Meta-analysis on the relevance of HLA-DRB1 gene polymorphisms in a Chinese population with pulmonary tuberculosis

Yong Su, Ying Li, Guixiang Sun

Abstract

Objective: The current meta-analysis determined the relevance of HLA-DRB1 gene polymorphisms in a Chinese population with pulmonary tuberculosis.

Methods: The CBM, CNKI, and MEDLINE databases were retrieved by computers. Domestic and international documents involving studies on the relevance of HLA-DRB1 gene polymorphisms in a Chinese population with pulmonary tuberculosis were collected from the database until May 2013, and statistical analysis was performed on all of the eligible results with RevMan5.0 software.

Results: Eleven case-control studies were collected, including 1364 cases in the pulmonary tuberculosis group and 1496 cases in the control group.

The consolidated OR values and 95% confidence intervals of the case and control groups of HLA-DRB1 *04, HLA-DRB1 *15, and HLA-DRB1 *16 were 1.32 (1.09–1.60), 1.40 (1.01–1.93), and 1.36 (1.01–1.83), respectively.

Conclusion: HLA-DRB1 *04, HLA-DRB1 *15, and HLA-DRB1 *16 may be susceptibility genes for pulmonary tuberculosis in a Chinese population.

Keywords: Pulmonary tuberculosis, HLA-DRB1 gene, Polymorphism, Meta-analysis

Introduction

Tuberculosis is a chronic infectious disease caused by complex groups of Mycobacterium tuberculosis. The incidence of tuberculosis is related to a number of factors, such as the social environment and genetic factors [1]. One-third of the global population is infected with M. tuberculosis, but only 10% develop disease. The incidence of tuberculosis in identical twins with the same genetic background is significantly higher than dizygotic twins [2, 3]. These phenomena fully demonstrate the important role of genetic factors in patients infected with M. tuberculosis.

The human leukocyte antigen (HLA) complex is characterized by genetic and immune polymorphisms [4]. It has been shown that there are >70 diseases associated with HLA gene polymorphisms. Immunity against tuberculosis is mainly of the cellular type [5, 6]. Therefore, studies on the relevance of the HLA complex with susceptibility to pulmonary tuberculosis (PTB) has become a focus with wide attention. The HLA-DRB1 gene is located on the short arm of the 6th human chromosome, and is the most polymorphic genetic system. In recent years, PTB susceptibility as a function of
HLA-DRB1 polymorphisms in different races and different geographic regions has been studied, but there are some differences and even contradictions in the results. In addition, the distribution of HLA genes is related to nationality, race, and the geographic environments [7]. Therefore, by adopting evidence-based medicine theory and methods, we have systematically evaluated the relevance of PTB in the Chinese population with 14 alleles of HLA-DRB1 from the perspective of hierarchical genotyping, and provide data for further study.

Materials and methods

Retrieval strategy

PubMed, EMBase, Chinese Biomedical Document Database (CBM), Chinese HowNet (CNKI), VIP Chinese scientific journals database (VIP), and Wanfang Data Platform were searched for relevant literature. The retrieval was performed from the database construction to May 2013.

The retrieval strategy of English language documents was as follows: [“pulmonary tuberculosis” “tuberculoses” OR “pulmonary tuberculoses” (MeSH)] AND [“human leukocyte antigen DRB” “HLA-DRB1” OR “HLA-DRB1” (MeSH)] AND [“polymorphism” “polymorphisms” OR “polymorphisms, genetic” (MeSH)]. The retrieval strategy of Chinese language documents was as follows: (pulmonary tuberculosis OR tuberculosis OR tuberculosis) AND (gene polymorphism OR polymorphisms) AND (human leukocyte antigen DRB1 OR HLA-DRB1). The relevant journals, conference proceedings, dissertation compilations, and science and technology reports were manually retrieved.

Inclusion and exclusion criteria

The study inclusion criteria were as follows: (1) well-designed domestic and international case-control studies with a focus on HLA-DRB1 gene polymorphisms and susceptibility to PTB; (2) the study subjects were Chinese who were diagnosed with PTB by bacteriologic and imaging examinations (case group) and healthy individuals without a genetic relationship (control group); (3) the data were complete, and the genotype frequency of HLA-DRB1 in each group was clear or could be obtained through extrapolation; and (4) the genotyping methods were reliable.

The exclusion criteria were as follows: (1) documents without rigorous experimental design; (2) documents without sufficient raw data; and (3) documents published repeatedly.

Data extraction

Two researchers independently conducted the screening and data extraction from the documents according to the inclusion and exclusion criteria, and any disagreement that arose was settled through consultation with a third researcher. The following information was extracted from the selected documents: document title; author; publication time; journal name; source of study subjects; study sample size; genetic testing methods; and genotype frequencies.

Quality evaluation on documents

Two evaluators independently performed the quality evaluation according to the STREGA standards [8]; in the case of disputes, a third evaluator was invited to intervene to reach a consensus through discussion. STREGA contained the following six elements: (1) adequacy of the sample size; (2) clear explanation of the diagnostic criteria; (3) grouping and matching conditions; (4) whether or not the control group was comparable with the case group; (5) gene detection method was reasonable; and (6) adequacy of data. For the aforementioned 6 elements, each qualified elements was assigned a score =1; if the total score was ≥3, the quality of the document was considered reliable.

Statistical analysis

RevMan5.0 software (provided by the Cochrane Collaboration website) was used for the meta-analysis. When statistical heterogeneity existed, a combined analysis was performed using a stochastic effect model rather than a fixed effect model. The OR and 95% CI were used for effect sizes and the test level was an α=0.05. When the number in the study was >9, funnel plots were used to determine publication bias.

Results

General characteristics and quality evaluation

Eighty-five English and 43 Chinese documents were retrieved, 11 of which were qualified [9–19]. The included documents involved control studies of non-familial cases on the relevance
of HLA-DRB1 gene polymorphisms with susceptibility to tuberculosis, with 1364 cases in the tuberculosis group and 1496 normal cases in the control group; a total of 14 alleles (HLA-DRB1 *01, 03, 04, 06, 07, 08, 09, 10, 11, 12, 13, 14, 15, and 16) were reported. The characteristics of the included studies are shown in Table 1.

In the sample size, the numerator was the number of cases and the denominator was the number of control cases.

Results of the meta-analysis
The 6 genotypes (HLA-DRB1 *03, 09, 11, 12, 13, and 15) had heterogeneity (the $X^2$ values were 20.20, 35.23, 29.69, 25.96, 24.90, and 29.56, respectively, and the $P$ values were all <0.05). A random effect model was adopted for consolidation; the remaining genotypes were analyzed with a fixed effect model (Table 2).

The HLA-DRB1 *04, 15, and 16 alleles may represent the susceptibility gene for PTB (OR=1.32, 95% CI=1.09–1.60, $P=0.005$; OR=1.40, 95% CI=1.01–1.93, $P=0.04$; and OR=1.36, 95% CI=1.01–1.83, $P=0.04$, respectively; Table 2, Figs. 1–3).

Sensitivity analysis and publication bias evaluation
When the documents with the greatest weights in the 14 observation indicators were removed one-by-one or an analysis was made by selecting different statistical models in software for analysis, the conclusions of the consolidated OR values were similar, which indicates that this conclusion had a strong stability and could not be easily influenced by correlation factors.

The funnel plots were drawn according to funnel plot command with REV5.0 software, which showed that the funnel plots of the HLA-DRB1 *03, *09, *11, *12, *13, and *15 alleles were asymmetric, suggesting the presence of publication bias (Fig. 4).

Discussion
The results of a meta-analysis showed that the HLA-DRB1 *04, 15, and 16 gene polymorphisms may be susceptible genes to PTB in a Chinese population; the consolidated OR values were 1.32, 1.40, and 1.36, respectively. This finding is consistent with international reports. Specifically, Dahe [20] reported that PTB in Syrians was positively correlated with HLA-DRB1 *04 (OR=1.77), Rajalingam [21] reported HLA-DRB1 *15 may be a susceptibility gene for PTB in an Indian population, and Dubaniewicz [22] in Poland and Cao et al. [23] in north-eastern Mexico independently found that PTB was positively correlated with HLA-DRB1 *16 in their population studies.

The specific role and mechanism of action of the HLA-DRB1 polymorphic alleles in PTB susceptibility is unclear, but

Table 1. Baseline characteristics of the studies

<table>
<thead>
<tr>
<th>Document</th>
<th>Author</th>
<th>Study site</th>
<th>Publication year</th>
<th>DNA typing methods</th>
<th>Sample size (case/control)</th>
<th>Quality rating/score</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Ye Shihui [10]</td>
<td>Xi’an</td>
<td>2007</td>
<td>PCR-SSO</td>
<td>80/105</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>Chen Zhuobin [13]</td>
<td>Guangxi</td>
<td>2010</td>
<td>PCR-SSO</td>
<td>100/105</td>
<td>4</td>
</tr>
<tr>
<td>6</td>
<td>Li Hong [14]</td>
<td>Zunyi</td>
<td>2010</td>
<td>PCR-SSP</td>
<td>36/100</td>
<td>5</td>
</tr>
<tr>
<td>8</td>
<td>Wang Xi [16]</td>
<td>Aksu,</td>
<td>2010</td>
<td>PCR-SSP</td>
<td>226/231</td>
<td>4</td>
</tr>
<tr>
<td>9</td>
<td>Liu Yucai [17]</td>
<td>Xinjiang</td>
<td>2010</td>
<td>PCR-SSP</td>
<td>228/231</td>
<td>4</td>
</tr>
<tr>
<td>10</td>
<td>G.L. Shi [18]</td>
<td>Beijing</td>
<td>2011</td>
<td>PCR-SSP</td>
<td>97/62</td>
<td>5</td>
</tr>
<tr>
<td>11</td>
<td>Li Suzhi [19]</td>
<td>Tibet</td>
<td>2011</td>
<td>PCR-SSP</td>
<td>176/189</td>
<td>4</td>
</tr>
</tbody>
</table>

Note: PCR-SSP = the polymerase chain reaction of sequence-specific primers; PCR - SSO = the amplification analysis of sequence-specific oligonucleotide.
Relevance of HLA-DRB1 Gene Polymorphisms in a Chinese Population with Pulmonary Tuberculosis

Table 2. Meta-analysis of the relationship between HLA-DRB1 gene polymorphisms and susceptibility to tuberculosis in the Chinese population

<table>
<thead>
<tr>
<th>Allele</th>
<th>Number of document</th>
<th>Heterogeneity test</th>
<th>Model selected F/R</th>
<th>OR (95% CI)</th>
<th>P-value</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>DRB1*01</td>
<td>11</td>
<td>8.80</td>
<td>0.550</td>
<td>F</td>
<td>1.17 (0.85, 1.61)</td>
<td>0.33</td>
</tr>
<tr>
<td>DRB1*03</td>
<td>11</td>
<td>20.20</td>
<td>0.030</td>
<td>R</td>
<td>0.90 (0.59, 1.38)</td>
<td>0.63</td>
</tr>
<tr>
<td>DRB1*04</td>
<td>10</td>
<td>7.16</td>
<td>0.620</td>
<td>F</td>
<td>1.32 (1.09, 1.60)</td>
<td>0.005</td>
</tr>
<tr>
<td>DRB1*06</td>
<td>2</td>
<td>0.16</td>
<td>0.690</td>
<td>F</td>
<td>0.82 (0.29, 2.32)</td>
<td>0.70</td>
</tr>
<tr>
<td>DRB1*07</td>
<td>11</td>
<td>5.27</td>
<td>0.870</td>
<td>F</td>
<td>1.04 (0.84, 1.28)</td>
<td>0.72</td>
</tr>
<tr>
<td>DRB1*08</td>
<td>11</td>
<td>8.85</td>
<td>0.550</td>
<td>F</td>
<td>1.12 (0.90, 1.40)</td>
<td>0.31</td>
</tr>
<tr>
<td>DRB1*09</td>
<td>11</td>
<td>35.23</td>
<td>&lt;0.001</td>
<td>R</td>
<td>1.44 (0.92, 2.26)</td>
<td>0.11</td>
</tr>
<tr>
<td>DRB1*10</td>
<td>11</td>
<td>11.00</td>
<td>0.360</td>
<td>F</td>
<td>1.33 (0.96, 1.83)</td>
<td>0.08</td>
</tr>
<tr>
<td>DRB1*11</td>
<td>11</td>
<td>29.69</td>
<td>0.001</td>
<td>R</td>
<td>0.60 (0.33, 1.09)</td>
<td>0.10</td>
</tr>
<tr>
<td>DRB1*12</td>
<td>11</td>
<td>25.96</td>
<td>0.004</td>
<td>R</td>
<td>1.17 (0.80, 1.69)</td>
<td>0.42</td>
</tr>
<tr>
<td>DRB1*13</td>
<td>11</td>
<td>24.90</td>
<td>0.006</td>
<td>R</td>
<td>0.78 (0.47, 1.28)</td>
<td>0.33</td>
</tr>
<tr>
<td>DRB1*14</td>
<td>11</td>
<td>11.16</td>
<td>0.350</td>
<td>F</td>
<td>1.16 (0.89, 1.50)</td>
<td>0.27</td>
</tr>
<tr>
<td>DRB1*15</td>
<td>11</td>
<td>29.56</td>
<td>0.001</td>
<td>R</td>
<td>1.40 (1.01, 1.93)</td>
<td>0.04</td>
</tr>
<tr>
<td>DRB1*16</td>
<td>11</td>
<td>9.30</td>
<td>0.500</td>
<td>F</td>
<td>1.36 (1.01, 1.83)</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Note: R refers to a random effect model; F refers to a fixed effect model.
*Indicates the meta-analysis results at a $P\leq0.05$.

Fig. 1. Results of a meta-analysis on the relationship between the HLA-DRB1 *04 gene polymorphism and susceptibility to tuberculosis.

Fig. 2. Results of a meta-analysis on the relationship between the HLA-DRB1 *15 gene polymorphism and susceptibility to tuberculosis.
studies have shown that relevant HLA molecules selectively deliver pathogenic peptides through its amino acid charge [24], making those carrying the relevant HLA molecules more apt to develop disease after infection with *M. tuberculosis*, such as HLA-DRB1 *04, 15, and 16 in the current study.

In 2009, Wang et al. [25] published a meta-analysis with the following findings: (1) the study only included studies involving PTB cases and was more representative; (2) the study explored the disease at the genotype level (excluding serotype) and the methods of detection were more clear; (3) the preliminary study was published for nearly 5 years and needs to be updated; and (4) the study included a large list of documents and carried out a rigorous quality evaluation. These measures give a better demonstration of the strength of this study with a more specific scope of application.

A sensitivity analysis of the results showed that the consolidated results of the current study had good stability. Because the relevant documents involving sites of DRB1 *03, 09, 11, 12, 13, and 15 have heterogeneity, the random effect model allowing the existence of heterogeneity was selected for the meta-analysis. The possible sources of heterogeneity are as follows: (1) the age difference of the study subjects; (2) the presence of publication bias; (3) the differences in HLA typing techniques (the PCR-SSO typing method was adopted in

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**Fig. 3.** Results of a meta-analysis on the relationship between the HLA-DRB1 *16* gene polymorphism and susceptibility to tuberculosis.

**Fig. 4.** Funnel plot of publication bias evaluation of HLA-DRB1 *03, *09, *11, *12, *13 and *15 alleles.

Note: a, b, c, d, e, and f are HLA-DRB1 *03, *09, *11, *12, *13, and *15 alleles, respectively.
documents 2 and 5, and the PCR-SSP method was utilized in other documents); and (4) the heterogeneity may be associated with gene–gene, gene–environment, pathogen–environment, and pathogen–host interactions.

In summary, based on the meta-analysis, the alleles of HLA-DRB1 *04, 15, and 16 may be susceptibility genes for PTB in a Chinese population, but the conclusion lacks consistency. In future studies multi-ethnic and multi-regional collaboration are needed with an expanded sample size for definitive exploration to carry out the analysis on stratifications and interactions. Moreover, the evaluation of combined effects of gene–environment interactions on PTB susceptibility will provide clues to the pathogenesis of PTB and ultimately provide the scientific basis for prevention and control of PTB.

Conflict of interest
The authors declare no conflict of interest.

References
hybridization analysis of HLA class II antigens in pulmonary tuberculosis: relevance to chemotherapy and disease severity.