original article

Association of TLR4 gene rs4986790 and rs4986791 polymorphisms with asthma susceptibility: meta-analysis and trial sequential analysis

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BACKGROUND: The current understanding of the correlation between TLR4 gene (toll-like receptor 4) rs4986790 and rs4986791 polymorphisms and asthma susceptibility is inconclusive, with studies and populations yielding conflicting results.

OBJECTIVES: Evaluate this relationship using meta-analysis and trial sequential analysis (TSA).

PATIENTS AND METHODS: Databases were systematically queried for relevant articles from the establishment of the database to 19 June 2023 adhering to predefined inclusion and exclusion criteria. Two authors independently conducted screening, data extraction, and quality evaluation. Meta-analysis and TSA were carried out using RevMan 5.4, StataMP 17.0, and TSA 0.9.5.10 Beta, with α=0.05. Subgroup analyses were conducted based on racial demographics. A sensitivity analysis was conducted employing a one-by-one exclusion method. Publication bias was assessed using the Begg and Egger tests.

MAIN OUTCOME MEASURES: Association of asthma susceptibility with TLR4 gene rs4986790 and rs4986791 polymorphisms.

SAMPLE SIZE: 23 articles included 22 studies on the rs4986790 polymorphism and 11 studies on the rs4986791 polymorphism on the TLR4 gene.

RESULTS: Out of 692 studies screened, 23 met the inclusion criteria. While the overall meta-analysis showed no significant association between the TLR4 rs4986790 polymorphism and asthma susceptibility, subgroup analysis revealed a significant link in the Caucasian population. A significant association was noted in the meta-analysis, particularly among Asian populations, on the rs4986791 polymorphism. The sensitivity analysis indicated that the meta-analysis results were relatively stable. Publication bias analysis revealed minimal influence from publication bias. However, TSA was underscored by the necessity for additional original studies to further validate specific outcomes.

CONCLUSIONS: Our study underscores the ethnicity-specific impact on the relationship between TLR4 polymorphisms and asthma susceptibility. While the overall findings for rs4986790 were not significant, the association with the Caucasian population merits further investigation. Furthermore, rs4986791 demonstrated a significant correlation with asthma susceptibility, specifically among Asian populations.

LIMITATIONS: Our study predominantly examined the rs4986790 and rs4986791 polymorphisms, overlooking the potential influence of other genetic variants within TLR4.

CONFLICT OF INTEREST: None.

Asthma, a chronic inflammatory condition characterized by persistent airway inflammation, hyperresponsiveness, and reversible restriction of airflow, represents a substantial global health burden.[1] Its prevalence has increased in recent years,[2] as evidenced by incidence rates in Beijing of 0.77%, 2.05%, and 2.55% in 1990, 2000 and 2010, respectively, indicating a clear upward trend.[3-5] Asthma is associated with genetics, environmental changes, obesity, smoking, aging, and other factors.[6-9] Among these, the influence of epigenetics has also been widely studied in recent years.[10,11] Additionally, a national cross-sectional study conducted in China in 2018 observed a significant increase in general sensitization to mites compared to the 2008 population, ranking first among all tested allergens.[12] The common symptoms of asthma include coughing, wheezing, and shortness of breath. Currently, corticosteroids, muscarinic receptor antagonists, β2 receptor agonists, and other drugs are primarily used for treatment. Additionally, sex hormones are believed to be effective in controlling asthma symptoms.[13] However, China lacks large-scale national registry data on asthma. Only four countries in Europe (Denmark, Sweden, Finland, and the United Kingdom) and Australia have national registries. Certain hospitals have single-center asthma registry databases.[14] Additionally, despite the increasing prevalence of asthma in China in recent years, there are still deficiencies in its diagnosis and treatment. One study indicated that only 28.8% of asthma patients are diagnosed.[15] This may be attributed to a lack of awareness of asthma among patients and doctors, as well as insufficient healthcare resources, particularly in less developed regions where only 5.6% of asthma patients use inhaled corticosteroid therapy.[15]

Toll-like receptors (TLR) are pattern recognition receptors expressed in dendritic cells and macrophages. Alongside CD14, TLRs can identify bacterial lipopolysaccharides, triggering inflammation and innate immune responses.[16] Given its importance in immune function, TLRs have emerged as a potential contributor to the complex interplay of asthma susceptibility. TLR4 genetic variants have been associated with various diseases, including pre-eclampsia, diabetes, tuberculosis, sepsis, and a range of cancers.[17-21] Several studies have investigated the relationship between polymorphisms of the TLR4 gene, including rs1927914, rs4986790, rs4986791, rs10983755, rs11536879, and rs1927907, and the risk of asthma.[22-24] Among these, rs4986790 and rs4986791 have received significant attention due to their association with asthma susceptibility. The pathogenesis of asthma is believed to be closely linked to immune responses and inflammation mediated by TLR4. Studies suggest that TLR4 gene polymorphisms may also be associated with decreased by IL-10 and IL-12 responses induced by lipopolysaccharides.[24] Furthermore, some propose that the rs4986790 polymorphism alters the body's responsiveness to endotoxins, potentially impacting the severity of asthma.[25] The single nucleotide polymorphisms at rs4986790 and rs4986791 are located in the exon of the TLR4 gene, with mutations A>G and C>T resulting in the amino acid changes of Asp299Gly and Ile399Thr, respectively.[26] In recent years, numerous articles on the TLR4 gene rs4986790 and rs4986791 polymorphisms and the association with asthma susceptibility have been published. The existing body of evidence on this association remains inconclusive, with conflicting findings across different studies and populations. Therefore, our study systematically evaluated the impact of TLR4 rs4986790 and rs4986791 polymorphisms on asthma susceptibility using meta-analysis to provide evidence for future research and medical practice. Moreover, we recognized the importance of going beyond conventional meta-analyses by incorporating trial sequential analysis (TSA) to provide a more reliable judgement of the association and enhance the robustness of our findings.[27]

METHODS

The protocol of our meta-analysis was established according to the PRISMA guideline. Our study was registered in PROSPERO with the ID CRD42023487246. Inclusion criteria were original case-control studies that evaluated the relationship between TLR4 rs4986790 and rs4986791 polymorphisms and asthma susceptibility. The control group were healthy people without asthma and the case group was clinically diagnosed asthma patients, regardless of gender, age, race and medical history. The total amount of samples of each allele and/or genotype in the control and case groups were reported in the original studies, which have high reliability, correct statistical methods, and high-quality data. The study did not require ethical review and permission nor patient consent because the data were taken from previously published articles.

Exclusion criteria were that the original authors could not be contacted to collect any missing or incomplete necessary data, study participants were not human, the data were not repeatedly published or repeatedly searched articles and articles whose language was not English or Chinese.

The online search was performed using the Embase, PubMed, Web of Science, Wanfang (Chinese), VIP (Chinese), and CNKI (Chinese) databases. The search

was ended on 19 June 2023. Tracing incorporated references to complement relevant articles. Among the search terms were TLR4 (toll-like receptor 4), asthma, gene polymorphism, and gene variants. During the screening process, the title and abstract were first read. After the removal of articles that were obviously unrelated, we read the full text to determine whether the remaining articles need to be included. For articles missing or with incomplete data, the corresponding author was contacted by email to obtain the necessary information. The first author's name, the publication year, the country, the number of samples in the case and control groups, and the number of samples for each genotype were extracted. The included articles were screened separately by two researchers, who also extracted the data and cross-checked them. Any disagreement was addressed with the help of third party.

The quality of the case-control studies that included was assessed using the Newcastle-Ottawa scale, with the following factors considered: Whether the case definition and diagnosis were adequate, whether the cases were representative, whether the selection and definition of controls were justified, whether there was comparability between the cases and the controls, whether the methods of investigation and evaluation of the exposures were the same, whether the methods of determining the exposures were the same in the cases and in the controls, and whether there was an adequate response rate.

Meta-analysis was performed using StataMP 17.0 (StataCorp LLC, Texas, USA) and RevMan 5.4 (The Cochrane Collaboration, London, UK). The odds ratio (OR), *P* value and 95% confidence interval (95% CI) of all the models we studied were reported (α=0.05). The chi-square test was used to examine the heterogeneity among the included studies (α=0.10). If there was no obvious statistical heterogeneity between the studies (*P*>.1), a fixed effect model was applied. Otherwise (*P*<.1), a random effects model was applied. Besides, a subgroup analysis was carried out to determine the source of potential heterogeneity and provide a meta-analysis of the results of different races. For the sensitivity analysis, the included studies were individually deleted to observe whether a certain study had a significant effect on the combined effect. Furthermore, publication bias among the original studies was judged using the Begg and Egger tests (α=0.05). During the process of conducting and updating of each meta-analysis, TSA examined the possibility of random error, which included false positives and false negatives, and determined the sample size required to reach definitive conclusions through TSA 0.9.5.10 Beta (Copenhagen Trial Unit, Copenhagen, Denmark), thus indicating whether definitive meta-analysis conclusions with no further validation by original studies required had been reached (α=0.05).[27]

RESULTS

A total of 692 related studies were initially found based on our search strategy. After screening, 23 articles were included, including 22 studies on the TLR4 gene rs4986790 polymorphism and 11 studies for the rs4986791 polymorphism (**Figure 1**).[23,25,28-48] **Tables 1 and 2** show the fundamental data for each study. The evaluation of article quality revealed that all the included studies had comparable patients and controls, appropriate diagnostic criteria, satisfactory genetic testing procedures, and clear results (**Table 3**).

Overall meta-analysis results

According to the overall meta-analysis of the rs4986790 polymorphism, there was no significant difference in asthma susceptibility among the codominant model AA vs AG group (*P*=.39), AA vs GG group (*P*=.52), the dominant model AA vs AG+GG group (*P*=.06), the recessive model AA+AG vs GG group (*P*=.09), the overdominant model AA+GG vs AG group (*P*=.82), and the allelic model A vs G group [(*P*=.06). The *P* values in all of the above models were higher than .05, indicating there was no significant association of the TLR4 gene rs4986790 A/G polymorphism with asthma susceptibility (**Table 4**).

According to overall meta-analysis of the rs4986791 gene polymorphism, there was no significant difference in asthma susceptibility among the dominant model TT vs TC+CC group (*P*=.49), the recessive model CC+CT vs TT group (*P*=.65), the overdominant model CC+TT vs CT group (*P*=.08), and the allelic model C vs T group (*P*=.46). However, a significant difference was found in the risk of asthma in the codominant model CC vs CT group (*P*=.02) (**Figure 2A**). People with genotype CT were more likely to develop asthma than those with CC. A significant difference was found in the risk of asthma in the codominant model CC vs TT group (*P*=.03) (**Figure 2B**). People with genotype TT were more likely to develop asthma than those with CC (**Table 3**).

Subgroup analysis results

According to overall meta-analysis results, all the models of the rs4986790 were not statistically significant. However, for the Caucasian population, the dominant model AA vs AG+GG group showed statistical significance (*P*=.01). People with genotype AG+GG were

more likely to develop asthma than those with genotype AA. The recessive model AA+AG vs GG group showed statistical significance (*P*=.03). People with genotype GG were more likely to develop asthma than those with genotype AA+AG. The allelic model A vs G group also showed statistical significance (*P*=.007). People with allele G were more likely to develop asthma than those with allele A. No significant statistical difference was found in the codominant model AA vs AG group (*P*=.09) and AA vs GG group (*P*=.10), nor in the overdominant model AA+GG vs AG group (*P*=.26) (**Table 5**).

For the Asian population, no significant association of the TLR4 gene rs4986790 A/G polymorphism with asthma variant was found according to the codominant model AA vs AG group (*P*=.86), AA vs GG group (*P*=.73), the dominant model AA vs AG+GG group (*P*=.84), the recessive model AA+AG vs GG group (*P*=.94), the overdominant model AA+GG vs AG group (*P*=.62), and the allelic model A vs G group (*P*=.76) (**Table 5**).

According to overall meta-analysis results, the codominant model CC vs CT and CC vs TT groups of rs4986791 were statistically significant. However, for Caucasian population, no significant association of the TLR4 gene rs4986791 C/T polymorphism with asthma susceptibility was found according to the codominant model CC versus CT group (*P*=.98). CC vs TT group (*P*=.97), the dominant model CC vs CT+TT group (*P*=.15), the recessive model CC+CT vs TT group (*P*=.14), the overdominant model CC+TT vs CT group (*P*=.75), and the allelic model C vs T group (*P*=.16) (**Table 6**).

For the Asian population, the codominant model CC vs CT group (*P*=.01) and CC vs TT group (*P*=.02) of rs4986791 had statistical significance. No significant statistical difference was found in the dominant model CC vs CT+TT group (*P*=.89), the recessive model CC+CT vs TT group (*P*=.87), the overdominant model CC+TT vs CT group (*P*=.08), nor the allelic model C vs T group (*P*=.86) (**Table 6**).

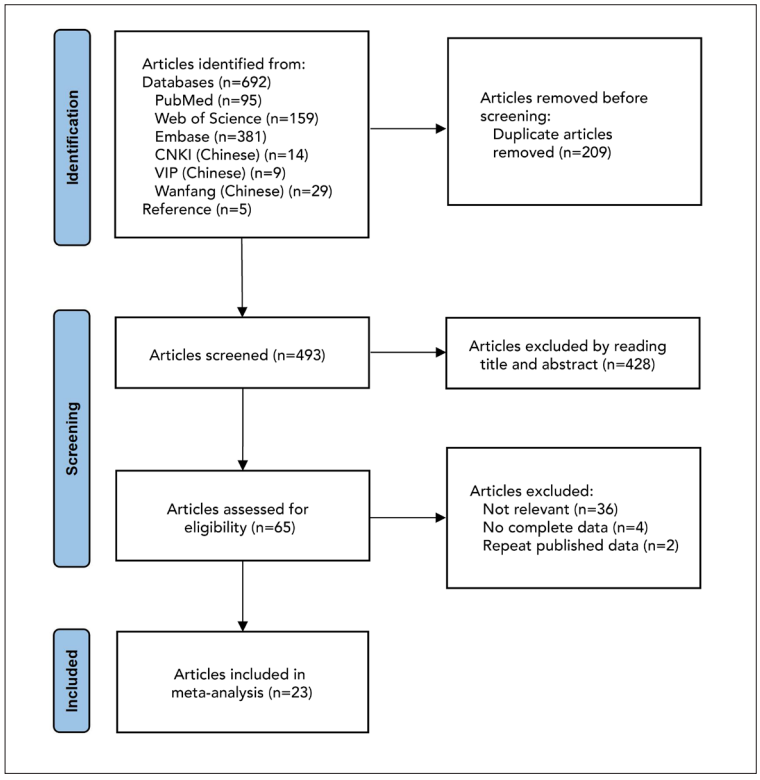

Figure 1. Article searching and screening results.

Sensitivity analysis and publication bias

The exclusion procedure was used one at a time for the sensitivity analysis. The findings of sensitivity analysis demonstrated that our results are comparatively stable and less prone to shift with the changes of a single original study.

The Begg and Egger tests for estimating the risk of publication bias indicated a significant publication bias (**Table 7**). Most of the groups showed no significant publication bias existed, except the AA vs AG+GG and AA+AG vs GG groups of rs4986790 according to the Egger test, indicating the reliability of our meta-analysis results was less affected by publication bias.

Trial sequential analysis results

Taking AA vs GG and A vs G groups of rs4986790 as examples, the Z curve had reached required information size or/and intersected with the TSA boundary, indicating the conclusions drawn by meta-analysis were definitive, therefore, no additional original study was required for further verification (**Figure 3A, 3B**). However, taking AA vs AG of rs4986790 as an example, the Z curve neither intersected with the TSA boundary nor reached the required information size, indicating the accumulated information size was too small for the required size, with a higher risk of false negatives or false positives, and more original studies are needed for further verification (**Figure 3C**). This situation was similar among AA vs AG+GG, AA+AG vs GG, AA+GG vs AG groups of rs4986790 and all the groups of rs4986791.

DISCUSSION

In this meta-analysis, we systematically reviewed 23 articles that investigated the association between TLR4 gene rs4986790 and rs4986791 polymorphisms and

Table 1. Characteristics of articles that studied the rs4986790 polymorphism.

Author and publication year	Country	Case			Control		
		AA	AG	GG	AA	AG	GG
Adjers et al, 2005[28]	Finland	202	39	2	334	65	2
Bahrami et al, 2015[29]	Iran	85	14	0	104	16	0
Bisyuk et al, 2017[31]	Ukraine	261	66	4	242	40	3
Davoodi et al, 2015[32]	India	55	43	2	29	20	1
Fageras Bottcher et al, 2004[33]	Sweden	24	6	0	80	5	0
Hussein et al, 2012[34]	Egypt	434	64	2	223	27	1
Kutsenko et al, 2012[35]	Ukraine	31	6	1	91	4	0
Lachheb et al, 2008[23]	Tunisia	209	1	0	223	0	1
Lau et al, 2017[36]	Australia	758	69	3	321	34	0
Liang et al, 2005[37]	Singapore	21	0	0	96	0	0
Liu et al, 2005[44]	China	161	29	7	128	23	5
Lyakhovska et al, 2017[30]	Ukraine	38	7	0	86	4	0
Sahin et al, 2014[38]	Turkiye	122	9	0	71	4	0
Sinha et al, 2014[40]	India	390	87	4	381	95	7
Sun et al, 2013[41]	China	50	0	0	30	0	0
Voron'ko et al, 2011[42]	Russia	245	31	7	200	27	0
Wang et al, 2014[43]	China	126	0	0	126	0	0
Yang et al, 2004[25]	UK	698	113	3	159	19	1
Yang, 2010[45]	China	132	0	0	132	0	0
Yang et al, 2023[46]	China	67	74	31	57	54	25
Zaborowski et al, 2011[47]	Poland	94	12	0	142	17	0
Zhang, 2006[48]	China	113	0	0	79	0	0

Abbreviations: A=Adenine; G=Guanine.

Table 2. Characteristics of included articles studied rs4986791.

Author and publication year	Country	Case			Control		
		TT	CT	CC	TT	CT	CC
Kutsenko et al, 2012[35]	Ukraine	0	3	35	0	1	94
Lachheb et al, 2008[23]	Tunisia	1	0	209	1	2	221
Liang et al, 2005[37]	Singapore	0	0	21	0	0	96
Liu et al, 2005[44]	China	2	4	191	1	5	150
Sahin et al, 2014[38]	Turkiye	0	11	120	0	4	71
Schurman et al, 2018[39]	USA	1	91	871	2	141	1598
Sinha et al, 2014[40]	India	5	68	408	7	92	384
Wang et al, 2014[43]	China	0	0	126	0	0	126

Table 2 (cont.). Characteristics of included articles studied rs4986791.

Author and publication year	Country	Case			Control		
		TT	CT	CC	TT	CT	CC
Yang et al, 2010[45]	China	0	0	132	0	0	132
Yang, 2023[46]	China	68	73	31	34	60	42
Zhang, 2006[48]	China	0	0	113	0	0	79

C: cytosine; T: thymine.

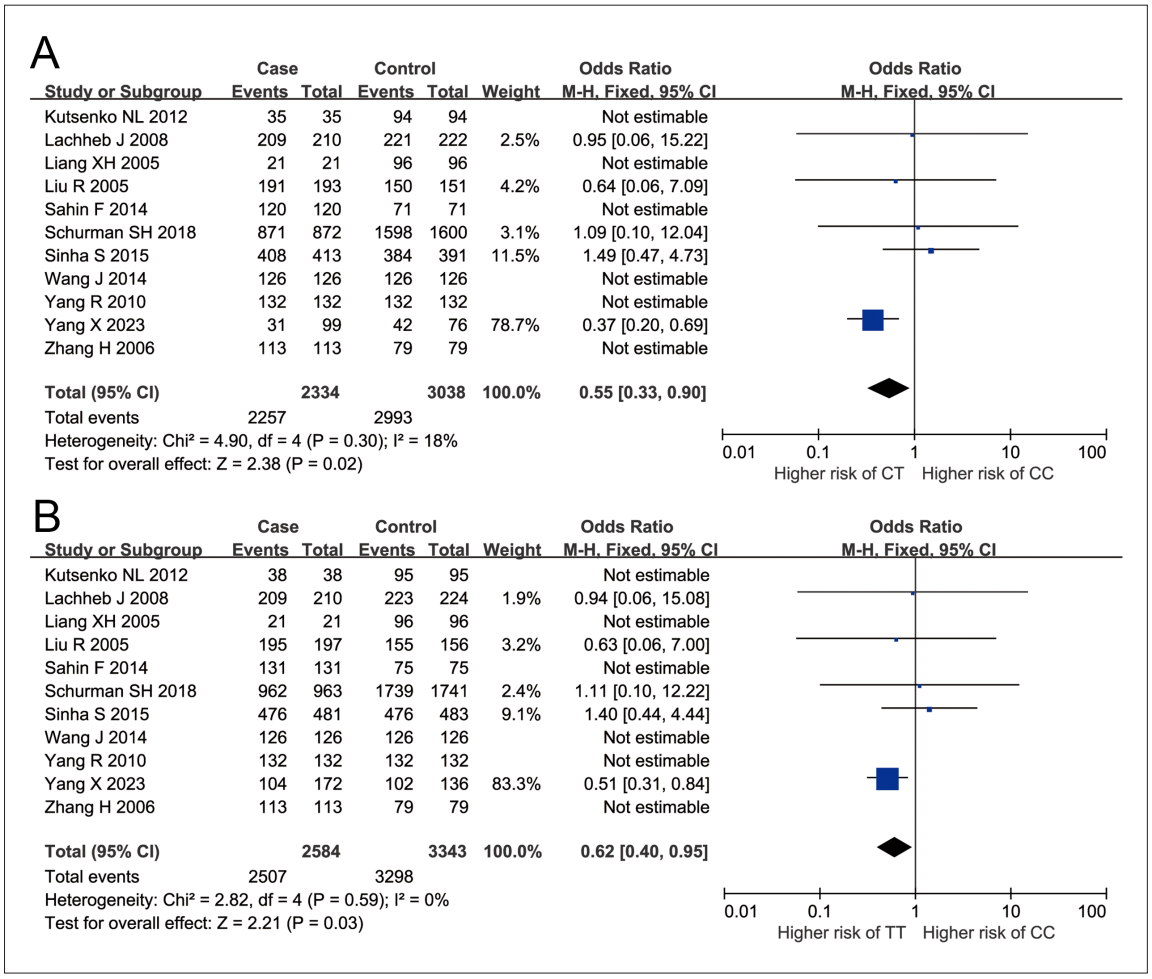

Figure 2. Association between rs4986791 gene polymorphism and asthma susceptibility. (A) CC vs CT group of rs4986791; (B) CC vs TT group of rs4986791.

Table 3. Quality assessment of included studies.

Author and publication year	Newcastle-Ottawa Scale									Total
	Selection			Comparability			Outcome			
	a	b	c	d	e	f	g	h	i	
Adjers et al, 2005[28]	1	1	1	1	1	0	1	1	1	8
Bahrami et al, 2015[29]	1	1	1	1	1	1	1	1	1	9
Bisyuk et al, 2017[31]	0	1	1	1	0	0	1	1	1	6
Davoodi et al, 2015[32]	1	1	1	1	1	0	1	1	1	8
Fagerås et al, 2004[33]	0	1	1	1	1	0	1	1	1	7
Hussein et al, 2012[34]	1	1	1	1	1	1	1	1	1	9
Kutsenko et al, 2012[35]	0	1	1	1	0	0	1	1	1	6
Lachheb et al, 2008[23]	1	1	1	1	1	1	1	1	1	9
Lau et al, 2017[36]	0	1	1	1	1	1	1	1	1	8
Liang et al, 2005[37]	1	1	1	1	1	1	1	1	1	9
Liu et al, 2005[44]	1	1	1	1	0	0	1	1	1	7
Lyahovskaya et al, 2013[30]	1	1	1	1	0	0	1	1	1	7
Sahin et al, 2014[38]	1	1	0	1	1	1	1	1	1	8
Sinha et al, 2014[40]	1	1	1	1	1	1	1	1	1	9
Sun et al, 2013[41]	1	1	1	1	1	0	1	1	1	8
Voron'ko et al, 2011[42]	0	1	1	1	0	0	1	1	1	6
Wang et al, 2014[43]	1	1	1	1	1	1	1	1	1	9
Yang et al, 2004[25]	0	1	1	1	1	0	1	1	1	7
Yang, 2010[45]	1	1	1	1	1	1	1	1	1	9
Yang et al, 2023[46]	1	1	1	1	1	1	1	1	1	9
Zaborowski et al, 2011[47]	1	1	1	1	1	1	1	1	1	9
Zhang, 2006[48]	1	1	1	1	1	0	1	1	1	8
Schurman et al, 2018[39]	1	1	1	1	1	1	1	1	1	9

a: Representativeness of the exposed cohort. b: Selection of the non-exposed cohort. c: Ascertainment of exposure. d: Demonstration that outcome of interest was not present at start of study. e: Comparability of cohorts on the basis of the design or analysis (adjusted for age). f: Comparability of cohorts on the basis of the design or analysis (adjusted for any other factor). g: Assessment of outcome. h: Was follow-up long enough for outcomes to occur. i: Adequacy of follow-up of cohorts.

Table 4. Summary of meta-analysis results.

	rs4986790			rs4986791		
	OR	95% CI	*P*	OR	95% CI	*P*
XX vs XY	0.84	0.56, 1.26	.39	0.55	0.33, 0.90	.02
XX vs YY	0.88	0.59, 1.30	.52	0.62	0.40, 0.95	.03
XX vs XY+YY	0.88	0.77, 1.01	.06	0.86	0.55, 1.33	.49
XX+XY vs YY	0.89	0.77, 1.02	.09	0.91	0.61, 1.37	.65
XX+YY vs XY	0.95	0.63, 1.45	.82	0.66	0.41, 1.05	.08
X vs Y	0.89	0.79, 1.01	.06	0.86	0.58, 1.28	.46

For rs4986790, X represents adenine, Y represents guanine; For rs4986791, X represents cytosine), Y represents thymine.

asthma susceptibility. For rs4986790, the results of our overall meta-analysis did not reveal any significant association with asthma susceptibility across the various genetic models. However, a subgroup analysis revealed a significant association among the Caucasian population, suggesting potential ethnicity-specific effects. For rs4986791, the overall meta-analysis did not reveal any significant association with asthma susceptibility, except for the codominant model CC vs CT and CC vs TT groups, indicating a higher asthma risk for individuals with genotype TT and CT, respectively, compared to CC. Subgroup analysis revealed that this significant association occurred only in the Asian populations, emphasizing potential ethnic disparities. These findings underscore the importance of considering ethnic diversity in genetic association studies because genetic

Table 5. Summary of subgroup analysis results of the rs4986790 polymorphism.

	Caucasian			Asian		
	OR	**95% CI**	***P***	**OR**	**95% CI**	***P***
AA vs AG	0.52	0.24, 1.11	.09	1.05	0.64, 1.71	.86
AA vs GG	0.53	0.25, 1.13	.10	1.09	0.68,1.74	.73
AA vs AG+GG	0.81	0.68, 0.96	.01	1.02	0.82, 1.28	.84
AA+AG vs GG	0.83	0.70, 0.98	.03	1.01	0.80, 1.27	.94
AA+GG vs AG	0.64	0.29, 1.39	.26	1.14	0.69, 1.88	.62
A vs G	0.8	0.68, 0.94	.007	1.03	0.85, 1.24	.76

A=adenine, G=guanine.

Table 6. Summary of subgroup analysis results of the rs4986791 polymorphism.

	Caucasian			Asian		
	OR	**95% CI**	***P***	**OR**	**95% CI**	***P***
CC vs CT	1.03	0.17, 6.28	.98	0.52	0.31, 0.87	.01
CC vs TT	1.03	0.17, 6.31	.97	0.60	0.39, 0.93	.02
CC vs CT+TT	0.82	0.63, 1.07	.15	0.95	0.42, 2.12	.89
CC+CT vs TT	0.82	0.63, 1.07	.14	1.06	0.54, 2.07	.87
CC+TT vs CT	0.73	0.11, 4.85	.75	0.65	0.41, 1.06	.08
C vs T	0.83	0.65, 1.08	.16	0.94	0.47, 1.90	.86

C=cytosine, T=thymine.

Table 7. Summary of publication analysis *P* values.

	rs4986790		rs4986791	
	Begg test	**Egger test**	**Begg test**	**Egger test**
XX vs XY	0.193	.228	1.000	0.278
XX vs YY	0.244	.196	1.000	0.260
XX vs XY+YY	0.064	.008	1.000	0.617
XX+XY vs YY	0.027	.004	1.000	0.732
XX+YY vs XY	0.304	.439	0.462	0.990
X vs Y	0.077	.016	0.764	0.769

For rs4986790, X represents adenine, Y represents guanine); For rs4986791, X represents cytosine), Y represents thymine.

factors contributing to asthma susceptibility may vary across populations.

The sensitivity analysis revealed that the results were less influenced by certain individual studies, indicating the stability of the findings of this meta-analysis. Most groups did not show any significant publication bias, except for specific comparisons involving rs4986790. The overall reliability of our meta-analysis results remained relatively unaffected by publication bias. The TSA is a valuable tool for estimating the size of the information required for definitive conclusions in meta-analyses. It reinforced the reliability of our meta-analysis findings for certain outcomes, such as the AA vs GG and A vs G groups of rs4986790, indicating that the sample size included in this meta-analysis met the minimum required information size to obtain a "definitive" conclusion and that further studies are unlikely to alter the conclusions derived from this meta-analysis. However, owing to insufficient information size for certain analyses, caution is suggested for some outcomes, such as AA vs AG of rs4986790, for which more original research is required for further verification.[27]

As a key component of the innate immune system, TLR4 is essential for identifying pathogen-associated molecular patterns.[49] The rs4986790 and rs4986791 polymorphisms within the TLR4 gene have been implicated in altering the functionality of this receptor, potentially impacting the immune response in the host body.[49]

The intricate interplay between genetic variations and environmental factors, such as exposure to allergens and pollutants may also be significant in modulating TLR4-mediated immune responses during asthma development.[50,51] Understanding how these polymorphisms influence TLR4 function at the molecular level and interact with environmental triggers is crucial for elucidating the etiology of asthma. Future investigations should explore gene-environment interactions, epigenetic modifications, and the role of TLR4 in modulating inflammatory responses to shed light on the nuanced associations between TLR4 gene polymorphisms and asthma susceptibility. Such insights may pave the way for personalized therapeutic strategies and targeted interventions in populations with specific genetic profiles.

Although this meta-analysis elucidated statistically significant associations between the TLR4 gene polymorphisms and asthma susceptibility, along with their ethnicity-specific patterns, it is still crucial to acknowledge certain limitations that warrant consideration. Firstly, our meta-analysis is relied on the currently available articles, and excluded unpublished studies. Additionally, this meta-analysis predominantly focused

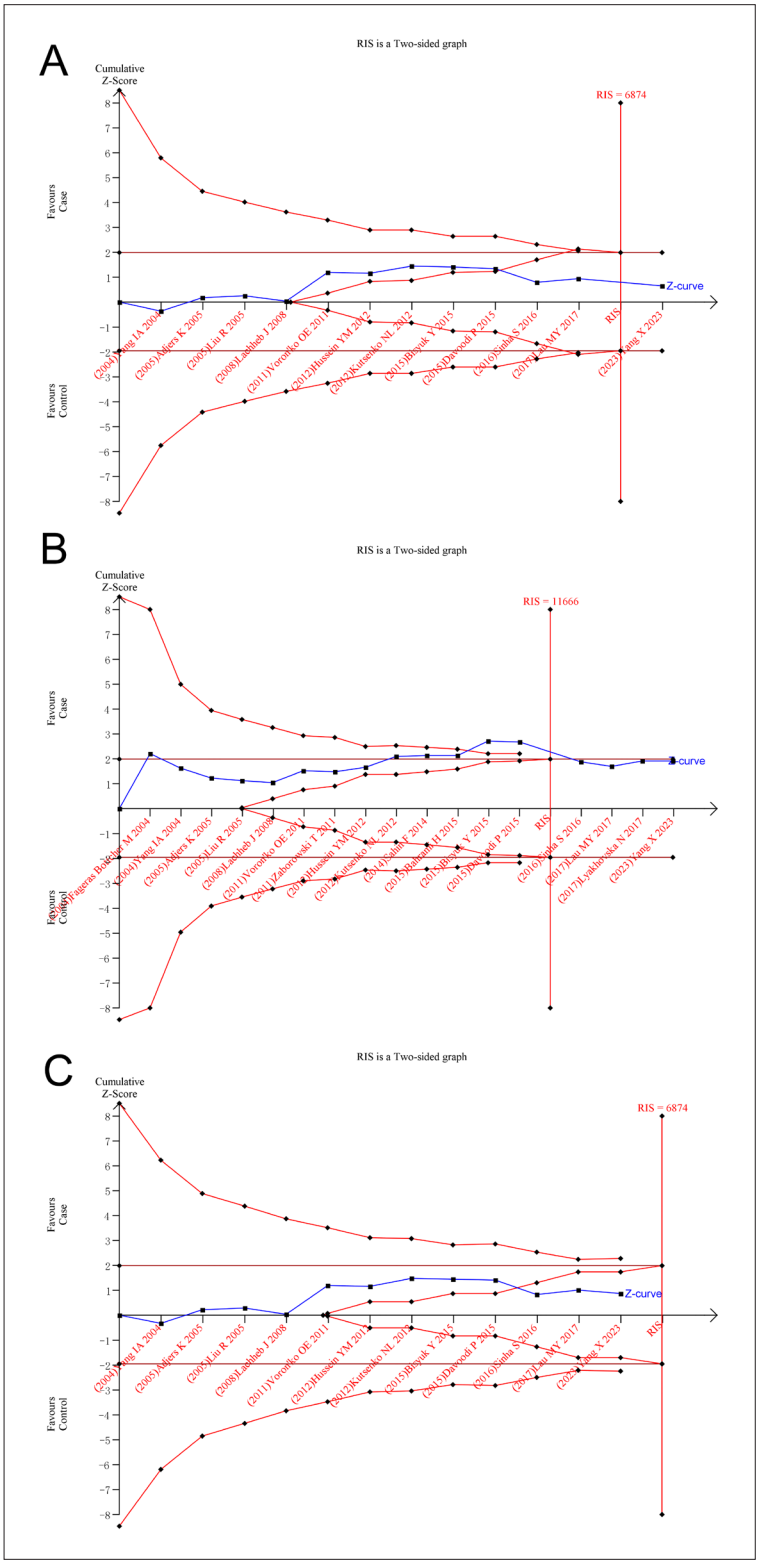

Figure 3. Trial sequential analysis results. (A) AA vs GG of rs4986790; (B) A vs G group of rs4986790; (C) AA vs AG group of rs4986790.

on the rs4986790 and rs4986791 polymorphisms, neglecting the potential influence of other genetic variants within the TLR4 gene. This narrow scope may limit our comprehensive understanding of the association between TLR4 gene polymorphisms and asthma susceptibility. Moreover, as indicated by the TSA, certain outcomes have reached the required information size, suggesting a high level of confidence in the drawn conclusions. However, for other outcomes, the TSA indicated that the accumulated information size might be insufficient, emphasizing the need for ongoing research to validate and consolidate our findings.[27]

In summary, based on the assessment of 23 included articles, this meta-analysis elucidates the association between TLR4 gene rs4986790 and rs4986791 variants and asthma susceptibility. Although the overall meta-analysis of rs4986790 did not reveal statistically significant differences, ethnicity-specific variations emerged in the subgroup analysis. Notably, a statistically significant relationship was observed in the Caucasian population, suggesting that the rs4986790 polymorphism contributes to asthma susceptibility in this ethnic group. However, this association was not evident in the Asian population. In contrast, for rs4986791, the overall meta-analysis provided robust statistical evidence for its association with asthma susceptibility. Nevertheless, when focusing on specific ethnic groups, a significant association was confined to the Asian population, with no statistical significance observed among Caucasians. We look forward to future research to further elucidate potential mechanisms, explore additional genetic markers, and enhance our understanding of the complex interactions between TLR4 gene polymorphisms and asthma susceptibility, which can assist in developing tailored interventions and precision healthcare for asthma management.

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