Association of TLR4 (896A/G and 1196C/T) Gene Polymorphisms with Asthma Risk: A Meta-Analysis

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Statistical Analysis C
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Background: Conflicting data have been reported on the association between Toll-like receptor 4 (TLR4) +896A/G and +1196C/T polymorphisms and the risk of asthma. Therefore, we conducted this meta-analysis to clarify the effect of TLR4 +896A/G and +1196C/T polymorphisms on the risk of asthma.

Material/Methods: An electronic literature search was performed using PubMed, Embase, Web of Science, Chinese National Knowledge Infrastructure (CNKI), and Wanfang Data to find relevant studies. Odds ratios (ORs) and 95% confidence intervals (CIs) were used to assess the strength of the associations. All statistical analyses were conducted using STATA software version 12.0.

Results: A total of 14 studies with 2873 asthma cases and 3110 controls were included. The pooled results indicated a significant association between TLR4 +1196C/T polymorphism and the risk of asthma (T *vs.* C: OR=0.79, 95%CI=0.63–0.99, P=0.04; TT+CT *vs.* CC: OR=0.76, 95%CI=0.59–0.96, P=0.03; CT *vs.* CC: OR=0.74, 95%CI=0.58–0.95, P=0.02). In subgroup analysis by ethnicity, TLR4 +1196C/T polymorphism was significantly associated with asthma risk in Asians (T *vs.* C: OR=0.73, 95%CI=0.54–0.98, P=0.04; TT+CT *vs.* CC: OR=0.70, 95%CI=0.51–0.96, P=0.03; CT *vs.* CC: OR=0.69, 95%CI=0.50–0.96, P=0.03), but not in whites. For TLR4 +896A/G polymorphism, no significant association was found between TLR4 +896A/G polymorphism and asthma risk under any genetic models.

Conclusions: The results of this meta-analysis suggest that T allele of the TLR4 +1196C/T might act as a protective factor against the development of asthma.

MeSH Keywords: Asthma • Meta-Analysis • Polymorphism, Genetic • Toll-Like Receptor 4

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Background

Asthma is a chronic inflammatory airway disorder character-ized by airway hyper-responsiveness, reversible airway ob-struction, and airway remodeling [1,2]. It is commonly thought that asthma is a multi-factorial disease resulting from com-plex interactions between genetic predisposition and environ-mental factors [3]. Studies have shown that genetic influences on asthma are substantial, with heritability estimates ranging between 35% and 95% [4]. Notably, a large number of poly-morphisms in genes positioned throughout the genome have been implicated in asthma causation, including the gene en-coding Toll-like receptor 4 (TLR4) [5].

Toll-like receptors (TLRs) are a class of evolutionarily conserved membrane-bound pattern recognition receptors (PRRs) pre-sented on the cell surface of innate immune cells. TLRs recog-nize pathogen-associated molecular patterns (PAMPs) exclu-sively expressed by microbial pathogens, and activate cellular signaling pathways to induce immune-response genes, includ-ing inflammatory cytokines [6]. Ten different human TLRs have been identified. TLR4, the best-studied TLR, is expressed on macrophages, dendritic cells, and other cell types, and mainly recognizes lipopolysaccharide (LPS) of gram-negative bacte-ria [7,8]. Interaction of LPS with TLR4 can activate the nuclear factor kappa B (NF-κB) signaling pathway, increase expression of inflammatory cytokines and disturb the Th1/Th2 balance in asthma [9]. TLR4 also regulates innate immune responses to respiratory syncytial virus infection, which is a risk factor for the development of asthma [10].

Human TLR4 gene is located on chromosome 9q32-q33 [11]. Two single-nucleotide polymorphisms (SNPs), TLR4 +896A/G (rs4986790, also known as Asp299Gly) and TLR4 +1196C/T (rs4986791, also known as Thr399Ile), have been demonstrat-ed to modify the receptor's response to endotoxin, which is an important trigger of asthma [12]. This genetically deter-mined alteration in endotoxin responsiveness may be involved in the development of asthma. Several studies have evaluat-ed the association of TLR4 +896A/G and +1196C/T polymor-phisms with asthma [13–31]. However, due to the limitation of subjects, the results were inconsistent and controversial. A previous meta-analysis has shown a marginal association of TLR4 +896A/G with asthma, and no association between TLR4 +1196C/T polymorphism and asthma [32], but it did not cover all eligible studies. The exact correlation between TLR4 +896A/G and +1196C/T polymorphisms and asthma has not been entirely established. Therefore, we performed a meta-analysis including all eligible case-control studies to clarify and quantify the authentic effect of TLR4 +896A/G and +1196C/T polymorphisms on the risk of asthma.

Material and Methods

This study was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.

Literature search strategy

A literature search was performed using PubMed, Embase, Web of Science, Chinese National Knowledge Infrastructure (CNKI), and Wanfang Data from inception to July 2015. The search strings was: ("asthma" or "asthmatic") and ("toll-like recep-tor 4" or "TLR4") and ("mutation" or "polymorphism" or "vari-ant"). The reference lists of the identified articles were also ex-amined. No restrictions were applied for language, population, sample size, publication date, or type of report.

Inclusion and exclusion criteria

Studies were included in the meta-analysis that met the fol-lowing criteria: 1) case-control study design, 2) evaluation of the association between the TLR4 +896A/G (Asp299Gly) and +1196C/T (Thr399Ile) polymorphisms and risk of asthma, 3) genotype frequency was available or sufficient data could be extracted to calculate odds ratios (ORs) and 95% confidence intervals (CIs), and 4) not animal studies. For overlapping stud-ies, the most recent article or the one with the largest sample size was selected. Studies were excluded if they did not meet these inclusion criteria. Unpublished data were not considered.

Data extraction

The following data were extracted: year of publication, first au-thor, country, ethnicity, age, atopic status, detection methods, sample size, and genotype frequencies in cases and controls. Two investigators independently extracted data. Disagreements were resolved by discussion and consensus.

Statistical analysis

All the statistical analyses were conducted using STATA software version 12.0 (STATA Corporation, College Station, TX, USA). We first evaluated Hardy-Weinberg equilibrium (HWE) in the con-trol group for each study using the chi-square test, and it was considered statistically significant when $P<0.05$. A statistical test was performed based on the Q statistic to assess hetero-geneity. The $P>0.10$ of the Q test indicated a lack of hetero-geneity among studies. If heterogeneity was observed among studies, the random-effects model was used. Otherwise, the fixed-effects model was adopted. The strength of association between TLR4 +896A/G and +1196C/T polymorphisms and the risk of asthma was assessed by calculating ORs with 95% CIs. Stratified analysis was performed by ethnicity, if possible.
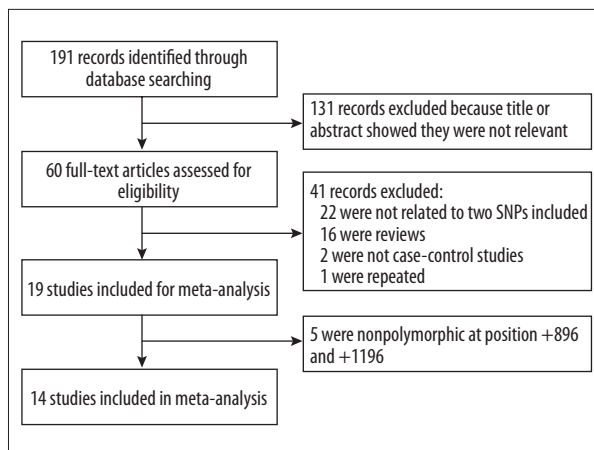
Figure 1. The process of study selection.

Potential publication bias was assessed by Begg's rank correlation test and Egger's linear regression test, and P<0.05 was considered significant publication bias.

Results

Study characteristics

The study selection process was shown in Figure 1. In total, 72 English articles and 119 Chinese articles met search criteria after we initially searched PubMed, Embase, Web of Science, CNKI, and Wanfang Data. After reading titles and abstracts, 131 articles were excluded because they did not refer to TLR4 gene polymorphisms and asthma risk. The remaining 60 articles were identified for full-text view; 22 were excluded because they investigated other gene polymorphisms, 16 were excluded because they were reviews, 2 were not case-control studies, and 1 was repeated. A total of 19 relevant studies investigating the TLR4 +896A/G and +1196C/T polymorphisms and asthma for meta-analysis were identified. However, 5 studies were monomorphic at site and were excluded from this meta-analysis [15,21,22,29,30]. Finally, 14 case-control studies were included in the present meta-analysis. The characteristics of the selected studies are listed in Table 1, with a total of 13 studies of 2649 asthma cases and 2542 controls for investigating +896A/G polymorphism and 7 studies of 1443 asthma cases and 1693 controls for +1196C/T polymorphism. As for ethnicity, 11 studies investigated white populations [13,17,19,20,23–28,31], 2 studies investigated Asian populations [16,18], and 1 study investigated the U.S. population [14]. Deviation from HWE was detected in the controls of 2 eligible studies [13,16]. The genotype frequencies of TLR4 +896A/G and +1196C/T polymorphisms in each study are presented in Table 2.

Main results of the meta-analysis

The main results of the relationship between TLR4 +896A/G and +1196C/T polymorphisms and asthma risk are listed in Table 3. Overall, no significant association between TLR4 +896A/G polymorphism and asthma risk was found in the allele model (G vs. A: OR=1.05, 95% CI=0.90–1.23, P=0.51), the dominant model (GG + AG vs. AA: OR=1.05, 95% CI=0.89-1.24, P=0.56), or the codominant models (AG vs. AA: OR=1.04, 95% CI=0.88–1.23, P=0.64). After categorizing subjects into different subgroups on the basis of ethnicity, the results remained non-significant (Figure 2). The recessive model (GG vs. AG+AA) and codominant model (GG vs. AA) were not performed due to the low frequency of the GG genotype in cases and controls.

As for TLR4 +1196C/T polymorphism, a protective association was found between TLR4 +1196C/T polymorphism and asthma in the allele model (T vs. C: OR=0.79, 95%CI=0.63–0.99, P=0.04), the dominant model (TT+CT vs. CC: OR=0.76, 95%CI=0.59–0.96, P=0.03), and the codominant model (CT vs. CC: OR=0.74, 95%CI=0.58–0.95, P=0.02). This association was not examined via the recessive model (TT vs. CT+CC) or codominant model (TT vs. CC) due to the low frequency of the TT genotype in cases and controls. The subgroup analysis by ethnicity showed that the T allele of TLR4 +1196C/T polymorphism was a significant protective gene for the development of asthma in Asians (T vs. C: OR=0.73, 95%CI=0.54-0.98, P=0.04; TT+CT vs. CC: OR=0.70, 95%CI=0.51–0.96, P=0.03; CT vs. CC: OR=0.69, 95%CI=0.50–0.96, P=0.03), but there was no statistically significant difference in whites (T vs. C: OR=0.87, 95%CI=0.60–1.26, P=0.46; TT+CT vs. CC: OR=0.84, 95%CI=0.57–1.24, P=0.39; CT vs. CC: OR=0.82, 95%CI=0.55–1.22, P=0.33) (Figures 3–5).

Test of heterogeneity, sensitivity analysis, and publication bias

There was no significant heterogeneity between any studies when analyzing the association of TLR4 +896A/G and +1196C/T polymorphisms and asthma risk in all genetic models, so we used fixed-effects models. Sensitivity analyses were conducted by altering the statistical models. No material alterations were detected, indicating that our results were statistically robust. Exclusion of the HWE-deviated studies did not meaningfully change the pooled estimates (data not shown). The funnel plot was used to evaluate publication bias, and there was no obvious asymmetry. Furthermore, no significant publication bias was detected by Begg's test and Egger's test (all P>0.05).

Discussion

Asthma is a chronic inflammatory airway disorder with complex etiologies involving both genetic and environmental

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

Study	Year	Country	Ethnicity	Age	Atopic status	Cases	Controls	Genotyping method
+896A/G								
Yang	2004	UK	Caucasian	Adults	Mixed	185	179	ARMS-PCR
Adjers	2005	Finland	Caucasian	Adults	Mixed	243	401	TaqMan
Liu	2005	China	Asian	Mixed	Atopic	197	156	PCR-RFLP
Larocca	2006	Venezuela	American	Mixed	Mixed	100	100	PCR-RFLP
Smit	2007	Denmark	Caucasian	Adults	Mixed	100	87	TaqMan
Carvalho	2008	UK	Caucasian	Adults	Atopic	14	80	Bi-PASA
Lachheb	2008	Tunisia	Caucasian	Children	Mixed	210	224	PCR-RFLP
Voronko	2011	Russia	Caucasian	Mixed	Atopic	283	227	MALDI-TOF-MS
Zaborowski	2011	Poland	Caucasian	Adults	Mixed	106	159	PCR-RFLP
Hussein	2012	Egypt	Caucasian	Children	Mixed	500	251	ARMS-PCR
Sahin	2014	Turkey	Caucasian	Adults	Mixed	131	75	RT-PCR
Sinha	2014	India	Asian	Mixed	Mixed	481	483	PCR-RFLP
Bahrami	2015	Iran	Caucasian	Adults	Mixed	99	120	PCR-RFLP
+1196C/T								
Liu	2005	China	Asian	Mixed	Atopic	197	156	PCR-RFLP
Larocca	2006	Venezuela	American	Mixed	Mixed	100	100	PCR-RFLP
Smit	2007	Denmark	Caucasian	Adults	Mixed	100	87	PCR-SSP
Lachheb	2008	Tunisia	Caucasian	Children	Mixed	210	224	PCR-RFLP
Smit	2009	France	Caucasian	Adults	Mixed	224	568	Taqman and Illumina Golden Gate assays
Sahin	2014	Turkey	Caucasian	Adults	Mixed	131	75	RT-PCR
Sinha	2014	India	Asian	Mixed	Mixed	481	483	PCR-RFLP

ARMS-PCR – amplification refractory mutation system-polymerase chain reaction; PCR-RFLP – polymerase chain reaction-restriction fragment length polymorphism; Bi-PASA – bidirectional polymerase chain reaction amplification of specific alleles; MALDI-TOF-MS – matrix-assisted laser desorption/ionization time of flight mass spectrometry; RT-PCR – real time polymerase chain reaction; PCR-SSP – polymerase chain reaction with sequence-specific primers.

contributions. Several candidate genes, such as TLR4, CD14, STAT6, ADAM33, and IL-13, have been reported to be associated with asthma susceptibility [18,33–36]. TLR4 is a principal receptor for LPS. Recognition of LPS by TLR4 plays a crucial role in the activation of subsequent immune and inflammatory responses against invaders. Numerous studies have indicated the role of TLR4 in the pathogenesis of asthma [37–40]. TLR4 is up-regulated in patients with asthma/allergic rhinitis [37]. Bortolatto et al. demonstrated that LPS impair the development of Th2 immunity, signaling via TLR4 and MyD88 molecules and via the IL-12/IFN-γ axis, and the synthetic TLR4 agonists protect against allergic asthma development [41].

Genetic polymorphisms of TLR4 gene have been demonstrated to be associated with diminished airway responsiveness to inhaled LPS [42], and to be closely involved in the susceptibility to many diseases, including asthma, juvenile spondyloarthritis, inflammatory bowel disease, and systemic lupus erythematosus [18,43–45].

Two co-segregating single-nucleotide polymorphisms, +896 A/G and +1196C/T, in human TLR4 gene that result in amino acid changes in the extracellular domain of the TLR4 protein have been widely studied. Environmental endotoxins are important triggers of asthma. These 2 variants have been reported

Table 2. Distribution of TLR4 +896A/G and +1196C/T polymorphisms among patients and controls.

Study	Cases			Controls			HWE(P)
+896A/G	AA	AG	GG	AA	AG	GG	
Yang	155	30	0	159	19	1	0.603
Adjers	202	39	2	334	64	3	0.973
Liu	161	29	7	128	23	5	0.006
Larocca	91	9	0	92	8	0	0.677
Smit	91	8	1	78	9	0	0.611
Carvalho	12	2	0	70	10	0	0.551
Lachheb	209	1	0	223	0	1	< 0.01
Voronko	245	31	7	200	27	0	0.341
Zaborowski	94	12	0	142	17	0	0.476
Hussein	434	64	2	223	27	1	0.851
Sahin	122	9	0	71	4	0	0.812
Sinha	390	87	4	381	95	7	0.699
Bahrami	85	14	0	104	16	0	0.434
+1196C/T	CC	CT	TT	CC	CT	TT	
Liu	191	4	2	150	5	1	< 0.01
Larocca	95	4	1	94	6	0	0.757
Smit	93	6	1	77	10	0	0.570
Lachheb	209	0	1	221	2	1	< 0.01
Smit	198	26	0	492	76	0	0.087
Sahin	120	11	0	71	4	0	0.812
Sinha	408	68	5	384	92	7	0.581

HWE – Hardy-Weinberg equilibrium.

to be associated with a blunted response to inhaled endotoxin on bronchial challenge testing and a reduced systemic inflammatory response to low-dose inhaled endotoxin [42,46]. Non-carriers of these polymorphisms have been found to be more frequently affected by asthma [12]. Several case-control studies have evaluated the association of TLR4 +896A/G and +1196C/T polymorphisms with asthma susceptibility. However, the results remain controversial. A previous meta-analysis has shown a marginal association of TLR4 +896A/G with asthma, and no association between TLR4 +1196C/T polymorphism and asthma [32]. However, the previous meta-analysis did not cover all eligible studies related to asthma. Therefore, to obtain a more precise conclusion we conducted this meta-analysis including all eligible case-control studies.

On the basis of 7 case-control studies including 1443 asthma cases and 1693 controls, the present meta-analysis found that TLR4 +1196C/T polymorphism might be a protective factor against the development of asthma. This result is different from the previous meta-analysis. The discrepancy stemmed from the fact that Tizaoui and coworkers only included 4 studies with 541 asthma cases and 486 controls. Next, we conducted the stratified analysis by ethnicity. A significant protective association between TLR4 +1196C/T polymorphism and asthma was detected in Asians. However, we found no significant relationship between TLR4 +1196C/T polymorphism and asthma in whites. These results suggest that the effect of TLR4 +1196C/T polymorphism on asthma risk might be influenced by ethnicity. More studies should be performed based on different ethnic groups.

Table 3. Summary ORs and 95%CI of TLR4 +896A/G and +1196C/T polymorphisms and asthma risk.

| Subgroup	Genetic model	Genotype/Allele	Type of model	Heterogeneity		Test of association		
				I²	P	OR	95%CI	P
+896A/G								
Overall	Dominant model	GG+AG *vs.* AA	Fixed	0.0%	0.99	1.05	0.89–1.24	0.56
	Codominant model	AG *vs.* AA	Fixed	0.0%	0.97	1.04	0.88–1.23	0.64
	Allele model	G *vs.* A	Fixed	0.0%	0.97	1.05	0.90–1.23	0.51
Caucasians	Dominant model	GG+AG *vs.* AA	Fixed	0.0%	1.00	1.14	0.92–1.41	0.22
	Codominant model	AG *vs.* AA	Fixed	0.0%	0.96	1.12	0.90–1.39	0.32
	Allele model	G *vs.* A	Fixed	0.0%	0.99	1.16	0.95–1.42	0.15
Asians	Dominant model	GG+AG *vs.* AA	Fixed	0.0%	0.62	0.91	0.69–1.19	0.48
	Codominant model	AG *vs.* AA	Fixed	0.0%	0.74	0.92	0.69–1.22	0.56
	Allele model	G *vs.* A	Fixed	0.0%	0.52	0.91	0.71–1.16	0.43
+1196C/T								
Overall	Dominant model	TT+CT *vs.* CC	Fixed	0.0%	0.83	0.76	0.59–0.96	0.03
	Codominant model	CT *vs.* CC	Fixed	0.0%	0.75	0.74	0.58–0.95	0.02
	Allele model	T *vs.* C	Fixed	0.0%	0.87	0.79	0.63–0.99	0.04
Caucasians	Dominant model	TT+CT *vs.* CC	Fixed	0.0%	0.52	0.84	0.57–1.24	0.39
	Codominant model	CT *vs.* CC	Fixed	0.0%	0.40	0.82	0.55–1.22	0.33
	Allele model	T *vs.* C	Fixed	0.0%	0.65	0.87	0.60–1.26	0.46
Asians	Dominant model	TT+CT *vs.* CC	Fixed	0.0%	0.84	0.70	0.51–0.96	0.03
	Codominant model	CT *vs.* CC	Fixed	0.0%	0.89	0.69	0.50–0.96	0.03
	Allele model	T *vs.* C	Fixed	0.0%	0.67	0.73	0.54–0.98	0.04
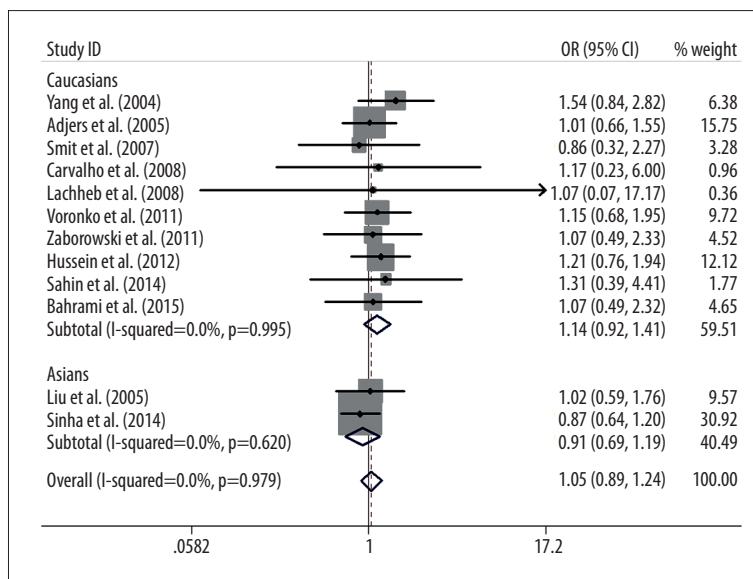
Figure 2. Forest plot of the association between TLR4 +896A/G polymorphism and asthma risk by ethnicity stratification under the dominant model (GG+AG *vs.* AA).
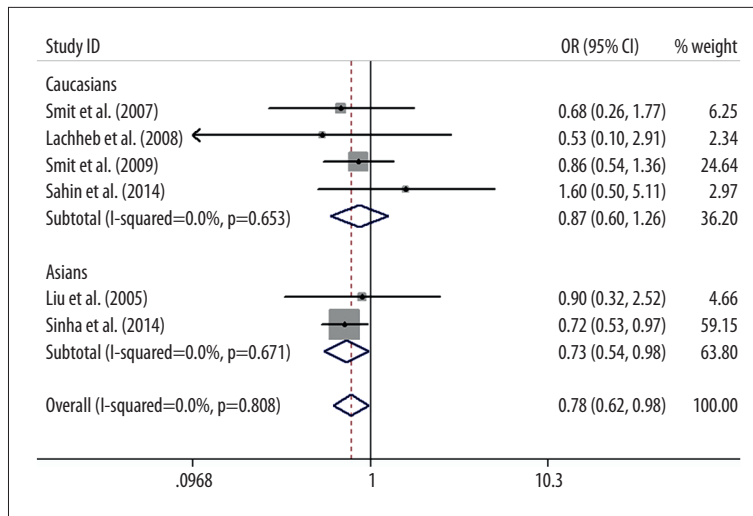
Figure 3. Forest plot of the TLR4 +1196C/T polymorphism associated with asthma risk by ethnicity stratification under the allele model (T *vs.* C).
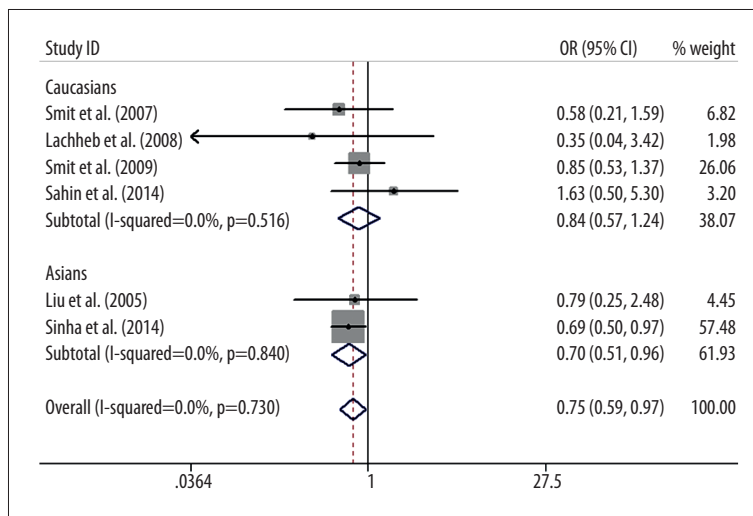

Figure 4. Forest plot of the TLR4 +1196C/T polymorphism associated with asthma risk by ethnicity stratification under the dominant model (TT+CT *vs.* CC).
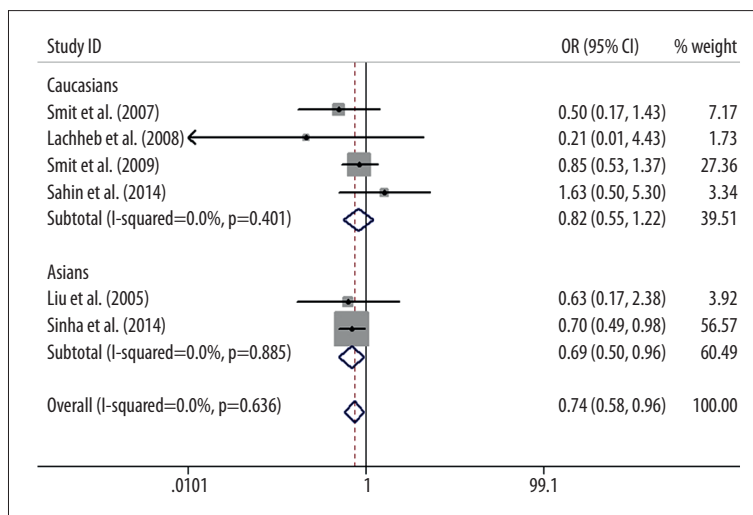

Figure 5. Forest plot of the TLR4 +1196C/T polymorphism associated with asthma risk by ethnicity stratification under the codominant model (CT *vs.* CC).

For TLR4 +896A/G polymorphism, no significant correlation was observed between TLR4 +896A/G polymorphism and asthma risk. The results remained non-significant after subgroup analysis by ethnicity. Our results are consistent with previous meta-analyses [32,47,48]. Therefore, TLR4 +896A/G polymorphism seemed not to be associated with the risk of asthma development. However, a study conducted in Turkish children with asthma observed that both TLR4 +896A/G and +1196C/T polymorphisms were statistically more frequent in the mild asthma group [49]. A strong association between TLR4 +896A/G and asthma course has been found in a Russian study, which reported that the minor G allele was associated with moderate/severe asthma [26]. In addition, the G allele has been suggested to be significantly associated with moderate-severe asthma compared to mild asthma in an Egyptian population study [27]. These findings indicate that TLR4 +896A/G polymorphism might be associated with the severity of asthma, but not susceptibility to asthma.

Several limitations in this study should be addressed. Firstly, the number of studies and subjects included in the present meta-analysis were relatively small. Secondly, only published studies with sufficient data were included, so the possibility of publication bias cannot be completely ruled out, even though funnel plot and Egger's test did not detect publication bias. Thirdly, the frequencies of GG genotype and TT genotype were low, which may undermine the findings. Moreover, the potential interactions between gene-gene and gene-environment during development of asthma were not conducted due to a lack of original data. Considering these limitations, the results of the meta-analysis should be interpreted with caution. Well-designed case-control studies with larger sample sizes and different population characteristics are needed to confirm these results.

Conclusions

In summary, this meta-analysis suggests that the T allele of the TLR4 +1196C/T might act as a protective factor against the development of asthma, but there was no significant association between TLR4 +896A/G polymorphism and risk of asthma. Larger well-designed studies based on different ethnic groups should be performed to confirm our findings.

Conflicts of interest

None of the authors have any conflicts of interest to declare.

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