Lack of association between polymorphisms in the P2X7 gene and tuberculosis in a Chinese Han population

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Abstract

Several studies have suggested that genetic factors may affect the susceptibility of a population to tuberculosis, and it has been found that P2X7 is linked to an increased risk for tuberculosis in some West African, Southeast Asian, North American, and North European populations. To explore the potential role of P2X7 in the susceptibility to tuberculosis among members of the Chinese Han population, we evaluated the association of the 1513A → C and −762T → C polymorphisms in P2X7 with the risk for tuberculosis. PCR amplification of genomic DNA was followed by restriction fragment length polymorphism analysis, and allele-specific PCR was used. We found no significant differences in the genotypic and allelic frequencies of 1513A → C polymorphisms in 96 patients with tuberculosis compared with 384 control subjects (P = 0.856 and 0.316, respectively; odds ratio (OR) for the C allele = 0.976; 95% confidence interval (CI) = 0.755–1.262). Similarly, no significant association was found between the −762T → C polymorphism and tuberculosis (P = 0.102 and 0.095 for the patients and controls, respectively; OR for the C allele = 0.924; 95% CI = 0.847–1.010). Thus, our analysis of P2X7 showed that the 1513A → C and −762T → C polymorphisms did not appear to be associated with the susceptibility of the Chinese Han population to tuberculosis.

Introduction

Tuberculosis is a major cause of morbidity and mortality worldwide, especially in Asia and Africa. Globally, an estimated 9.2 million new cases of and 1.7 million deaths from tuberculosis were recorded in 2006, including 0.7 million new cases and 0.2 million deaths among HIV-positive individuals (World Health Organization, 2008). HIV infection is the strongest risk factor for activation of tuberculosis, and the risk factors associated with disease progression are not clear in the case of most non-HIV-infected patients. Genetic variability and some environmental factors are expected to contribute to the risk of developing active tuberculosis (Cooke & Hill, 2001). Some genetic variations such as Mendelian-inherited mutations in the genes encoding interferon (IFN)-γ, interleukin-12, and signal transducers are rare and are associated with severe mycobacterial infection. Other genetic variations such as polymorphisms in the genes encoding human leukocyte antigen (HLA) type, P2X7 receptor, the solute carrier family 11 member 1 protein (SLC11A1, formerly known as NRAMP1), and vitamin D3 receptor (VDR), which occur more commonly, are considered to account for the susceptibility of the general population to tuberculosis (Döffinger et al., 2001; Bellamy, 2003).

Mycobacterium tuberculosis is a facultative intracellular pathogen. Macrophages, as the principal host cells for the intracellular replication of mycobacteria, act as antigen-presenting cells during the activation of lymphocytes at the sites of infection; they are responsible for killing internalized bacilli through the generation of reactive nitrogen and oxygen intermediates and, importantly, through the promotion of phagolysosomal fusion (Schaible et al., 1999). Many studies have shown that extracellular ATP, through
the activation of the P2X7 purinergic receptor, induces both macrophage apoptosis and the killing of intracellular mycobacteria in infected human macrophages (Kusner & Barton, 2001; Franco-Martinez et al., 2006).

The human P2X7, which encodes the P2X7 receptor, comprises 13 exons and is localized on chromosome 12q24; it encodes a 595-aa-long polypeptide with two transmembrane stretches (Buell et al., 1998). The P2X7 receptor is a ligand-gated cation channel that is highly expressed on human and murine macrophages and is further upregulated by IFN-γ (Nicke et al., 1998; Gu et al., 2001). Activation of P2X7 by ATP causes immediate opening of a cation-selective channel, allowing the influx of Ca²⁺ and Na⁺ and the efflux of K⁺, initiating a number of downstream signaling events, such as the caspase cascade, which results in apoptosis; and activating phospholipase D, which promotes phagosome–lysosome fusion and thus leads to mycobacterial death (Humphreys et al., 2000; Kusner & Barton, 2001; Coutinho-Silva et al., 2003).

P2X7 is highly polymorphic and several single nucleotide polymorphisms (SNPs) that lead to the loss of receptor function have been described (Fernando et al., 2005; Shemon et al., 2006). The most common is the 1513A → C polymorphism, because of which glutamic acid at position 496 changes to alanine, the function of the P2X7 receptor in macrophages from subjects homozygous for the 1513C allele is ablated, and the function of P2X7 receptor in macrophages from heterozygous subjects is significantly impaired. Additionally, the –762T → C SNP in the promoter region of P2X7 has been described to have a protective effect against tuberculosis in the Gambian population (Li et al., 2002).

It appears that there are ethnic and racial differences in the susceptibility of humans to tuberculosis and in the P2X7 polymorphisms observed (Li et al., 2002; Fernando et al., 2007; Niño-Moreno et al., 2007; Mokrousov et al., 2008). This study is the first in which the allelic distribution of these two P2X7 polymorphisms and its possible link to tuberculosis susceptibility are assessed in the Chinese population. We compared our results with the data available on these SNPs in other human populations.

Materials and methods

Study population

We identified 96 tuberculosis patients admitted to Beijing Children’s Hospital on the basis of their clinical manifestations, culture and X-ray results, etc. The control group included 384 children who were sampled from the surgical outpatient department by random sampling; their gender, age, and region distribution information was matched with that of the tuberculosis patients. Members of both groups were of Chinese Han ancestry. Information on the patients and their clinical features was obtained from the patients’ files, and all the participants and their parents provided written, informed consent.

Data analysis

Statistical analyses were carried out using SPSS software, version 11.5 (SPSS Inc., Chicago, IL). The Hardy–Weinberg equilibrium (HWE), which indicates the absence of a discrepancy between the genotypic and the allele frequencies, was determined in the case of both control subjects and patients. The overall genotypic frequency was compared between the groups using a 3 × 2 χ² test, and the allelic frequency was compared using a 2 × 2 χ² test.

DNA extraction and genotyping

DNA samples were extracted using the standard salting-out procedure. The concentration and purity of DNA were estimated spectrophotometrically. The 1513A → C SNP was genotyped by PCR-restriction fragment length polymorphism (RFLP) with the following primers: 5’-AGACCTGGGATGG ACTTCACAG-3’ (forward) and 5’-GCCAGGGTCGATGG ACCTG-3’ (reverse) (Niño-Moreno et al., 2007). The PCR conditions were as follows: initial denaturation at 95°C for 3 min; three cycles of 94.5°C for 45 s, 65°C for 1 min, and 72°C for 30 s; three cycles of 94°C for 50 s, 64°C for 45 s, and 72°C for 30 s; 27 cycles of 94°C for 45 s, 63°C for 40 s, and 72°C for 30 s; and final elongation at 72°C for 4 min. The PCR products were digested at 37°C for 3 h with 5.0 U of HaeII (Promega, Milwaukee, WI). The digested products were run on a 1.5% agarose gel that was stained with ethidium bromide and visualized using a UV transilluminator.

The –762T → C polymorphism was genotyped using an allele-specific PCR assay (Mokrousov et al., 2008). Two outer primers – P2X7,3 (5’-GGTGTCGGGTGGTGC TGCC-3’, forward) and P2X7,4 (5’-TGGTGGGGGTGGGAGGG GC-3’, reverse) – as well as two inner primers – P2X7,5 (5’-GGGTGCCCTGAATTGAACTG-3’, forward) and P2X7,6 (5’-GGCAGGCTCCAAAGGTTAGTTGGTTC-3’, reverse) – were used. The two outer primers amplified a 373-bp fragment in all cases. For the –762T allele, a 186-bp fragment was amplified using primers P2X7,4 and P2X7,5; for the –762C allele, a 235-bp fragment was amplified using primers P2X7,3 and P2X7,6. The cycling conditions were as follows: initial denaturation at 95°C for 5 min; 10 cycles of 94°C for 20 s, 65°C for 30 s, and 72°C for 30 s; 30 cycles of 94°C for 20 s, 63°C for 30 s, and 72°C for 30 s; and final elongation at 72°C for 10 min. The amplified PCR fragments were subjected to electrophoresis in 1.5% standard agarose gels.
Results

This study included 96 tuberculosis patients and 384 control subjects belonging to the Chinese Han ethnic group. The mean age was 5.5 years (SD, 4.5; range, 3 months–15 years) in the case of the tuberculosis patients and 5.9 years (SD, 4.0; range, 3 months–16 years) in the case of the control subjects. The proportion of female patients was 44.8% in the patient group and 41.1% in the control group. Table 1 lists the basic characteristics of the patients and control subjects.

We analyzed the genotypic and allelic frequencies of the two SNPs of the \( P2X7 \) gene. We detected the 1513A→C polymorphism using RFLP with the restriction enzyme HaeII. We found that the A and C alleles were in HWE (Table 2). The frequency of the 1513A allele in the tuberculosis patients was 0.724, whereas that of 1513C was 0.276, and no significant differences were noted in comparison with the frequencies in the case of the control subjects (\( \chi^2 = 0.033, P = 0.856 \), odds ratio (OR) for the C allele of the 1513A→C SNP = 0.976, 95% confidence interval (CI) = 0.755–1.262; Table 2). Analysis of genotypic distribution using a 3×2 \( \chi^2 \) test revealed no significant difference between the two groups (\( \chi^2 = 2.306, P = 0.316 \)). Moreover, no significant associations were found between the genotypic or the allelic distributions and pulmonary or extrapulmonary tuberculosis (Table 2).

The polymorphism at position –762 in the promoter of \( P2X7 \) was studied by allele-specific PCR; the T and C alleles were also found to be in HWE (Table 3). When the allelic and genotypic frequencies were analyzed via a \( \chi^2 \) test, no significant differences were detected between the groups (\( \chi^2 = 2.670 \) and 4.717, \( P = 0.102 \) and 0.095, respectively; OR for the C allele of –762T→C SNP = 0.924; and 95% CI = 0.847–1.010; Table 3), indicating the lack of any association between this SNP and tuberculosis (either pulmonary or extrapulmonary) in the Chinese Han population (Table 3).

Discussion

This study was conducted to obtain insights into the role of the human \( P2X7 \) in host susceptibility to tuberculosis in

### Table 1. Characteristics of patients and control subjects belonging to the Chinese Han population

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patients</th>
<th>Control subjects</th>
<th>All enrolled subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years ± SD (range)</td>
<td>5.5 ± 4.5 (0.25–15)</td>
<td>5.9 ± 4.0 (0.25–16)</td>
<td>5.8 ± 4.1 (0.25–16)</td>
</tr>
<tr>
<td>Sex, no. (% female)</td>
<td>43 (44.8)</td>
<td>158 (41.1)</td>
<td>201 (41.9)</td>
</tr>
<tr>
<td>Address, no. (% town)</td>
<td>31 (32.3)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>BCG vaccination, no. (%)</td>
<td>33 (34.4)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Exposure history, no. (%)</td>
<td>54 (56.3)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Tuberculin skin test results &gt; 10 mm, no. (%)</td>
<td>70 (72.9)</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND, not determined.

### Table 2. Frequency of the 1513A→C polymorphism of \( P2X7 \) in the Chinese population

<table>
<thead>
<tr>
<th>Subjects (n)</th>
<th>Genotype</th>
<th>Allele</th>
<th>HWE (( P^* ))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AA (%)</td>
<td>AC (%)</td>
<td>CC (%)</td>
</tr>
<tr>
<td>Control (384)</td>
<td>221 (57.6)</td>
<td>119 (31.0)</td>
<td>44 (11.5)</td>
</tr>
<tr>
<td>Patients (96)</td>
<td>51 (53.1)</td>
<td>37 (38.5)</td>
<td>8 (8.3)</td>
</tr>
<tr>
<td>Pulmonary TB (41)</td>
<td>21 (51.2)</td>
<td>18 (43.9)</td>
<td>2 (4.9)</td>
</tr>
<tr>
<td>Extrapulmonary TB (55)</td>
<td>30 (54.5)</td>
<td>19 (34.5)</td>
<td>6 (10.9)</td>
</tr>
</tbody>
</table>

\( P^* \) is the significance of correspondence to Hardy–Weinberg proportions according to Pearson’s \( \chi^2 \) test.

### Table 3. Frequency of the –762T→C polymorphism of the \( P2X7 \) gene in the Chinese population

<table>
<thead>
<tr>
<th>Subjects (n)</th>
<th>Genotype</th>
<th>Allele</th>
<th>HWE (( P^* ))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TT (%)</td>
<td>TC (%)</td>
<td>CC (%)</td>
</tr>
<tr>
<td>Control (384)</td>
<td>41 (10.7)</td>
<td>135 (35.2)</td>
<td>208 (54.2)</td>
</tr>
<tr>
<td>Patients (96)</td>
<td>10 (10.4)</td>
<td>23 (24.0)</td>
<td>63 (65.6)</td>
</tr>
<tr>
<td>Pulmonary TB (38)</td>
<td>4 (10.5)</td>
<td>11 (28.9)</td>
<td>23 (60.5)</td>
</tr>
<tr>
<td>Extrapulmonary TB (58)</td>
<td>6 (10.3)</td>
<td>12 (20.7)</td>
<td>40 (69.0)</td>
</tr>
</tbody>
</table>

\( P^* \) is the significance of correspondence to Hardy–Weinberg proportions according to Pearson’s \( \chi^2 \) test.
the Chinese Han population. We found that neither the 1513A → C nor the −762T → C variant of P2X7 was significantly associated with tuberculosis. It was previously shown that the −762T → C SNP in the promoter region conferred a significant protective effect against tuberculosis in a Gambian population, but no association was found between the 1513A → C SNP and tuberculosis (Li et al., 2002). While a significant association was found between the 1513A → C variant of P2X7 and pulmonary tuberculosis, no association was found between the −762T → C polymorphism and tuberculosis in the Mexican mestizo population (Niño-Moreno et al., 2007) or the Russian Caucasian population (Mokrousov et al., 2008). Researchers from Australia, meanwhile, found an association between the 1513C allele and extrapulmonary rather than pulmonary tuberculosis in the Australian Vietnamese population (Fernando et al., 2007).

Many authors have analyzed and studied the putative mechanism of the SNPs in P2X7. The 1513C polymorphism occurs in a region of exon 13, which encodes the carboxy terminal tail of the P2X7 protein, and the truncation of this domain prevents the influx of large cations that affect the functioning of ATP-induced pores, eventually leading to the loss of receptor function (Saunders et al., 2003; Shemon et al., 2006). A polymorphism in the promoter region, such as −762T → C, might act by influencing the degree to which the receptor is downregulated via other host- or pathogen-generated inhibitory factors; accordingly, Li et al. (2002) observed not only that the expression of the P2X7 receptor on the mononuclear cells of Gambian patients with active tuberculosis was reduced but also that the expression levels recovered during chemotherapy.

Although the mechanism by which these genotypes protect against or support the pathogenicity of tuberculosis has not been completely elucidated, we consider these apparently discrepant results to be of interest. The data in the above studies indicate that the importance of individual SNPs within genes may vary markedly among different racial groups. Therefore, it is rational to conclude that in a Gambian population, the −762T → C variant of P2X7 has an important effect on the regulation of the expression of this gene, while in a Mexican, Caucasian, or Asian population, this is not the case; the same would apply to the −1513A → C position. Therefore, further study of the association of these P2X7 polymorphisms with the development of clinical tuberculosis in different populations is warranted.

The overall impact that SNPs have on the susceptibility of a particular population to tuberculosis depends on the frequency of the allele in the study population. Consider the 1513A → C polymorphism as an example: in a study on a Gambian population (Li et al., 2002), the 1513A → C polymorphism was observed to have a lower frequency (7.6%) than that in the Australian Caucasian population (17.2%) or the Australian Vietnamese population (25%) (Fernando et al., 2007). Therefore, this polymorphism may confer significant tuberculosis susceptibility to Australian Caucasians and Australian Vietnamese. With regard to the −762T → C SNP in the Gambian population, the −762C allele was found in 32.9% and 25.4% and the CC genotype in only 12.7% and 7.1% of the control subjects and tuberculosis patients, respectively. Further, the −762C allele and the CC genotype were found more frequently in the control subjects (P = 0.03 and P = 0.003, respectively) (Li et al., 2002). In contrast, the Russian Caucasian population showed much higher and almost equal frequencies in terms of both the −762C allele (69.3% vs. 68.2%) and the CC homozygotes (51.2% vs. 45.3%) in the control subjects and tuberculosis patients, respectively (Mokrousov et al., 2008). The observations with regard to the Russian population were similar to those with regard to the Chinese Han population in our study; thus, the −762T → C SNP appeared to confer a protective effect against tuberculosis in the Gambian population but not in the Russian Caucasian and Chinese populations.

In summary, our data indicate that the 1513A → C and −762T → C polymorphisms of P2X7 are not associated with an increased susceptibility to M. tuberculosis infection in the Chinese population. Because host susceptibility to tuberculosis is likely to be under polygenic control, and the risk attributable to each polymorphism is modest, the precise mechanism(s) underlying susceptibility or protection, as well as its possible clinical relevance, remains an interesting topic to be explored.

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Authors’ contribution

J.X. and L.S. contributed equally to this study.

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