

Original Article

Relationship between toll-like receptor 4 gene polymorphism and the susceptibility to pulmonary tuberculosis

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Abstract: Objective: To evaluate the susceptibility of pulmonary tuberculosis based on the single nucleotide polymorphism (SNP) of Toll like receptor 4 (TLR4) gene. Methods: We searched PubMed, Web of science, EMBASE, and Chinese National Knowledge Infrastructure (CNKI) databases using mesh words: “tuberculosis”, “pulmonary”, “TLR4”, “SNP”, “Toll like receptor 4”, “nucleotide polymorphism” for studies on the relations between TLR4 SNP polymorphism and the risk of pulmonary tuberculosis that were published before September 1st, 2021. Papers were selected according to the inclusion and exclusion criteria established in advance. The allele and genotype data of the four most widely studied SNP loci (rs4986791, rs4986790, rs11536889, rs10759932) in TLR4 gene were extracted and analyzed by Review Manager 5.3 software. Results: 20 studies including a total of 24727 patients were included in the final meta-analysis. Results of the meta-analysis showed that the C allele of rs10759932 increased the risk of pulmonary tuberculosis (odds ratio - OR: 1.144; 95% confidence interval (CI) 1.043-1.254, P = 0.004). Compared with TT genotype, CC+CT genotype of rs10759932 and CT alone genotype significantly increased the risk of pulmonary tuberculosis (OR = 1.218, 95% CI 1.084-1.369, P = 0.001; OR = 1.227, 95% CI 1.085-1.387, P = 0.001). However, rs4986790, rs4986791 and rs11536889 had no significant correlation with the susceptibility of pulmonary tuberculosis (P > 0.05). Conclusion: G allele GG+GA genotype, and the GA genotype of rs4986790; C allele, CC+CT genotype, and the CC genotype of rs10759932 increased the risk of pulmonary tuberculosis, and may potentially be used as a marker for pulmonary tuberculosis diagnosis and monitoring.

Keywords: Pulmonary tuberculosis, single nucleotide polymorphism, Toll like receptor 4, meta-analysis

Introduction

Tuberculosis is a common infectious disease, and it has high rate of patient mortality and morbidity. According to the statistics of the World Health Organization global tuberculosis report in 2018, there are about 11 million new cases of tuberculosis every year in the world. Approximately one third of the global population is in the latent infection state of tuberculosis, and 5-10% of them may develop active pulmonary tuberculosis [1]. Different individuals have different susceptibility to tuberculosis, which may be related to the differences of environment, diet, drug use and genetic back-

ground. Among those factors, the relationship between genetic polymorphism of Toll like receptor (TLR) family genes and the susceptibility to pulmonary tuberculosis have been widely studied in the recent years [2]. As one of the main components of host pattern recognition receptor, TLRs play key role in innate immune response of various infectious agents by recognizing pathogenic molecular patterns such as lipopolysaccharide, phosphoteichoic acid and surface lipoproteins, and activating nuclear factor kappa B signaling pathway [3]. As one of the most studied TLRs, TLR4 can recognize lipopolysaccharide and initiate MyD88 dependent or independent pathways. The abnormali-

ty of TLR4 can significantly affect the host's susceptibility to mycobacterium tuberculosis [4], and TLR4 knockout models have also proven the essential role of TLR4 in host's resistance to mycobacterium tuberculosis infection [5, 6].

In the past decade, scholars studied the correlation of TLR4 gene polymorphism and the patient susceptibility for pulmonary tuberculosis in different groups of populations, and found that single nucleotide polymorphism (SNP) site of TLR4 might be associated with the susceptibility to pulmonary tuberculosis. In the meanwhile, outcomes of those studies are not all consistent. Although various authors have carried out meta-analysis on the relations of SNP site of TLR4 and the susceptibility to pulmonary tuberculosis [7-9], with the emerging new data that have been published in the recent years, it is necessary to include those data to reach more reliable conclusion. Here we carried out a meta-analysis to systematically analyze the association of genetic variations of TLR4 gene and patient susceptibility to pulmonary tuberculosis.

Materials and methods

Data collection

Inclusion criteria: 1) study type: prospective and retrospective case control studies; 2) patient allocation: patients with confirmed diagnosis of pulmonary tuberculosis were located to the experimental group, while healthy population with no genetic relationship with the experimental group were located to the control group; 3) risk factor: SNP of TLR-4 gene; 4) outcome parameter: incidence of pulmonary tuberculosis. Exclusion criteria: 1) pedigree based studies; 2) incomplete or unclear data; 3) the gene distribution of the control group did not conform to the Hardy-Weinberg Equilibrium (HWE); 4) allele or genotype frequency was not provided in the literature; 5) Newcastle Ottawa Scale (NOS) score less than 6.

Literature search

Two authors independently searched PubMed, Web of science, EMBASE, and Chinese National Knowledge Infrastructure (CNKI) databases using mesh words: "tuberculosis", "pulmonary", "TLR4", "SNP", "Toll like receptor 4",

and "nucleotide polymorphism" for studies on the relations between TLR4 SNP polymorphism and the risk of pulmonary tuberculosis that were published before September 1st, 2021. When there was inconsistency between the two reviewers, the third author made the last decision on study inclusion. Final inclusion of studies was decided according to the inclusion and exclusion criteria established in advance.

Evaluation of study quality and data extraction

Two researchers independently evaluated the quality of the included studies using Newcastle Ottawa Scale (NOS) [10]. When the disagreement between the two researchers could not be solved by discussion, the third author made the last decision. Author's name, year of publication, population, diagnosis, human immunodeficiency virus (HIV) infection, sample size, age, and SNP locus type were collected and recorded for each eligible literature.

Statistical analysis

The data on allele and genotype frequency in the two groups were extracted and were analyzed using Review Manager 5.3 software. The statistical difference was determined by Z test, the test level was $\alpha = 0.05$, and the relationship between SNP and TB susceptibility was evaluated by odds ratio and 95% confidence interval (CI). Egger test was used to analyze the potential publication bias ($P < 0.05$).

Results

Study inclusion

Initial screening of Web of science, PubMed, CNKI and EMBASE resulted in 937 papers on the relationship between SNP and TB. 906 papers were included in the title screening after the removal of duplications. 854 papers were further removed after title and abstract screening, and the full texts of the rest 52 papers were analyzed for further inclusion. According to the preset criteria, 20 papers including 24727 patients were analyzed by RevMan5.3 software (**Figure 1; Table 1**) [11-30].

Quality assessment of the included studies

Among the included 20 studies, SNP was measured by different typing methods, such as

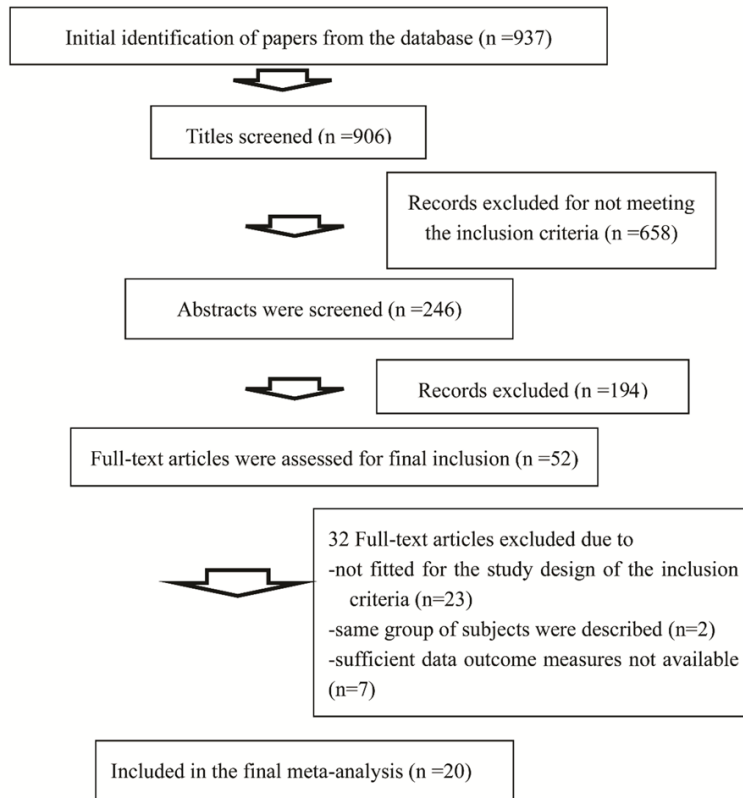


Figure 1. Schematic chart for the process of patient inclusion. Among the 937 papers initially included in title screening, 20 papers with 24727 patients were included in the final analysis.

ARMS-PCR, PCR-RFLP, TaqMan probe, MALDI-TOFMS and sequencing. The average NOS score of the included studies was more than 7 (Table 1).

Results of meta-analysis

rs4986790 locus: 16 papers including 17122 patients studied the rs4986790 locus of TLR4 gene. In the allele (A allele vs G allele) and dominant model (AA vs GG+GA), heterogeneity was significant ($I^2 > 50\%$) between the groups, therefore, random effect analysis was carried out. In recessive (GG vs GA+AA), homozygous (GG vs AA) and heterozygote models (GA vs AA), heterogeneity was not significant ($I^2 < 50\%$), and fixed effect analysis was performed on the data. Results of the analysis in allele model (G vs A) showed significant differences [OR = 1.15, 95% CI (1.03, 1.29), $P = 0.01$] between the case and control groups (Figure 2A). Significant differences were also found concerning dominant model (GG+GA vs AA, Figure 2B) [OR = 1.36, 95% CI (1.04, 1.80),

$P = 0.03$] and heterozygote model (GA vs AA, Figure 2C) [OR = 1.27, 95% CI (1.02, 1.59), $P = 0.03$]. Rs4986790 polymorphism showed no significant difference between the case and the control groups in the recessive model (GG vs GA+AA, Figure 2D) and homozygous model (GG vs AA, Figure 2E) ($P > 0.05$).

rs4986791 locus: 11 papers including 13207 patients studied the rs4986791 locus of TLR4 gene. Heterogeneity was significant ($I^2 > 50\%$) among the included studies in the allele model (C allele vs T allele, Figure 3A), dominant model (CC vs TT+TC, Figure 3B), and heterozygote model (TC vs CC, Figure 3C). Therefore, random effect models analysis was applied. In the recessive model (TC+CC vs TT, Figure 3D) and homozygous model (CC vs TT, Figure 3E), heterogeneity was not significant ($I^2 < 50\%$), and fixed

effect model was used for meta-analysis. Results of the meta-analysis showed that rs4986791 polymorphism was not significant between pulmonary tuberculosis patient group and the control group ($P > 0.05$).

rs10759932 locus: 4 papers including 10304 patients studied rs10759932 locus of TLR4 gene. There was no significant heterogeneity ($I^2 < 50\%$) concerning allele model (C vs T, Figure 4A), recessive model (CC vs CT+TT, Figure 4B) and homozygous model (CC vs TT, Figure 4C), and fixed effect model was used for meta-analysis for those genetic models. There was significant heterogeneity ($I^2 > 50\%$) concerning dominant model (TT vs CC+CT, Figure 4D) and heterozygote model (CT vs TT, Figure 4E), therefore random effect model was used for meta-analysis. Result of the meta-analysis in allele model (C vs T, Figure 4A) showed significant differences [OR = 1.15, 95% CI (1.06, 1.26), $P = 0.002$] concerning C and T allele of TLR-4 SNP rs10759932 in pulmonary tuberculosis patient group and control group. Similar

Table 1. Demographic characteristics of patients in the included studies, and the quality assessment of those studies

Studies	Country, region and ethnicity	Patient number		Age		Method of genotyping	HIV tests	NOS score
		Cases	Controls	Cases	Controls			
Fitness 2004 [11]	Malawi	162	427	NA	NA	PCR-RFLP	Positive	7
Jafari 2016 [12]	Iran	96	122	51 ± 31	48 ± 27	ARMS-PCR	Negative	8
Jahantigh 2013 [13]	Southeastern Iran	124	149	51.1 ± 20.0	48.4 ± 14.7	PCR-RFLP	Negative	7
Li 2016 [14]	Chinese Han	238	310	52.0 ± 11.9	58.7 ± 11.5	TaqMan	Negative	7
Li 2019 [15]	Chinese Han	1601	1727	52.1 ± 17.7	55.0 ± 17.7	TaqMan	Negative	8
Ma 2007 [16]	USA (African origin)	339	194	46.5 ± 12.8	45.5 ± 10.6	DNA Sequencing	Negative	7
Ma 2007 [16]	USA (Caucasian origin)	180	110	51.7 ± 13.6	50.0 ± 10.0	DNA Sequencing	Negative	7
Ma 2007 [16]	USA (Hispanic origin)	375	114	42.3 ± 17.2	43.4 ± 11.0	DNA Sequencing	Negative	7
Najmi 2010 [17]	Indian	135	250	27.2 ± 11.4	NA	PCR-RFLP	Negative	7
Newport 2004 [18]	Gambian	307	298	36 (19, 58)	32 (18, 50)	ARMS-PCR	Negative	8
Olesen 2007 [19]	Guinea-Bissau	315	337	37.246	38.116	TaqMan	Positive	7
Rosas-Taraco 2007 [20]	Mexico	104	114	39.5 ± 17.9	26.3 ± 8.5	PCR-RFLP	Negative	7
Salie 2015 [21]	South Africa	421	287	35.0 ± 12.8	32.6 ± 11.1	ARMS-PCR	Negative	8
Sanchez 2012 [21]	Colombia	466	300	39 (26, 51)	42 (2, 54)	MALDITOFMS	Negative	8
Selvaraj 2010 [23]	Southern India	206	212	34.9 ± 11.4	32.3 ± 9.8	PCR-RFLP	NA	7
Singh 2018 [24]	India	309	96	34.1 ± 13.5	34.2 ± 13.4	PCR-RFLP	Negative	8
Torres-García 2013 [25]	Mexico	90	90	46.9 ± 17.8	42.9 ± 15.9	TaqMan	NA	8
Varzari 2019 [26]	Moldova	272	251	40.7 ± 12.7	47.6 ± 14.1	MALDI-TOF	Negative	8
Wang 2016 [27]	Chinese Han	1601	1526	52.1 ± 17.7	52.4 ± 17.0	TaqMan	Negative	7
Wang 2017 [28]	Chinese Han	310	622	58.3 ± 14.9	57.6 ± 15.3	PCR-RFLP	Negative	7
Wu 2015 [29]	Chinese Han	109	422	45.0 ± 12.6	45.0 ± 18.7	PCR-RFLP	Negative	8
Zaki 2012 [30]	Sudan	207	395	30.0 ± 11.6	28.5 ± 6.1	PCR-RFLP	Negative	8

Age of patients was recorded as mean ± standard deviation. PTBL: pulmonary tuberculosis; HWEP: Hardy-Weinberg equilibrium *P* value; PCR-RFLP: polymerase chain reaction restriction fragment length polymorphism; ARMS-PCR: amplification refractory mutation system polymerase chain reaction; MALDITOFMS: matrix-assisted laser desorption/ionization time of flight mass spectrometry; MALDI-TOF: matrix-assisted laser desorption/ionization time-of-flight.

significance was observed in the recessive model (CT+TT vs CC, **Figure 4B**) [OR = 0.50, 95% CI (0.41, 0.62), *P* < 0.001] and homozygous model (CC vs TT, **Figure 4C**) model [OR = 1.27, 95% CI (1.02, 1.59), *P* = 0.03]. There was no significant difference in the dominant model (TT vs CC+CT, **Figure 4D**) and heterozygote model (CT vs TT, **Figure 4E**) (*P* > 0.05).

rs11536889 locus: 5 papers including 12102 patients studied the *rs11536889* locus of TLR4 gene. Heterogeneity was significant (I^2 > 50%) between the studies in the allele model (C allele vs G allele, **Figure 5A**), dominant model (GG vs CC+CG, **Figure 5B**), and heterozygote model (GG vs CG, **Figure 5C**), therefore random effect model was used for meta-analysis. In the recessive model (CG+GG vs CC, **Figure 5D**) and homozygous model (CC vs GG, **Figure 5E**), heterogeneity was not significant (I^2 < 50%), and fixed effect model was used for meta-analysis. Results of the meta-analysis showed no significant difference (*P* > 0.05) concerning *rs11536889* polymorphism between the pulmonary tuberculosis patient group and control group.

Discussion

There are numerous studies in the current literature on the connections between TLR4 gene polymorphism and susceptibility to pulmonary tuberculosis in different group of populations. However, each study included small number of patients, and the conclusions were inconsistent. Therefore, in order to get a more comprehensive and objective conclusion, here we carried out the current meta-analysis to further confirm the relationship between the susceptibility of pulmonary tuberculosis and polymorphism of the four most widely studied SNP loci in TLR4 gene.

Results of the current meta-analysis revealed that SNP locus of TLR4 gene G allele of *rs4986790* increased the risk of pulmonary tuberculosis (OR = 1.15, *P* = 0.01), proving G allele of *rs4986790* to be an independent risk factor for pulmonary tuberculosis, and may potentially be used as a marker for pulmonary tuberculosis diagnosis and monitoring. Our meta-analysis on dominant (GA vs AA+GG) and heterozygous model (AA vs GA) of *rs4986790*

TLR-4 gene polymorphism and pulmonary tuberculosis

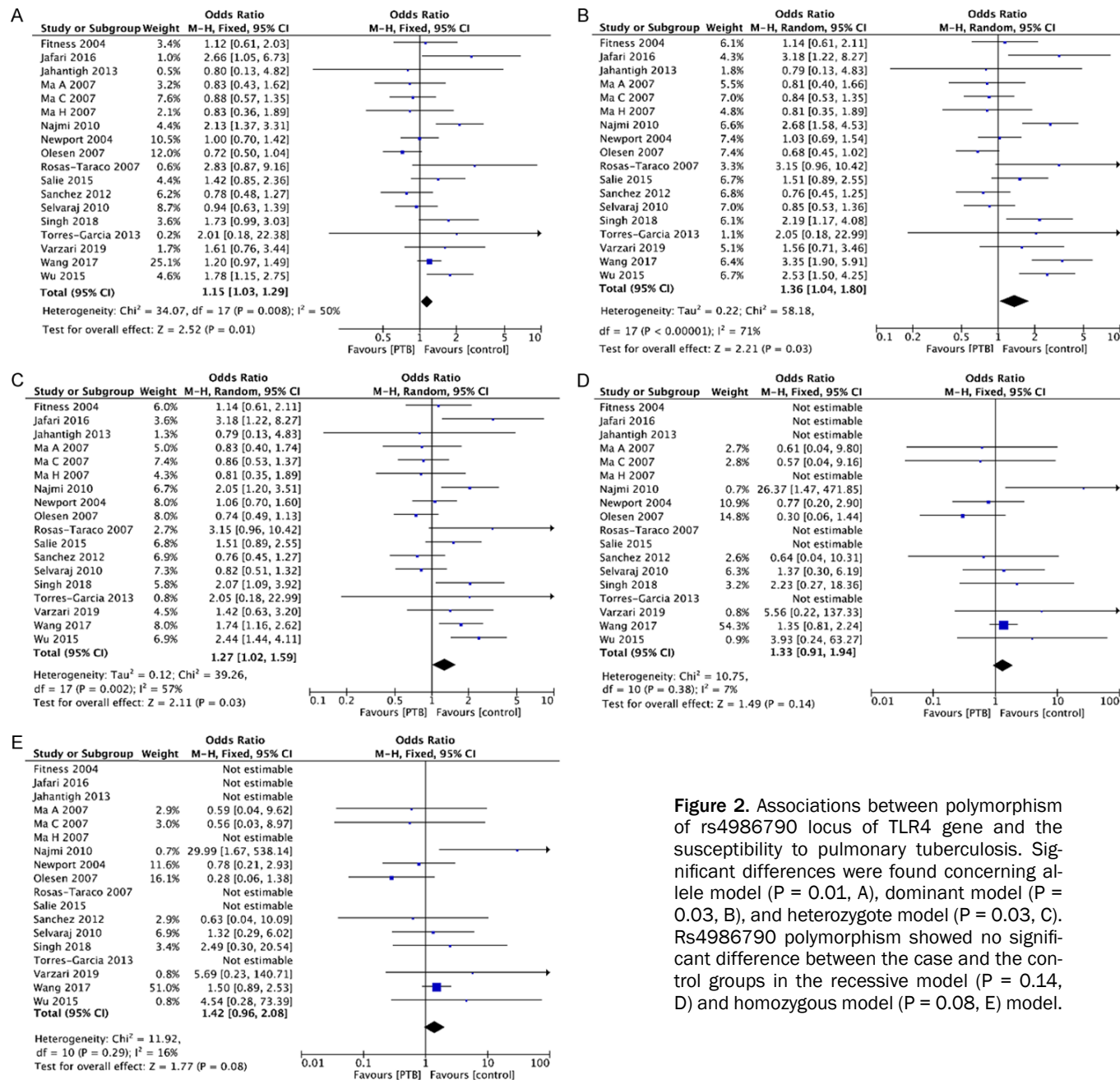


Figure 2. Associations between polymorphism of rs4986790 locus of TLR4 gene and the susceptibility to pulmonary tuberculosis. Significant differences were found concerning allele model ($P = 0.01$, A), dominant model ($P = 0.03$, B), and heterozygote model ($P = 0.03$, C). Rs4986790 polymorphism showed no significant difference between the case and the control groups in the recessive model ($P = 0.14$, D) and homozygous model ($P = 0.08$, E) model.

TLR-4 gene polymorphism and pulmonary tuberculosis

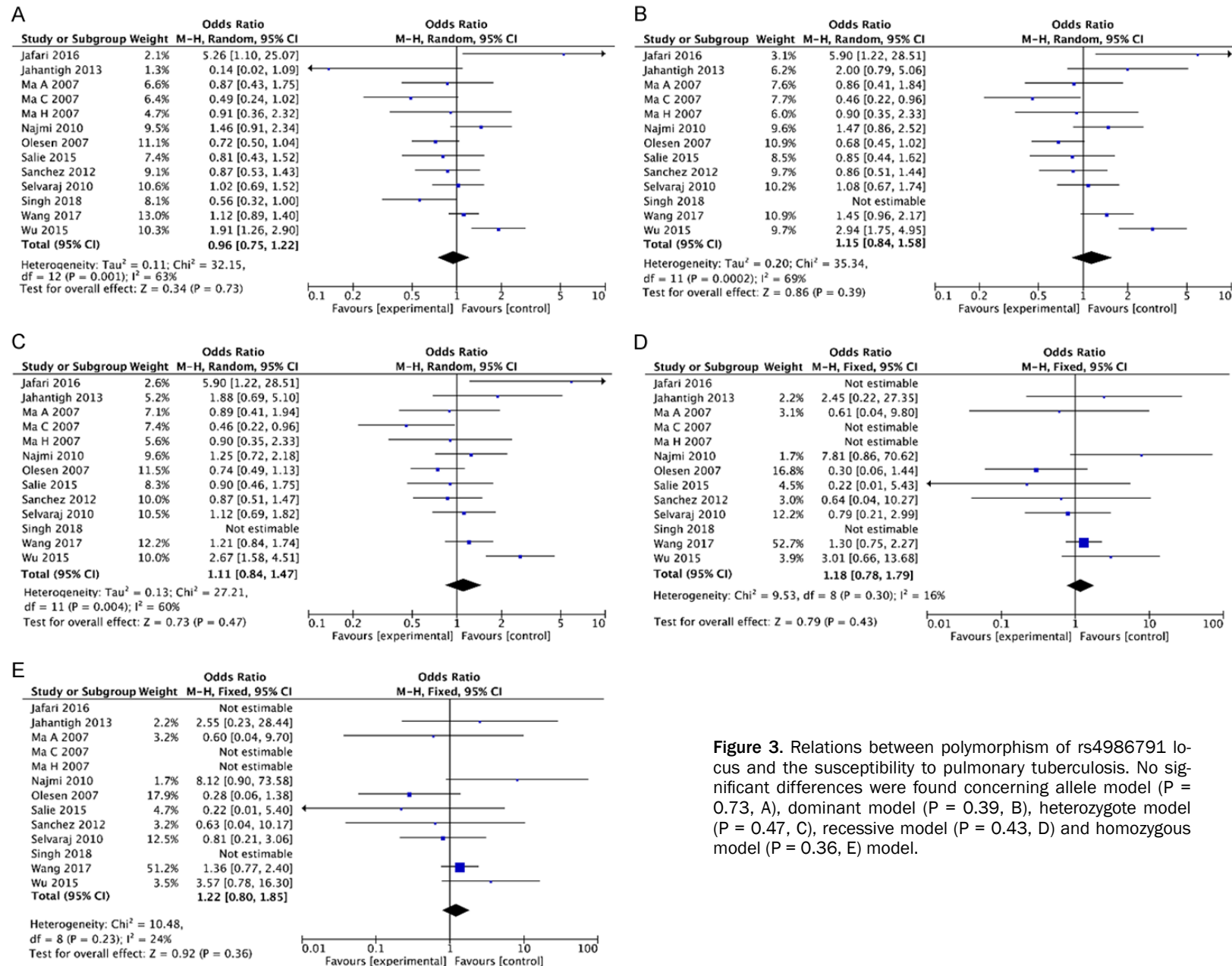


Figure 3. Relations between polymorphism of rs4986791 locus and the susceptibility to pulmonary tuberculosis. No significant differences were found concerning allele model ($P = 0.73$, A), dominant model ($P = 0.39$, B), heterozygote model ($P = 0.47$, C), recessive model ($P = 0.43$, D) and homozygous model ($P = 0.36$, E) model.

TLR-4 gene polymorphism and pulmonary tuberculosis

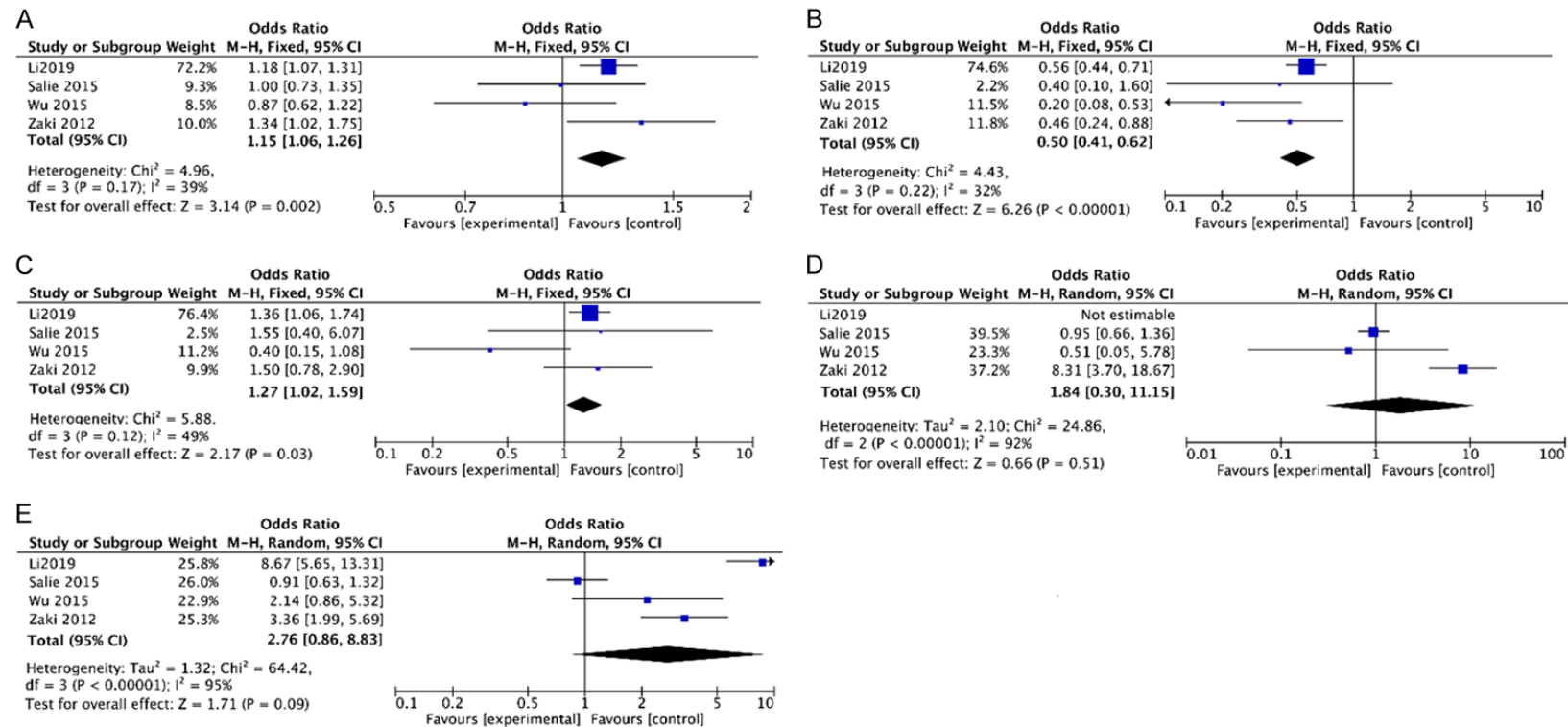


Figure 4. Relations between polymorphism of rs10759932 locus and the susceptibility to pulmonary tuberculosis. Significant differences were found concerning allele model ($P = 0.002$, A), recessive model ($P < 0.0001$, B), and homozygous model ($P = 0.03$, C). Rs4986790 polymorphism showed no significant difference between the experimental and the control groups in the dominant model ($P = 0.51$, D) and heterozygote model ($P = 0.09$, E).

TLR-4 gene polymorphism and pulmonary tuberculosis

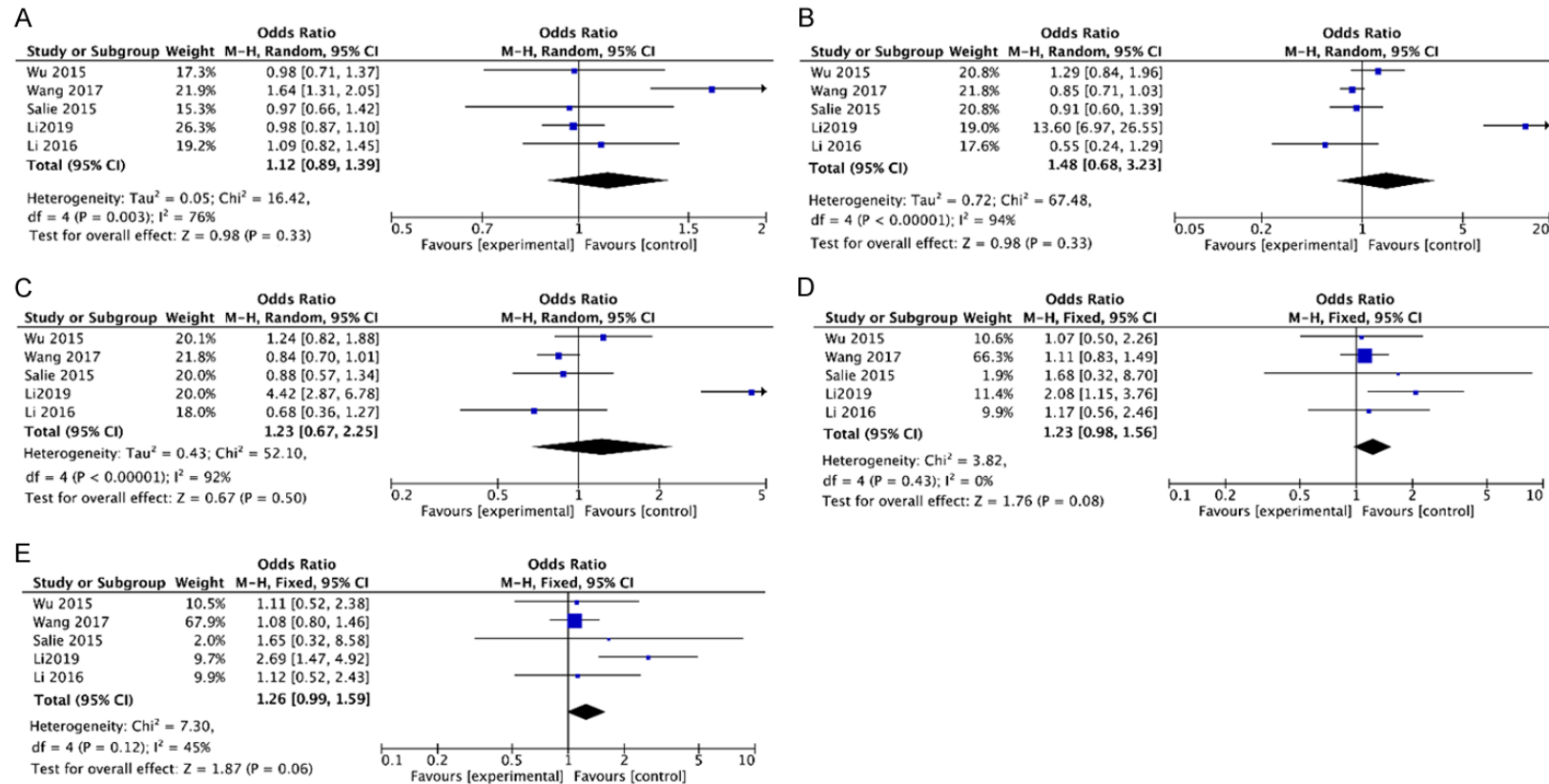


Figure 5. Relationship between polymorphism of rs11536889 locus and the susceptibility to pulmonary tuberculosis. No significant differences were found concerning allele model ($P = 0.33$, A), dominant model ($P = 0.33$, B), heterozygote model ($P = 0.50$, C), recessive model ($P = 0.08$, D) and homozygous model ($P = 0.06$, E) model.

also showed that GG+GA genotype increases the susceptibility to pulmonary tuberculosis (OR = 1.36, $P = 0.03$), and the GA genotype alone increases the susceptibility to tuberculosis compared with AA genotype (OR = 1.27, $P = 0.03$), indicating that GG+GA genotype and GA genotype of rs4986790 may also be the potential markers for diagnosis and monitoring of pulmonary tuberculosis. In the meanwhile, no significant differences were found in any allele or genetic model of rs4986791. While the results of rs4986791 locus are similar to the previous publications, results concerning rs4986790 locus are inconsistent from some authors. Zhao and Schurz et al. [31, 32] showed that polymorphism of rs4986790 and rs4986791 had no significant correlation with pulmonary tuberculosis. In the meanwhile, Najmi et al. [17] reported that the G allele of rs4986790 increased the susceptibility to pulmonary tuberculosis. Jafari et al. [12] also reported that the gene polymorphism of rs4986790 and rs4986791 is significantly correlated with pulmonary tuberculosis. Our results, with significantly more participants than the previous reports, provide new information on the correlation between polymorphism of rs4986790 and pulmonary tuberculosis.

Results of the current study also showed that C allele of rs10759932 increased the risk of pulmonary tuberculosis (OR = 1.15, $P = 0.002$), suggesting that the C allele of rs10759932 may be an independent risk factor for pulmonary tuberculosis, and may potentially be used as a marker for pulmonary tuberculosis diagnosis and monitoring. However, studies carried out by Zaki and Wu et al. [29, 30] found no significant difference in the distribution of rs10759932 alleles C and T between the pulmonary tuberculosis patients and normal controls. It could be because of the small patient size in those studies that made them susceptible to patient selection bias. In the study of Wang et al. [27], there were 1601 cases with tuberculosis and 1526 cases in the control group. Meta-analysis on rs10759932 recessive (CT+TT vs CC) and homozygous model (TT vs CC) showed that CC+CT genotype is related to higher risk of pulmonary tuberculosis (OR = 2.0, $P < 0.001$), and the CC genotype alone increased the susceptibility of tuberculosis compared with TT genotype (OR = 1.27, $P = 0.03$), indicating that CC+CT genotype and CC genotype of rs1075-

9932 locus can be used as indicators in diagnosing and monitoring pulmonary tuberculosis. There was no significant difference in the distribution of any genetic models of rs11536889 locus, suggesting that polymorphism of rs11536889 may not be related to the risk of pulmonary tuberculosis. Wu and Wang et al. [27, 29] also found no correlation between rs11536889 and pulmonary tuberculosis, which were similar to our results. However, there seem to be difference concerning recessive (CG+GG vs CC) ($R = 1.23$, $P = 0.08$) and homozygous model (GG vs CC) ($R = 1.87$, $P = 0.06$) of rs11536889. It is possible that the difference could be significant when more patients are included.

Despite relatively large patient size, reliability of our study may suffer from several disadvantages. Only the papers published in English and Chinese were analyzed in our study, and the unaccountability of literature in other languages or unpublished literature could lead to publication bias. There are various SNP typing methods such as PCR-RFLP, ARMSPCR, TaqMan, MALDI-TOFMS and Sequencing used in the literature included in the study. Sensitivity of those techniques could be different and may lead to heterogeneity. Without subgroup analysis of potential confounding factors such as race, age, other illnesses, HIV infection status as well as SNP typing techniques, the real cause of heterogeneity may be missed, affecting the reliability of the meta-analysis. Therefore, it is necessary that conclusions of this study be treated with caution. In the meanwhile, rigorous large sample case-control studies can be carried out to confirm the conclusions of our study before using them in clinical practice.

Conclusion

Results of the current study indicate that, G allele GG+GA genotype and the GA genotype of rs4986790, C allele and CC+CT genotype, as well as the CC genotype of rs10759932 increased the risk of pulmonary tuberculosis, while rs4986791 and rs11536889 polymorphism may not be a risk factor for pulmonary tuberculosis.

Disclosure of conflict of interest

None.

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