P2X7R Gene Polymorphisms Are Associated with Increased Risk of Pulmonary Tuberculosis in the Tibetan Chinese Population

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Abstract. In this study, we aim to explore the correlation between single nucleotide polymorphisms (SNPs) in the *P2X7R* gene and pulmonary tuberculosis (PTB) susceptibility in the Tibetan Chinese population in China. We examined 467 patients with active PTB and 504 healthy controls living in Xi'an and the surrounding area. Eight *P2X7R* SNPs were genotyped, and association analysis was performed. Odds ratios (ORs) and 95% confidence intervals (CIs) were tested by unconditional logistic regression analysis to evaluate the effects of the polymorphisms on PTB risk. *P2X7R* SNP association analyses were performed using SPSS 17.0 statistical packages and Microsoft Excel, SNP statistics software, Haploview software package (version 4.2), and SHEsis software platform. The results show that the “C” allele of rs656612 in the *P2X7R* gene was associated with an increased PTB risk by the additive model (OR = 1.307, 95% CI = 1.088–1.570, $P = 0.004$) and dominant model (rs656612, OR = 1.490, 95% CI = 1.153–1.926, $P = 0.002$). The “A” allele of rs208290 showed an increased PTB risk by the additive model (OR = 1.418, 95% CI = 1.179–1.706, $P < 0.001$) and dominant model (OR = 1.680, 95% CI = 1.297–2.177, $P < 0.001$), whereas the “A” allele of rs7958311 showed an increased risk by the additive model (rs7958311, OR = 1.260, 95% CI = 1.055–1.505, $P = 0.011$) and recessive model (OR = 1.609, 95% CI = 1.200–2.158, $P = 0.001$). After Bonferroni correction, rs208290 was found to be associated with PTB in the allele, dominant, and genotype models. In conclusion, our study revealed a significant association between three *P2X7R* gene polymorphisms (rs656612, rs208290, and rs7958311) and PTB in a Tibetan Chinese population.

INTRODUCTION

Pulmonary tuberculosis (PTB) is an infectious disease caused by the bacillus *Mycobacterium tuberculosis* (MTB).¹ It is still a public health challenge worldwide, especially in developing countries. The disease is spread in the air, for example, by an infected subject coughing. In 2014, there were an estimated 9.6 million incident cases of PTB worldwide: 5.4 million among males, 3.2 million among females, and 1.0 million among children.² A 2014 World Health Organization report estimated that even though there are so many people infected with MTB, only one-third of the global population has been latently infected without clinical symptoms, and about 10% of infected individuals develop into active PTB during their lifetime.³ Apart from bacterial variability, genetic host factors may also play a crucial role in an individual's predisposition to PTB.⁴ Stead and others reported that individuals with African ancestry were twice as likely to be infected with MTB as individuals with European ancestry, even though they shared the same environment.⁵ There has been dramatic progress in our understanding of the innate and adaptive immunity pathways involved in the human host defense to PTB,⁶ and some single nucleotide polymorphisms (SNPs) in innate immunity genes are reported candidate biomarkers associated with PTB susceptibility. It was reported that the rs2393799 polymorphism in the *P2X7R* gene was associated with increased risk of

PTB in an Iranian population.⁷ *P2X7R* is located on chromosome 12q24.31, and in this study, we genotyped eight SNPs: rs656612, rs208290, rs208305, rs7958311, rs2857590, rs1718119, rs11065468, and rs891781. To investigate potential relationships between *P2X7R* SNPs, genotypes, haplotypes, and their roles in PTB etiology, we performed a comprehensive association analysis in a case-control study in a Tibetan Chinese population.

MATERIALS AND METHODS

Study participants. A total of 467 Tibetan PTB patients were recruited from the Department of Respiratory Physicians of the Xi'an Chest and Tuberculosis Hospital and the School of Medicine, Xizang Minzu University, from October 2012 to September 2013 in Xi'an, China. Patients were diagnosed based on the criteria of sputum smear or culture positive for MTB pathogen, clinical-radiological findings, and histological evidence of PTB.⁸ We excluded individuals who were infected with nontuberculous *Mycobacterium*, treated with immunosuppressants, or exhibited only extrapulmonary tuberculosis without PTB.

We also recruited a random sample of 504 healthy controls at the medical center of Xi'an Chest and Tuberculosis Hospital from June 2012 to July 2013; they were all Tibetan Chinese living in Xi'an and the surrounding region. The subjects had no previous clinical history or laboratory criteria suggestive of PTB infection.

Cases and controls with any of the following conditions were excluded from the study: human immunodeficiency virus (HIV) positive or known to have any autoimmune, chronic inflammatory, or other diseases. Informed consent was obtained from all participants, and the study protocol

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was approved by the Human Research Committee of the Xi'an Chest and Tuberculosis Hospital.

SNP selection and genotyping. We selected eight *P2X7R* gene SNPs with minor allele frequencies > 5% for further genotyping in the HapMap Asian population.⁹ Blood samples were collected from each patient during a laboratory examination after their recruitment. DNA was extracted from whole blood samples using the Gold Mag-Mini Whole Blood Genomic DNA Purification Kit (GoldMag Ltd., Xi'an, China).¹⁰ DNA concentrations were measured by spectrometry (DU530 UV/VIS spectrophotometer; Beckman Instruments, Fullerton, CA). We designed a Multiplexed SNP Mass EXTENDED assay using Sequenom MassARRAY Assay Design 3.0 Software (Sequenom, Inc., San Diego, CA) and performed SNP genotyping with a Sequenom MassARRAY RS1000 (Sequenom, Inc.) using the standard protocol.¹¹ The eight SNPs selected for genotyping were rs656612, rs208290, rs208305, rs7958311, rs2857590, rs1718119, rs11065468, and rs891781. Their genetic information is shown in Table 1.

Statistical analysis. All statistical analyses were performed using SPSS version 17.0 statistical package (SPSS, Chicago, IL) and Microsoft Excel (Microsoft, Redmond, WA). All *P* values presented in this study are two sided, and *P* < 0.05 was considered statistically significant. We used Pearson's χ^2 tests to compare the distribution of categorical variables and Student's *t* tests to compare continuous variables.¹² The Hardy-Weinberg equilibrium (HWE) of each SNP was tested by an exact test to compare the expected frequency in controls. Allele frequencies and genotype frequencies for each SNP of PTB patients and control subjects were compared using χ^2 test. Odds ratios (ORs) and 95% confidence intervals (CIs) were tested by unconditional logistic regression analysis to evaluate the SNPs' effects on the risk of PTB.¹³ We used (<http://pngu.mgh.harvard.edu/Purcell/plink/>), a website software to test the associations between certain SNPs and the risk of PTB in three models (dominant, recessive, and additive).

We used the Haploview software package (version 4.2) and SHEsis software platform (<http://www.nhgg.org/analysis/>) to analyze linkage disequilibrium (LD), haplotype construction, and genetic association at polymorphism loci.

RESULTS

A total of 467 cases and 504 controls were enrolled in our study. Detailed information about the selected SNPs is shown in Table 1. The minor allele of each SNP, a risk factor,

was compared with the wild-type allele. All the tested SNPs were in HWE in the control group (*P* > 0.05). Comparing the differences in frequency distributions of alleles between cases and controls by χ^2 tests, we found a correlation between three loci (rs656612, OR = 1.320, 95% CI = 1.096–1.590, *P* = 0.003; rs208290, OR = 1.430, 95% CI = 1.188–1.722, *P* < 0.001; rs7958311, OR = 1.264, 95% CI = 1.057–1.511, *P* = 0.010) and increased risk for PTB under the allele model. We performed Bonferroni corrections and found that rs208290 (*P* = 0.004) was associated with an increased risk of PTB (Table 1). The other SNPs had no connection with PTB susceptibility.

Further model association analyses were performed by unconditional logistic regression analysis (Table 2). We found that rs656612 and rs208290 were associated with increased PTB risk by dominant model analyses (rs656612, OR = 1.490, 95% CI = 1.153–1.926, *P* = 0.002; rs208290, OR = 1.680, 95% CI = 1.297–2.177, *P* < 0.001). In the recessive model, rs7958311 was associated with increased PTB risk (rs7958311, OR = 1.609, 95% CI = 1.200–2.158, *P* = 0.001). Interestingly, rs656612, rs208290, and rs7958311 increased the risk of PTB in the additive model (rs656612, OR = 1.307, 95% CI = 1.088–1.570, *P* = 0.004; rs208290, OR = 1.418, 95% CI = 1.179–1.706, *P* < 0.001; rs7958311, OR = 1.260, 95% CI = 1.055–1.505, *P* = 0.011). After Bonferroni correction, we found that rs208290 was associated with PTB in two models (dominant model: *P* = 0.004; additive model: *P* = 0.008).

Association results between *P2X7R* genotypes and the risk of PTB are listed in Table 3. We identified three SNPs associated with PTB risk (rs656612, Genotype "CC", OR = 1.577, 95% CI = 1.066–2.335, *P* = 0.023; Genotype "CA", OR = 1.464, 95% CI = 1.114–1.923, *P* = 0.006; rs208290, Genotype "AA", OR = 1.826, 95% CI = 1.234–2.703, *P* = 0.003; Genotype "AG", OR = 1.637, 95% CI = 1.243–2.154, *P* < 0.050; rs7958311, Genotype "AA", OR = 1.595, 95% CI = 1.117–2.276, *P* = 0.010) (Table 3). After Bonferroni correction, we found that rs208290 (Genotype "AG", *P* = 0.016) was associated with PTB.

Finally, a haplotype-based association study was performed to identify associations between *P2X7R* haplotype and PTB risk (Table 4). We found that the SNPs in the *P2X7R* gene showed strong linkage. Haplotype blocks were defined according to the criteria laid out by Gabriel and others.¹⁴ Two LD blocks were detected in the control group (Figure 1). Block 1 consisted of two closely linked SNPs (rs656612 and

TABLE 1
SNPs in this study

rs number	Nucleotide position	Allele A/B	MAF frequency		HWE <i>P</i> value	Allele model		
			Case	Control		OR (95% CI)	<i>P</i> value	<i>P</i> value*
rs656612	121576652	C/A	0.392	0.328	0.129	1.320 (1.096–1.590)	0.003	0.120
rs208290	121594056	A/G	0.409	0.326	0.129	1.430 (1.188–1.722)	< 0.001	0.004
rs208305	121603469	C/A	0.105	0.092	0.294	1.151 (0.854–1.552)	0.357	1
rs7958311	121605355	A/G	0.526	0.467	0.325	1.264 (1.057–1.511)	0.010	0.400
rs2857590	121606059	A/C	0.105	0.092	0.294	1.154 (0.856–1.552)	0.350	1
rs1718119	121615103	A/G	0.106	0.092	0.294	1.164 (0.864–1.568)	0.320	1
rs11065468	121615369	C/T	0.106	0.092	0.294	1.164 (0.864–1.568)	0.320	1
rs891781	121618438	T/C	0.105	0.092	0.294	1.151 (0.854–1.552)	0.360	1

CI = confidence interval; HWE = Hardy-Weinberg equilibrium; MAF = minor allele frequency; OR = odds ratio; SNPs = single nucleotide polymorphisms. *P* values were calculated with Pearson's χ^2 tests; *P* < 0.05 indicates statistical significance. The bold *P* value indicates statistical significance.
**P* values adjusted with Bonferroni corrections.

TABLE 2
Unconditional logistic regression analysis of the association between the SNPs and PTB risk

SNP	Model	Genotype	Cases	Controls	OR (95% CI)	P value	P value*
rs656612	Dominant model	A/A	173	235	1	0.002	0.080
		C/C-C/A	294	268	1.490 (1.153–1.926)		
	Recessive model	A/A-A/C	395	441	1	0.164	1
rs208290	Additive model	C/C	72	62	1.297 (0.899–1.869)		
		–	–	–	1.307 (1.088–1.570)	0.004	0.160
	Dominant model	G/G	161	236	1	< 0.001	0.004
		A/A-A/G	306	267	1.680 (1.297–2.177)		
	Recessive model	G/G-G/A	391	442	1	0.065	1
rs208305	Additive model	A/A	76	61	1.408 (0.979–2.026)		
		–	–	–	1.418 (1.179–1.706)	< 0.001	0.008
	Dominant model	A/A	373	412	1	0.420	1
		C/C-C/A	94	91	1.141 (0.828–1.572)		
	Recessive model	A/A-A/C	463	501	1	0.374	1
rs7958311	Additive model	C/C	4	2	2.164 (0.395–11.870)		
		–	–	–	1.158 (0.853–1.571)	0.347	1
	Dominant model	G/G	114	137	1	0.316	1
		A/A-A/G	353	366	1.159 (0.869–1.546)		
	Recessive model	G/G-G/A	329	399	1	0.001	0.040
rs2857590	Additive model	A/A	138	104	1.609 (1.200–2.158)		
		–	–	–	1.260 (1.055–1.505)	0.011	1
	Dominant model	C/C	372	412	1	0.411	1
		A/A-A/C	94	91	1.144 (0.830–1.576)		
	Recessive model	C/C-C/A	462	501	1	0.373	1
rs1718119	Additive model	A/A	4	2	2.169 (0.395–11.900)		
		–	–	–	1.160 (0.855–1.575)	0.339	1
	Dominant model	G/G	372	412	1	0.374	1
		A/A-A/G	95	91	1.156 (0.840–1.592)		
	Recessive model	G/G-G/A	463	501	1	0.374	1
rs11065468	Additive model	A/A	4	2	2.164 (0.395–11.870)		
		–	–	–	1	0.374	1
	Dominant model	T/T	372	412	1	0.374	1
		C/C-C/T	95	91	1.156 (0.840–1.592)		
	Recessive model	T/T-T/C	463	501	1	0.374	1
rs891781	Additive model	C/C	4	2	2.164 (0.395–11.870)		
		–	–	–	1.172 (0.864–1.589)	0.308	1
	Dominant model	C/C	373	412	1	0.420	1
		T/T-T/C	94	91	1.141 (0.828–1.572)		
	Recessive model	C/C-C/T	463	501	1	0.374	1
	Additive model	T/T	4	2	2.164 (0.395–11.870)		
		–	–	–	1.158 (0.853–1.571)	0.347	1

CI = confidence interval; OR = odds ratio; PTB = pulmonary tuberculosis; SNPs = single nucleotide polymorphisms. *P* values were calculated with Pearson's χ^2 tests; *P* < 0.05 indicates statistical significance. The bold *P* value indicates statistical significance.

**P* values adjusted with Bonferroni corrections.

rs208290), and block 2 included five closely linked SNPs (rs7958311, rs2857590, rs1718119, rs11065468, and rs891781). We found two haplotypes associated with an increased PTB risk (Genotype “CA”, OR = 1.37, 95% CI = 1.14–1.65,

P = 0.001; Genotype “AA”, OR = 5.43, 95% CI = 2.02–14.61, *P* < 0.001), whereas genotype “GCGTC” was associated with decreased risk Genotype “GCGTC”, OR = 0.74, 95% CI = 0.61–0.89, *P* = 0.002).

DISCUSSION

This case-control study analyzed the association between eight *P2X7R* gene SNPs and the risk of PTB among Tibetan Chinese. It was reported that *P2X7R* can mediate the apoptosis of immune cells including monocytes, macrophages, and lymphocytes,¹⁵ but others hypothesized that *P2X7R* gene does not play a relevant role in controlling PTB in humans.¹⁶ Still, ethnic and racial differences in vulnerability to PTB with respect to *P2X7R* polymorphisms have been reported,^{9,17–19} including in Iranian, Asian Indian, and Chinese Han populations. In the present study, we examined whether *P2X7R* was associated with the incidence of PTB in the Tibetan Chinese population.

The results showed that the “C” allele of rs656612, “A” allele of rs208290, and “A” allele of rs7958311 in the *P2X7R* gene were associated with an increased risk of PTB. This phenomenon might result from deficient *P2X7R* function in

TABLE 3

Frequency distributions of *P2X7R* genotypes and their associations with the risk of developing PTB

Genotype	Cases	Controls	OR (95% CI)	P value	P value*
rs656612					
	AA	173	235	1.00 (Ref)	
	CA	222	206	1.464 (1.114–1.923)	0.006
rs208290					
	CC	72	62	1.577 (1.066–2.335)	0.023
	GG	161	236	1.00 (Ref)	
rs7958311					
	AG	161	206	1.637 (1.234–2.154)	< 0.01
	AA	76	61	1.826 (1.234–2.703)	0.003
rs891781					
	GG	114	137	1.00 (Ref)	
	AG	215	262	0.986 (0.726–1.341)	0.930
	AA	138	104	1.595 (1.117–2.276)	0.010

CI = confidence interval; OR = odds ratio; PTB = pulmonary tuberculosis; SNPs = single nucleotide polymorphisms. *P* values were calculated with Pearson's χ^2 tests; *P* < 0.05 indicates statistical significance. The bold *P* value indicates statistical significance.

**P* values adjusted by Bonferroni corrections.

TABLE 4
Haplotype frequencies and their associations with PTB risk

Block	SNPs	Haplotype	Frequency		OR	95% CI	P value
			Case	Control			
1	rs656612 rs208290	AG	0.586	0.667	1.00	(Ref)	–
		CA	0.387	0.321	1.37	1.14–1.65	0.001
		AA	0.023	0.005	5.43	2.02–14.61	< 0.001
2	rs7958311 rs2857590 rs1718119 rs11065468 rs891781	ACGTC	0.526	0.467	1.00	(Ref)	–
		GCGTC	0.368	0.44	0.74	0.61–0.89	0.002
		GAACT	0.105	0.092	1.03	0.75–1.41	0.860

CI = confidence interval; OR = odds ratio; PTB = pulmonary tuberculosis; SNPs = single nucleotide polymorphisms. $P < 0.05$ indicates statistical significance; P values were calculated with unconditional logistic regression. The bold p value indicates statistical significance.

the immune cells of PTB patients, which may prevent mycobacteria from being eliminated from the patient's body.⁷ Bahari and others had reported that the rs2393799 polymorphism in the *P2X7R* gene might contribute to patient susceptibility to PTB in the Iranian population.⁷ Sharma and others also suggested that hosts who are homozygous or heterozygous for the +1513C allele of *P2X7R* experience apoptosis of infected macrophages, which further increases the risk of PTB.²⁰

In this study, a protective effect of PTB was also observed for the *P2X7R* haplotypes "AG" and "GCGTC," which is in accordance with Saunders's conclusion that *P2X7R* is involved in host defense systems against MTB and adenosine triphosphate (ATP) stimulation of this receptor, resulting in mycobacterial death via apoptosis of infected macrophage cells.¹⁵ In addition, Palomino and others suggested that rs656612 located in intron 1 of the *P2X7R* gene had a small but significant effect on blood pressure in individuals with northern and western European ancestry.²¹ Ide and others reported that the functional rs7958311 G/A SNP located in the gap between the present LD4 and LD5 regions was asso-

ciated with lower chronic pain intensity in two human cohort studies.²² It was also suggested that *P2X7R* gene is associated with anxiety, bipolar, and unipolar disorders.²³

PTB produced by MTB is an infectious disease, and with increasing antibiotic resistance and coinfection with HIV, it is an increasing world health problem with a high impact on morbidity and mortality.²⁴ Macrophages are the principal host cells responsible for controlling intracellular mycobacterial replication. They act as antigen-presenting cells during reactivation of lymphocytes at infection sites. Numerous genes and pathways associated with PTB risk have been verified, with validation of polymorphisms in *IFNG*, *NRAMP1*, and *NOS2A* and equivocal results for *IL10*, *CCL2*, *DC-SIGN*, *P2X7R*, *VDR*, *TLR2*, *TLR9*, and *SP110*²⁵; however, specific *P2X7R* receptor gene SNPs associated with PTB had not been identified.

The human *P2X7R* gene is located on chromosome 12q24.31 and consists of 13 exons, with exon 12 and 13 coding for the C-terminal tail.²⁶ It is a member of the purinergic family of cellular receptors expressed in the blood and immune system, which is gated by extracellular ATP and involved in killing of intracellular mycobacteria followed by apoptosis of infected macrophages.²⁷ Additional studies suggested that *P2X7R* might have a key role in the inflammatory response, mediating the release of interleukin-1b and tumor necrosis factor- α .²⁸ Moreover, *P2X7R* is highly polymorphic, with several SNPs affecting its function.⁷

To the best of our knowledge, this was the first investigation into a correlation between the above three *P2X7R* gene polymorphisms and PTB susceptibility in a Tibetan Chinese population, and we obtained useful results. Of course, these findings simply provide preliminary evidence of ethnic and racial differences. The vulnerability of different populations to PTB with respect to *P2X7R*⁹ should also be examined in further studies.

Certainly, there were a number of reasons for these potentially conflicting data. First, we did not consider the effects of age and sex of the PTB group, which could reduce the results' credibility. Second, this was a hospital-based study and inherent biases can lead to unreliable results. All the participants of the cases were restricted to Tibetan Chinese living in Xi'an City and nearby. Third, environmental factors such as tobacco, diet, and alcohol consumption and the possibility of inaccurate exposure were not considered. Fourth, our sample size was not large enough for association studies; more subjects would need assessment to confirm our findings. Finally, statistical error might also have influenced the results. We will endeavor to improve these shortcomings in future investigations.

FIGURE 1. Haplotype block map for *P2X7R* single nucleotide polymorphisms (SNPs). The measure of linkage disequilibrium (D') among all possible pairs of SNPs is shown graphically according to the shading, where white and black represent very low and very high D' , respectively. The numbers in squares are D' values ($D' \times 100$).

CONCLUSION

In conclusion, our study revealed significant associations between three polymorphisms (rs656612, rs208290, and rs7958311) in the *P2X7R* gene and PTB in a Tibetan Chinese population. *P2X7R* gene polymorphisms may be a novel underlying mechanism of PTB development and a new personal treatment target.

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