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Association of Polymorphisms in Toll-Like Receptors 4 and 9 with Risk of Pulmonary Tuberculosis: A Meta-Analysis

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Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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Background: Findings regarding the association of the single-nucleotide polymorphisms (SNPs) rs4986790 and rs4986791 in Toll-like receptor 4 and rs187084, rs574386, and rs352139 in Toll-like receptor 9 (TLR9) with pulmonary tuberculosis (PTB) susceptibility are inconsistent. We conducted a meta-analysis to systematically summarize and clarify the association between these SNPs and PTB susceptibility.

Material/Methods: A systematic literature search for relevant studies up to December, 2014 was performed in PubMed, EMBASE, Web of Science, Chinese National Knowledge Infrastructure (CNKI), and Wanfang databases. Information was gathered from each eligible study. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were used to pool the effect size.

Results: Finally, a total of 16 case-control studies on these polymorphisms were enrolled in this meta-analysis. The meta-analysis results suggest there was no association between these polymorphisms and PTB risk in all the genetic models overall. However, for TLR4 rs4986791, a significant increased PTB risk was found in Africans, and for TLR9 rs352139 a significant increased PTB risk was found in Asians after subgroup analysis by ethnicity, although the enrolled studies were limited.

Conclusions: There was no association between the polymorphisms in TLR4 and 9 and PTB risk overall, but TLR4 rs4986791 and TLR9 rs352139 might be associated with increased PTB risk in Africans and Asians, respectively. Additional well-designed, larger-scale epidemiological studies are needed to validate our results.

MeSH Keywords: **Meta-Analysis • Polymorphism, Single Nucleotide • Toll-Like Receptors • Tuberculosis, Pulmonary**

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Background

Tuberculosis (TB) caused by *Mycobacterium tuberculosis* (*M. tb*) still is a leading cause of morbidity and mortality worldwide [1]. *M. tb* infects one-third of the world population; however, only 10% of them progress to active TB, possibly due to complex environmental, genetic, and immunological interactions [2]. Host genetics are considered to play a critical role in the apparent differences in disease progression [3,4].

Toll-like receptors (TLRs), which are pathogen recognition receptors (PRRs), play an essential role in host innate response in the pathogenesis of TB. Currently, 11 mammalian TLRs have been identified and TLR 1-10 are functional in humans [5]. TLR1, TLR2, TLR4, TLR6, TLR8, and TLR9 are thought to be involved in the recognition of *M. tb*.

Activation of TLRs may result in several possible biological outcomes such as cytokine secretion, modulation of the adaptive immune response, rapid cellular differentiation, apoptosis, and direct antimicrobial activity [6–8]. *M. tb* cell-surface ligands interact with TLRs that can result in NF- κ B activation and the production of proinflammatory cytokines, chemokines, and nitric oxide through myeloid differentiation primary response protein 88 (MyD88)-dependent or independent pathways [9–12].

It has been proposed that SNPs of TLR4 and TLR9 genes are associated with PTB susceptibility. TLR-knockout mouse studies indicate that TLR2, TLR4, and TLR9 contribute to host resistance to *M. tb* infection [13–17]. Recently, a series of studies have been conducted to investigate the association between the TLR4 and TLR9 polymorphisms and the risk of pulmonary tuberculosis (PTB) in diverse populations, but the results were mixed and inconclusive. Therefore, we performed a meta-analysis to evaluate the association of TLR4 and TLR9 SNPs with the susceptibility to PTB.

Material and Methods

Publication search

A systematic search was performed for published studies on the relationship between TLR9 polymorphisms and PTB susceptibility, without language restriction. PubMed, EMBASE, Web of Science, and 2 Chinese databases (Chinese National Knowledge Infrastructure and Wanfang databases) were utilized to search the available articles, with the last search update on September 30, 2014. The following terms were used: “Toll-like receptor 4 OR Toll-like receptor 9 OR TLR4 OR TLR9”, “tuberculosis”, and “polymorphism OR polymorphisms”, without any limitation applied. The articles retrieved were screened and selected independently by the 2 authors (L.Y. and K.X.J.)

using eligibility criteria. The reference lists of selected articles and review articles were also examined to identify additional eligible studies.

Inclusion and exclusion criteria

Studies in this meta-analysis met the following inclusion criteria: (1) evaluated the relationship between TLR4 rs4986790 (Asp299Gly) and rs4986791 (Thr399Ile) and TLR9 rs352139, rs187084, and rs5743836 polymorphisms with tuberculosis susceptibility; (2) case-control study; (3) detailed genotype frequency data could be acquired to calculate the odds ratios (ORs) and 95% confidence intervals (CIs); and (4) original articles published in peer-reviewed journals. Exclusion criteria were: (1) studies that did not specify sample origins; (2) studies with no detailed genotype data; (3) studies with insufficient or duplicate data; (4) studies with same author from same country of origin. Two independent investigators selected the studies according to the inclusion and exclusion criteria by screening the title, abstract, and full text. Any dispute was resolved by discussion.

Data extraction

The following information was collected in duplicate by 2 investigators independently (Z.L.L. and L.K.H.) according to the criteria described above: name of first author, year of publication, the characteristics of cases and controls, country of origin, the detective sample, ethnicity, genotyping methods, Hardy-Weinberg equilibrium, number of cases and controls, and genotype frequency in cases and controls of the selected polymorphisms. For those studies that included subjects of PTB, extra-pulmonary tuberculosis, and tuberculosis meningitis, only the data on PTB cases and healthy controls were collected. If there was a disagreement about data, the 2 investigators rechecked the original data of the included studies and had a discussion to reach consensus; otherwise, the third investigator adjudicated the disagreements (K.X.J.).

Quality score assessment

Two investigators (T.Z.X. and W.Y.X.) assessed the methodological quality of each eligible article included in this meta-analysis according to the Newcastle Ottawa Scale (NOS) based on 3 aspects: selection, comparability, and exposure, with scores ranging from 0 to 9 [18]. The standard of high quality was NOS score ≥ 7 .

Statistical analysis

Initially, Hardy-Weinberg equilibrium (HWE) for each study was assessed by chi-square test in the control groups, and $P < 0.05$ was considered as deviation from HWE. The crude odds ratios

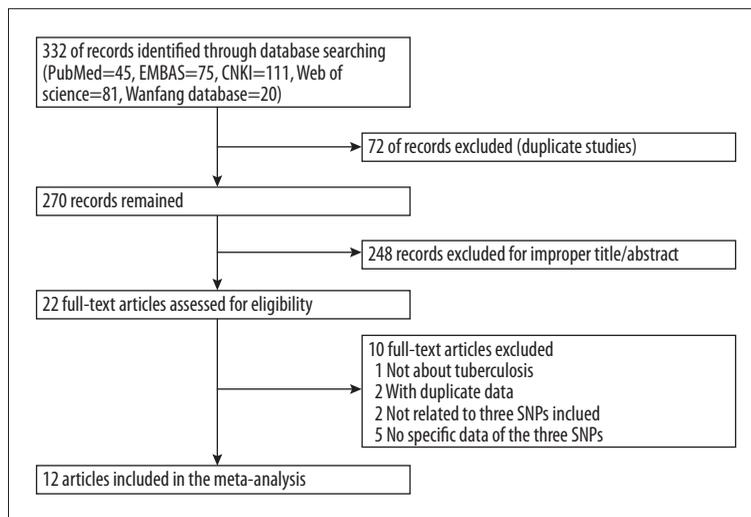


Figure 1. Flow chart of study selection.

(ORs) and 95% confidence intervals (95% CIs) were calculated to assess the strength of association between the included polymorphisms and PTB risk. A p -value <0.05 was considered to indicate statistically significant association. Studies with zero value in both groups were excluded from meta-analysis, for such studies do not provide any indication of either the direction or magnitude of gene and disease association. Pooled ORs were performed for allelic comparison, homozygote model, heterozygote model, dominant model, and recessive model. The statistical significance level was determined by Z-test with P value less than 0.05. The chi-square based on Q statistic test was used for the assessment of heterogeneity and $P < 0.1$ was considered statistically significant. When the effect was assumed to be lack of heterogeneity, the fixed-effects model (Mantel-Haenszel method) was used to calculate the pooled OR and 95% CIs; otherwise, the random-effects model (DerSimonian-Laird method) was used [19]. Sensitivity analysis was also conducted to evaluate the effect of each study on the combined ORs by omitting each study in each turn. Subgroup analysis was also performed to assess the ethnic-specific effect. Potential publication bias was checked by Begg's funnel plots and Egger's test [20,21]. An asymmetric plot and the P value of Egger's test less than 0.05 was considered representative of statistically significant publication bias. All analyses were done using Stata 12.0 software (StataCorp, College Station, TX, USA). A 2-tailed $P < 0.05$ was considered significant except for specific conditions, where a certain P value would be declared.

Results

Study characteristics

The literature selection process is shown in Figure 1. We initially identified 332 relevant articles. After excluding overlap between

the databases, 273 abstracts were evaluated. Subsequently, 22 articles with full texts that met the inclusion criteria were assessed. Then 10 of the remaining 27 articles were excluded, among which, 1 had no relation to tuberculosis [22], 2 had duplicate data [23,24], 2 were not related to the included SNPs [25,26], and 4 did not present specific data on the included SNPs genotype [27–31]. Finally, a total of 12 eligible articles were included in the current meta-analysis [32–43].

The characteristics of each included study are listed in Table 1. There were 32 case-control studies published from 2004 to 2013. We finally analyzed 14 studies containing 2801 cases and 2590 controls for rs4986790, 8 studies containing 2137 cases and 1656 controls for rs4986791, 4 studies containing 1103 cases and 1301 controls for rs352139, 3 studies containing 635 cases and 696 controls for rs187084, and 3 studies containing 608 cases and 633 controls for rs5743836. Different genotyping methods, including polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), TaqMan, DigiTag2, and Illumina GoldenGate genotyping assay were utilized. The genotyping distribution was in agreement with HWE in all studies. The methodological quality scores of all included studies ranged from 7 to 9, with all the studies identified to be high quality.

Tests of heterogeneity

Significant heterogeneities for rs4986790 were found in the overall analyses under allelic model and for rs352139 in the overall analyses under 5 genetic models (Figure 2, Table 2). After subgroup analysis by ethnicity, for rs4986790, significant heterogeneities only existed in Asians, and for rs352139 most of the heterogeneity could be eliminated through ethnicity-specific analyses. For the other polymorphisms – rs4986791, rs187084, and rs5743836 – no heterogeneity was found in the overall analyses using 5 genetic models.

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

| Study ID | Year | Country | Ethnicity | Case | | | Control | | | P for HWE | Quality |
|-----------------------|------|---------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|---------|
| | | | | AA | AG | GG | AA | AG | GG | | |
| TLR4 rs4986790 | | | | AA | AG | GG | AA | AG | GG | | |
| Newport et al. | 2004 | Gambia | African | 241 | 62 | 4 | 235 | 58 | 5 | 0.814 | 8 |
| Fitness et al. | 2004 | Malawi | African | 146 | 16 | 0 | 389 | 38 | 0 | 0.629 | 8 |
| Rosas Taraco et al. | 2007 | Mexico | Caucasian | 94 | 10 | 0 | 110 | 4 | 0 | 0.982 | 7 |
| Ma et al. | 2007 | USA | African | 281 | 57 | 1 | 157 | 36 | 1 | 0.783 | 8 |
| Ma et al. | 2007 | USA | Caucasian | 159 | 20 | 1 | 95 | 14 | 1 | 0.84 | 7 |
| Ma et al. | 2007 | USA | Caucasian | 353 | 22 | 0 | 106 | 8 | 0 | 0.927 | 7 |
| Olesen et al. | 2007 | Guinea Bissau | African | 262 | 51 | 2 | 265 | 65 | 7 | 0.459 | 7 |
| Najmi et al. | 2010 | India | Asian | 95 | 34 | 6 | 206 | 44 | 0 | 0.312 | 8 |
| Selvaraj et al. | 2010 | India | Asian | 153 | 47 | 4 | 151 | 53 | 3 | 0.791 | 8 |
| Sanchez et al. | 2012 | Colombia | Caucasian | 429 | 36 | 1 | 270 | 29 | 1 | 0.973 | 9 |
| Jahantigh et al. | 2013 | Iran | Asian | 122 | 2 | 0 | 146 | 3 | 0 | 0.992 | 8 |
| Torres-garcia et al. | 2013 | Mexico | Caucasian | 88 | 2 | 0 | 89 | 1 | 0 | 0.989 | 7 |
| TLR4 rs4986791 | | | | CC | CT | TT | CC | CT | TT | | |
| Ma et al. | 2007 | USA | African | 325 | 14 | 0 | 178 | 16 | 0 | 0.836 | 8 |
| Ma et al. | 2007 | USA | Caucasian | 161 | 18 | 1 | 97 | 12 | 1 | 0.675 | 8 |
| Ma et al. | 2007 | USA | Caucasian | 357 | 18 | 0 | 108 | 6 | 0 | 0.959 | 8 |
| Olesen et al. | 2007 | Guinea Bissau | African | 262 | 51 | 2 | 265 | 65 | 7 | 0.459 | 9 |
| Najmi et al. | 2010 | India | Asian | 105 | 26 | 4 | 206 | 43 | 1 | 0.731 | 7 |
| Selvaraj et al. | 2010 | India | Asian | 150 | 49 | 4 | 152 | 46 | 5 | 0.798 | 8 |
| Sanchez et al. | 2012 | Colombia | Caucasian | 429 | 36 | 1 | 272 | 26 | 1 | 0.905 | 9 |
| Jahantigh et al. | 2013 | Iran | Asian | 112 | 10 | 2 | 141 | 7 | 1 | 0.054 | 8 |
| TLR9 rs352139 | | | | AA | AG | GG | AA | AG | GG | | |
| Kobayashi et al. | 2011 | Indonesia | Asian | 199 | 279 | 59 | 259 | 233 | 68 | 0.386 | 8 |
| Kobayashi et al. | 2011 | Vietnamese | Asian | 123 | 125 | 28 | 232 | 183 | 40 | 0.902 | 8 |
| Torres-Garcia et al. | 2013 | Mexico | Caucasian | 23 | 48 | 19 | 14 | 41 | 35 | 0.942 | 7 |
| Yang et al. | 2013 | China | Asian | 70 | 89 | 41 | 68 | 95 | 33 | 1.000 | 8 |
| TLR9 rs187084 | | | | CC | CT | TT | CC | CT | TT | | |
| Olesen et al. | 2007 | Guinea-Bissau | African | 25 | 122 | 171 | 21 | 132 | 186 | 0.931 | 8 |
| Selvaraj et al. | 2010 | India | Asian | 27 | 91 | 75 | 32 | 92 | 84 | 0.718 | 8 |
| Jahantigh et al. | 2013 | Iran | Asian | 10 | 51 | 63 | 8 | 59 | 82 | 0.822 | 9 |
| TLR9 rs5743836 | | | | CC | CT | TT | CC | CT | TT | | |
| Olesen et al. | 2007 | Guinea-Bissau | African | 62 | 154 | 104 | 66 | 175 | 101 | 0.818 | 8 |
| Selvaraj et al. | 2010 | India | Asian | 1 | 29 | 168 | 2 | 32 | 167 | 0.945 | 8 |
| Torres-Garcia et al. | 2013 | Mexico | Caucasian | 0 | 8 | 82 | 0 | 12 | 78 | 0.795 | 7 |

HWE – Hardy-Weinberg equilibrium.

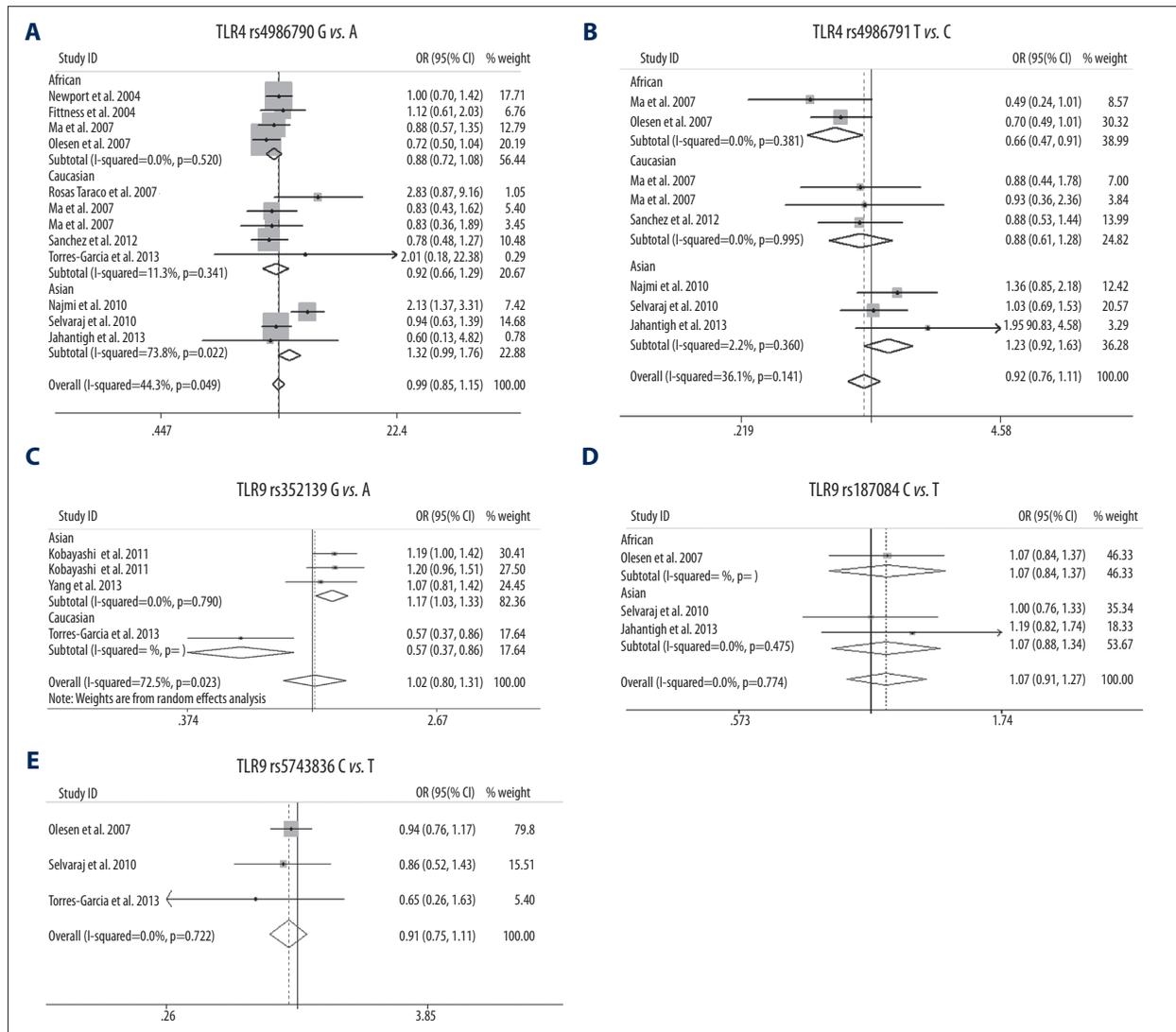


Figure 2. Forest plot of allele comparison of rs4986790 and rs4986791 in TLR4 and rs352139, rs187084, and rs5743836 in TLR9.

Meta-analysis results

The association between the included polymorphisms and the susceptibility to PTB was first analyzed. As shown in Figure 2 and Table 2, no significant association was identified for any polymorphisms in overall analyses in any genetic model. Next, we performed subgroup analysis according to ethnicity, and significant association was still not found between the polymorphisms rs4986790, rs187084, and rs573836 and PTB risk in diverse populations. For TLR4 rs4986791, a significant decreased risk was observed in Africans in allele and dominant models. However, there were only 2 studies that analyzed in Africans, suggesting the results was not very convincing and further studied are need to verify the conclusion. For TLR9 rs352139, the association with PTB risk was found to be significant in allelic, heterozygote, and dominant models.

Publication bias

Begg's funnel plot and Egger's test were carried out to assess the publication bias among the selected studies for the polymorphisms rs4986790 and rs4986791 in TLR4 and rs352139 in TLR9. Symmetrical funnel plots were obtained in all the genetic models (Figure 3). The Egger's test was also performed to provide the statistical evidence of publication bias. The findings showed lack of publication bias for all the included polymorphisms among all comparison genetic models except for TLR9 rs352139 in dominant model ($P_{Begg} = 0.089$, $P_{Egger} = 0.002$). No funnel plot or Egger's test were performed for the association between rs187084 and rs5743836 and PTB susceptibility due to the limited number of included studies.

Table 2. Summary of pooled ORs in the meta-analysis.

| Genotype | Studies | Comparison model | N | OR (95%CI) | | P _{OR} | M | I ² (%) | P _H |
|----------------|-----------|------------------|----|------------|-----------------|-----------------|---|--------------------|----------------|
| TLR4 rs4986790 | Overall | G vs. A | 12 | 1.012 | (0.812–1.261) | 0.915 | R | 44.3 | 0.049 |
| | | GG vs. AA | 7 | 1.098 | (0.588–2.049) | 0.770 | F | 29.1 | 0.206 |
| | | GA vs. AA | 12 | 0.981 | (0.832–1.157) | 0.819 | F | 0.0 | 0.469 |
| | | GG vs. GA+AA | 7 | 1.098 | (0.578–2.054) | 0.769 | F | 24.8 | 0.240 |
| | | GG+GA vs. AA | 12 | 0.985 | (0.838–1.157) | 0.853 | F | 26.8 | 0.182 |
| | African | G vs. A | 4 | 0.884 | (0.720–1.085) | 0.520 | F | 0.0 | 0.520 |
| | | GG vs. AA | 3 | 0.502 | (0.198–1.273) | 0.147 | F | 0.0 | 0.638 |
| | | GA vs. AA | 4 | 0.930 | (0.742–1.165) | 0.525 | F | 0.0 | 0.728 |
| | | GG vs. GA+AA | 3 | 0.510 | (0.201–1.293) | 0.156 | F | 0.0 | 0.666 |
| | | GG+GA vs. AA | 4 | 0.902 | (0.723–1.125) | 0.360 | F | 0.0 | 0.614 |
| | Caucasian | G vs. A | 5 | 0.923 | (0.661–1.288) | 0.341 | F | 11.3 | 0.341 |
| | | GG vs. AA | 2 | 0.613 | (0.086–4.378) | 0.626 | F | 0.0 | 0.979 |
| | | GA vs. AA | 5 | 0.941 | (0.663–1.337) | 0.735 | F | 11.8 | 0.339 |
| | | GG vs. GA+AA | 2 | 0.626 | (0.088–4.465) | 0.640 | F | 0.0 | 0.978 |
| | | GG+GA vs. AA | 5 | 0.931 | (0.658–1.3160) | 0.684 | F | 13.1 | 0.330 |
| | Asian | G vs. A | 3 | 1.313 | (0.655–2.634) | 0.443 | R | 73.8 | 0.022 |
| | | GG vs. AA | 2 | 4.824 | (0.213–109.508) | 0.808 | R | 73.4 | 0.053 |
| | | GA vs. AA | 3 | 1.144 | (0.821–1.159) | 0.427 | F | 45.3 | 0.161 |
| | | GG vs. GA+AA | 2 | 4.590 | (0.235–89.760) | 0.315 | R | 70.9 | 0.064 |
| | | GG+GA vs. AA | 3 | 1.249 | (0.651–2.397) | 0.503 | R | 64.8 | 0.059 |
| TLR4 rs4986791 | Overall | T vs. C | 8 | 0.919 | (0.764–1.107) | 0.374 | F | 36.1 | 0.141 |
| | | TT vs. CC | 6 | 0.946 | (0.465–1.926) | 0.879 | F | 23.9 | 0.255 |
| | | TC vs. CC | 8 | 0.921 | (0.749–1.131) | 0.432 | F | 0.0 | 0.474 |
| | | TT vs. CT+CC | 6 | 0.946 | (0.464–1.926) | 0.878 | F | 20.4 | 0.280 |
| | | TT+CT vs. CC | 8 | 0.922 | (0.755–1.127) | 0.427 | F | 20.4 | 0.267 |
| | African | T vs. C | 2 | 0.656 | (0.474–0.908) | 0.011 | F | 0.0 | 0.381 |
| | | TT vs. CC | 1 | | – | | | | |
| | | TC vs. CC | 2 | 0.709 | (0.497–1.011) | 0.058 | F | 27.2 | 0.241 |
| | | TT vs. CT+CC | 1 | | – | | | | |
| | | TT+CT vs. CC | 2 | 0.678 | (0.479–0.960) | 0.028 | F | 5.8 | 0.303 |
| | Caucasian | T vs. C | 3 | 0.884 | (0.609–1.285) | 0.519 | F | 0.0 | 0.995 |
| | | TT vs. CC | 2 | 0.618 | (0.087–4.412) | 0.631 | F | 0.0 | 0.980 |
| | | TC vs. CC | 3 | 0.890 | (0.599–1.321) | 0.562 | F | 0.0 | 0.997 |
| | | TT vs. CT+CC | 2 | 0.625 | (0.088–4.457) | 0.639 | F | 0.0 | 0.980 |
| | | TT+CT vs. CC | 3 | 0.878 | (0.595–1.296) | 0.513 | F | 0.0 | 0.997 |

Table 2 continued. Summary of pooled ORs in the meta-analysis.

| Genotype | Studies | Comparison model | N | OR (95%CI) | P _{OR} | M | I ² (%) | P _H |
|----------------|--------------|------------------|---------------------|---------------------|-----------------|------|--------------------|----------------|
| TLR9 rs352139 | Asian | T vs. C | 3 | 1.225 (0.921–1.631) | 0.163 | F | 2.2 | 0.360 |
| | | TT vs. CC | 3 | 1.780 (0.685–4.628) | 0.237 | F | 36.6 | 0.206 |
| | | TC vs. CC | 3 | 1.184 (0.851–1.647) | 0.317 | F | 0.0 | 0.661 |
| | | TT vs. CT+CC | 3 | 1.736 (0.669–4.505) | 0.257 | F | 36.0 | 0.210 |
| | | TT+CT vs. CC | 3 | 1.233 (0.898–1.694) | 0.195 | F | 0.0 | 0.500 |
| | Overall | G vs. A | 4 | 1.021 (0.798–1.307) | 0.866 | R | 72.5 | 0.012 |
| | | GG vs. AA | 4 | 0.977 (0.621–1.539) | 0.921 | R | 62.0 | 0.048 |
| | | GA vs. AA | 4 | 1.193 (0.890–1.600) | 0.237 | R | 55.4 | 0.081 |
| | | GG vs. GA+AA | 4 | 0.901 (0.601–1.349) | 0.612 | R | 61.9 | 0.049 |
| | | GG+GA vs. AA | 4 | 1.137 (0.841–1.538) | 0.404 | R | 62.1 | 0.048 |
| Asian | G vs. A | 3 | 1.170 (1.033–1.326) | 0.014 | F | 0.0 | 0.790 | |
| | GG vs. AA | 3 | 1.196 (0.907–1.578) | 0.204 | F | 0.0 | 0.898 | |
| | GA vs. AA | 3 | 1.338 (1.118–1.602) | 0.002 | F | 52.4 | 0.113 | |
| | GG vs. GA+AA | 3 | 1.049 (0.811–1.358) | 0.714 | F | 0.0 | 0.480 | |
| | GG+GA vs. AA | 3 | 1.313 (1.107–1.558) | 0.002 | F | 23.1 | 0.272 | |
| TLR9 rs187084 | Overall | C vs. T | 3 | 1.070 (0.906–1.265) | 0.425 | F | 0.0 | 0.774 |
| | | CC vs. TT | 3 | 1.172 (0.792–1.736) | 0.428 | F | 0.0 | 0.600 |
| | | CT vs. TT | 3 | 1.059 (0.843–1.330) | 0.625 | F | 0.0 | 0.905 |
| | | CC vs. CT+TT | 3 | 1.122 (0.772–1.630) | 0.546 | F | 0.0 | 0.527 |
| | | CC+CT vs. TT | 3 | 1.079 (0.868–1.340) | 0.494 | F | 0.0 | 0.908 |
| Asian | C vs. T | 2 | 1.068 (0.850–1.342) | 0.575 | F | 0.0 | 0.475 | |
| | CC vs. TT | 2 | 1.095 (0.657–1.823) | 0.728 | F | 0.0 | 0.356 | |
| | CT vs. TT | 2 | 1.115 (0.807–1.541) | 0.509 | F | 0.0 | 0.963 | |
| | CC vs. CT+TT | 2 | 1.026 (0.636–1.655) | 0.917 | F | 0.0 | 0.739 | |
| | CC+CT vs. TT | 2 | 1.113 (0.819–1.513) | 0.492 | F | 0.0 | 0.334 | |
| TLR9 rs5743836 | Overall | C vs. T | 3 | 0.195 (0.753–1.111) | 0.371 | F | 0.0 | 0.722 |
| | | CC vs. TT | 2 | 0.893 (0.579–1.378) | 0.61 | F | 0.0 | 0.627 |
| | | CT vs. TT | 3 | 0.893 (0.579–1.378) | 0.61 | F | 0.0 | 0.815 |
| | | CC vs. CT+TT | 2 | 0.986 (0.674–1.443) | 0.944 | F | 0.0 | 0.580 |
| | | CC+CT vs. TT | 3 | 0.850 (0.649–1.112) | 0.235 | F | 0.0 | 0.818 |

P_H – P value of heterozygote.

Sensitivity analysis

Sensitivity analysis was performed to examine the influence of any individual study on the pooled ORs for the polymorphisms

rs4986790 and rs4986791 in TLR4 and rs352139 in TLR9 by deleting each study once in every genetic model, and we arrived at almost the same results (Figure 4 and data not shown).

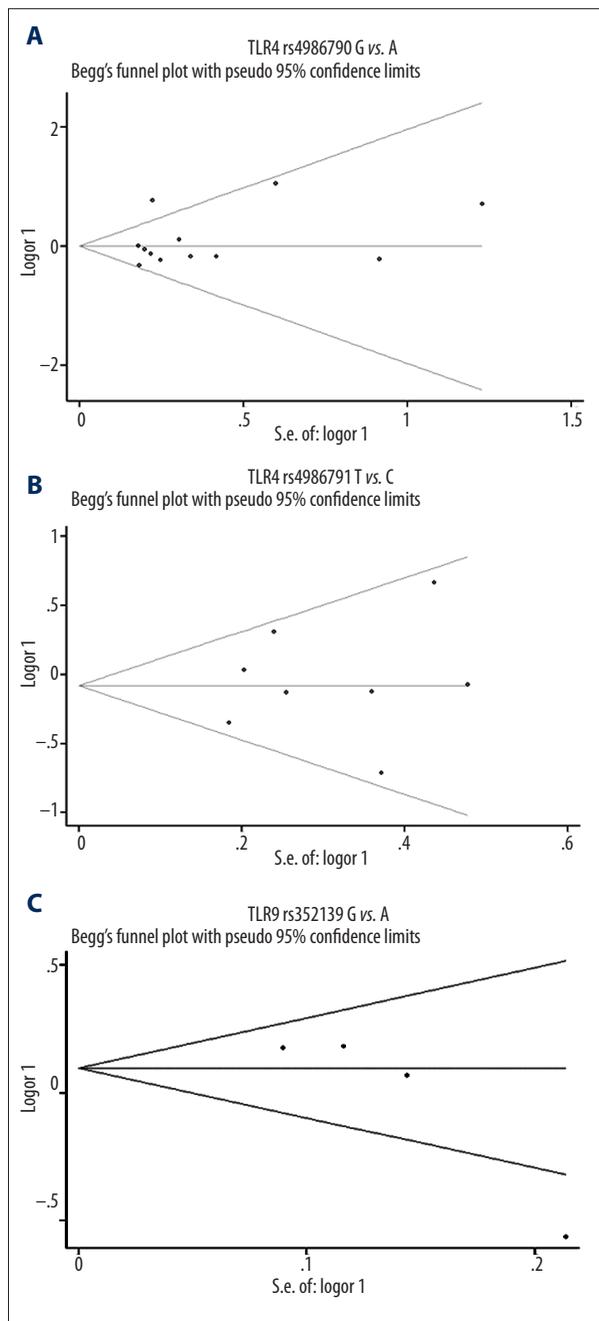


Figure 3. Publication bias analysis for rs4986790 and rs4986791 in TLR4 and rs352139, rs187084, and rs5743836 in TLR9 in allele model.

Discussion

In the present meta-analysis, we pooled a total of 32 case-control studies to evaluate the association of rs4986790 and rs4986791 polymorphisms in TLR4 and rs352139, rs187084, and rs5743836 polymorphisms in TLR9 with PTB susceptibility,

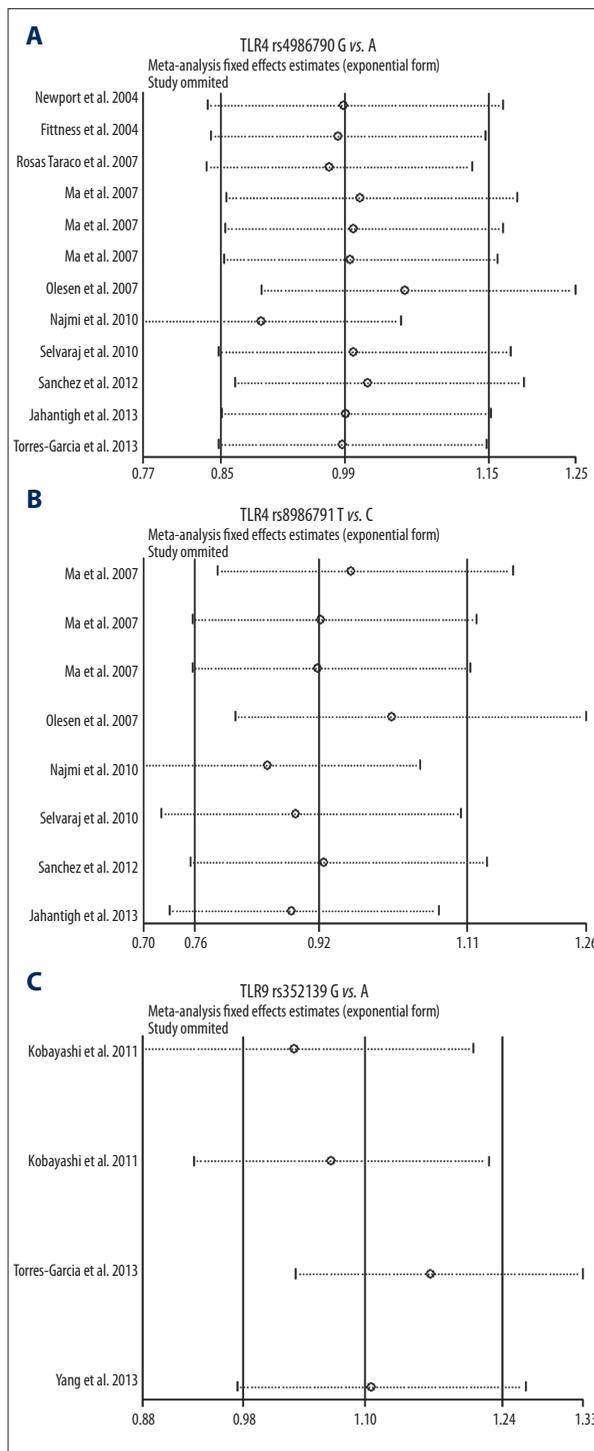


Figure 4. Sensitivity analysis for rs4986790 and rs4986791 in TLR4 and rs352139 in TLR9 in allele model.

and found that none the included polymorphisms were associated with PTB risk in overall analysis under all 5 genetic models. After subgroup analysis according to ethnicity, we found that TLR4 rs4986791 and TLR9 rs352139 might contribute to PTB infection in Africans and Asians, respectively.

Both innate and adaptive immune responses determine the development and outcomes of PTB. Toll-like receptors, including TLR4 and TLR9, have been considered to be involved in response to *M. tb*. TLR4 can recognize lipopolysaccharide, mycobacterial cell wall components, and heat-labile soluble mycobacterial factor to initiate the innate responses against tuberculosis, while TLR9 can recognize unmethylated CpG motifs in bacterial DNA and is essential for cellular responses to mycobacterial CpG DNA [44]. It has been reported that TLR2 or/and TLR9 deficiency makes mice susceptible to *M. tb* infection [17]. TLR4 and TLR9 activation is essential for the maintenance of *M. tb* Ag elicited pulmonary granulomatous response; however, the underlying mechanism is unknown. A series of SNPs have been identified, including rs4986790 (Asp299Gly, +896A>G) and rs4986791 (Thr399Ile, +1196C>T) in TLR4 and rs187084 (C-1486T), rs5743836 (C-1237T), rs352139 (G+1174A), and rs352140 (G+2848A) in TLR9 in different ethnicities (45). Rs4986790 and rs4986791 polymorphisms in TLR4 are the most widely studied and have been implicated in several diseases, including carcinomas. Rs187084 and rs5743836 polymorphisms of TLR9 are located within the putative promoter region of the gene that may influence the transcriptional regulation of the TLR9 gene by altering the binding of transcription factors. In contrast, rs352139 and rs352140 in the introns may affect mRNA splicing and/or enhance TLR9 gene transcription.

The functional evidence supports that TLR4 and TLR9 are genetic components in the risk of PTB, and the association of several SNPs with PTB susceptibility has been determined. However, the recent studies on TLR4 and TLR9 gene polymorphisms with PTB sociability are still contradictory because a single case-control study only provides a small test power due to a limited sample size. In this study, we performed a systematic search in several electronic databases, including PubMed, Web of Science, CNKI, and Wanfang database, on the association of TLR4 and TLR9 polymorphisms with PTB up to December, 2014. Finally, 16 articles with a total of 32 case-control studies containing a total of 14 160 participants were enrolled in this meta-analysis. After pooling independent analyses, we did not find any associations of TLR4 and TLR9 polymorphisms with the susceptibility to PTB overall under all the genetic models. However, further subgroup analysis suggested TLR4 rs4986791 was correlated with PTB risk in Africans under allelic and dominant models, and TLR9 rs352139 polymorphism was correlated with PTB risk in Asians under allelic, heterozygote, and dominant models.

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Recently, several genome-wide association studies have been performed to identify the SNPs that influence PTB susceptibility [46,47]. However, no polymorphisms in TLR4 and TLR9 were identified to be significantly related to PTB risk, which might be due to sample size, ethnicity, or geographical limitations. Unfortunately, we could not obtain the genotype frequencies in the GWAS studies related to the polymorphisms in TLR4 and TLR9. To the best of our knowledge, the present study is the most recent meta-analysis to address the association of TLR4 polymorphisms and PTB risk. In a previous meta-analysis that enrolled the articles related to TLR4 polymorphism and PTB risk up to February 2012, the researchers found no association between TLR4 rs4986790 and rs4986791 polymorphism and PTB; however, subgroup analysis was not performed. In the present meta-analysis, we included the related studies up to December 2014 and verified the conclusions. Furthermore, we performed subgroup analysis according to ethnicity and found a decreased risk of PTB in Africans who harbor rs4986791 polymorphism, although there were few enrolled studies, suggesting more attention should be paid to the association of rs4986791 and PTB risk. In contrast, this is the first meta-analysis to investigate the association of TLR 9 polymorphisms with PTB susceptibility. There are some limitations in our meta-analysis that should be considered. Firstly, the number of the included studies limited further analysis and made the results tentative. Secondly, we did not perform an evaluation of potential interactions such as polymorphism-polymorphism, gene-gene, and gene-environment due to the lack of sufficient data. Further well-designed case-control studies with larger sample sizes focusing on diverse populations should be conducted to confirm the results.

Conclusions

Our comprehensive conclusion from the current meta-analysis is that TLR4 rs4986791 might correlate with the decreased risk of PTB in Africans, and TLR9 rs352139 polymorphism might associate with the increased risk of PTB in Asians, but rs4986790, rs187084, and rs5743836 were not independent risk factors for PTB susceptibility.

Conflict of interest

We declare no conflict of interest.

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