients of neurology department with normal chest x-ray results, without history and clinical manifestations of tuberculosis, without history of autoimmune disease, primary immunodeficiency and receipt of immunosuppressive therapy. Control subjects came from the same district where the TB group lived, with similar socio-economic status.

All individuals under study were of the Han Chinese ethnicity and all came from North China.

Blood samples, DNA isolation and polymerase chain reaction restriction fragment length polymorphism

Blood samples from TB and controls were collected and stored at -80°C. Genomic DNA was extracted from EDTA-anticoagulated peripheral venous blood from each individual using the QIAamp DNA blood kit (Qiagen). The genotype typing was performed using polymerase chain reaction restriction fragment length polymorphism (PCRRFLP) technique by means of an Applied Biosystems® 2720 Thermal Cycler. PCR optimization for each primer set was validated by temperature gradient and primers are listed in **Table 1**. The thermocycling procedure consists of initial denaturation step at 95°C for 4 min, denaturation at 95°C for 30 s, annealing temperatures (58°C for 30 s for CCL-1 rs2072069) and (60°C for 30 s for TLR2 T597C) with 35 cycles, extension at 72°C for 30 s), and final extension (72°C for 7 min). CCL-1 rs2072069 polymorphism was identified by *MvaI* restriction enzyme and restricted on this region. The genotype GG yielded four bands with 129, 68, 66 and 17 bp in size, while the genotype GA yielded five bands with 195, 129, 68, 66 and 17 bp in size (**Figure 1**). TLR2 T597C polymorphism was identified by *TaiI* restriction enzyme and restricted on this region. The TLR2 T597C generated three bands with 361, 258 and 103 bp, while TLR2 C597C showed two bands with 258 and 103 bp (**Figure 2**). Digested PCR products were resolved by 3% agarose gel electrophoresis, stained by using ethidium bromide and photographed under UV illumination. Genotyping was conducted in a blinded fashion.

We re-genotyped approximately 20% of the samples to verify the initial results. The verification confirmed the previous genotyping results by 100%. Ten percent of individuals from each

Table 2. Gender and age distribution of study population and their comparisons

	Gender		Age (years)	Gender comparison		Age comparison	
Group	Male	Female		χ^2	p	t	p
Control	234 (0.606)	152 (0.394)	59.0±13.9	1		1	
All TB	230 (0.674)	111 (0.326)	36.8±17.4	3.655	0.056	[a]18.859	<0.001
PTB	161 (0.700)	69 (0.300)	36.8±17.9	5.51	0.019	[a]16.178	<0.001
TBM	69 (0.622)	42 (0.378)	36.8±16.5	0.086	0.769	[a]12.925	<0.001

Each group was compared with the control group. All TB, pulmonary tuberculosis and tuberculous meningitis; PTB, pulmonary tuberculosis; TBM, tuberculous meningitis. [a]Separate variance estimation t-test was used because of equal variances not assumed.

study group were randomly chosen, directly sequenced and used as genotyping positive controls and the results were 100% concordant (**Figures 3** and **4**).

Statistical analysis

Statistical analysis was conducted using the SPSS 19.0. Independent *t*-test was used to examine the statistical significance of the difference of age and sex in different groups. Differences of genotype distribution and allele frequency were tested by chi-square analysis. Fisher's exact test was used when the expected number of samples in a group was less than five. Two sided testing was used to evaluate statistical significance. Multivariate logistic regression analysis was applied when adjusting age and sex to compare genotype distribution and allele frequency. Odds ratios (OR) with 95% confidence intervals (95% CI) were calculated to identify the relationship between SNPs and TB groups.

Results

TB and controls

The TB group (n=341) included 230 (67%) cases of active PTB and 111 (33%) cases of TBM. The detailed information about TB and control groups was shown in **Table 2**. There was significant difference between control and TB groups in age comparison (P<0.001) (**Table 2**). Subjects in TB group, PTB group and TBM group were younger than in control group.

Analysis of HWE and the associations between SNPs with TB and subgroups of PTB or TBM

The genotypes of TB and control were in Hardy-Weinberg equilibrium (HWE) (P>0.05).

The genotype distribution and allele frequency of polymorphisms in TB and control groups were summarized in **Table 3**. There was significant difference between control and TB groups for age. The genotype distribution and allele frequency adjusted for age and sex were shown in **Table 4**.

The wild-type genotype GG at rs2072069 in the CCL1 gene was observed in 101 (31.6%) of the TB patients, whereas the frequencies of variant genotypes AG and AA were 151 (47.2%) and 68 (21.3%) respectively. In the control group, the frequencies of genotypes were 106 (30.0%) for GG, 164 (46.5%) for AG and 83 (23.5%) for AA. There was no significant difference between the TB and control group with regard to the frequency of genotype or allele (**Table 4**). We further analyzed subgroups of TB. In PTB group, the frequencies of genotypes were 70 (32.4%) for GG, 97 (44.9%) for AG and 49 (22.7%) for AA. In TBM group, the frequencies of genotypes were 31 (29.8%) for GG, 54 (51.9%) for AG and 19 (18.3%) for AA. There were no statistical differences in allele frequency or genotype distribution at rs2072069 in the CCL1 gene when comparing the control group with the PTB and TBM groups, respectively (**Table 4**).

This study showed that the TLR2 T597C polymorphism was significantly associated with susceptibility to PTB in recessive tested inheritance model (OR=2.218, 95% CI: 1.180-4.169, P=0.013) while there was no statistical difference in dominant tested inheritance model (**Table 4**). The choice of model is based on Bayesian information criterion (BIC) values [18]. The model with the smallest BIC was considered as the best fit. The results suggested that the recessive model was better than the dominant model (BIC of recessive model = 354.350 < BIC of dominant model = 403.896).

Table 3. The genotype distribution and allele frequency of SNPs in cases (TB, PTB and TBM) and control when the data were not adjusted for age and sex

					All TB vs. Control		PTB vs. Control		TBM vs. Control	
Polymorphism	All TB	PTB	TBM	Control	OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P
rs2072069 (CCL1)										
Additive										
GG	101 (0.316)	70 (0.324)	31 (0.298)	106 (0.300)	1		1		1	
AG	151 (0.472)	97 (0.449)	54 (0.519)	164 (0.465)	0.966 (0.680-1.373)	0.848	0.896 (0.605-1.326)	0.582	1.105 (0.666-1.833)	0.699
AA	68 (0.213)	49 (0.227)	19 (0.183)	83 (0.235)	0.860 (0.565-1.310)	0.482	0.894 (0.562-1.423)	0.636	0.783 (0.413-1.483)	0.452
Dominant										
GG	101 (0.316)	70 (0.324)	31 (0.298)	106 (0.300)	1		1		1	
AG+AA	219 (0.684)	146 (0.676)	73 (0.702)	247 (0.700)	0.931 (0.671-1.291)	0.667	0.895 (0.622-1.289)	0.551	1.011 (0.627-1.630)	0.966
Recessive										
GG+AG	252 (0.788)	167 (0.773)	85 (0.817)	270 (0.765)	1		1		1	
AA	68 (0.213)	49 (0.227)	19 (0.183)	83 (0.235)	0.878 (0.610-1.263)	0.482	0.954 (0.638-1.427)	0.82	0.727 (0.417-1.266)	0.259
Alleles										
G	353 (0.552)	237 (0.549)	116 (0.558)	376 (0.533)	1		1		1	
A	287 (0.448)	195 (0.451)	92 (0.442)	330 (0.467)	0.926 (0.747-1.148)	0.485	0.937 (0.737-1.192)	0.599	0.904 (0.662-1.233)	0.523
T597C (TLR2)										
Additive										
TT	157 (0.460)	104 (0.452)	53 (0.477)	166 (0.430)	1		1		1	
TC	147 (0.431)	94 (0.409)	53 (0.477)	183 (0.474)	0.849 (0.624-1.155)	0.298	0.820 (0.578-1.163)	0.265	0.907 (0.587-1.401)	0.66
CC	37 (0.109)	32 (0.139)	5 (0.045)	37 (0.096)	1.057 (0.638-1.752)	0.829	1.380 (0.810-2.352)	0.235	0.423 (0.158-1.132)	0.079
Dominant										
TT	157 (0.460)	104 (0.452)	53 (0.477)	166 (0.430)	1		1		1	
TC+CC	184 (0.540)	126 (0.548)	58 (0..523)	220 (0.570)	0.884 (0.660-1.186)	0.411	0.914 (0.658-1.270)	0.592	0.826 (0.541-1.261)	0.375
Recessive										
TT+TC	304 (0.891)	198 (0.861)	106 (0.955)	349 (0.904)	1		1		1	
CC	37 (0.109)	32 (0.139)	5 (0.045)	37 (0.096)	1.148 (0.710-1.857)	0.573	1.524 (0.921-2.524)	0.099	0.445 (0.171-1.161)	0.09
Alleles										
T	461 (0.676)	302 (0.657)	159 (0.716)	515 (0.667)	1		1		1	
C	221 (0.324)	158 (0.343)	63 (0.284)	257 (0.333)	0.961 (0.771-1.196)	0.72	1.048 (0.822-1.338)	0.704	0.794 (0.572-1.102)	0.167

Each group was compared with the control group. All TB, pulmonary tuberculosis and tuberculous meningitis. PTB, pulmonary tuberculosis. TBM, tuberculous meningitis. SNP, single nucleotide polymorphisms. OR, odds ratio. CI, confidence interval.

Table 4. The genotype distribution of SNPs in cases (TB, PTB and TBM) and control when the data were adjusted for age and sex

					All TB vs. Control		PTB vs. Control		TBM vs. Control	
Polymorphism	All TB	PTB	TBM	Control	[a]OR (95%CI)	P	[a]OR (95%CI)	P	[a]OR (95%CI)	P
rs2072069 (CCL1)										
Additive										
GG	101 (0.316)	70 (0.324)	31 (0.298)	106 (0.300)	1		1		1	
AG	151 (0.472)	97 (0.449)	54 (0.519)	164 (0.465)	0.823 (0.531-1.274)	0.381	0.690 (0.422-1.129)	0.140	1.111 (0.597-2.066)	0.740
AA	68 (0.213)	49 (0.227)	19 (0.183)	83 (0.235)	0.929 (0.557-1.547)	0.776	1.041 (0.596-1.817)	0.888	0.686 (0.317-1.487)	0.340
Dominant										
GG	101 (0.316)	70 (0.324)	31 (0.298)	106 (0.300)	1		1		1	
AG+AA	219 (0.684)	146 (0.676)	73 (0.702)	247 (0.700)	0.858 (0.572-1.286)	0.458	0.802 (0.512-1.256)	0.335	0.960 (0.534-1.726)	0.893
Recessive										
GG+AG	252 (0.788)	167 (0.773)	85 (0.817)	270 (0.765)	1		1		1	
AA	68 (0.213)	49 (0.227)	19 (0.183)	83 (0.235)	1.044 (0.674-1.618)	0.846	1.292 (0.799-2.087)	0.296	0.642 (0.330-1.249)	0.192
T597C (TLR2)										
Additive										
TT	157 (0.460)	104 (0.452)	53 (0.477)	166 (0.430)	1		1		1	
TC	147 (0.431)	94 (0.409)	53 (0.477)	183 (0.474)	0.777 (0.530-1.138)	0.194	0.745 (0.483-1.148)	0.182	0.782 (0.460-1.328)	0.782
CC	37 (0.109)	32 (0.139)	5 (0.045)	37 (0.096)	1.493 (0.799-2.792)	0.209	1.928 (0.993-3.742)	0.052	0.611 (0.192-1.943)	0.611
Dominant										
TT	157 (0.460)	104 (0.452)	53 (0.477)	166 (0.430)	1		1		1	
TC+CC	184 (0.540)	126 (0.548)	58 (0.523)	220 (0.570)	0.878 (0.612-1.259)	0.478	0.909 (0.607-1.360)	0.641	0.760 (0.454-1.271)	0.295
Recessive										
TT+TC	304 (0.891)	198 (0.861)	106 (0.955)	349 (0.904)	1		1		1	
CC	37 (0.109)	32 (0.139)	5 (0.045)	37 (0.096)	1.421 (0.975-2.072)	0.067	2.218 (1.180-4.169)	0.013	0.689 (0.223-2.127)	0.689

Each group was compared with the control group. All TB, pulmonary tuberculosis and tuberculous meningitis. PTB, pulmonary tuberculosis. TBM, tuberculous meningitis. SNP, single nucleotide polymorphisms; OR, odds ratio; CI, confidence interval. [a]Adjusted for age and sex.

Table 5. The genotype distribution of rs2072069 in the CCL1 gene in cases (TB, PTB and TBM) and control, stratified by sex

					All TB vs. Control		PTB vs. Control		TBM vs. Control	
Polymorphism	All TB	PTB	TBM	Control	[a]OR (95%CI)	P	[a]OR (95%CI)	P	[a]OR (95%CI)	P
rs2072069 (CCL1)										
Male										
Additive										
GG	63 (0.289)	44 (0.289)	19 (0.288)	64 (0.299)	1		1		1	
AG	107 (0.491)	72 (0.474)	35 (0.530)	100 (0.467)	0.898 (0.525-1.535)	0.693	0.757 (0.416-1.379)	0.363	1.248 (0.574-2.713)	0.575
AA	48 (0.220)	36 (0.237)	12 (0.182)	50 (0.234)	1.019 (0.546-1.899)	0.954	1.186 (0.603-2.333)	0.622	0.703 (0.267-1.849)	0.475
Dominant										
GG	63 (0.289)	44 (0.289)	19 (0.288)	64 (0.299)	1		1		1	
AG+AA	155 (0.711)	108 (0.711)	47 (0.712)	150 (0.701)	0.937 (0.569-1.544)	0.800	0.891 (0.515-1.543)	0.681	1.050 (0.504-2.188)	0.896
Recessive										
GG+AG	170 (0.780)	116 (0.763)	54 (0.818)	164 (0.766)	1		1		1	
AA	48 (0.220)	36 (0.237)	12 (0.182)	50 (0.234)	1.089 (0.642-1.847)	0.753	1.401 (0.787-2.492)	0.252	0.610 (0.267-1.395)	0.241
Female										
Additive										
GG	38 (0.373)	26 (0.406)	12 (0.316)	42 (0.302)	1		1		1	
AG	44 (0.431)	25 (0.391)	19 (0.500)	64 (0.460)	0.714 (0.333-1.529)	0.386	0.606 (0.254-1.444)	0.258	0.886 (0.313-2.508)	0.819
AA	20 (0.196)	13 (0.203)	7 (0.184)	33 (0.237)	0.785 (0.319-1.930)	0.598	0.808 (0.299-2.183)	0.674	0.657 (0.180-2.395)	0.525
Dominant										
GG	38 (0.373)	26 (0.406)	12 (0.316)	42 (0.302)	1		1		1	
AG+AA	64 (0.627)	38 (0.594)	26 (0.684)	97 (0.698)	0.737 (0.365-1.490)	0.396	0.673 (0.306-1.480)	0.325	0.809 (0.304-2.153)	0.671
Recessive										
GG+AG	82 (0.804)	51 (0.797)	31 (0.816)	106 (0.763)	1		1		1	
AA	20 (0.196)	13 (0.203)	7 (0.184)	33 (0.237)	0.954 (0.435-2.093)	0.906	1.065 (0.443-2.560)	0.888	0.708 (0.230-2.178)	0.548

Each group was compared with the control group. All TB, pulmonary tuberculosis and tuberculous meningitis. PTB, pulmonary tuberculosis. TBM, tuberculous meningitis. SNP, single nucleotide polymorphisms; OR, odds ratio. CI, confidence interval. [a]Adjusted for age and sex.

Table 6. The genotype distribution of TLR2 T597C polymorphism in cases (TB, PTB and TBM) and control, stratified by sex

					All TB vs. Control		PTB vs. Control		TBM vs. Control	
Polymorphism	All TB	PTB	TBM	Control	[a]OR(95%CI)	P	[a]OR(95%CI)	P	[a]OR(95%CI)	P
T597C (TLR2)										
Male										
Additive										
TT	104 (0.452)	75 (0.466)	29 (0.420)	103 (0.440)	1		1		1	
TC	101 (0.439)	65 (0.404)	36 (0.522)	110 (0.470)	0.794 (0.500-1.263)	0.33	0.677 (0.404-1.136)	0.14	0.957 (0.491-1.863)	0.897
CC	25 (0.109)	21 (0.130)	4 (0.058)	21 (0.090)	1.724 (0.802-3.708)	0.163	1.850 (0.820-4.174)	0.138	1.169 (0.317-4.305)	0.815
Dominant										
TT	104 (0.452)	75 (0.466)	29 (0.420)	103 (0.440)	1		1		1	
TC+CC	126 (0.548)	86 (0.534)	40 (0.580)	131 (0.560)	0.915 (0.591-1.419)	0.693	0.829 (0.512-1.343)	0.445	0.981 (0.515-1.869)	0.953
Recessive										
TT+TC	205 (0.891)	140 (0.870)	65 (0.942)	213 (0.910)	1		1		1	
CC	25 (0.109)	21 (0.130)	4 (0.058)	21 (0.090)	1.929 (0.928-4.010)	0.079	2.220 (1.020-4.828)	0.044	1.195 (0.339-4.212)	0.781
Female										
Additive										
TT	53 (0.477)	29 (0.420)	24 (0.571)	63 (0.414)	1		1		1	
TC	46 (0.414)	29 (0.420)	17 (0.405)	73 (0.480)	0.755 (0.383-1.490)	0.418	0.949 (0.427-2.109)	0.897	0.558 (0.226-1.374)	0.204
CC	12 (0.108)	11 (0.159)	1 (0.024)	16 (0.105)	1.080 (0.357-3.265)	1.08	2.103 (0.656-6.745)	0..211	0.125 (0.010-1.524)	0.103
Dominant										
TT	53 (0.477)	29 (0.420)	24 (0.571)	63 (0.414)	1		1		1	
TC+CC	58 (0.523)	40 (0.580)	18 (0.429)	89 (0.586)	0.810 (0.427-1.537)	0.519	1.138 (0.540-2.397)	0.735	0.484 (0.200-1.166)	0.106
Recessive										
TT+TC	99 (0.892)	58 (0.841)	41 (0.976)	136 (0.895)	1		1		1	
CC	12 (0.108)	11 (0.159)	1 (0.024)	16 (0.105)	1.229 (0.425-3.553)	0.703	2.158 (0.720-6.473)	0.17	0.164 (0.014-1.910)	0.149

Each group was compared with the control group. All TB, pulmonary tuberculosis and tuberculous meningitis. PTB, pulmonary tuberculosis. TBM, tuberculous meningitis. SNP, single nucleotide polymorphisms; OR, odds ratio. CI, confidence interval. [a]Adjusted for age and sex.

Table 7. The genotype distribution of SNPs in cases (TBM grade 1 and TBM grade 2) and control, stratified TBM severity grade

				TBM grade 1 vs. Control		TBM grade 2 vs. Control	
Polymorphism	TBM grade 1	TBM grade 2	Control	[a]OR (95%CI)	P	[a]OR (95%CI)	P
rs2072069 (CCL1)							
Additive							
GG	27 (0.370)	4 (0.129)	106 (0.300)	1		1	
AG	36 (0.493)	18 (0.581)	164 (0.465)	0.832 (0.416-1.665)	0.603	2.587 (0.804-8.326)	0.111
AA	10 (0.137)	9 (0.290)	83 (0.235)	0.437 (0.171-1.115)	0.083	2.440 (0.678-8.775)	0.172
Dominant							
GG	27 (0.370)	4 (0.129)	106 (0.300)	1		1	
AG+AA	46 (0.630)	27 (0.871)	247 (0.700)	0.696 (0.361-1.343)	0.28	2.535 (0.821-7.831)	0.106
Recessive							
GG+AG	63 (0.863)	22 (0.710)	270 (0.765)	1		1	
AA	10 (0.137)	9 (0.290)	83 (0.235)	0.488 (0.211-1.131)	0.094	1.218 (0.505-2.936)	0.66
T597C (TLR2)							
Additive							
TT	38 (0.481)	15 (0.469)	166 (0.430)	1		1	
TC	38 (0.481)	15 (0.469)	183 (0.474)	0.821 (0.448-1.507)	0.525	0.638 (0.276-1.477)	0.294
CC	3 (0.038)	2 (0.063)	37 (0.096)	0.425 (0.100-1.808)	0.247	0.896 (0.183-4.387)	0.892
Dominant							
TT	38 (0.481)	15 (0.469)	166 (0.430)	1		1	
TC+CC	41 (0.519)	17 (0.531)	220 (0.570)	0.767 (0.424-1.388)	0.381	0.669 (0.301-1.490)	0.326
Recessive							
TT+TC	76 (0.962)	30 (0.938)	349 (0.904)	1		1	
CC	3 (0.038)	2 (0.063)	37 (0.096)	0.470 (0.114-1.931)	0.295	1.100 (0.234-5.161)	0.904

Each group was compared with the control group. OR, odds ratio. CI, confidence interval. [a]Adjusted for age and sex.

While there were no statistical differences in allele frequency or genotype distribution when the TB group or TBM group was compared with the control group (**Table 4**).

Association of SNPs with TB groups stratified by sex

Genotype distribution was analyzed with stratification by sex (**Tables 5**, **6**). We identified statistically significant difference of T597C genotype distribution between the PTB and control group among male subjects in recessive tested inheritance model (OR=2.220, 95% CI: 1.020-4.828, *P*=0.044), while there was no statistical difference between the PTB and control group among female subjects under recessive tested inheritance model (OR=2.158, 95% CI: 0.720-6.473, *P*=0.170) (**Tables 5**, **6**).

However, there were no statistical differences in variant genotype distribution at rs2072069 in the CCL1 gene when comparing the control group with these TB groups stratified by sex compared with control group.

Association of SNPs with TBM groups stratified by TBM severity grade

We analyzed the genotype comparison across subgroups stratified by TBM severity grade to determine whether the polymorphisms were associated with the severity of TBM. TBM patients were grouped into those belonged to grade 1 (no coma and no focal neurological deficits), grade II (reduced consciousness or focal neurological deficit) and grade III (deep coma, with or without focal deficit) [18]. Subgroups analysis revealed no statistical difference between SNPs and severity of TBM (**Table 7**).

Discussion

CCL1, one of members of the CC chemokine family, is an inflammatory mediator that stimulates the migration of human monocytes [21]. CCR8, present on lymphocytes and monocytes, is the receptor of CCL1 [22]. CCL1 expression was up-regulated in *Mycobacterium bovis* purified protein derivative (PPD) induced granulo-

mas in the lungs [23]. In our study, we did not find association between polymorphism rs2072069 in the CCL1 gene and different clinical forms of TB including PTB or/and TBM in our population. While Thuong et al [2] have found that polymorphism rs2072069 of CCL1 gene was associated with TBM rather than TB or PTB in Vietnamese population, which is the first identification of CCL1 as a gene involved in host susceptibility to TB. The inconsistent results may be in part explained by racial difference in TB susceptibility. One probable reason resulting in the said inconsistence may lie in the selection criteria of control subjects given that their study has provided cord-blood samples as controls. They didn't consider the possibility that these samples would become TB subjects if exposed to *M. tuberculosis* after birth. Our study avoided the risk of this form of misclassification. ÖZDEMİR FA et al [24] have found no association between CCL1 rs159294 T/A polymorphism and susceptibility to PTB or extra PTB in Turkish population.

On the other hand, our study showed that TLR2 T597C polymorphism was a predisposing risk factor for PTB rather than TBM, which was not in keeping with the previous observation [18]. While NTT Thuong et al [18] found that TLR2 genotype 597CC was associated with susceptibility to TB. Further subgroup analysis showed TLR2 genotype 597CC was associated with TBM rather than PTB in Vietnamese population. The contrary results may be resulting from the racial background or the selection criteria as we previously described. NTT Thuong et al [18] also demonstrated that the association increased with the severity of neurologic symptoms while subgroups analysis in our study revealed no statistical difference between polymorphism T597C and severity of TBM. In their study, the severity of subjects with TBM included grade 1, grade 2 and grade 3 while in our study, there was only grade 1 and grade 2. One limitation of our study was the small sample size of TBM. In agreement with our finding, Mohammad et al [19] found that T597C polymorphism of TLR2 gene was associated with susceptibility to PTB in Iranian population but they did not enrolled TBM as TB subjects.

Nazan et al [25] have found that the Arg753Gln polymorphism of the TLR2 gene influences pathogenic progression from infection to the onset of TB disease in Turkish children. D. Sa'nchez et al [26] found that TLR2 Asn199Asn and Arg753Gln polymorphisms in TLR2 were not involved as risk factors for PTB in a Colombian population. Xue et al [27] suggested that the S/M genotype of the guanine-thymine (GT) repeat microsatellite polymorphism in intron 2 of the TLR2 was associated with susceptibility to PTB in the Chinese population.

Abrar-ul-Haq Khan et al [28] have revealed that (-196 to -174 del) polymorphism of TLR2 gene was associated with susceptibility to PTB in Pakistan population. Moreover, males with heterozygous genotype (I/D) were more prone to have TB than females with the same genotype were. Our study has found that genotype 597CC was only associated with male subjects with PTB. Above all, gender may affect the outcome of *M. tuberculosis* infection.

As a result, this discrepancy may be due to differences in TB diagnostic and selection criteria, population genetic differences, small sample size, analytic approach and analysis of subgroups. Larger scale study will be needed in the future.

Our patients with TBM are diagnosed by modified Ziehl-Neelsen stain and clinical manifestation. All the subjects with TBM were Ziehl-Neelsen smear positive. Modified Ziehl-Neelsen stain not only significantly improves the rate of detection of extracellular *M. tuberculosis* significantly but also identifies intracellular *M. tuberculosis* in the neutrophils, monocytes, and lymphocytes [29]. In our study the potential misclassification of TBM group is low given that all of the subjects with TBM were Ziehl-Neelsen smear positive.

Our study demonstrates that T597C polymorphism of TLR2 is a risk factor for susceptibility to PTB rather than to TBM in a sample of Chinese adult population. Patients' gender may affect the outcome of *M. tuberculosis* infection. TLR2 gene may influence the development of different clinical forms of TB disease by different immune mechanisms given that the polymorphism of TLR2 was associated with PTB rather than with TBM. We further validate the hypothesis that different macrophage responses to *M. tuberculosis* are associated with distinct clinical outcomes that are genetically regulated. The most important thing is that mapping the host genetic background and geo-

graphical variation in Chinese population may provide knowledge and evidence on how the *M. tuberculosis* infect certain population in certain clinical form and by certain means of TBM pathogenesis.

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Disclosure of conflict of interest

None.

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