Associations between TNF-α Polymorphisms and Pneumonia: A Meta-Analysis

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Abstract

Background: Several studies evaluated the associations of tumor necrosis factor-α (TNF-α) polymorphisms with pneumonia in different populations. However, the results were conflicting and controversial.

Methods: Databases including PubMed, Embase, Web of Science, and China National Knowledge Infrastructure (CNKI) were searched to find relevant studies. Data were extracted independently by two investigators. Crude odds ratios (ORs) and corresponding 95% confidence intervals (CIs) were estimated.

Results: Twelve case-control studies and one cohort study were included. Overall, no association between TNF-α –308A/G polymorphism and pneumonia risk was observed for AA +AG vs. GG (OR = 1.13; 95% CI 0.99–1.30; P = 0.07). In addition, TNF-α –308A/G polymorphism was not associated with pneumonia mortality (OR = 1.96; 95% CI 0.94–4.09; P = 0.07). Furthermore, there was no association of TNF-α –238A/G polymorphism with the risk of pneumonia (OR = 1.38; 95% CI 0.84–2.28; P = 0.20).

Conclusions: TNF-α –308A/G, –238A/G polymorphisms were not associated with pneumonia risk. Moreover, TNF-α –308A/G polymorphism did not play a role in the pneumonia mortality risk.

Introduction

Previous studies evidenced the importance of individual genetic differences on the risk of developing or dying from infection [1]. For example, the familial risk of death from infection has been suggested to be greater than that from either cancer or cardiovascular disease [2]. Pneumonia is a common infectious disease associated with high morbidity and mortality. Therefore, host genetic susceptibility may play a key role in the pathogenesis of pneumonia. So far, a lot of studies have focused on this field, and the tumor necrosis factor-α (TNF-α) gene has been studied extensively.

TNF-α is a potential proinflammatory cytokine that plays a critical role in inflammatory and immune responses. Excessive TNF-α production contributed to lung injury in a variety of diseases [3,4] and increased the risk of sepsis [5]. In mechanically ventilated patients, Montón and colleagues found that serum TNF-α level was significantly higher in patients with pneumonia compared with controls [6]. In addition, Bauer et al. showed that levels of serum TNF-α was strongly associated with the degree of lung injury [7]. Furthermore, Puren et al. indicated that plasma levels of TNF-α may be a marker of severity of pneumonia [8]. Collectively, these results suggested that TNF-α might have an important role in the pathophysiology of pneumonia.

TNF-α gene is located on chromosome 6, within the class III region of MHC. Several polymorphisms in the promoter region of TNF-α, such as –238A/G (rs361525), –308A/G (rs1800629), –857G/A (rs1799724), and –1031T/C (rs1799964), have been identified. These polymorphisms could alter the expression of TNF-α [9–11]. Previous studies have assessed the associations between TNF-α polymorphisms and the risk and outcomes of pneumonia [12–24]. However, the results were inconclusive and remained contradictory. Since most studies only included a modest sample size, each of them might not achieve a reliable conclusion. Meta-analysis is a good method to synthesize data from different studies on the same topic. Therefore, we did this meta-analysis to determine whether TNF-α polymorphisms were associated with an increased risk of pneumonia or higher pneumonia mortality. To our knowledge, this was the first meta-analysis of the associations between TNF-α polymorphisms and pneumonia risk and mortality.

Methods

Publication search

The electronic databases of PubMed, Embase, Web of Science, and China National Knowledge Infrastructure (CNKI) were searched. The following search terms were used: pneumonia or community-acquired pneumonia or hospital-acquired pneumonia.
or ventilator-associated pneumonia) and (tumor necrosis factor or TNF or tumor necrosis factor-α or TNF-α) and (polymorphism or mutation or variant). Last search was updated in January 23, 2013. Additional relevant references cited in searched articles were also retrieved. No language restrictions were applied.

### Inclusion and exclusion criteria

The included studies should meet the following criteria: (1) evaluation of the TNF-α polymorphisms and risk of pneumonia or pneumonia mortality, (2) using a case-control design or cohort design, (3) sufficient data for estimating an odds ratio (OR) and 95% confidence interval (CI). Studies were excluded if one of the

### Table 1. Characteristics of the studies included in the meta-analysis.

| First author | Year | Ethnicity group | Age | Pneumonia type | Disease severity | Mortality reported | Case number (n) | Control number (n) | Genotyping method | Polymorphisms | Genotyping score |
|--------------|------|-----------------|-----|----------------|------------------|-------------------|----------------|-------------------|-----------------|---------------|-----------------|
| Waterer [12] | 2001 | Mixed           | Adult | CAP            | Mixed            | Yes               | 280            | NA                | PCR-RFLP        | −308A/G       | 5               |
| Schaab [13]  | 2003 | Mixed           | Pediatric | CAP      | NA               | Yes               | 64             | 50                | PCR-RFLP        | −308A/G       | 5               |
| Hedberg [14] | 2004 | Mixed           | Pediatric | HAP      | Severe           | No                | 12             | 161               | PCR-RFLP        | −308A/G       | 3               |
| Cipriano [15] | 2005 | Caucasian       | Adult | CAP            | Mixed            | Yes               | 20             | 53                | PCR-RFLP        | −308A/G       | 5               |
| Kinder [16]  | 2007 | Mixed           | Mixed | NA             | No               | Yes               | 42             | 217               | PCR-RFLP        | −308A/G, −238A/G, +488A/G | 4               |
| Yuan [17]    | 2008 | Asian           | Adult | CAP            | Mixed*           | Yes               | 67             | 50                | PCR-RFLP        | −308A/G, −238A/G, −1031T/C | 3               |
| Sole-Violan [18] | 2009 | Caucasian      | Adult | CAP            | Mixed            | Yes               | 1136           | 1152              | PCR-RFLP        | −308A/G, −863C/A, −857C/T | 5               |
| Zhang [19]   | 2010 | Asian           | Adult | HAP            | Mixed*           | Yes               | 167            | 110               | PCR-RFLP        | −308A/G       | 5               |
| Endeman [20] | 2011 | Caucasian       | Adult | CAP            | Mixed            | No                | 200            | 313               | TaqMan           | −238A/G       | 5               |
| Antonopoulou [21] | 2012 | Caucasian    | Mixed | NA             | No               | Yes               | 27             | 108               | PCR-RFLP        | −308A/G, −376G/A | 6               |
| Kotsaki [22] | 2012 | Caucasian       | Adult | VAP            | Severe           | No                | 213            | 212               | PCR-RFLP        | −308A/G, −238A/G | 6               |
| Song [23]    | 2012 | Asian           | Adult | Mixed          | Mixed*           | Yes               | 551            | 600               | Direct sequencing | −308A/G, −238A/G, −857G/A | 6               |
| Saliukova [24] | 2012 | Caucasian     | Adult | CAP            | NA               | No                | 321            | 452               | PCR              | −308A/G       | 6               |

*Data for severe pneumonia and non-severe pneumonia patients could be extracted.

CAP, community-acquired pneumonia; HAP, hospital-acquired pneumonia; VAP, ventilator-associated pneumonia; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; NA, not available.
Table 2. Distribution of TNF $-$308A/G and $-$238A/G genotype among cases and controls.

| Studies        | Case (AA+AG) | Case (GG) | Control (AA+AG) | Control (GG) | Hardy-Weinberg equilibrium |
|----------------|--------------|-----------|-----------------|--------------|----------------------------|
| $-$308A/G      |              |           |                 |              |                            |
| Schaaf         | 20           | 44        | 17              | 33           | Yes                        |
| Hedberg        | 3            | 9         | 42              | 111          | Yes                        |
| Cipriano       | 8            | 12        | 11              | 42           | Yes                        |
| Kinder         | 9            | 33        | 63              | 154          | Yes                        |
| Yuan           | 11           | 56        | 3               | 47           | Yes                        |
| Sole-Violan    | 280          | 856       | 282             | 870          | Yes                        |
| Zhang          | 47           | 120       | 20              | 90           |                            |
| Antonopoulou   | 5            | 22        | 16              | 92           | Yes                        |
| Kotsaki        | 41           | 172       | 29              | 183          | Yes                        |
| Song           | 58           | 493       | 40              | 560          | Yes                        |
| Salnikova      | 73           | 248       | 105             | 347          | No                         |
| $-$238A/G      |              |           |                 |              |                            |
| Kinder         | 11           | 31        | 19              | 198          | Yes                        |
| Yuan           | 0            | 67        | 1               | 49           |                            |
| Sole-Violan    | 159          | 976       | 156             | 1016         |                            |
| Endeman        | 13           | 187       | 30              | 286          |                            |
| Antonopoulou   | 7            | 20        | 4               | 104          |                            |
| Kotsaki        | 6            | 207       | 9               | 203          | Yes                        |
| Song           | 59           | 492       | 48              | 550          | Yes                        |

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following criteria existed: (1) not relevant to TNF-$\alpha$ polymorphisms, TNF-$\alpha$, pneumonia, or pneumonia mortality, (2) non-clinical studies, (3) reviews or abstracts, and (4) not reported genotype frequencies or numbers. For the overlapping studies, only the one with the largest sample size was included in our study.

Qualitative assessment

Two investigators (Li and Nie) assessed the quality of each study independently. Any disagreement was resolved by consensus. The predetermined quality assessment criteria were modified from a previous review [25]. This quality scoring system was based on both traditional epidemiologic considerations and genetic issues. Total scores ranged from 0 (worst) to 9 (best) for cohort studies and 0 (worst) to 10 (best) for case-control studies. Case-control studies scoring $<5$ were defined as low quality, and those $\geq5$ were defined as high quality. Cohort studies scoring $<4$ were defined as low quality, and those $\geq4$ were defined as high quality.

Data extraction

The following data were collected from each study: the first author’s surname, publication year, ethnicity, age group, type of pneumonia, severity of disease, outcome (mortality), sample size, genotyping method, TNF-$\alpha$ polymorphisms, and genotype numbers in cases and controls. Two independent reviewers (Li and Nie) collected these data and any discrepancy was resolved by discussion. Authors of the included studies were contacted via E-mail if further study details were needed.

Statistical analysis

Where the data from at least three similar studies were available, meta-analysis was performed. Thus, we evaluated the strength of associations between TNF-$\alpha$ $-$308A/G, $-$238A/G polymorphisms and the risk of pneumonia and mortality. For this meta-analysis, we examined the dominant genetic model (AA+AG vs. GG) because data were most commonly either presented in this format or convertible to this format. Crude ORs with 95% CIs were computed to assess the strength of the associations. The statistical significance of OR was determined with $z$ test.

Departure from Hardy-Weinberg equilibrium (HWE) in controls was tested by the Chi-square test. The $Q$ statistic and the $F$ statistic were used to assess the degree of heterogeneity among the studies included in the meta-analysis. A $P$ value greater than 0.10 for the $Q$-test indicated a lack of heterogeneity among studies, so that the pooled OR estimate of each study was calculated by the fixed-effects model. Otherwise, the random-effects model was used. Subgroup analyses were carried out by ethnicity, type of pneumonia, and severity of disease. Sensitivity analysis was performed by excluding the low quality studies and excluding the study not in HWE. Publication bias was evaluated with the funnel plot and the linear regression asymmetry test by Egger et al. [26] when there were at least 10 studies included in the meta-analysis [27]. All statistical tests were performed by using STATA 11.0 software (Stata Corporation, College Station, TX) and Revman 5.1 software (Nordic Cochrane Center, Copenhagen, Denmark). A $P$ value $<0.05$ was considered statistically significant. Bonferroni correction of critical $P$ values was applied when performing a high number of comparisons. In order to avoid spurious positives, the alpha value was lowered to account for the number of comparisons being performed.

Results

Study characteristics

Our search strategy yielded 306 articles for review. Figure 1 shows the literature search and the selection flow chart. Our final pool of eligible studies included 12 case-control studies [13–24] and 1 cohort study [12]. Table 1 presents a summarized description of the 13 ultimately selected articles. Six studies were conducted in Caucasian populations [15,18,20–22,24], three in Asian populations [17,19,23], and four in mixed ethnic groups [12–14,16]. Most of the studies comprised adult patients [12,15,17–20,22–24], whereas two studies focused on pediatric patients [13,14]. Pneumonia was classified as community-acquired pneumonia (CAP) [12,13,15,17,18,20,24], hospital-acquired pneumonia (HAP) [14,19], ventilator-associated pneumonia (VAP) [22], and mixed pneumonia [16,23]. Two studies included severe pneumonia patients [14,22]. Three studies included both severe and non-severe pneumonia patients but the data for these patients could be separately extracted [17,19,23]. Seven studies reported pneumonia-related mortality rate [12,13,15,17–19,23]. There were twelve studies on $-$308A/G [12–19,21–24], seven studies on $-$238A/G [16–18,20–23]. No more than two studies on $+$408A/G, $-1031T/C$, $-863C/A$, $-573C/T$, and $-376G/A$. The quality scores of studies ranged from 3 to 6. Three studies were defined as low quality studies [14,16,17]. One study was not in HWE [24]. Genotype numbers and HWE examination results are presented in Table 2.

TNF-$\alpha$ $-$308A/G polymorphism

Eleven studies determined the association between TNF-$\alpha$ $-$308A/G polymorphism and pneumonia risk [13–19,21–24]. Total sample sizes for case group and control group were 2620...
and 3157, respectively. The pooled OR was 1.13 (95% CI 0.99–1.30; \(P = 0.07\)) (Figure 2). In the subgroup analysis by ethnicity, a significant association was found among Asians (OR = 1.76; 95% CI 1.26–2.45; \(P = 0.0008\)) but not among Caucasians (OR = 1.04; 95% CI 0.89–1.21; \(P = 0.63\)). Subgroup analysis was also performed by the type of pneumonia. No increased risk was found among CAP patients (OR = 1.03; 95% CI 0.88–1.21; \(P = 0.68\)). In the subgroup analysis by the severity of pneumonia, significant increase of severe pneumonia risk was found among the A allele carriers (OR = 2.20; 95% CI 1.32–3.66; \(P = 0.002\)). No significant association was found between TNF-\(\alpha\)238A/G polymorphism and non-severe pneumonia risk (OR = 1.24; 95% CI 0.82–1.86; \(P = 0.30\)). Seven studies identified the association between TNF-\(\alpha\)–308A/G polymorphism and pneumonia mortality risk [12,13,15,17–19,23]. Total sample size of patients was 2302. Figure 3 shows that there is not a significant association between TNF-\(\alpha\)–308A/G polymorphism and mortality with an OR of 1.96 (95% CI 0.94–4.09; \(P = 0.07\)). In the subgroup analysis by ethnicity, no association was found among Asian (OR = 4.25; 95% CI 0.85–21.33; \(P = 0.08\)). Stratification by pneumonia type showed that CAP patients carrying A allele were not associated with mortality risk (OR = 1.15; 95% CI 0.73–1.83; \(P = 0.55\)). Furthermore, there was no significant association between TNF-\(\alpha\)–308A/G polymorphism and severe pneumonia mortality risk (OR = 3.43; 95% CI 0.60–19.95; \(P = 0.17\)). Summary results of comparisons are listed in Table 3.

TNF-\(\alpha\) –238A/G polymorphism

Seven studies (2235 cases and 2673 controls) studied the association between TNF-\(\alpha\)–238A/G polymorphism and pneumonia risk [16–18,20–23]. The pooled OR was 1.38 (95% CI 0.84–2.28, \(P = 0.20\)) (Figure 4). Subgroup analyses performed according to ethnicity and pneumonia type still did not find significant association between –238A/G polymorphism and pneumonia risk (Table 3). There were only two studies investigated the association between this polymorphism and mortality risk. Thus, meta-analysis was not performed.

Sensitivity analysis and Heterogeneity analysis

In order to assess the stability of the results of the meta-analysis, sensitivity analyses were performed by omitting the low quality studies [14,16,17] and the HWE-violating study [24], respectively. All the results were not altered which suggested the robustness of our results (Table 3).

For TNF-\(\alpha\)–308A/G polymorphism and pneumonia risk, no significant between-study heterogeneity was observed in the dominant genetic model (\(P_{\text{heterogeneity}} = 0.13\) and \(I^2 = 33\%\)). For TNF-\(\alpha\)–308A/G polymorphism and mortality risk, there was statistically significant between-study heterogeneity in the dominant genetic model (\(P_{\text{heterogeneity}} = 0.01\) and \(I^2 = 64\%\)). In the CAP subgroup, no significant heterogeneity was found (\(P_{\text{heterogeneity}} = 0.19\) and \(I^2 = 34\%\)). For TNF-\(\alpha\)–238A/G polymorphism and pneumonia risk, there was also statistically significant between-study heterogeneity (\(P_{\text{heterogeneity}} = 0.0009\) and \(I^2 = 74\%\)). As
shown in Table 3, the heterogeneity was significantly decreased in the CAP subgroup ($P_{\text{heterogeneity}} = 0.30$ and $I^2 = 16\%$).

**Publication bias**

Publication bias was examined by funnel plot qualitatively and estimated by Egger’s test quantitatively. The shape of the funnel plot seemed almost symmetrical (Figure 5). Egger’s test did not show evidence of publication bias ($P = 0.191$).

**Discussion**

This meta-analysis of 13 studies systematically evaluated the associations between TNF-$\alpha -308A/G$, $-238A/G$ polymorphisms and risk and mortality of pneumonia. We found that $-308A/G$ polymorphism was not a risk factor for developing pneumonia in the overall study population. In the subgroup analysis, however, we noted that individuals carrying A allele had increased pneumonia risk in Asians, but not in Caucasians. This result indicated that interactions between different ethnicities and genetic variants may contribute to pneumonia risk. We also carried out subgroup analysis for type of pneumonia. No evidence supported the association between this polymorphism and risk of CAP. However, we found that patients with A allele had increased severe pneumonia risk, suggesting a possible role of $-308A/G$ polymorphism in severe pneumonia pathogenesis. Although subgroup analyses found significant associations among Asians and patients with severe pneumonia, we should exercise great caution in interpreting apparent results. Chance is the explanation for spurious subgroup analyses. Thus, these results should be validated by future studies.

A non-significant result was observed for the association of $-308A/G$ polymorphism with pneumonia-related mortality. Teuffel and colleagues also did not find an association between this polymorphism and mortality from sepsis in their meta-analysis [5]. When stratified for ethnicity, type of pneumonia, and disease severity, no increased risk for fatal outcome was found among Asians, CAP patients, and severe pneumonia patients. There were no more than 2 studies using Caucasians evaluated the association between $-308A/G$ polymorphism and mortality risk. The positive association between this condition and mortality risk could not be ruled out, because studies with small sample size may have insufficient statistical power to detect a slight effect. Therefore, more studies with Caucasian population are needed to assess the effect of $-308A/G$ polymorphism on pneumonia mortality risk.

Results from our meta-analysis showed the lack of association between $-238A/G$ polymorphism and pneumonia risk. Subgroup analyses also did not detect significant association between this polymorphism and pneumonia risk. These results suggested that $-238A/G$ polymorphism might have no role in the etiology of pneumonia. However, the study from Pappachan et al. [28] showed a significant association between the $-238A/G$ polymorphism and mortality from systemic inflammatory response syndrome (SIRS). They found that $-238A$ was significantly higher in patients who died in ICU compared to those who survived [28]. Thus, TNF-$\alpha -238A/G$ polymorphism may play a possible role in the pneumonia-related mortality. However, there were only two studies estimated this association and meta-analysis could not be conducted. This issue is needed to be further studied.

Pneumonia is a complex inflammatory disease. Moderate degree of local inflammation is required to control infection;
however, excessive release of inflammatory cytokines favours intracellular and extracellular bacterial growth and organ dysfunction [29,30]. Lee et al. [31] found that TNF-α augmented *Escherichia coli* growth both *in vitro* and *in vivo*. In addition, systemic administration of TNF-α in rat worsened pneumonia by reducing alveolar neutrophil recruitment and bacterial clearance [32,33]. Moreover, Yende et al. [34] indicated that preinfection systemic level of TNF-α was associated with higher risk of CAP requiring hospitalization. Therefore, TNF-α may play a pivotal role in the development of pneumonia. Early study demonstrated that a functional mutation (−308A/G) in the TNF-α gene could influence the expression of TNF-α [35]. The presence of the A substitution led to increase in the binding of nuclear factors and enhanced transcription of the gene [36]. Recently, Song et al. [23] showed severe sepsis patients with AA+AG genotypes had significantly higher TNF-α serum concentrations than those with GG genotype. Therefore, it was biologically plausible that −308A/G polymorphism which could affect TNF-α production in pneumonia patients. Thus, −308A/G polymorphism influenced the susceptibility to pneumonia and mortality risk. Subgroup analysis was used to explore the sources of heterogeneity. After subgroup analysis by the type of pneumonia, the heterogeneity was effectively decreased in the CAP subgroup. Therefore, it could be speculated that the large heterogeneity mainly resulted from the type of pneumonia. In addition, funnel plot and Egger’s test were performed to find potential publication bias. No significant publication bias was noted. Furthermore, Huizinga and coworkers revealed that the multiple sclerosis patients with −238 GA produced lower TNF-α compared to those with −238 GG [9]. Nevertheless, no published studies analyzed the relationship between −238A/G polymorphism and TNF-α production in pneumonia patients. Thus, whether −238A/G polymorphism influenced the pneumonia risk was still unclear. The functional studies of −238A/G polymorphism in pneumonia are required.

We should address the importance of heterogeneity and publication bias, which might influence the results of meta-analysis. In our meta-analysis, significant heterogeneity was observed in −308A/G and mortality risk and also in −238A/G and pneumonia risk. Subgroup analysis was used to explore the sources of heterogeneity. After subgroup analysis by the type of pneumonia, the heterogeneity was effectively decreased in the CAP subgroup. Therefore, it could be speculated that the large heterogeneity mainly resulted from the type of pneumonia. In addition, funnel plot and Egger’s test were performed to find potential publication bias. No significant publication bias was noted. Furthermore, we carried out the sensitivity analysis. Removal of the low quality studies or the HWE-violating study did not change the conclusions of meta-analysis. These procedures suggested that our results were stable and robust.

Several limitations of this meta-analysis should be considered when interpreting results. First, the number of included studies was moderate. It was possible that some relevant published studies or unpublished studies with negative results were missed. Second, most of the studies were conducted in Caucasian and Asian populations. Therefore, our results may be applicable only to these ethnic groups. Third, because insufficient data could be extracted

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### Table 3. Summary of meta-analysis results.

| Comparisons | Study | No. of studies | Test of association | Heterogeneity |
|-------------|-------|----------------|---------------------|---------------|
| −308A/G and pneumonia risk | | | | |
| AA+AG vs. GG Overall | 11 | 1.13 (0.99–1.30) | 1.82 | 0.07 | F | 15.01 | 0.13 | 33.0 |
| AA+AG vs. GG Asian | 3 | 1.76 (1.26–2.45) | 3.35 | 0.0008 | F | 0.77 | 0.68 | 0.0 |
| AA+AG vs. GG Caucasian | 5 | 1.04 (0.89–1.21) | 0.63 | 0.63 | F | 5.87 | 0.21 | 32.0 |
| AA+AG vs. GG CAP | 4 | 1.05 (0.88–1.26) | 0.55 | 0.58 | F | 5.28 | 0.15 | 43.0 |
| AA+AG vs. GG Severe | 5 | 2.20 (1.32–3.66) | 3.03 | 0.002 | R | 8.60 | 0.01 | 53.0 |
| AA+AG vs. GG Non-severe | 3 | 1.23 (0.82–1.86) | 1.00 | 0.32 | F | 0.85 | 0.65 | 0.0 |
| AA+AG vs. GG High quality | 8 | 1.14 (0.99–1.31) | 1.87 | 0.06 | F | 11.00 | 0.14 | 36.0 |
| AA+AG vs. GG HWE | 10 | 1.17 (1.01–1.35) | 2.05 | 0.04 | F | 14.12 | 0.12 | 36.0 |
| −308A/G and mortality risk | | | | |
| AA+AG vs. GG Overall | 7 | 1.96 (0.94–4.09) | 1.80 | 0.07 | R | 16.74 | 0.01 | 64.0 |
| AA+AG vs. GG Asian | 3 | 4.25 (0.85–21.35) | 1.76 | 0.08 | R | 9.66 | 0.008 | 79.0 |
| AA+AG vs. GG CAP | 5 | 1.15 (0.73–1.83) | 0.60 | 0.55 | F | 6.10 | 0.19 | 34.0 |
| AA+AG vs. GG Severe | 3 | 3.45 (0.60–19.95) | 1.38 | 0.17 | R | 9.78 | 0.008 | 80.0 |
| AA+AG vs. GG High quality | 6 | 1.74 (0.82–3.68) | 1.44 | 0.15 | R | 14.58 | 0.01 | 66.0 |
| −238A/G and pneumonia risk | | | | |
| AA+AG vs. GG Overall | 7 | 1.38 (0.84–2.28) | 1.27 | 0.20 | R | 22.71 | 0.0009 | 74.0 |
| AA+AG vs. GG Caucasian | 4 | 1.21 (0.58–2.54) | 0.50 | 0.62 | R | 12.87 | 0.005 | 77.0 |
| AA+AG vs. GG CAP | 3 | 0.94 (0.67–1.33) | 0.33 | 0.74 | F | 2.40 | 0.30 | 16.0 |
| AA+AG vs. GG High quality | 5 | 1.19 (0.74–1.93) | 0.72 | 0.47 | R | 14.22 | 0.007 | 72.0 |

Bonferroni correction was applied (P<0.00294). Bold indicates statistically significant P values. vs., versus; CAP, community-acquired pneumonia; HWE, Hardy-Weinberg equilibrium; R, random-effects model; F, fixed-effects model.

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from the primary publications, we could not assess the effects of +888A/G, −1051T/C, −863C/A, −857C/T, and −376G/A polymorphisms on pneumonia risk and mortality risk. Fourth, the overall outcome was based on unadjusted data, whereas a more precise analysis could be performed if individual data were available to allow adjustment. Finally, pneumonia is a complex disease, and many genes are related to pneumonia [38]. However, we could not address gene-gene interactions in our study due to the lack of the related information.

Figure 4. Meta-analysis for the association between pneumonia risk and the TNF-α −238A/G polymorphism. doi:10.1371/journal.pone.0061039.g004

Figure 5. Funnel plot for publication bias in the association between pneumonia risk and the TNF-α −308A/G polymorphism. doi:10.1371/journal.pone.0061039.g005
In conclusion, our results suggested that TNF-α −308A/G polymorphism and −238A/G polymorphism were not associated with the risk of pneumonia in the overall population. In addition, TNF-α −308A/G polymorphism was not associated with pneumonia mortality. Future large sample size studies with more ethnic groups are needed to confirm our findings. Moreover, other TNF-α polymorphisms and gene-gene interactions should also be considered in future studies.

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Author Contributions
Conceived and designed the experiments: LL WN. Performed the experiments: LL WN WFL WFY WJH. Analyzed the data: LL WN WFL. Contributed reagents/materials/analysis tools: LL WN WFL. Wrote the paper: LL WN WFL WJH.