Original Article

Association of toll-like receptor 2 gene polymorphism with susceptibility to pulmonary tuberculosis and tuberculous meningitis: a meta-analysis

Kan Zhang1,2, Lingling Yi1,2, Dan Cheng1,2, Yuqing Mo1,2, Guohua Zhen1,2

1Division of Respiratory and Critical Care Medicine, Department of Internal Medicine, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, China; 2Key Laboratory of Respiratory Diseases, National Health and Family Planning Commission of The People’s Republic of China, Wuhan, China

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Abstract: Background: Toll-like receptor 2 (TLR2) gene plays an important role in the pathogenesis of pulmonary tuberculosis and tuberculous meningitis. The association between TLR2 T597C polymorphism and the susceptibility to pulmonary tuberculosis has been extensively studied. However, the results of these studies remain inconsistent. Therefore, we performed a meta-analysis to evaluate the association between TLR2 T597C polymorphism and the susceptibility to pulmonary tuberculosis and tuberculous meningitis. Methods: PubMed, Embase, CNKI, Wanfang, Weipu databases were searched for case-control studies on TLR2 polymorphisms and the risks of tuberculosis, published up to Nov 31, 2014. To assess the strength of the association between TLR2 polymorphism and pulmonary tuberculosis and tuberculous meningitis, the odds ratios (ORs) with 95% confidence intervals (CIs) were used. The meta-analysis of the associations between the TLR2 T597C polymorphism and pulmonary tuberculosis and tuberculous meningitis were carried out under different genetic models. Results: Fourteen published studies with 4381 cases and 5082 controls were included. Overall, there are significant association between TLR2 T597C polymorphism and the risk of tuberculosis (CC vs. TC OR = 1.26, 95% CI = 1.10-1.43; CC vs. TT OR = 1.20, 95% CI = 1.04-1.38; CC vs. TC+TT OR = 1.23, 95% CI = 1.08-1.39). When stratified by ethnicity, we found a significant association between this polymorphism and tuberculosis risks in Asian (CC vs. TC OR=1.23, 95% CI = 1.02-1.48; CC vs. TT OR = 1.22, 95% CI = 1.12-1.47; CC vs. TC+TT OR = 1.22, 95% CI = 1.02-1.46) and Caucasians (CC vs. TC OR = 1.31, 95% CI = 1.01-1.62; CC vs. TC+TT OR = 1.24, 95% CI = 1.01-1.52). We also found significant the association between this polymorphism and pulmonary tuberculosis (CC vs. TC OR = 1.16, 95% CI = 1.01-1.34) and the tuberculous meningitis (CC vs. TC OR = 3.16, 95% CI = 2.00-5.00; CC vs. TT OR = 3.56, 95% CI = 2.27-5.58; CC vs. TC+TT OR = 3.37, 95% CI = 2.19-5.19; CC+TC vs. TT OR = 1.36, 95% CI = 1.05-1.77; C vs. T OR = 1.52, 95% CI = 1.25-1.85), respectively. Conclusions: TLR2 T597C polymorphism associated with the susceptibility to pulmonary tuberculosis and tuberculous meningitis.

Keywords: Toll-like receptor 2 T597C, TLR2, single nucleotide polymorphism, tuberculosis risk, pulmonary tuberculosis, tuberculosis meningitis, meta-analysis

Introduction

According to WHO statistics report in 2010, about one third people in the world have infected with Mycobacterium tuberculosis, in some developing countries, adults who have carried Mycobacterium tuberculosis even up to 80%, about 5% to 10% of these carriers may develop to active tuberculosis. A number of genes participate in the progression from M. tuberculosis infection to tuberculosis diagnosis. Toll-like receptors (TLR) family plays essential roles in the innate responses against M. tuberculosis [3-5]. Toll-like receptors (TLRs) mediate the each stage of the inflammatory response, for example the first line of host defense and the immune activation [6-11]. Therefore, the polymorphisms of TLR1, TLR2, TLR4, TLR6 and TLR9 have been reported that they have the susceptibility to pulmonary tuberculosis (PTB) with distinct nationality [12-16]. In contrast, some studies showed that there was no association between the polymorphism of TLRs and the susceptibility to tuberculosis [16-19].
TLR2 gene locates in human’s chromosome 4q32 and consists of 4 exons and 3 introns. It contains numerous polymorphisms, in vitro and in vivo studies; TLR2 plays the critical role in the recognition of M. tuberculosis [20-22].

Up to now, a number of studies have evaluated the association between TLR2 T597C (rs3804099) polymorphism and risk of different types of tuberculosis in diverse populations. However, results from published studies remain conflicting. Therefore, we performed a meta-analysis on all eligible case-control studies to elucidate the association between TLR2 T597C polymorphism and the susceptibility to pulmonary tuberculosis and tuberculous meningitis.

Materials and methods

Literature search

We conducted literature search by using the PubMed, Embase, CNKI, Wanfang, Weipu databases (update to Nov 31, 2014) with the following search terms: “TLR2” or “Toll-like receptor 2” and “polymorphism” or “polymorphisms” and “tuberculosis” or “pulmonary tuberculosis” or “tuberculous meningitis”. In addition, the reference lists of reviews and retrieved studies were identified by manual search.

Inclusion and exclusion criteria

Basing on the published articles [23], the inclusion criteria were: (1) the study evaluated the association between TLR2 polymorphism and pulmonary tuberculosis or tuberculous meningitis risk in human; (2) a case-control study; (3) the genotype distributions in both cases and controls were available for estimating the odds ratio with 95% confidence interval (CI) and P value; (4) the genotype distributions of cases and controls must be consistent with Hardy-Weinberg equilibrium (HWE). The main exclusion criteria of studies were as follows: (1) case reports, reviews, letters and editorial articles; (2) only have the case population; (3) duplicate of previous publication; and (4) the distribution of genotypes among controls are consistent with HWE.

Data extraction

The data were extracted from every eligible study by two authors independently, and then the authors discussed to reach a consensus. In each study, the first author’s name, year of publication, country of origin, ethnicity, the definition of case, source of control selection and genotype frequencies in cases and controls were extracted.

Statistical analysis

For each study, the genotype distributions in the control group were first examined to determine if it is consistent with Hardy-Weinberg. The heterogeneity of each study was evaluated by the $\chi^2$ based Q-statistic which was considered statistically significant at $P$ value < 0.10. To measure the percentage of variability in the studies that due to heterogeneity rather than chance, the $I^2$ value was used. If the $I^2$ value < 70%, the effects were assumed to be homogenous, the fixed-effects model was used (the Mantel-Haenszel method); otherwise, the random-effects model (DerSimonian and Laird method) were used [24-26]. The subgroup analysis by ethnicity was performed, to explore the source of heterogeneity. Hardy-Weinberg equilibrium (HWE) was also tested by Pearson’s chi-square test. Comprehensively define the strength of associations between TLR2 poly-
**Table 1.** Distribution of *TLR2* genotype and allele among Pulmonary Tuberculosis and Tuberculous Meningitis patients and controls

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Country</th>
<th>Disease</th>
<th>Race</th>
<th>Case</th>
<th>Control</th>
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<td>PTB</td>
<td>African American</td>
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<td>194</td>
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<td>PTB</td>
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<tr>
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<td>TBM</td>
<td>Asian</td>
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<tr>
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<td>China</td>
<td>PTB</td>
<td>Asian</td>
<td>115</td>
<td>156</td>
</tr>
<tr>
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<td>China</td>
<td>PTB</td>
<td>Asian</td>
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<td>262</td>
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<tr>
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<td>China</td>
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<td>Asian</td>
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<tr>
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<td>Asian</td>
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<td>20</td>
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<tr>
<td>Shi</td>
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<td>China</td>
<td>TBM</td>
<td>Asian</td>
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<td>Asian</td>
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<td>377</td>
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<td>TBM</td>
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<tr>
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<td>TBM</td>
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</table>

**Results**

**Literature search and studies characteristics**

According to the inclusion and exclusion criteria (Figure 1), 14 publications [5, 18, 21, 22, 28-37] including 4381 cases and 5082 controls were included for this meta-analysis. The main characteristics of these studies are summarized in Table 1. There were 12 studies of Asian populations, 6 studies of Caucasians population, and 1 study of the African American population. In this analysis, two types of tuberculosis were addressed: 16 studies focused on pulmonary tuberculosis, and 4 studies on tuberculous meningitis. The diagnoses of most of the cases were based on clinical, sputum Acid Fast Bacillus (AFB) or pathology. Healthy subjects who matched for age and sex served as controls. Polymerase chain reaction (PCR) or Mass-Array was performed for genotypes.

**Quantitative data synthesis**

A total of fourteen case-control studies, including 4381 cases and 5082 population, and 1 study of the African American population. In this analysis, two types of tuberculosis were addressed: 16 studies focused on pulmonary tuberculosis, and 4 studies on tuberculous meningitis. The diagnoses of most of the cases were based on clinical, sputum Acid Fast Bacillus (AFB) or pathology. Healthy subjects who matched for age and sex served as controls. Polymerase chain reaction (PCR) or Mass-Array was performed for genotypes.
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= 2.00-5.00; CC vs. TT OR = 3.56, 95% CI = 2.27-5.58; CC vs. TC+TT OR = 3.37, 95% CI = 2.19-5.19; CC+TC vs. TT OR = 1.36, 95% CI = 1.05-1.77; C vs. T OR = 1.52, 95% CI = 1.25-1.85), respectively.

Test of heterogeneity

In the overall analysis, the P showed stable variation and the Q-statistic was significant under the models (CC vs. TC OR = 0.79, P = 0.03, I² = 42%; CC vs. TT OR = 0.0001, P = 64%; TC vs. TT OR = 0.00001, P = 79%; CC vs. TC+TT OR = 0.001, P = 57%; CC+TC vs. TT OR = 0.03, P = 42%; C vs. T P = 0.002, P = 55%). In the subgroup analysis by ethnicity, the P and I² are different. In the subgroup analysis by tuberculosis, the P and I² are also different. In the subgroup of pulmonary tuberculosis, P and I² are (CC vs. TC P = 0.53, I² = 0%). And in the subgroup of tuberculous meningitis (CC vs. TC P = 0.79, P = 0%; CC vs. TT P = 0.22, I² = 32%; CC vs. TC+TT P = 0.46, I² = 0%; CC+TC vs. TT P = 0.21, I² = 33%; C vs. T P = 0.10, I² = 52%), respectively.

Sensitivity analysis

By excluding each study at a time, we can estimate the influence of a single study on the overall meta-analysis. There are not any significant changes through the omission of any study. This indicated that the results of our meta-analysis were statistically reliable.

Publication bias

To assess the publication bias of the literatures, we used the Begg's funnel plot and

Figure 2. The association between TLR2 T597C polymorphism and tuberculosis risk (CC vs. TC).
Egger’s regression asymmetry test. We analyzed the symmetrical shapes of the Begg’s funnel plot of TLR2 T597C polymorphism; the Begg’s funnel plot did not suggest any evidence of publication bias (Table 2). Meanwhile, the result of Egger’s test also showed no publication bias.

Discussion

In the present meta-analysis, we show that TLR2 T597C polymorphism is associated with susceptibility to pulmonary tuberculosis and tuberculous meningitis. In subgroup by ethnicity, we found significant associations between this polymorphism and tuberculosis risk in Asian and Caucasians.

The TLR2 gene plays the important role in the pathogenesis of tuberculosis. Toll-like receptors (TLRs) involve in linking innate and adaptive immunity by recognizing the microbial patterns, and the proinflammatory immune response. Up to now, many studies have investigated whether the TLR2 T597C polymorphism confers susceptibility to pulmonary tuberculosis and tuberculous meningitis. However, the results of the published studies were still controversial. Therefore we conducted this meta-analysis including in 4381 cases and 5082 controls from 14 case-control studies to evaluate the association between TLR2 T597C gene polymorphism and the tuberculosis risks.

There is a significant association between TLR2 T597C polymorphism and tuberculosis risks under the homozygote model (CC vs. TT), heterozygote model (CC vs. TC), and recessive model (CC vs. TC+TT) genetic model in the over-
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all population. In the subgroup analysis by ethnicity, we found that the TLR2 T597C polymorphism is associated with tuberculosis risk in Asian and Caucasians. Similar to the total population, there are many genetic models shown this susceptibility. Compared with the T genotype, the C genotype was the recessive phenotype in human. Our meta-analysis suggests that individuals with the recessive phenotype (C genotype) appear to be associated with increased risk of tuberculosis, particularly with tuberculous meningitis. That indicates that the

Table 2. Summary of different model meta-analysis results

| Genetic model | Participants (case/control) | OR [95% CI] | P value | P% | Effect mode | Begg's test P > |z| | Egger's test P > |t|
|---------------|-----------------------------|-------------|---------|----|-------------|----------------|---------|----------------|
| TC vs. TT     | 3647/4521                   | 0.91 [0.72, 1.14] | < 0.00001 | 79 | R           | 0.441          | 0.826   |
| CC vs. TC     | 2746/2853                   | 1.26 [1.10, 1.43] | 0.03    | 42 | F           | 0.162          | 0.215   |
| CC vs. TT     | 2379/2790                   | 1.20 [1.04, 1.38] | < 0.00001 | 64 | F           | 0.050          | 0.102   |
| CC vs. TT+TC  | 4381/5082                   | 1.23 [1.08, 1.39] | 0.001   | 57 | F           | 0.108          | 0.183   |
| TC+CC vs. TT  | 4381/5082                   | 1.02 [0.93, 1.11] | 0.03    | 42 | F           | 0.093          | 0.152   |
| C vs. T       | 8762/10164                  | 1.06 [1.00, 1.13] | 0.002   | 55 | F           | 0.162          | 0.177   |

Figure 4. The association between TLR2 T597C polymorphism and tuberculosis risk (CC vs. TC+TT).
TLR2 T597C polymorphism may contribute to pathogenesis of tuberculosis or mediate immune process, and also exert a regulatory effect of TLR2 gene expression.

The heterogeneity was found in all comparisons in our meta-analysis. To get more complete and accurate detail of the precious data, we try to use the random-effect models. The results are stable when analysis their sensitivity, which does not change the results of the meta-analysis. Meanwhile, there are no publication biases for the risks of tuberculosis in the TLR2 T597C polymorphism studies.

There were certain limitations in our meta-analysis. First, all the included studies were from Caucasians, Asian and African American populations in 8 country, further studies are necessary to contain more findings of other ethnic populations and nationality. Second, the synergistic effect of the gene has not been premediated in our study. Third, tuberculosis is a multifactorial disease; the immune response has a pivotal position in the human resisting against the Mycobacterium tuberculosis. The environmental and genetic factors may contribute. Due to lack of original data, we could not evaluate the potential interactions of gene-gene and gene-environment.

In conclusion, TLR2 T597C polymorphism is associated with susceptibility to pulmonary tuberculosis and tuberculous meningitis. Future studies with more stringent design and a larger sample size are required to further validate this conclusion.

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Disclosure of conflict of interest

None.

Address correspondence to: Dr. Guohua Zhen, Division of Respiratory and Critical Care Medicine, Department of Internal Medicine, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China. E-mail: ghzhen@tjh.tjmu.edu.cn

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