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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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 s 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-

philes 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.
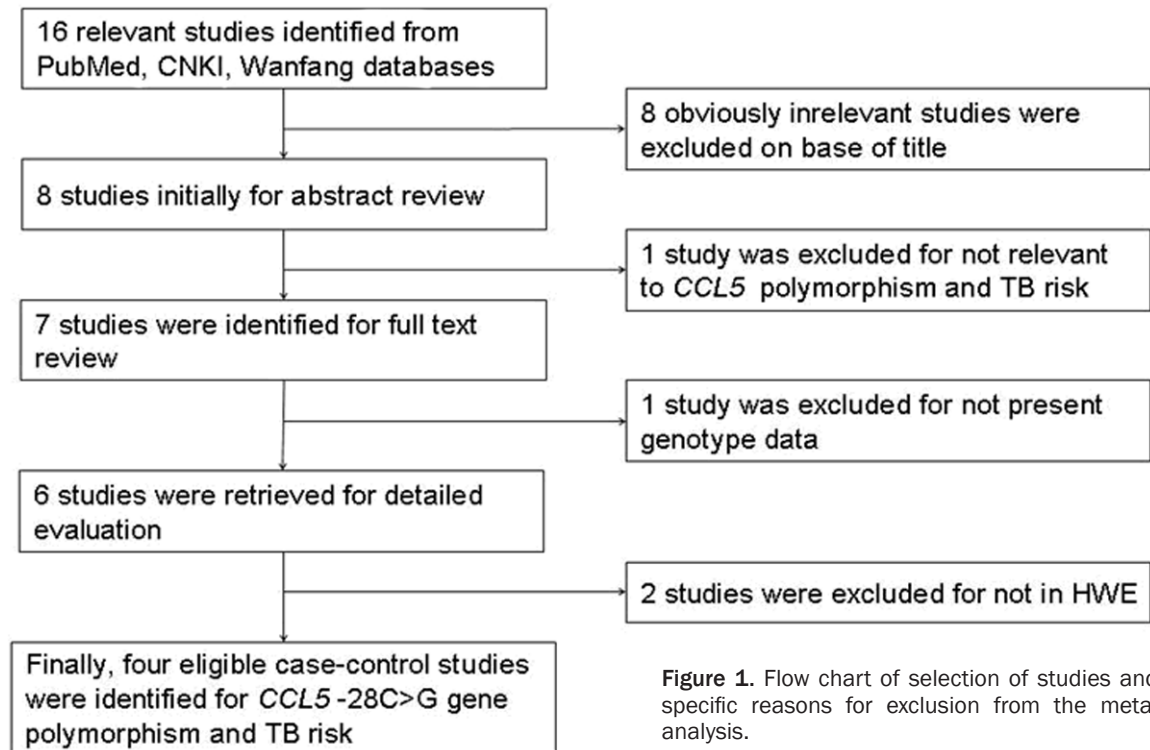
Figure 1. Flow chart of selection of studies and specific reasons for exclusion from the meta-analysis.

OR1, OR2 and OR3 were calculated for the genotypes GG vs. CC (OR1), CG vs. CC (OR2) and GG vs. CG (OR1). These pairwise differences were used to indicate the most appropriate genetic model as follows: if OR1 = OR3 ≠ 1 and OR2 = 1, then a recessive model was suggested; if OR = OR2 ≠ 1 and OR3 = 1, then a dominant model was suggested; if OR2 = 1/OR3 ≠ 1 and OR1 = 1, then a complete over dominant model was suggested; if OR1 > OR2 > 1 and OR1 > OR3 > 1 (or OR1 < OR2 < 1 and OR1 < OR3 < 1), then a codominant model was suggested [22, 23]. HWE for the controls was tested by Pearson's χ^2 test ($P < 0.05$ means deviated from HWE). Heterogeneity was estimated by Cochrane's Q test and the I^2 statistic. If P > 0.10, which indicated significant heterogeneity across studies, the random-effects model (DerSimonian-Laird method) was used. Otherwise, the fixed-effects model (Mantel-Haenszel method) was applied [24, 25]. Additionally, I^2 was used to quantify the proportion of the total variation that was due to heterogeneity. $I^2 >$ 50% suggested high heterogeneity across studies. Begg's funnel plot and Egger's linear regression test were applied to assess the potential publication bias ($P < 0.05$ was considered statistically significant). The software Stata 10.0 (STATA Corporation, College Station, Texas, USA) was used for all statistical analyses.

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 χ^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

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

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.

Table 2. Genotype distribution of studies included in this meta-analysis

Author	Case			Control		
	CC	CG	GG	CC	CG	GG
Sánchez-Castañón	56	14	6	141	16	0
Selvaraj	211	1	0	208	4	0
Ben-Selm	29	89	105	90	50	10
Mhmoud	183	1	7	202	4	0

Table 3. Overall and stratified analyses of the CCL5 28C/G gene polymorphism on TB risk

| Comparisons	Test of association				Test of heterogeneity	
	OR	95% CI	*P*-value	Model	*P*-value	I^2
G vs. C	3.75	1.76-7.99	0.001	R	0.015	71.2
GG vs. CC (OR1)	30.26	14.28-64.12	0	F	0.901	0
CG vs. CC (OR2)	1.46	0.42-5.05	0.55	R	0.002	79
GG vs. CG (OR3)	6.91	3.46-13.80	0	F	0.451	1

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**).

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-

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 creditability of the conclusion. It is necessary for us to perform a meta-analysis that excludes the results that deviated from the HWE to increase creditability 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
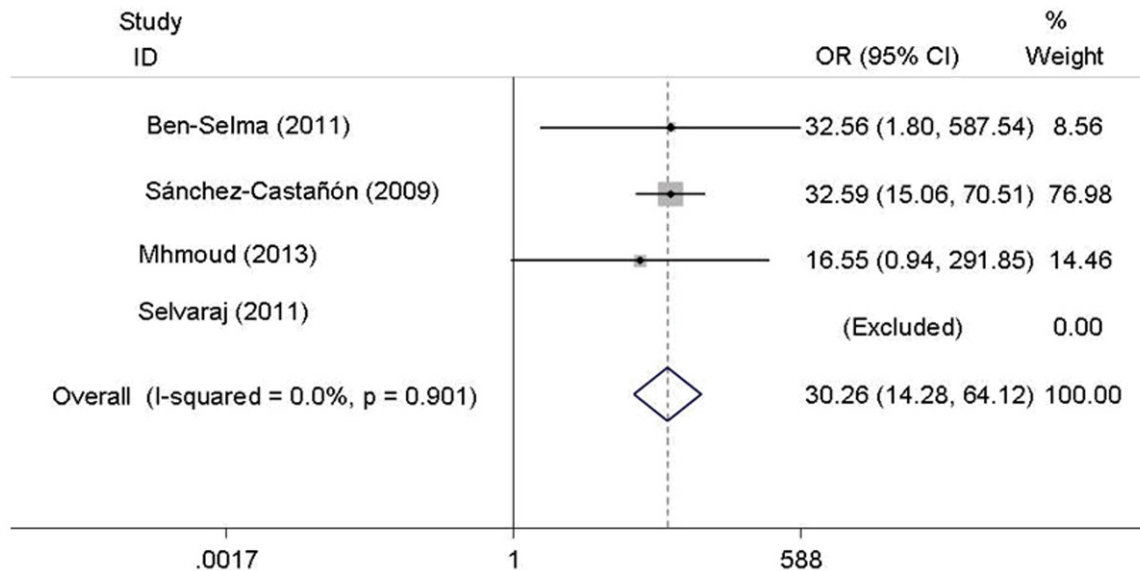
Figure 2. Meta-analysis for the association between the CCL5 -28C/G gene polymorphisms and the risk of TB. GG *vs.* CC.
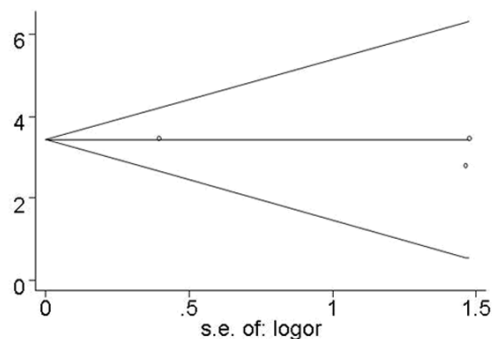
Figure 3. Funnel plot for publication bias of the meta-analysis of TB risk and CCL5 -28C/G polymorphism. GG vs. CC.

calculation. The HWE of Chu et al was 0.02 instead of 0.28, which deviated from the HWE. To perform a meta-analysis with all available studies to derive precise association between *CCL5* polymorphisms (-28C>G) and TB, our study choose four reports that were in accordance with HWE. However, our study showed that the *CCL5* -28C>G polymorphism was significantly associated with an increased risk of TB. Second, there were two genotyping methods used in the study of Alqumber MA et al, while all studies involved in our study used the RFLP-PCR method, thus our conclusion may be more reliable.

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 be 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 homogenous. 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

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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