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The relationship between toll like receptor 4 gene rs4986790 and rs4986791 polymorphisms and sepsis susceptibility: A meta-analysis

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Accumulating evidences have demonstrated that lipopolysaccharide (LPS) represents the important etiologic factor for sepsis. Some previous studies have reported the relationship between common polymorphisms rs4986790 and rs4986791 in the coding gene for this receptor and the susceptibility to sepsis, but there were distinct divergences between those findings. We therefore designed this meta-analysis incorporated 28 published articles containing 6,537 sepsis patients and 8,832 controls for a more comprehensive conclusion on this matter. Odds ratios (ORs) and 95% confidence interval (95% CIs) were calculated to evaluate the association of toll like receptor 4 gene polymorphisms rs4986790 and rs4986791 with sepsis risk. Heterogeneity between included studies was inspected using Q test, and sensitivity analysis was implemented via sequential deletion of each included study to investigate the stability of overall estimates. Funnel plot and Egger's test were adopted to examine publication bias across selected studies. We found no significant association for either the polymorphism rs4986790 or rs4986791 with sepsis susceptibility in total analysis under any genetic models. Neither did we after combining these two polymorphisms. The results of this meta-analysis suggest that the rs4986790 and rs4986791 polymorphisms in toll like receptor 4 gene may have no statistically significant influence on sepsis susceptibility.

Sepsis is a systemic inflammatory response syndrome (SIRS) induced by organism infections from pathogenic microorganisms¹. Organisms identify pathogen microorganisms through innate immunity system, thereby starting protective inflammatory response to eliminate pathogenic microorganisms, but over-response of systemic inflammation in organism may cause sepsis or septic shock². Thousands of patients undergo target organ damage and multiple organ dysfunction caused by severe sepsis and septic shock annually, and the death rate of sepsis is still increasing constantly despite the development of medical condition and technology³. Although the precise etiology of sepsis remains unclear, relevant studies have shown that gene polymorphism plays important role in affecting individual susceptibility to sepsis^{4–6}. Consequently, key gene polymorphisms involved in immune response pathways have been detected their influence on sepsis onset^{7,8}.

Innate immune system depends on pattern recognition receptors (PRRs) to detect conserved structures of pathogenic microorganisms (like bacteria, virus, fungus and protozoa), which are called pathogen-associated molecular patterns (PAMPs)⁹. Toll like receptors (TLRs) play an important role in autoimmunity, and one of them, toll like receptor 4, occupies an extremely significant position as a receptor recognizing PAMPs¹⁰.

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PRISMA 2009 Flow Diagram

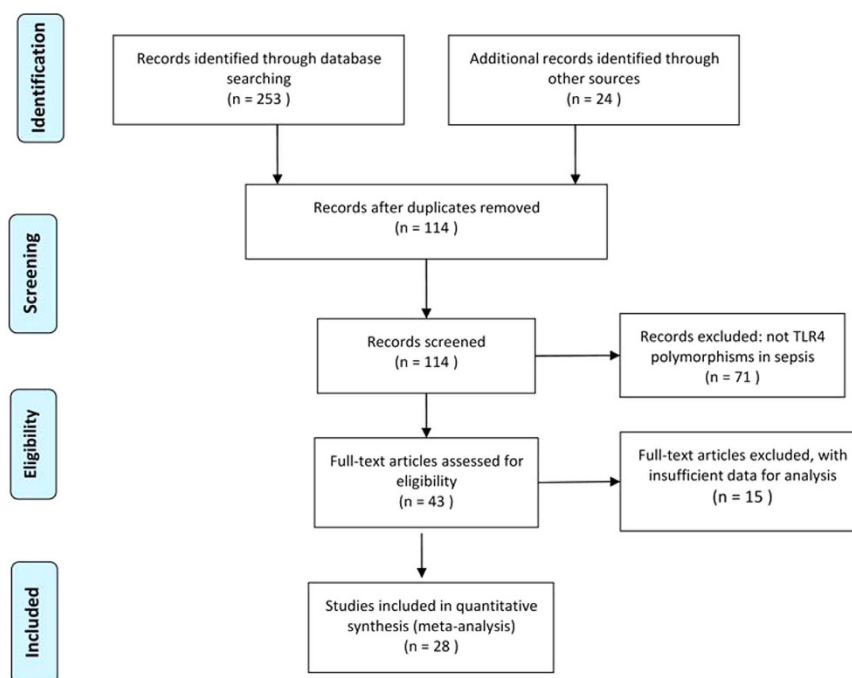


Figure 1. Flow diagram for study selection.

Reportedly, polymorphisms in the gene coding for this receptor may change the extracellular structures of the protein and affect the combinations with pathogen ligands, especially the combination with lipopolysaccharide (LPS) of gram negative bacteria¹¹, thereby changing the progression of sepsis. Studies have demonstrated that toll like receptor 4 gene polymorphism rs4986790 (also known as Asp299Gly or +896 A > G) increased the risk of severe sepsis^{12,13}. In addition, Shalhub *et al.* indicated that apart from the polymorphism rs4986790, another one rs4986791 (also known as Thr399Ile) in this gene was also related to the severity of post-traumatic sepsis¹⁴. Whereas Jessen *et al.* showed that there were no obvious association between polymorphisms in the gene toll like receptor 4 and sepsis caused by gram negative bacteria in their study¹⁵. Feterowski *et al.* found that the morbidity and mortality rate of sepsis didn't correlate with toll like receptor 4 gene mutations in their study on postoperative sepsis induced by various microorganism infections¹⁶.

It is important in terms of clinical value and theoretical significance to get clearer perspective on the relationship of toll like receptor 4 gene polymorphisms rs4986790 and rs4986791 with sepsis risk. Consequently, we systematically analyzed this correlation via the method of meta-analysis in this study.

Materials and Methods

Literature search and selection criteria. We searched potentially relevant studies from MEDLINE, EMBASE, Google Scholar and CNKI up to January 2016. The key terms adopted in search strategy included “toll like receptor 4 or *TLR4*”, “polymorphism or mutation or variant” and “sepsis”. What's more, we also searched for additional articles through manually screening the reference lists of relevant articles or reviews to minimize the possibility of missing potent papers. Eligible studies were enrolled in this meta-analysis based on the following criteria: they estimated the correlation of the rs4986790 and/or rs4986791 polymorphisms in toll like receptor 4 gene with sepsis and had a case-control design; they adopted valid genotyping method; and they provided information about genotype distribution in case and control group for calculating pooled odds ratios (ORs) with the corresponding 95% confidence intervals (95% CIs). Meanwhile, publications were excluded if they were case-only studies, with irrelevant title or abstract, without sufficient genotype data or focusing on animals.

Data extraction. The following information were extracted by two investigators from included articles: the first author's name, publication year, original country, ethnicity, control source, genotyping method, numbers of cases and controls, genotype frequency and *P* values for Hardy-Weinberg equilibrium (HWE) in control group. All disagreements over extracted data were resolved through discussion between the two investigators to reach a consensus.

Statistical analysis. The ORs and 95% CIs were calculated to assess the association of sepsis susceptibility with toll like receptor 4 rs4986790 polymorphism under five genetic models: GG vs. AA, GG + GA vs. AA, GG

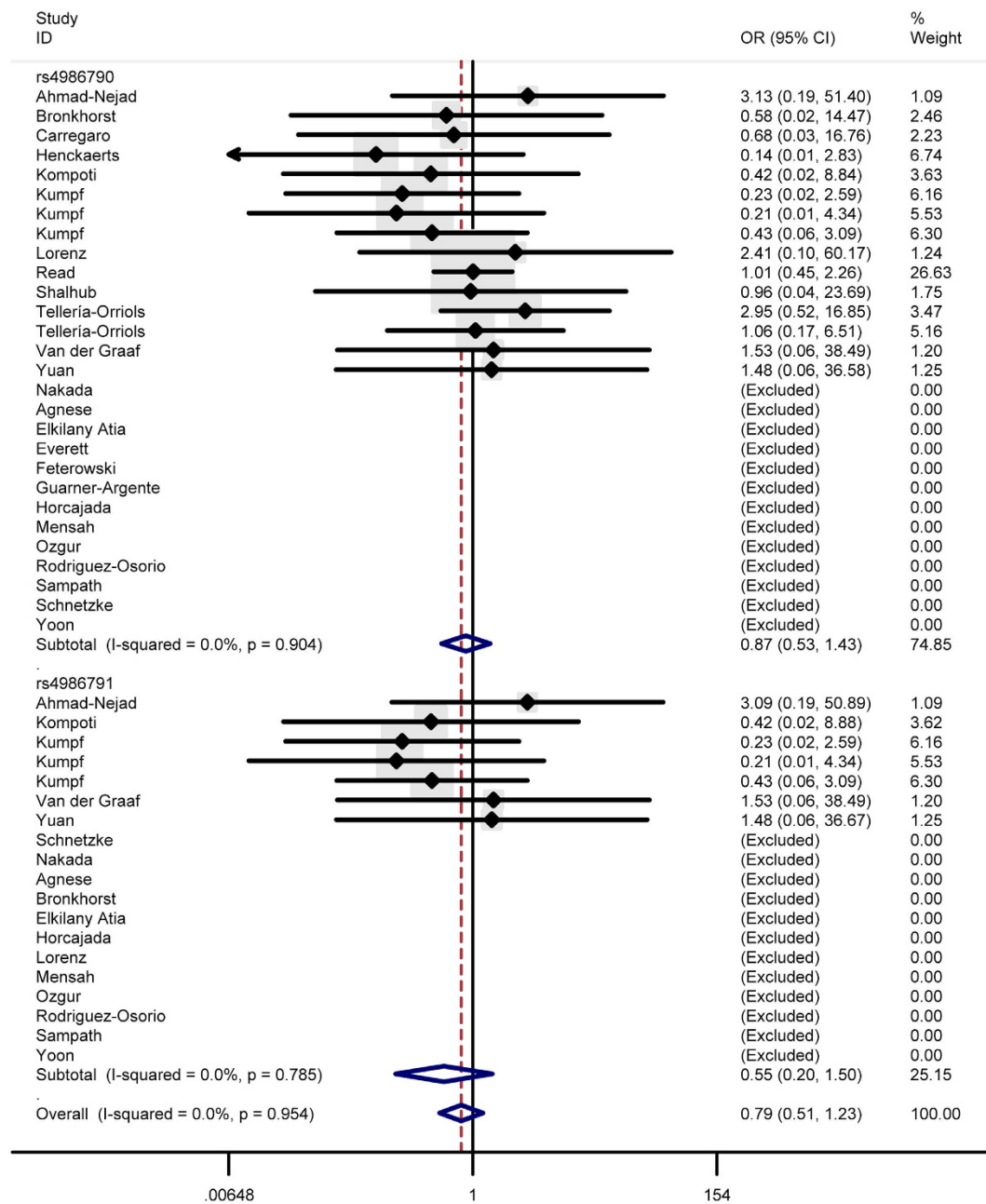


Figure 2. Forest plot of sepsis susceptibility associated with the polymorphisms rs4986790 and rs4986791 of toll like receptor 4 under the homozygous model.

vs. GA+AA, allele G vs. allele A, and GA vs. GG, as well as with the polymorphism rs4986791 under the five genetic models: TT vs. CC, TT+CT vs. CC, TT vs. CC+CT, allele T vs. allele C and CT vs. CC. In addition, subgroup analysis was implemented according to ethnicity. Between-study heterogeneity was evaluated through the χ^2 -based Q test. The fixed-effect model was adopted to calculate the pooled ORs when $P > 0.05$ in Q test which indicated the lack of heterogeneity; otherwise, the random-effect model was selected. HWE was checked in the control group via χ^2 test. Publication bias was examined with Begg's funnel plot and Egger's test. Sensitivity analysis was performed to reflect the effect of each individual dataset on pooled results through sequentially omitting each included study. Statistical analysis was conducted using STATA version 12.0 (STATA Corp). $P < 0.05$ was considered statistically significant for all tests.

Results

Characteristics of studies. According to the search strategy, 277 potentially relevant studies were initially identified from MEDLINE, EMBASE, Google Scholar and CNKI, and 163 of them were excluded due to irrelevant titles or abstracts. Additionally, consequent screening removed 86 more reports for not concerning on the association between the polymorphism rs4986790 or rs4986791 of toll like receptor 4 and sepsis (71) and insufficient

| SNP | First author-Year | Ethnicity | Control source | Genotyping method | Sample size | | Genotype and allele distribution | | | | | | | | | | HWE |
|-------------------------------------|-------------------------------------|-----------------|------------------------|------------------------|-------------|-----|----------------------------------|-----|-----|------|-----|---------|----|-----|------|--------|--------|
| | | | | | | | Case | | | | | Control | | | | | |
| | | | | | | | AA | AG | GG | A | G | AA | AG | GG | A | G | |
| rs4986790 | Ahmad-Nejad-2011 ¹⁷ | Caucasian | Hospital | PCR-RFLP | 38 | 112 | 31 | 6 | 1 | 68 | 8 | 99 | 12 | 1 | 210 | 14 | 0.3640 |
| | Nakada-2005 ²² | Asian | Population | PCR-RFLP | 86 | 214 | 86 | 0 | 0 | 172 | 0 | 214 | 0 | 0 | 428 | 0 | / |
| | Agnese-2002 ²² | Multi-ethnic | Population | PCR-RFLP | 33 | 39 | 21 | 12 | 0 | 54 | 12 | 34 | 5 | 0 | 73 | 5 | 0.6690 |
| | Bronkhorst-2013 ²⁷ | Caucasian | Hospital | PCR | 79 | 139 | 68 | 11 | 0 | 147 | 11 | 118 | 20 | 1 | 256 | 22 | 0.8800 |
| | Carregaro-2010 ¹⁸ | Multi-ethnic | Population | PCR-RFLP | 97 | 205 | 88 | 9 | 0 | 185 | 9 | 178 | 26 | 1 | 382 | 28 | 0.9620 |
| | Elkilany Atia-2015 ²⁶ | Caucasian | Hospital | PCR | 20 | 21 | 17 | 3 | 0 | 37 | 3 | 19 | 2 | 0 | 40 | 2 | 0.8190 |
| | Everett-2007 ³⁸ | Undefined | Hospital | PCR | 85 | 167 | 71 | 14 | 0 | 156 | 14 | 145 | 22 | 0 | 312 | 22 | 0.3620 |
| | Feterowski-2003 ¹⁶ | Caucasian | Hospital | PCR | 153 | 154 | 143 | 10 | 0 | 296 | 10 | 135 | 19 | 0 | 289 | 19 | 0.4150 |
| | Guarner-Argente-2010 ³⁵ | Undefined | Hospital | PCR-RFLP | 6 | 105 | 4 | 2 | 0 | 10 | 2 | 97 | 8 | 0 | 202 | 8 | 0.6850 |
| | Henckaerts-2009 ⁴⁰ | Caucasian | Population | PCR | 407 | 293 | 393 | 14 | 0 | 800 | 14 | 264 | 27 | 2 | 555 | 31 | 0.1690 |
| | Horcajada-2009 ²⁹ | Caucasian | Hospital | PCR | 57 | 114 | 48 | 9 | 0 | 105 | 9 | 100 | 14 | 0 | 214 | 14 | 0.4850 |
| | Kompoti-2015 ²⁵ | Caucasian | Hospital | PCR-RFLP | 108 | 245 | 102 | 6 | 0 | 210 | 6 | 213 | 30 | 2 | 456 | 34 | 0.4170 |
| | Kumpf-2010 ⁴¹ | Caucasian | Population | PCR-RFLP | 375 | 176 | 325 | 49 | 1 | 699 | 51 | 150 | 24 | 2 | 324 | 28 | 0.3620 |
| | Kumpf-2010 ⁴¹ | Caucasian | Population | PCR-RFLP | 159 | 176 | 147 | 12 | 0 | 306 | 12 | 150 | 24 | 2 | 324 | 28 | 0.3620 |
| | Kumpf-2010 ⁴¹ | Caucasian | Population | PCR-RFLP | 415 | 176 | 350 | 63 | 2 | 763 | 67 | 150 | 24 | 2 | 324 | 28 | 0.3620 |
| | Lorenz-2002 ³³ | Caucasian | Population | PCR | 91 | 73 | 80 | 10 | 1 | 170 | 12 | 65 | 8 | 0 | 138 | 8 | 0.6200 |
| | Mensah-2009 ²³ | Multi-ethnic | Hospital | PCR | 15 | 48 | 11 | 4 | 0 | 26 | 4 | 42 | 6 | 0 | 90 | 6 | 0.6440 |
| | Ozgur-2009 ³⁰ | Undefined | Hospital | PCR | 16 | 70 | 16 | 0 | 0 | 32 | 0 | 66 | 4 | 0 | 136 | 4 | 0.8060 |
| | Rodriguez-Osorio-2013 ²⁸ | Mexican-Mestizo | Population | PCR-RFLP | 44 | 126 | 43 | 1 | 0 | 87 | 1 | 122 | 4 | 0 | 248 | 4 | 0.8560 |
| | Read-2001 ³¹ | Caucasian | Population | TaqMan | 1047 | 879 | 924 | 110 | 13 | 1958 | 136 | 787 | 81 | 11 | 1655 | 103 | 0.8250 |
| | Sampath-2013 ¹⁹ | Multi-ethnic | Hospital | multiplexed SBE | 89 | 318 | 74 | 15 | 0 | 163 | 15 | 287 | 31 | 0 | 605 | 31 | 0.3610 |
| | Schnetzke-2015 ²¹ | Caucasian | Hospital | PCR | 74 | 81 | 61 | 13 | 0 | 135 | 13 | 76 | 5 | 0 | 157 | 5 | 0.7740 |
| | Shalhub-2009 ¹⁴ | Caucasian | Hospital | PCR | 147 | 451 | 139 | 8 | 0 | 286 | 8 | 400 | 50 | 1 | 850 | 52 | 0.6650 |
| | Telleria-Orriols-2014 ²⁰ | Caucasian | Hospital | PCR | 51 | 66 | 38 | 9 | 4 | 85 | 17 | 60 | 4 | 2 | 124 | 8 | 0.0001 |
| Telleria-Orriols-2014 ²⁰ | Caucasian | Hospital | PCR | 102 | 66 | 85 | 14 | 3 | 184 | 20 | 60 | 4 | 2 | 124 | 8 | 0.0001 | |
| Van der Graaf-2006 ³⁷ | Undefined | Population | PCR-RFLP | 43 | 166 | 32 | 11 | 0 | 75 | 11 | 148 | 17 | 1 | 313 | 19 | 0.5120 | |
| Yoon-2006 ²⁴ | Asian | Hospital | PCR-RFLP | 154 | 179 | 154 | 0 | 0 | 308 | 0 | 179 | 0 | 0 | 358 | 0 | / | |
| Yuan-2008 ³⁹ | Caucasian | Population | PCR-RFLP | 85 | 409 | 82 | 3 | 0 | 167 | 3 | 364 | 44 | 1 | 772 | 46 | 0.7850 | |
| Smirnova-2003 ³⁴ | Caucasian | Hospital | mutationseeker surveys | 197 | 127 | 186 | 11 | | / | / | 114 | 13 | | / | / | / | |
| Ahrens-2004 ³⁶ | Caucasian | Hospital | PCR-RFLP | 50 | 306 | 45 | 5 | | / | / | 277 | 29 | | / | / | / | |
| Barber-2004 ¹³ | Multi-ethnic | Hospital | PCR | 36 | 123 | 29 | 7 | | / | / | 112 | 11 | | / | / | / | |
| rs4986791 | | | | | | | CC | CT | TT | C | T | CC | CT | TT | C | T | |
| | Schnetzke-2015 ²¹ | Caucasian | Hospital | PCR | 74 | 81 | 60 | 14 | 0 | 134 | 14 | 76 | 5 | 0 | 157 | 5 | 0.7740 |
| | Nakada-2005 ²² | Asian | Population | PCR-RFLP | 86 | 214 | 86 | 0 | 0 | 172 | 0 | 214 | 0 | 0 | 428 | 0 | / |
| | Agnese-2002 ²² | Multi-ethnic | Population | PCR-RFLP | 33 | 39 | 21 | 12 | 0 | 54 | 12 | 34 | 5 | 0 | 73 | 5 | 0.6690 |
| | Ahmad-Nejad-2011 ¹⁷ | Caucasian | Hospital | PCR-RFLP | 38 | 112 | 31 | 6 | 1 | 68 | 8 | 98 | 13 | 1 | 209 | 15 | 0.4520 |
| | Bronkhorst-2013 ²⁷ | Caucasian | Hospital | PCR | 79 | 138 | 69 | 10 | 0 | 148 | 10 | 119 | 19 | 0 | 257 | 19 | 0.3850 |
| | Elkilany Atia-2015 ²⁶ | Caucasian | Hospital | PCR | 20 | 21 | 18 | 2 | 0 | 38 | 2 | 19 | 2 | 0 | 40 | 2 | 0.8190 |
| | Horcajada-2009 ²⁹ | Caucasian | Hospital | PCR | 57 | 114 | 48 | 9 | 0 | 105 | 9 | 101 | 13 | 0 | 215 | 13 | 0.5190 |
| | Kompoti-2015 ²⁵ | Caucasian | Population | PCR-RFLP | 108 | 245 | 102 | 6 | 0 | 210 | 6 | 214 | 29 | 2 | 457 | 33 | 0.3660 |
| | Kumpf-2010 ⁴¹ | Caucasian | Population | PCR | 375 | 176 | 325 | 49 | 1 | 699 | 51 | 150 | 24 | 2 | 324 | 28 | 0.3620 |
| | Kumpf-2010 ⁴¹ | Caucasian | Population | PCR | 159 | 176 | 147 | 12 | 0 | 306 | 12 | 150 | 24 | 2 | 324 | 28 | 0.3620 |
| | Kumpf-2010 ⁴¹ | Caucasian | Population | PCR | 415 | 176 | 350 | 63 | 2 | 763 | 67 | 150 | 24 | 2 | 324 | 28 | 0.3620 |
| | Lorenz-2002 ³³ | Caucasian | Population | PCR | 91 | 73 | 85 | 6 | 0 | 176 | 6 | 65 | 8 | 0 | 138 | 8 | 0.6200 |
| | Mensah-2009 ²³ | Multi-ethnic | Hospital | PCR | 15 | 48 | 11 | 4 | 0 | 26 | 4 | 42 | 6 | 0 | 90 | 6 | 0.6440 |
| | Ozgur-2009 ³⁰ | Undefined | Hospital | PCR | 16 | 70 | 16 | 0 | 0 | 32 | 0 | 63 | 7 | 0 | 133 | 7 | 0.6600 |
| | Rodriguez-Osorio-2013 ²⁸ | Mexican-Mestizo | Population | PCR | 44 | 126 | 43 | 1 | 0 | 87 | 1 | 122 | 4 | 0 | 248 | 4 | 0.8560 |
| | Sampath-2013 ¹⁹ | Multi-ethnic | Hospital | multiplexed SBE | 89 | 318 | 77 | 12 | 0 | 166 | 12 | 298 | 20 | 0 | 616 | 20 | 0.5630 |
| | Van der Graaf-2006 ³⁷ | Undefined | Population | PCR | 43 | 166 | 32 | 11 | 0 | 75 | 11 | 148 | 17 | 1 | 313 | 19 | 0.5120 |
| | Yoon-2006 ²⁴ | Asian | Hospital | PCR-RFLP | 154 | 179 | 154 | 0 | 0 | 308 | 0 | 179 | 0 | 0 | 358 | 0 | / |
| | Yuan-2008 ³⁹ | Caucasian | Population | PCR | 85 | 409 | 82 | 3 | 0 | 167 | 3 | 365 | 43 | 1 | 773 | 45 | 0.8210 |
| | Smirnova-2003 ³⁴ | Caucasian | Hospital | mutationseeker surveys | 197 | 127 | 186 | 11 | | / | / | 114 | 13 | | / | / | / |

Table 1. Principal characteristics of the studies included in this meta-analysis. Notes: HB, hospital-based; PCR, polymerase chain reaction; PCR-RFLP, PCR-restriction fragment length polymorphism; SBE, single-base extension; HWE, Hardy-Weinberg equilibrium.

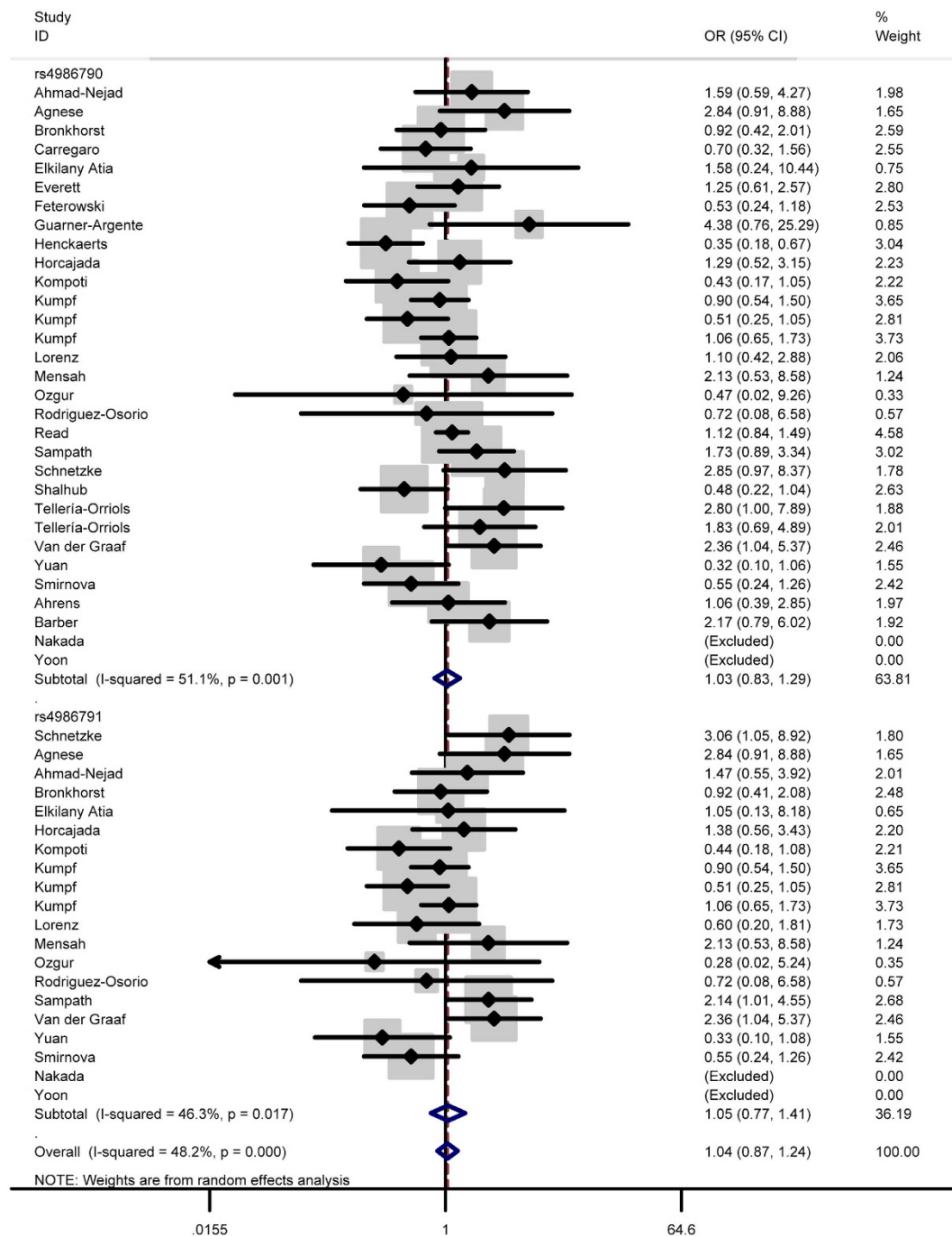


Figure 3. Forest plot of sepsis susceptibility associated with the polymorphisms rs4986790 and rs4986791 of toll like receptor 4 under the dominant model.

data (15). Therefore, as showed in Fig. 1, this meta-analysis finally included 28 eligible articles, containing 51 independent case-control studies (31 for the polymorphism rs4986790 and 20 for rs4986791)^{13,14,16-41}. The characteristics of included studies are summarized in Table 1. Apart from studies not providing sufficient information for χ^2 test, genotype distributions in controls were consistent with HWE in all studies for the polymorphism rs4986791 ($P > 0.05$, with a range of 0.3620 to 0.8560); while such distributions deviated from HWE expectation in two studies from the report by Telleria-Orrriols *et al.*²⁰ for the polymorphism rs4986790 ($P = 0.0001$), and perfectly fit in with the expectation in the other studies with P values ranging from 0.1690 to 0.9620.

Meta-analysis results. In total analysis, the polymorphism rs4986790 of toll like receptor 4 gene showed no significant association with sepsis susceptibility under genetic models GG vs. AA (OR = 0.87, 95% CI = 0.53–1.43) (Fig. 2), GG + AG vs. AA (OR = 1.03, 95% CI = 0.83–1.29) (Fig. 3), GG vs. AA + AG (OR = 0.85, 95% CI = 0.52–1.41) (Fig. 4), allele G vs. allele A (OR = 1.03, 95% CI = 0.81–1.31) (Fig. 5) and AG vs. AA (OR = 1.05,

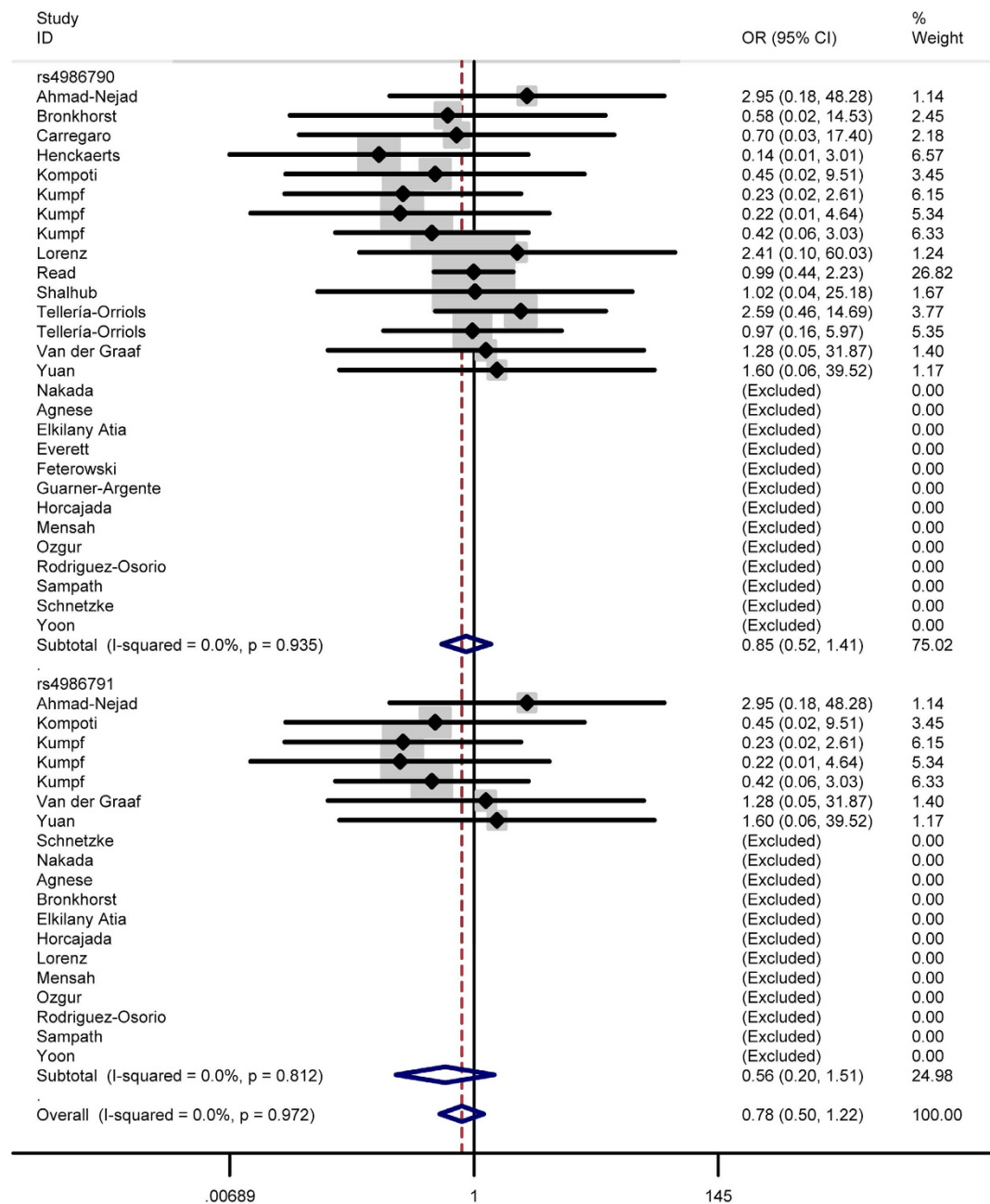


Figure 4. Forest plot of sepsis susceptibility associated with the polymorphisms rs4986790 and rs4986791 of toll like receptor 4 under the recessive model.

95% CI=0.83–1.33) (Fig. 6). Whereas, after stratification analysis by ethnicity, an increasing effect thereof on the sepsis risk was uncovered in other-ethnicity subgroup under GG + AG vs. AA (OR = 1.59 95% CI = 1.15–2.20) (Fig. 7), allele G vs. allele A (OR = 1.54 95% CI = 1.06–2.23) and AG vs. AA (OR = 1.56 95% CI = 1.09–2.24) comparisons.

As for the polymorphism rs4986791, no significant influence thereof was detected in total analysis under the contrasts of TT vs. CC (Fig. 2), TT + CT vs. CC (Fig. 3), TT vs. CC + CT (Fig. 4), allele T vs. allele C (Fig. 5) and CT vs. CC (Fig. 6) (OR = 0.55, 95% CI = 0.20–1.50; OR = 1.05, 95% CI = 0.77–1.41; OR = 0.56, 95% CI = 0.20–1.51; OR = 1.08, 95% CI = 0.79–1.48; OR = 1.12, 95% CI = 0.82–1.52) either. However, this polymorphism significantly elevated the sepsis susceptibility in other-ethnicity subgroup under TT + CT vs. CC (Fig. 8), allele T vs. allele C and CT vs. CC models (OR = 2.10, 95% CI = 1.33–3.30; OR = 2.08, 95% CI = 1.34–3.22; OR = 2.13, 95% CI = 1.35–3.36) after stratified analysis by ethnicity.

In addition, we also examined possible effects of the combination between the polymorphisms rs4986790 and rs4986791 on the sepsis onset. As a result, the combination of these two polymorphism still lacked significant impact on the disease risk under homozygous model (OR = 0.79, 95% CI = 0.51–1.23) (Fig. 2), dominant model

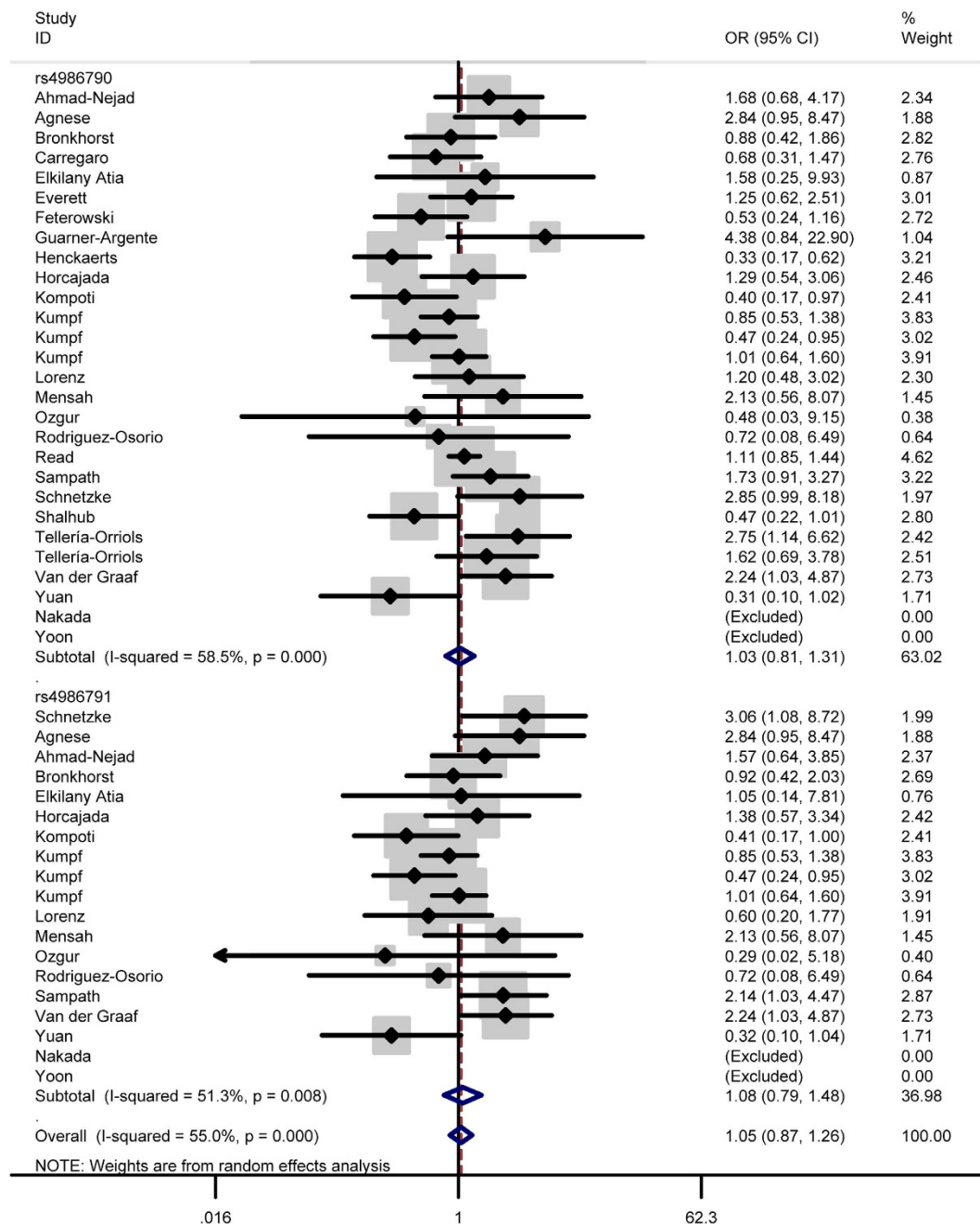


Figure 5. Forest plot of sepsis susceptibility associated with the polymorphisms rs4986790 and rs4986791 of toll like receptor 4 under the allele model.

(OR = 1.04, 95% CI = 0.87–1.24) (Fig. 3), recessive model (OR = 0.78, 95% CI = 0.50–1.22) (Fig. 4), allele model (OR = 1.05, 95% CI = 0.87–1.26) (Fig. 5) and heterozygous model (OR = 1.07, 95% CI = 0.89–1.29) (Fig. 6).

Test of heterogeneity. Significant heterogeneity was observed for the polymorphism rs4986790 under GG + GA vs. AA model, allele G vs. allele A model and GA vs. GG model ($P = 0.001$; $P < 0.001$; $P = 0.002$) as well as for the polymorphism rs4986791 under TT + CT vs. CC, allele T vs. allele C and CT vs. CC contrasts ($P = 0.017$; $P = 0.008$; $P = 0.034$), thus the random-effects model was applied to calculate pooled ORs in these cases. As for the other comparisons, the fixed-effects model was utilized considering the absence of significant heterogeneity (for rs4986790: $P = 0.904$ under GG vs. AA; $P = 0.935$ under GG + AG vs. AA; for rs4986791: $P = 0.785$ under TT vs. CC; $P = 0.812$ under TT + CT vs. CC).

As for the OR values for the effects of the combination between the two polymorphism, they were summarized applying the random-effects model under the dominant, allele and heterozygous models ($P < 0.001$; $P < 0.001$; $P = 0.001$) in view of the existence of significant heterogeneity, and pooled with the fixed-effects model under the homozygous and recessive models ($P = 0.954$; $P = 0.972$) because of the lack of significant heterogeneity.

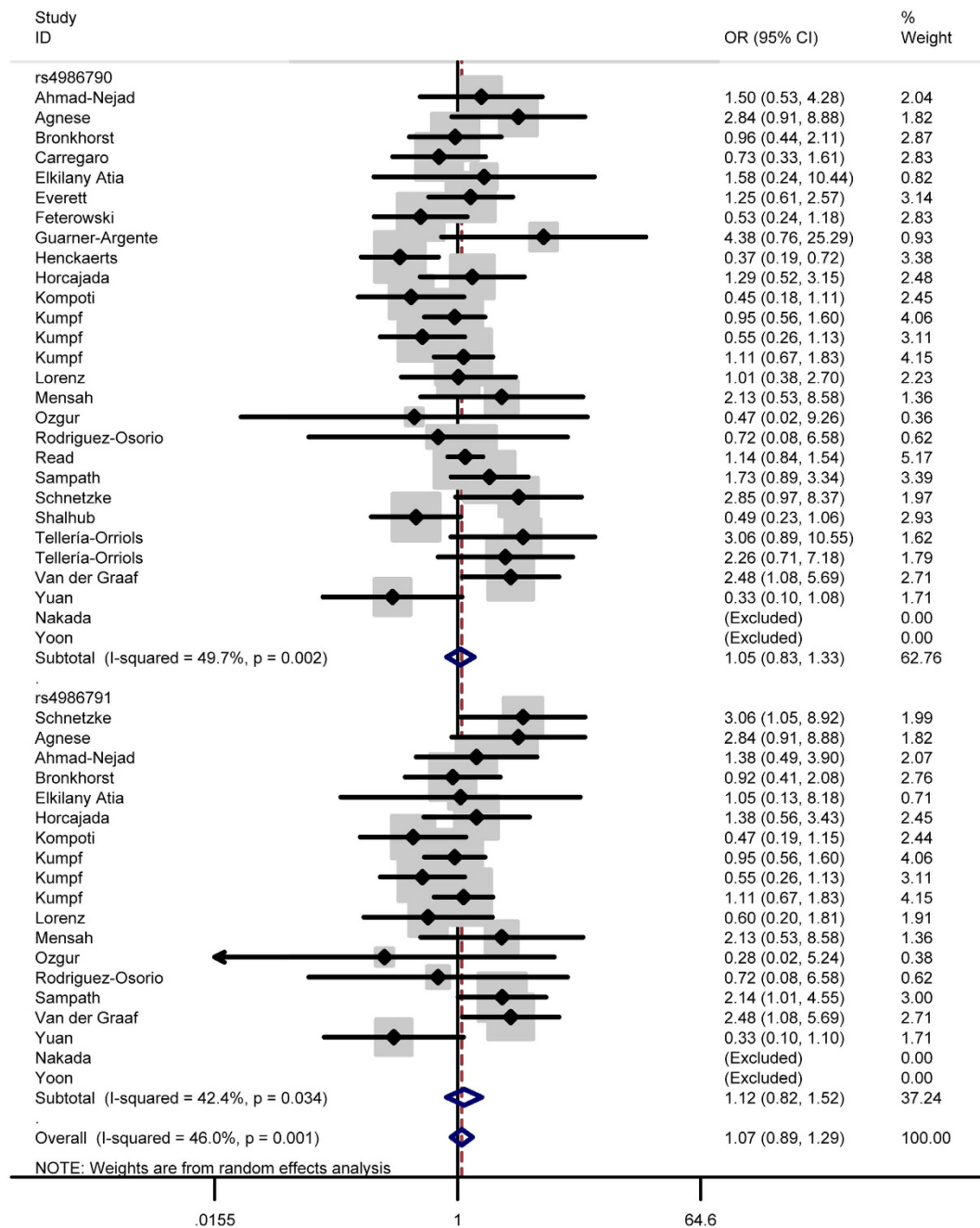


Figure 6. Forest plot of sepsis susceptibility associated with the polymorphisms rs4986790 and rs4986791 of toll like receptor 4 under the heterozygous model.

Sensitivity analysis and publication bias. We implemented sensitivity analysis to evaluate the stability of pooled results. We excluded each study one by one and observed that no pooled ORs was substantially affected (Fig. 9, with a range of lower CI from 0.42 to 0.56 and of upper CI from 1.30–1.57), indicating high stability of the meta-analysis results. Additionally, Begg's funnel plot and Egger's test were employed to evaluate publication bias between included studies. Consequently, all funnel plots seemed symmetrical (Fig. 10), implying no significant publication bias. What's more, Egger's test showed statistical evidence for these results ($P = 0.674$).

Discussion

Sepsis is caused by infections and characterized by acute onset, rapid progression and high fatality rate, being a common postoperative complication of severe traumas and burns. SIRS may develop to septic shock and multiple organ dysfunction syndrome (MODS), and studies on SIRS, sepsis, severe sepsis, septic shock and MODS indicate significant differences in different individuals. Specifically, inflammatory responses may develop easily in some people, which is difficult to be controlled and then become MODS, while different results may occur in other people with the same SIRS. Moreover, people with similar SIRS or sepsis can show different prognosis

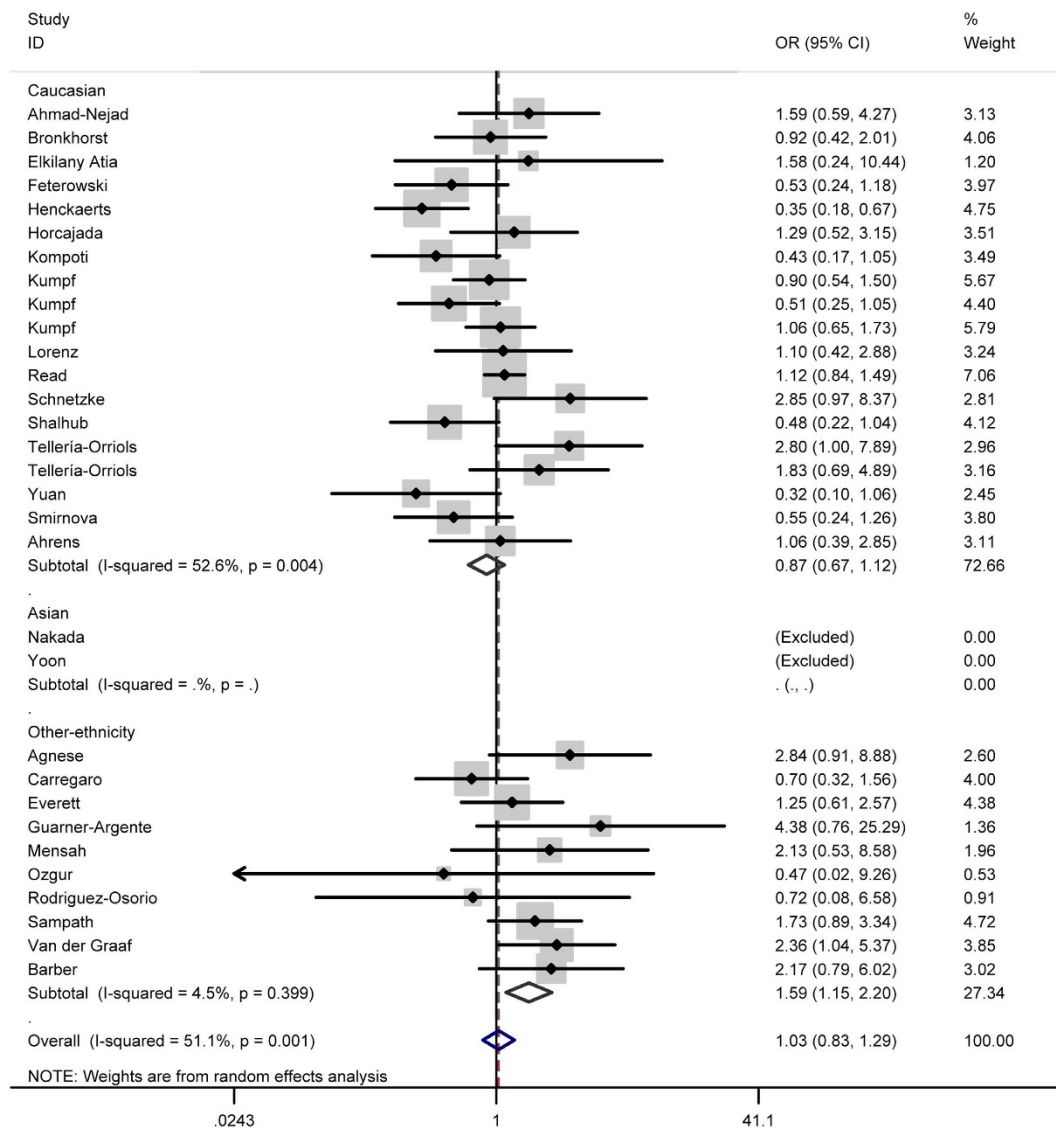


Figure 7. Forest plot of sepsis susceptibility associated with the polymorphism rs4986790 of toll like receptor 4 under GG + AG vs. AA model after stratification analysis by ethnicity.

when they adopt same therapeutic measures. All these evidence indicate that sepsis is affected by genetic factors as well. Therefore, it's important to explore the roles of sepsis-related genes, which provides theoretical basis to understand sepsis pathogenesis.

TLRs, a group of main PRRs, can recognize pathogenic microorganisms through PAMPs, activate intracellular signal transduction pathways and induce the generation of innate immunity. Therefore, TLRs are involved in the pathogenic courses of numerous diseases and closely correlated with communicable diseases, tumors, cardiovascular diseases, autoimmune diseases and allergy. According to their positions in chromosome, genetic structures and amino acid sequences, 11 members of TLRs family are divided to 5 subfamilies. Among members in this family, toll like receptor 4, first found and reported by Medzhitov *et al.* in 1997⁴², is a transmembrane protein producing homologization to drosophila toll protein which is composed of extracellular region, transmembrane domain and intracellular region of leucine-rich-repeat (LRR), distributing mainly on the surface of cells (such as monocyte). This protein can recognize LPS of gram negative bacteria, mannan of fungus and soluble components of mycobacterium tuberculosis, and is the endogenous ligand of some heat shock proteins and fibronectins. Therefore, polymorphisms in coding gene for the protein may greatly affect systemic inflammation and immunoreaction.

For example, Chen *et al.* found that the polymorphism T-2242C in toll like receptor 4 gene might be related to higher sepsis morbidity rate and organ dysfunction⁴³. Besides, Mansur *et al.* put forward in their study that the polymorphism rs11536889 in this gene was associated with renal, coagulation and hepatic organ failure in sepsis patients⁴⁴. In addition, a study by Nachtigall *et al.* reported that the polymorphism rs4986790 might shorten the time-to-onset of severe sepsis or septic shock in patients permitted to intensive care units⁴⁵. In the study by Child *et al.*, the polymorphism rs4986790 was demonstrated to be involved in the severity of SIRS⁴⁶. Additionally, this

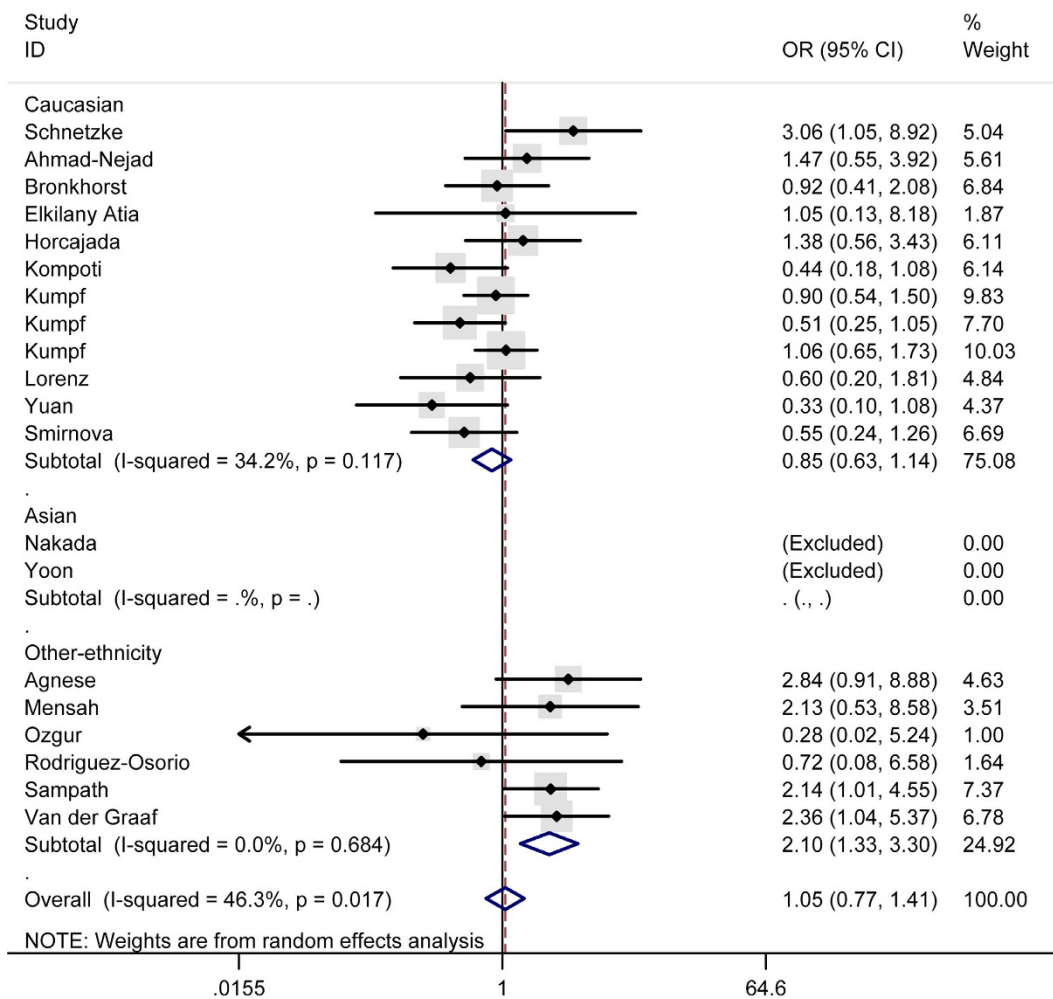


Figure 8. Forest plot of sepsis susceptibility associated with the polymorphism rs4986791 of toll like receptor 4 under the TT + CT vs. CC model after stratification analysis by ethnicity.

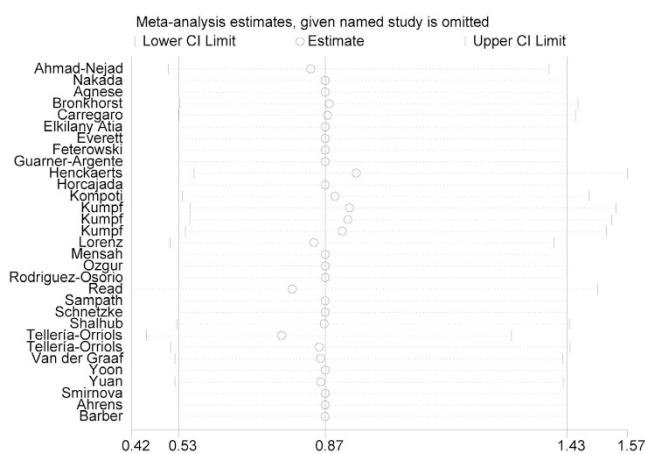


Figure 9. Sensitivity analysis for the polymorphism rs4986790.

polymorphism was found to be correlated with septic shock induced by gram-negative bacteria³³. However, there was research suggesting that such mutation might not influence the incidence of postoperative sepsis¹⁶. Such an inconclusive status was also true for another common polymorphism in the toll like receptor 4 gene, rs4976891.

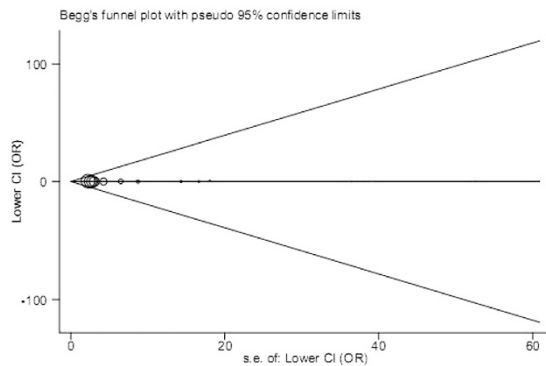


Figure 10. Begg's funnel plot of publication bias examination on the studies for the polymorphism rs4986790 (model: GG vs. AA). Each point represents a separate study for the indicated association. Log(OR), natural logarithm of OR. Horizontal line, mean effect size.

And these discrepancies might be attributed to but not limited to such aspects as various genetic backgrounds, different selection criteria for participants and uneven sample sizes.

Considering those discrepancies, we performed this meta-analysis to comprehensively analyze the associations of the rs4986790 and rs4986791 polymorphisms in toll like receptor 4 gene with sepsis susceptibility involving 51 relevant case-control studies. The results of this study showed that our studied polymorphisms, overall, had no significant association with the susceptibility of sepsis, but that both polymorphisms were related to increased risk of developing sepsis in other-ethnicity subgroups under corresponding genetic models after stratified analysis by ethnicity. Based on 6,537 sepsis patients and 8,832 controls, our findings had certain reliability, and sensitivity analysis also verified their stability. Compared to the previous individual case-control studies, our meta-analysis had a larger sample size and a more detailed stratification analysis by ethnicity, so our results were more reliable and comprehensive. In a recent meta-analysis about rs4986790 polymorphism and sepsis susceptibility published by Zhu *et al.* in 2012⁴⁷, 17 eligible articles were included, while our meta-analysis collected 28 related ones. Therefore, the present meta-analysis was an up-dated one with more eligible studies, suggesting our results could represent latest research results. However, there were some limitations in this meta-analysis, which might affect the accuracy of the result. For example, source limitation in literature searching might miss some relevant articles in other sources, thus leading to possible publication bias not detected even with Begg's funnel plot or Egger's test. In addition, possible effects from other relevant factors and gene-gene or gene-environment interactions were not incorporated in this study due to the insufficient data. Therefore, the results of the present meta-analysis should be applied with cautious.

In conclusion, the results of this meta-analysis showed that toll like receptor 4 gene rs4986790 and rs4986791 polymorphisms might not have independent association with sepsis susceptibility. In view of the advantages and disadvantages in this meta-analysis, the results should be further verified by better-designed studies based on larger sample size and more consideration of gene-gene and gene-environment interactions.

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Author Contributions

R.L. and L.L. conceived and designed the experiments; Y.M. and H.W. conceived and performed the experiments; Y.T. and X.W. prepared figures. M.D. and H.Y. wrote the main manuscript text. All authors reviewed the manuscript.

Additional Information

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