Original Article
Chemokine (C-C motif) ligand 5 -28C>G is significantly associated with an increased risk of tuberculosis: a meta-analysis

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Received May 19, 2015; Accepted July 10, 2015; Epub August 15, 2015; Published August 30, 2015

Abstract: Objective: Chemokine (C-C motif) ligand 5 (CCL5) has been shown to play an important role in antimycobacterial immune responses. Previous studies have extensively reported that the CCL5 -28C>G gene polymorphism is associated with susceptibility to tuberculosis (TB). However, the results of these studies have been inconsistent. To investigate the relationship between the CCL5 -28C>G and the risk of TB, we performed a meta-analysis.

Methods: We searched articles published before June 6, 2014 from PubMed, CNKI, and Wanfang databases. Data were extracted from all eligible publications independently by two investigators and statistically analyzed. Odds ratios (OR) with 95% confidence intervals (CI) were calculated to assess the strength of the association between CCL5 polymorphism and TB. Results: Four case-control studies including 647 TB cases and 726 controls were involved in the meta-analysis. Our meta-analysis indicated the CCL5 -28C>G gene polymorphism was significantly associated with increased risk of TB (G vs. C: 3.75, 95% CI = 1.76-7.99; GG vs. CC: OR = 30.26, 95% CI = 14.28-64.12). Conclusion: Our results suggested that the -28C>G polymorphism is significantly associated with higher TB risk, which is opposite from previously published reports. However, the number of the study is limited, additional well-designed studies are required to elucidate the association between the CCL5 -28C>G gene polymorphism and TB.

Keywords: CCL5, polymorphism, TB, meta-analysis

Introduction
Tuberculosis (TB) remains a major global health problem. It causes ill health among millions of people each year and ranks as the second leading cause of death from an infectious disease worldwide, after the human immunodeficiency virus (HIV) [1]. Epidemiological studies have demonstrated that one third of the world’s population is infected with Mycobacterium tuberculosis, whereas among those who are infected, only approximately 10% will ever develop clinical disease [2]. TB occurs predominantly in parts of the world such as Africa and South Asia. The occurrence of TB at different rates among particular races, ethnicities, and families indicates a genetic predisposition to TB susceptibility. Several lines of evidence, including twin studies, genome-wide linkage studies, and recently published genome-wide association studies (GWAS), demonstrate that several classes of candidate genes that are critical to the susceptibility of TB [3, 4]. These candidate genes include cytokines and their receptors expressed by macrophages, the Toll-like and Nod-like receptor families of genes, genes expressed by T-cells and key TB candidate genes [5].

Chemokine (C-C motif) ligand 5 (CCL5) is one of candidate genes that are implicated in the pathogenesis of TB [3]. CCL5 is located on chromosome 17q11.2-q12; the candidate gene region encoding for several chemokines might be responsible for genetic susceptibility to mycobacterial infections, such as leprosy and TB in several studies [6]. As it is chemotactic for eosinophils, mononuclear phagocytes, baso-
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philic and mast cells, it has been postulated to be important in inflammatory reactions [7]. CCL5 has been shown to play a major role in the antimycobacterial immune responses by suppressing intracellular growth of Mycobacterium tuberculosis [8]. Furthermore, it can recruit early IFN-γ-producing, antigen-specific T cells and mononuclear cells to the site of infection and promote lymphocyte-rich granulomas, which control M. tuberculosis growth [9]. Anti-CCL5 antibodies have been shown to decrease pulmonary granuloma lesion size in Mycobacterium bovis BCG strain-infected mice suggesting a functional role for CCL5 in murine mycobacterial granulomas [7].

The -28C/G polymorphism in the CCL5 promoter region polymorphism is an extensively studied single nucleotide polymorphism (SNP). Position -28 was once designated as position -96 according to different numbering system [10]. The variant allele -28G was found to increase levels of CCL5 transcription [11]. A number of molecular epidemiological studies have shown that the SNP in the CCL5 gene (-28C>G) is associated with TB risk; however the results remain inconsistent [12-18].

In 2013, Alqumber MA et al performed a meta-analysis to investigate the association between CCL5 -28C>G polymorphism and the risk of pulmonary TB, but no association was found [19]. However, their meta-analysis did not exclude the results that deviated from HWE, which may bias the results of genetic association studies. Furthermore, we found one result of Hardy-Weinberg equilibrium (HWE) from Chu et al involved in the meta-analysis of Alqumber MA is faulty, perhaps their authors made a mistake in their calculations. Therefore, it is necessary to carry out this meta-analysis again.

Methods

Search strategy

A literature search was conducted using online databases, including PubMed, Wanfang (www.wanfangdata.com.cn) and CNKI (China National Knowledge Infrastructure, www.cnki.net). The following keywords were used for searching: “CCL5” or “RANTES (Regulated upon activation, normal T-cell expressed and secreted)” and “polymorphism” or “mutation” or “variant” and “tuberculosis”. Unpublished reports were not considered. Additionally, the reference lists of all retrieved articles were tracked to find other eligible studies that have not been identified as aforementioned.

Inclusion and exclusion criteria

Abstracts of all citations and retrieved studies were reviewed. Studies included in this meta-analysis were required to meet the following criteria: (1) published case-control study; (2) evaluated the association between CCL5 polymorphisms and TB risk; (3) provided available genotype data of CCL5 -28C>G for calculating odds ratio (OR) with 95% confidence interval (CI). Studies were excluded for the following exclusion criteria: (1) not relevant to CCL5 polymorphisms and TB risk; (2) the control of the study deviated from HWE.

Data extraction

Two investigators (Hu L and Yao L) independently assessed and extracted the data from all eligible publications according to the inclusion criteria. The results were compared and discrepancies were resolved by consensus. The following information was collected from each study: first author's name, year of publication, country of origin, ethnicity, characteristics of controls, TB definition, genotyping methods and the distribution of genotypes in cases and controls.

Quality score assessment

Two authors (Hu L and Yao L) used the Newcastle-Ottawa quality assessment scale (NOS) to independently assess the quality of each study and reached consensus on NOS [20]. The NOS contained eight items, categorized into three dimensions, including selection, comparability and exposure. The NOS ranges between zero and nine stars [21]. The NOS of the studies included in the meta-analysis ranged between six to eight stars. The average NOS was seven stars, which suggests that the studies included in this meta-analysis were of high quality.

Statistical analysis

The strength of association between the CCL5 -28C>G polymorphism and TB risk was measured by pooled OR and 95% CI. The significance of pooled OR was determined by Z-test.
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**Results**

**Study characteristics**

Based on our inclusion and exclusion criteria, six eligible studies relevant to CCL5 polymorphism (-28C>G) and TB risk were involved in our meta-analysis. The flow diagram details the excluded reasons (Figure 1). The characteristics, HWE for control and the quality of each case-control study assessed by NOS are listed in Table 1. Genotype distribution of studies included in this meta-analysis is shown in Table 2. HWE for the controls was tested by Pearson's $\chi^2$ test. $P < 0.05$ means deviated from HWE. The genotype distributions among the controls of all studies included in the meta-analysis followed HWE. There were three studies of Asian population, two studies of African population and one study of Caucasian population. One study used the ARMS-PCR method, whereas the others used the RFLP-PCR method for genotyping (Table 1). The TB and control groups for each case-control study were matched for age, sex and ethnicity except for one study performed by Ben-Selma et al. and controls of all studies were selected from healthy population.
**Table 1.** Individual characteristics of studies included studies in this meta-analysis

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Country</th>
<th>Ethnicity</th>
<th>Sample size (case/control)</th>
<th>The diagnoses of TB patients</th>
<th>Control source</th>
<th>Genotyping method</th>
<th>HWE</th>
<th>NOS score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chu SF</td>
<td>2007</td>
<td>China</td>
<td>Asian</td>
<td>412/465</td>
<td>Smear, histopathology</td>
<td>PB</td>
<td>PCR-RFLP</td>
<td>0.02</td>
<td>7</td>
</tr>
<tr>
<td>Sánchez-Castañón M</td>
<td>2009</td>
<td>Spain</td>
<td>Caucasian</td>
<td>76/157</td>
<td>Clinical symptoms, radiography, Smear, culture</td>
<td>PB</td>
<td>PCR-RFLP</td>
<td>0.50</td>
<td>7</td>
</tr>
<tr>
<td>Selvaraj P</td>
<td>2011</td>
<td>India</td>
<td>Asian</td>
<td>212/213</td>
<td>Clinical symptoms, radiography, smear, culture</td>
<td>PB</td>
<td>PCR-RFLP</td>
<td>0.89</td>
<td>7</td>
</tr>
<tr>
<td>Ben-Selma W</td>
<td>2011</td>
<td>Tunisia</td>
<td>African</td>
<td>168/150</td>
<td>Clinical symptoms, radiography, smear, culture, histopathology</td>
<td>PB</td>
<td>PCR-RFLP</td>
<td>0.40</td>
<td>6</td>
</tr>
<tr>
<td>Mishra G</td>
<td>2012</td>
<td>India</td>
<td>Asian</td>
<td>215/215</td>
<td>Smear</td>
<td>PB</td>
<td>ARMS-PCR</td>
<td>&lt; 0.01</td>
<td>7</td>
</tr>
<tr>
<td>Mhmoud N</td>
<td>2013</td>
<td>Sudan</td>
<td>African</td>
<td>191/206</td>
<td>Smear and culture</td>
<td>PB</td>
<td>PCR-RFLP</td>
<td>0.89</td>
<td>8</td>
</tr>
</tbody>
</table>

PB: population-based of control; PCR-RFLP: polymerase chain reaction-restriction fragment length polymorphism; ARMS-PCR: amplification refraction mutation system-polymerase chain reaction; HWE: Hardy-Weinberg equilibrium.
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Quantitative data synthesis

The significance of pooled OR was determined by Z-test. As for the -28C>G polymorphism, the OR1, OR2 and OR3 and respective 95% CI were 30.265 (95% CI = 14.284-64.122), 1.46 (95% CI = 0.422-5.046) and 6.91 (95% CI = 3.46-13.80) (Table 3). As the argument discussed above, because OR1 > OR2 > 1 and OR1 > OR3 > 1, the codominant model was identified as the best genetic model. Our meta-analysis indicated that the CCL5 -28C>G gene polymorphism was significantly associated with an increased risk of TB (GG vs. CC: OR = 30.26, 95% CI = 14.28-64.12) (Figure 2).

Publication bias

The Begg’s funnel plots and Egger’s linear regression test were conducted to assess the publication bias of the literature. The shape of the funnel plots seem to be asymmetric, and the statistical results of Egger’s regression test showed that there was no evidence of publication bias in the codominant model (t = 2, P = 0.30, Figure 3).

Test of heterogeneity

We performed Cochrane’s Q test and the I^2 statistic to assess the heterogeneity among the four studies. According to the value of P, the random-effects model based on DerSimonian-Laird method or the fixed-effects model based Mantel-Haenszel method was chosen to combine values of the studies. There was no heterogeneity observed in the homozygous genotype model (GG vs. CC P = 0.901, I^2 = 0), thus the fixed-effects model was applied (Table 3). Heterogeneity was observed in the allele (G vs. C: P = 0.015, I^2 = 71.2%) and heterozygous (CG vs. CC: P = 0.002, I^2 = 79%) genotype models; the random effects model was applied to assess allele and heterozygous genotype models (Table 3).

Discussion

It has been previously shown that the -28G allele elevates promoter activity and enhances CCL5 production in the functional study. As CCL5 plays an important role in immune responses against TB, it is plausible that SNPs regulating CCL5 levels might be associated with TB [12]. A series of studies have investigated the association between the CCL5 -28C>G gene polymorphism and TB risk, but their results are still inconclusive and controversial.

A previous meta-analysis showed that genetic polymorphism -28C>G in CCL5 is not associated with increased TB risk, while we found a significant association between this polymorphism and TB risk in our study. There are two reasons that is account for the different results between our study and the study of Alqumber MA et al. First, the authors failed to exclude the data that deviated from HWE in previous meta-analysis. As we know, the quality of the studies included in the meta-analysis is crucial to credibility of the conclusion. It is necessary for us to perform a meta-analysis that excludes the results that deviated from the HWE to increase credibility of the conclusion. We calculated the HWE of the control study again, we found one HWE faulty result in the study of Alqumber MA et al; perhaps they made a mistake in their
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<table>
<thead>
<tr>
<th>Study ID</th>
<th>OR (95% CI)</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ben-Selma (2011)</td>
<td>32.56 (1.80, 587.54)</td>
<td>8.56</td>
</tr>
<tr>
<td>Sánchez-Castañón (2009)</td>
<td>32.59 (15.06, 70.51)</td>
<td>76.98</td>
</tr>
<tr>
<td>Mhmoud (2013)</td>
<td>16.55 (0.94, 291.85)</td>
<td>14.46</td>
</tr>
<tr>
<td>Selvaraj (2011)</td>
<td>(Excluded)</td>
<td>0.00</td>
</tr>
<tr>
<td>Overall</td>
<td>30.26 (14.28, 64.12)</td>
<td>100.00</td>
</tr>
</tbody>
</table>

Figure 2. Meta-analysis for the association between the CCL5 -28C/G gene polymorphisms and the risk of TB. GG vs. CC.

Figure 3. Meta-analysis for the association between the CCL5 -28C/G gene polymorphisms and the risk of TB. GG vs. CC.

Due to the limited studies, we did not perform a subgroup analyses or sensitivity analyses by ethnicity to explore the source of heterogeneity and assess the stability of our results. There might be some potential contributors to the significant heterogeneity in the allele (G vs. C) and heterozygous (CG vs. CC) genotype models in our meta-analysis. First, there were two studies of Asian population, one study of African population and one study of Caucasian population, the studied populations came from different regions with different genetic backgrounds which could contribute to genetic heterogeneity. Secondly, because the case group of some studies refers to patients with pulmonary TB (PTB), whereas others refer to patients with PTB or extra-pulmonary, the case groups included in the meta-analysis were not homogeneous. Thirdly, TB is a complex infectious disease that involves gene environment interactions. Different environmental exposures may also influence genetic susceptibility.

There are some limitations in our meta-analysis. First, the results should be interpreted with caution because the limited number of studies that were included in this meta-analysis might decrease the statistical power to reveal a reliable association. Better-designed studies are required to verify the association between the CCL5 -28C>G polymorphism and TB risk. Second, being a multi-factorial disease, TB's...
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pathogenesis depends not only on the host-pathogen interactions, but also on the gene-gene interactions and gene-environment interactions [16]. In this meta-analysis, the effect of gene-gene and gene-environment interactions was not considered. Third, although we did not restrict the language during our literature searching, only English publications were included in this meta-analysis. Therefore, a potential publication bias might exist. Fourth, because the case group of some studies refers to patients with pulmonary TB (PTB), whereas others refer to patients with PTB or extra-pulmonary, the case groups included in the meta-analysis were not homogenous and the results should be interpreted with caution.

In conclusion, the present meta-analysis indicated that the CCL5 -28C>G polymorphism was significantly associated with a higher risk for TB. In the future, much better-designed and larger scale studies in different populations are needed to help clarify the association between CCL5 -28C>G polymorphisms and TB risk.

Disclosure of conflict of interest

None.

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