Original Article
Allergy Asthma Immunol Res. 2015 March;7(2):130-140.
http://dx.doi.org/10.4168/aair.2015.7.2.130
pISSN 2092-7355 • eISSN 2092-7363

AAIR
Allergy, Asthma & Immunology Research

Association of Single Nucleotide Polymorphisms in Toll-like Receptor Genes With Asthma Risk: A Systematic Review and Meta-analysis

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Purpose: Asthma is a complex disease, with contributions from multiple genes, various genetic backgrounds, and environmental factors. Many human epidemiological studies have demonstrated that single nucleotide polymorphisms (SNPs) in Toll-like receptor (TLR) genes are inconsistently associated with asthma risk. Some have demonstrated differences concerning the study design and effect size, and conflicting results have been reported. A meta-analysis is necessary to determine the magnitude of this association. **Methods:** Following the Preferred Reporting Items for Systematic Reviews and Meta-analyses guidelines, a systematic search and meta-analysis of the literature was conducted to estimate the association of SNPs in *TLR* genes with asthma risk. We screened the medical literature based on the following keyword searches in MEDLINE and EMBASE databases: 'TLR', 'polymorphism', 'asthma', and their combinations. **Results:** Meta-analysis of eight studies on *TLR4 Asp299Gly* showed a marginal association of *TLR4* with asthma risk (odds ratio [OR]=0.814 [95% confidence interval [CI], 0.652-1.016; *P*=0.069]) in the recessive model. *TLR4 Thr399Ile* was not associated with asthma risk under any genetic model. Meta-analysis of four studies on *TLR2 Arg753Gln* indicated that *TLR2* might be significantly associated with asthma in the dominant and codominant models (*P*=0.029, *P*=0.030, and *P*=0.009, respectively). *TLR9 -1237* was marginally associated with asthma risk (OR=0.408 [95% CI, 0.163-1.021; *P*=0.065]) in the codominant model. Analysis using the allele contrast model showed that the major *TLR9 -1237 T* allele tended to be a significant protective factor with OR=0.689 (95% CI, 0.471-1.007; *P*=0.055). **Conclusions:** The results showed that *TLR4 Asp299Gly*, *TLR2 Arg753Gln*, and *TLR9-1237* might contribute significantly to asthma susceptibility. Future genetic association studies would consolidate these findings.

Key Words: Asthma; *TLR* genes; SNPs; association; meta-analysis

INTRODUCTION

Asthma is a chronic and complex disorder of the respiratory system characterized by airway obstruction and inflammation,[1] and its prevalence is increasing in both developed and developing countries.[2,3] This increased prevalence may reflect increased exposure to environmental risk factors. Although environmental factors are important determinants of asthma, numerous studies have revealed that asthma has a strong genetic component. Susceptibility genes have been identified by candidate gene association studies, genome-wide linkage studies, and genome-wide association studies.[4] Many genes have been shown to make small contributions to the overall phenotype.

Toll-like receptors are an essential family of innate immune pattern recognition receptors that play a pivotal role in host defense against microbes.[5] TLRs can modulate the immune system through cellular activation, modulation of cytokine secretion[6] and production of soluble factors to local dendritic networks.[7] Human epidemiological studies have supported the common clinical perception that TLRs are associated with asthma. These studies have varied in design, population composition, asthma definition and size. Macrophages, one of the main immune cell types involved in asthma, express various TLRs, including *TLR2, 4, 5, 6, 7, 8,* and *9*.[8] Each TLR recognizes different classes of molecules expressed by pathogens and recogniz-

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Received: January 9, 2014; Revised: April 13, 2014; Accepted: May 2, 2014
• There are no financial or other issues that might lead to conflict of interest.

es structurally conserved motifs.[9]

TLR1 and *6* recognize multiple diacyl peptides, and *TLR1/2* dimers can recognize lipopeptides.[10] *TLR2*, which has been found to be expressed by various cell types,[11] is related to protection against allergies and allergic asthma by sensing pathogen-associated patterns in lipoproteins and lipopeptides. The *TLR2/6* dimer is involved in the recognition of diacylated lipopeptides.[12] Long-term stimulation of *TLR3* upregulates the production of inflammatory cytokines and cellular recruitment to the airways.[13] *TLR4* encodes a macrophage cell-surface receptor, which is principally activated by bacterial endotoxin, also known as lipopolysaccharide (LPS). Few studies of the role of *TLR5* in asthma have been reported. However, a significant decrease in the expression of *TLR5* was observed in asthma patients,[14] and TLR5 recognizes bacterial flagellin when complexed with *TLR4*.[15] Similar to *TLR5*, few data exist on the role of *TLR6* in allergic asthma.[12] *TLR6* is expressed on mast cells, which play important roles in allergy.[16] *TLR7* and *TLR8*, which are localized on the sex chromosome Xp22,[17] recognize single-stranded RNA, and induce interferons (IFNs) to protect the host from viral infection.[18] *TLR9* detects bacterial or viral DNA with unmethylated cytosine and guanine.[8] *TLR10* is the most recently identified gene in the TLR family whose product recognizes pathogen-associated molecular patterns (PAMPs).[19] TLRs are also involved in various signaling pathways of the immune system that protect from asthma or develop asthma phenotypes.[20-25]

Most studies published since 2004 have comprised genetic association investigations in various clinical settings, and have found positive associations of SNPs in *TLR2*,[26-28] *TLR4*,[29] *TLR6*,[30] *TLR7/8*,[31] *TLR9*,[32] and *TLR10*[19] with asthma. Recent insights into the complex mechanisms of human innate immunity have suggested that genetic variability in genes may play a role in the development of asthma and related diseases.[34-38]

Due to the important contribution of TLR genes to asthma, an updated meta-analysis was conducted to estimate the effect of SNPs in TLR genes on asthma susceptibility.

MATERIALS AND METHODS

Identification of eligible studies

The review process followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines.[39] We performed a literature search using the MEDLINE and EMBASE databases to identify articles that examined associations between TLR polymorphisms and asthma. Combinations of keywords, such as 'TLR', 'polymorphism', and 'asthma' were entered as medical subject heading (MeSH) and text words. References in the identified studies were used to identify additional studies not indexed by MEDLINE or EMBASE databases. No language or date restrictions were applied.

Inclusion and exclusion criteria

Studies identified from the searches were screened and excluded from further analysis if one of the following reasons was satisfied: a review article, lack of information, animal research, not case-control or nested case-control study design, or unreported genotype frequencies.

For inclusion, the studies must have met the following criteria: (1) they evaluated TLR gene polymorphisms and asthma; (2) were case-control studies; (3) supplied the number of individual genotypes in asthmatic cases and in controls, respectively; or (4) they had an asthma outcome definition that followed accepted diagnostic guidelines.

Data extraction

Information was extracted carefully from all of the eligible publications independently by 2 authors, based on the inclusion criteria above. The following information was extracted from each study: author, year of study publication, ethnicity of the study population, demographics (age), asthma status, and number of cases and controls for each TLR polymorphism.

Statistical analysis

Data from the studies were combined to provide a summary odds ratio (OR). Summary ORs were represented as a point estimate with 95% confidence intervals (CIs) on a forest plot.[40] The heterogeneity of the data was evaluated using the *Q* statistic.[41] The stability of the summary risk estimate was evaluated using a sensitivity analysis in which each study was removed individually, and the OR was recalculated. Egger's regression test was used to identify publication bias.[42]

RESULTS

Study inclusion and characteristics

The initial search using the headings 'TLR', 'polymorphism' 'asthma', and their combinations resulted in approximately 6,395 journal articles. Based on the titles, 110 were potentially relevant. Nineteen studies investigating general atopic and allergic phenotypes were excluded. An additional 25 studies on TLR polymorphism involvement in signaling pathways were excluded. Review articles (n=28) were also excluded. Seventeen studies were excluded because they did not meet the inclusion criteria: eight did not report genotypic frequencies in cases and/or in controls[24,25,38,43-47]; two were cross-sectional studies[48,49]; four were family-based studies,[50-53] and 3 were abstracts.[54-56] Twenty case-control studies were eligible for meta-analysis,[19,26-33,57-67] one study was in Portuguese,[62] and another was in Chinese.[64] Among the eligible studies, 11 were excluded because the number of studies of the same polymorphism was less than 3.

The controls for the studies by Lachheb *et al.*[32] and Zhang *et al.*[29] deviated from Hardy-Weinberg equilibrium (HWE) in the

controls ($P<0.01$). The study by Caravalho *et al.*[61] was small, and the definition of asthma was restricted to severe asthma with fungal sensitization. In the study by Palikhe *et al.*,[65] the definition of asthma was limited to aspirin-tolerant asthma. In the study by Yang *et al.*,[58] three sets of patients (first-affected sibling, second-affected sibling and asthmatic parents) were compared with hyper-normal controls. A meta-analysis was conducted when at least 3 studies on the same polymorphism were available. Fig. 1 provides a summary of the search results. Table 1 summarizes the characteristics of the case-control studies.

Data analysis

Association of TLR4 Asp299Gly with asthma risk

Analyses in the random effects model showed that the *TLR4 Asp299Gly* polymorphism was marginally associated with asthma under the recessive (0.069) (Fig. 2) and allele contrast (0.055) models. In the allele contrast model, sensitivity analysis by exclusion of any one particular study[60] in the influence analysis significantly changed the results. The recalculated OR was 0.803 (95% CI, 0.647-0.997; $P=0.047$), indicating that the major A allele might protect against asthma (Table 2).

The estimated OR1 (AA vs GG), OR2 (AG vs GG) and OR3 (AA vs AG) were 0.897, 0.831, and 0.972, respectively. These estimates were likely to suggest a codominant genetic model. The pooled OR1 was 0.897 (95% CI, 0.597-1.346; $P=0.599$). Heterogeneity was absent (0.00%). No evidence of publication bias was detected by Egger's test ($P=0.12$). Sensitivity analysis by the removal of one study did not significantly change the results (Table 2).

Association of TLR4 Thr399Ile C>T with asthma risk

Analyses in the random-effects model showed that the *TLR4 Thr399Ile* polymorphism was not associated with asthma under any of the genetic models.

The estimated OR1 (CC vs TT), OR2 (CT vs TT) and OR3 (CC vs TC) were 1.223, 1.306, and 0.398, respectively. These estimates did not fit any genetic model. Analysis in the allele contrast model showed no significant association of the *TLR4 Thr399Ile* C allele with asthma risk (OR=1.223 [95% CI, 0.655-2.286; $P=0.528$]) (Fig. 3). Heterogeneity was absent in all of the models (0.00%). Sensitivity analysis by the removal of one study did not significantly change the results. Publication bias was detected by Egg-

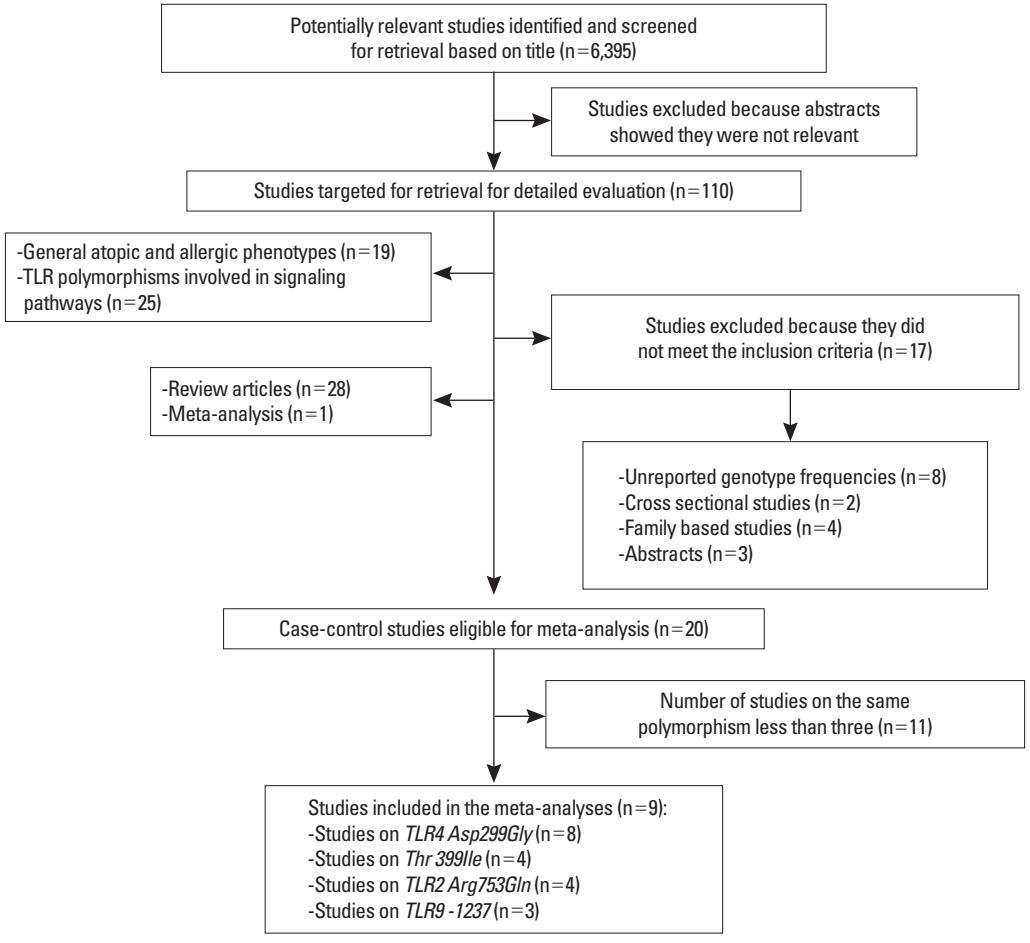

Fig. 1. Flow diagram of the systematic review and meta-analysis literature search results.

Table 1. Characteristics of the reviewed studies on SNPs in *TLR* genes and asthma risk

Reference	Ethnicity	Age: Case/control	Gender	Gene	Polymorphism	Case	Control	Results
Lazarus 2003	European–American	Matched	Matched	*TLR9*	rs5743836 T/C	64	114	SA
Noguchi 2004	Japanese (Chinese)	Children/46.9	Matched	*TLR2*	c. -191 G>A	133	186	NS
					c. 597 T>C	133	188	NS
					c. 1350 T>C	134	190	NS
				TLR3	-7A	135	189	NS
					IVS3 +71 C>A	134	188	NS
					c. 1377 C>T	133	189	NS
				TLR9	c. 1635 G>A	132	189	NS
Tantisira 2004	African–American	Matched	Matched	*TLR6*	Ser249Pro C>T	56	97	SA
Lazarus 2004	European–American	Matched	Matched	*TLR10*	c.+1031 G>T	506	514	SA
					c.+2322 A>G	505	513	SA
Yang 2004	English (Caucasian)	Matched	Matched	*TLR4*	rs4986790 A>G	320	184	NS
		Matched	Matched	*TLR4*	rs4986790 A>G	309	184	NS
		Matched	Matched	*TLR4*	rs4986790 A>G	185	184	NS
Hoffjan 2005	German (Caucasian)	38/59	Matched	*TLR6*	Ser249Pro C>T	68	212	NS
		9.5/59			Ser249Pro C>T	132	212	NS
Smit 2007	Danish (Caucasian)	19.2/matched	Matched	*TLR2*	rs4696480 A>T	100	87	NS
					rs5743704 C>A	100	87	NS
					rs5743708 C>T	100	87	NS
				TLR4	rs4986790 A>G	100	87	NS
					rs4986791 C>T	100	87	NS
Caravalho 2008	European (Caucasians)	57.6±11.8/not mentioned	Matched	*TLR2*	rs5743708 G>A	14	80	NS
				TLR4	rs4986790 A>G	14	80	NS
				TLR9	rs5743836 T>C	14	80	NS
Lachheb 2008	African Caucasian	10.5/8	Matched	*TLR2*	rs5743708 C>T	210	224	NS
				TLR4	rs4986790 A>G	210	224	NS
					rs4986791 C>T	210	224	NS
				TLR9	rs5743836 T>C	210	224	SA
					rs187084 T>C	210	224	NS
Larocca 2008	Venezuelan (American)	44.6±15.22/42.63±13.89	Matched	*T LR4*	rs4986790 A>G	100	100	NS
					rs4986791 C>T	100	100	NS
Bjørnvold 2009	Norwegian (Caucasian)	Children	Matched	*TLR2*	Rs3804100 T>C	108	494	SA
				TLR2	Rs3804099T>C	108	494	NS
Hseih 2009	Taiwanese (Asian)	7.2±2.4/Children	Matched	*TLR4*	rs10983755 A>G	117	60	NS
					rs1927914 C>T	117	60	NS
Zhang 2009	Chinese (Asian)	39.80±14.23/34.27±13.31	Male	*TLR7/8*	rs5935436 C>T	135	200	SA
					rs3761623 A>G	135	200	NS
					rs3764880 G>A	135	200	NS
		39.80±14.23/34.27±13.31	Female	*TLR7/8*	rs5935436 C>T	183	152	NS
					rs3761623 A>G	183	151	SA
					rs3764880 G>A	182	152	SA
Qian 2010	Chinese (Asian)	39.80/38.26	Matched	*TLR1*	rs4833095 C>T	318	351	NS
				TLR2	rs7656411 G>T	317	351	SA
				TLR6	rs5743831 A>G	318	352	NS
					rs5743808 T>C	318	351	NS
					rs2381289 C>T	318	352	NS
				TLR10	rs11466651 G>A	318	352	NS
					rs11466655 A>G	318	351	NS
					rs4504265 C>A	317	349	NS

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Table 1. Continued

Reference	Ethnicity	Age: Case/control	Gender	Gene	Polymorphism	Case	Control	Results
Zhang 2011	Chinese (Asian)	39.80/38.26	Matched	*TLR4*	rs1927914 T>C	318	350	SA*
					rs10983755 G>A	318	351	
					rs11536879 A>G	304	348	
					rs1927907 G>A	314	334	
Palikhe 2011	Korean (Asian)	43.1±15.2/32.1±12.7	Matched	*TLR3*	rs3775296 G>T	254	274	NS
					rs3775291 G>A	254	274	NS
Vorvonko 2011	Russian (Caucasian)	Adults/38.5±10.4	Matched	*TLR4*	rs4986790 A>G	283	227	NS
Qian 2011	Chinese (Asian)	1.9-11.6/matched	Matched	*TLR9*	rs187084 C/T	312	339	NS
Hussein 2012	African Caucasian	8.4±2.6/10.1±2.3	Matched	*TLR2*	rs5743708 G>A	500	250	SA*
				TLR4	rs4986790 A>G	500	251	
Şahin 2014	Turkish (Caucasian)	36±12.42/43±7.68	Matched	*TLR4*	rs4986790 A>G	131	75	NS
					rs4986791 C>T	131	75	SA

NS, non significant association; SA, significant association; SA*, significant association with asthma severity.

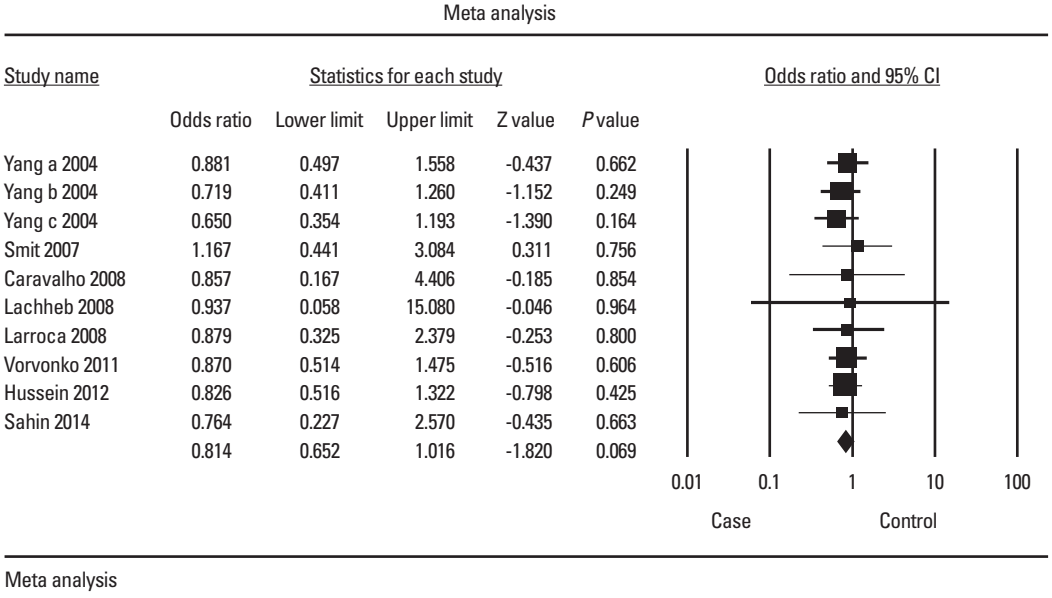

Fig. 2. Forest plot of the association between *TLR4 Asp299Gly* and asthma risk: AA vs AG+GG. The forest plot shows the odds ratios (ORs) and respective 95% confidence intervals (CIs) for the studies included in the meta-analysis. For each study in the forest plot, the area of the black square is proportional to the study weight, and the horizontal bar represents the 95% CI. Z score: the standardized expression of a value in terms of its relative position in the full distribution of values.

er's test in the dominant and CC vs TC codominant models (P=0.05 and P=0.03, respectively) (Table 2).

Association of TLR2 Arg753Gln G/A with asthma risk

Analyses showed that the *TLR2 Arg753Gln* polymorphism was not associated with asthma under the recessive, homozygous (Fig. 4) and allele contrast models. In the homozygous model, sensitivity analysis by the removal of one study[61] significantly changed the results. The recalculated OR was 4.460 (95% CI, 1.213-16.396), indicating that the homozygous genotypes might be a significant risk factor (P=0.024). In the GG vs GA model, when one study was removed,[61] the recalculated OR was 4.365 (95% CI, 1.227-15.536), suggesting that the homozygous genotype might be a significant risk factor (P=0.023). Sensitivity analysis by the removal of one study[60] in the allele contrast model significantly changed the results. The recalculated OR was 0.402 (95% CI, 0.199-0.811), indicating that the major G allele might protect significantly against asthma (P=0.011).

The estimated OR1 (GG vs AA), OR2 (GA vs AA) and OR3 (GG vs GA) were 0.102, 0.01, and 2.451, respectively. These estimates did not fit any genetic model. Only three studies were available; therefore, estimates were not sufficiently powerful. However, these estimates provided a first indication that genotypes with at least one copy of the major G allele might be protective. No

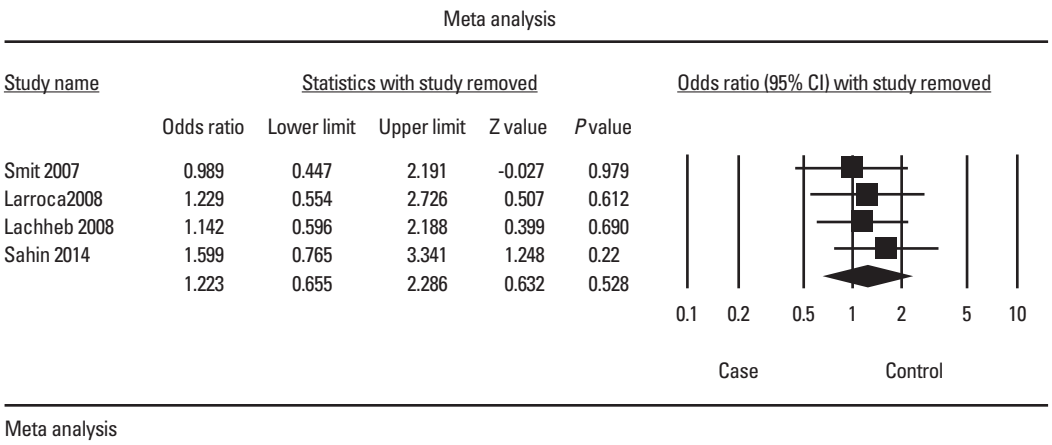

Fig. 3. Forest plot of the association between *TLR4 Thr399Ile* and asthma risk: C allele vs T allele. The forest plot shows the ORs and respective 95% CIs for the studies included in the meta-analysis. For each study in the forest plot, the area of the black square is proportional to the study weight, and the horizontal bar represents the 95% CI. Z score: the standardized expression of a value in terms of its relative position in the full distribution of values.

Table 2. Association of *TLR4 Asp299Gly A/G*, *TLR4 Thr399Ile C>T*, *TLR2 Arg753Gln G/A* and *TLR9 -1237 T/C* with asthma risk

TLRs	Genetic model		OR	*P*-value	I^2	Egger *P*
TLR4 Asp299Gly A>G (n=8)	Recessive	AA vs AG+GG	0.814 (0.652–1.016)	**0.069**	0.000	0.25
	Homozygous	GG+AA vs AG	0.835 (0.666–1.047)	0.118	0.000	0.40
	Dominant	AA+AG vs GG	0.857 (0.297–2.477)	0.776	0.000	0.42
	Codominant*	AA vs GG	0.897 (0.597–1.346)	0.599	0.000	0.12
	Codominant*	AG vs GG	0.831 (0,663–1.042)	0.110	0.000	0.40
	Codominant*	AA vs AG	0.972 (0.320–2.953)	0.960	0.000	0.28
	Allele contrast	A allele vs G allele	0.814 (0.659–1.005)	**0.055**	0.000	0.05
TLR4 Thr399Ile C>T (n=4)	Recessive	CC vs TC+TT	0.784 (0.571–1.075)	0.130	0.00	0.15
	Homozygous	CC+TT vs TC	1.315 (0.688–2.513)	0.408	0.00	0.45
	Dominant	CC+TC vs TT	0.708 (0.111–4522)	0.715	0.00	0.05
	Codominant	CC vs TT	1.223 (0.655–2.286)	0.527	0.00	0.34
	Codominant	CT vs TT	1.306 (0.683–2.497)	0.419	0.00	0.45
	Codominant	CC vs TC	0.398 (0.053–3.006)	0.372	0.00	0.03
	Allele contrast	C allele vs T allele	1.132 (0.623–2.056)	0.684	0.00	0.34
TLR2 Arg753Gln G>A (n=4)	Recessive	GG vs GA+AA	0.968 (0.441–2.123)	0.935	18.71	0.37
	Homozygous	GG+AA vs GA	2.476 (0.483–12.698)	0.277	57.34	0.30
	Dominant	GG+GA vs AA	0.100 (0.013–0.789)	**0.029**	0.00	-
	Codominant	GG vs AA	0.102 (0.013–0.803)	**0.030**	0.00	-
	Codominant	GA vs AA	0.01 (0.001–0.312)	**0.009**	24.25	-
	Codominant	GG vs GA	2.451 (0.483–12.441)	0.279	56.82	0.30
	Allele contrast	G allele vs A allele	0.635 (0.228–1.771)	0.385	52.24	0.47
TLR9 -1237 T>C (n=3)	Recessive	TT vs TC+CC	0.950 (0.570–1.583)	0.223	33.33	0.41
	Homozygous	TT+CC vs TC	1.267 (0.867–1.852)	0.222	0.00	0.39
	Dominant	TT+TC vs CC	0.684 (0.090–5.192)	0.714	85.96	-
	Codominant*	TT vs CC	0.408 (0.163–1.021)	**0.065**	30.95	-
	Codominant*	TC vs CC	0.498 (0.206–1.202)	0.121	16.83	-
	Codominant*	TT vs TC	0.876 (0.584–1.315)	0.525	0.00	0.12
	Allele contrast	T allele vs C allele	0.689 (0.471–1.007)	**0.055**	18.73	0.27

TLR4 Asp299Gly (rs 4986790); *TLR4 Thr399Ile* (rs4986791); *TLR2 Arg753Gln* (rs5743708); *TLR9 -1237* (rs187084); Bold: significant *P* value (<0.05). *TLR4 Asp299Gly* polymorphism: one study removed,[60] OR=0.803 (0.647-0.997); *P*=0.047 in the allele contrast model. *TLR2 Arg753Gln* polymorphism: one study removed,[61] OR=4.460 (1.213-16.396); *P*=0.024 in the homozygous model; one study removed,[61] OR=4.365 (1.2265-15.536); *P*=0.023 in the GG vs GA codominant model; one study removed,[60] OR=0.402 (0.199-0.811); *P*=0.011 in the allele contrast model.
n, number of studies; OR, odds ratio; I^2, heterogeneity test.
*best-fitted genetic model.

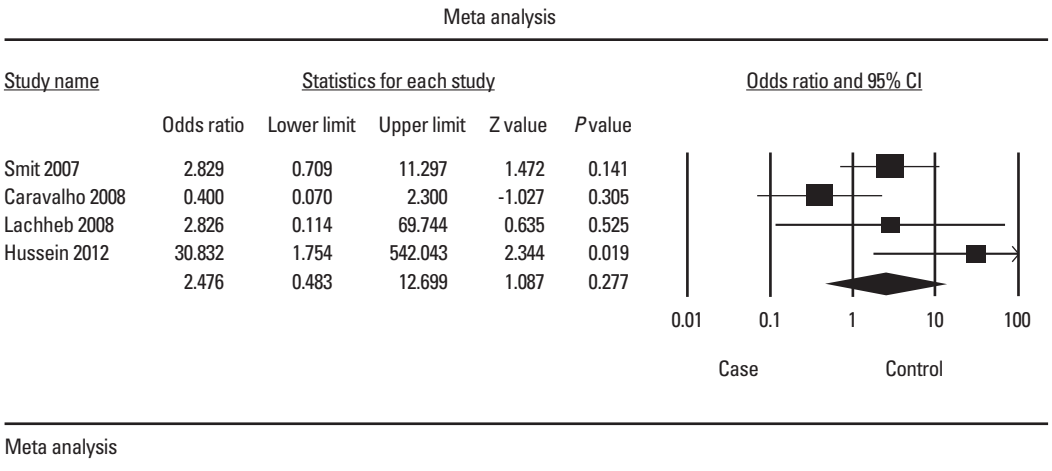

Fig. 4. Forest plot of the association between *TLR2 Arg753Gln* and asthma: GG+AA vs GA. The forest plot shows the ORs and respective 95% CIs for the studies included in the meta-analysis. For each study in the forest plot, the area of the black square is proportional to the study weight, and the horizontal bar represents the 95% CI. Z score: the standardized expression of a value in terms of its relative position in the full distribution of values.

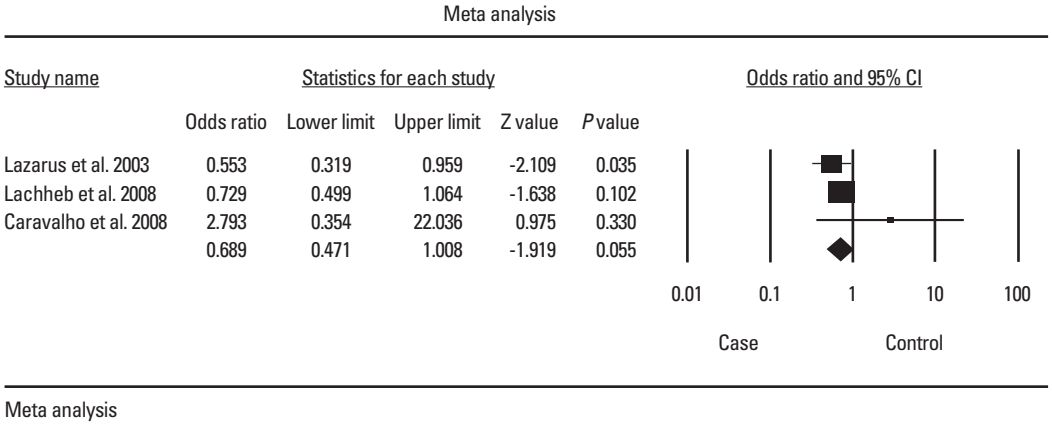

Fig. 5. Forest plot of the association between *TLR9 -1237* and asthma: T allele vs C allele. The forest plot shows the (ORs and respective 95% CIs for the studies included in the meta-analysis. For each study in the forest plot, the area of the black square is proportional to the study weight, and the horizontal bar represents the 95% CI. Z score: the standardized expression of a value in terms of its relative position in the full distribution of values.

evidence of publication bias was detected by Egger's test in all of the genetic models (Table 2).

Association of TLR9 -1237 T/C with asthma risk

Analyses showed that the *TLR9 -1237 T/C* polymorphism was not associated with asthma under the recessive, homozygous and codominant models. In the allele contrast model, the major T allele was marginally associated with asthma with OR=0.689 (95% CI, 0.471-1.007; *P*=0.055), suggesting that the major T allele might be a significant protective factor (Table 2 and Fig. 5).

The estimated OR1 (TT vs CC), OR2 (TC vs CC) and OR3 (TT vs TC) were 0.408, 0.01, and 0.498, respectively. These estimates likely suggested a codominant genetic model. Only three studies on *TLR9 -1237 T/C* were available; therefore, estimates were not sufficiently powerful. However, these estimates indicated that the TT genotype tended to be a significant protective factor (*P*=0.065). No evidence of publication bias was detected by Egger's test in all of the genetic models (Table 2).

DISCUSSION

During the last decade, the function of TLRs in asthma pathogenesis has been investigated largely by comparing the incidence of disease among persons with different polymorphisms. The magnitude of the association of polymorphisms with asthma varies depending on genetics, demographics, environmental factors, and study methodologies. Many association studies have reported that TLR polymorphisms predispose to asthma.[26,47,49,52,56,59,61,68] However, some polymorphisms might not be associated with asthma susceptibility, but rather with asthma severity, such as *TLR4* polymorphisms.[48,51,58] Conflicting results were also reported, demonstrating a protective effect of some polymorphisms.[12,45,46] However, other studies reported no association between TLR polymorphisms and asthma risk.[44,50,53,60,61,

[64,69,70] Given the conflicting results yielded by genetic association studies, a systematic review and meta-analysis is of great value. The current meta-analysis provides a comprehensive examination of the available evidence concerning the association of SNPs in *TLR* genes with asthma and refines their risk profiles. This analysis represents the first meta-analysis concerning the impact of some *TLR* polymorphisms on asthma and assesses their role as modifiers of asthma risk.

Estimates of combined effect sizes revealed that the major *TLR9 -1237 T* allele was significantly associated with a decreased asthma risk. Additionally, for *TLR2 Arg753Gln G>A*, sensitivity analysis in the allele contrast model revealed that the major G allele was significantly associated with a decreased asthma risk. For *TLR4 Asp299Gly A>G*, the AA homozygote genotype and A allele tended to be associated with a decreased asthma risk. These results implicate that the major allele in *TLR* polymorphisms might be generally associated with a protective effect. However, *TLR4 Thr399Ile* showed no significant association with asthma risk. In a previous meta-analysis, Chen[71] reported no direct association between *TLR4 Asp299Gly* and asthma. This result is consistent with our findings in the dominant, homozygous and codominant models. In a recent systematic review, Klassen *et al.*[72] reported that *TLR 2, TLR6, TLR9*, and *TLR10* appear to have some association with childhood asthma in Caucasians. However, no convincing evidence for a role of TLRs in relation to childhood asthma exists. Polymorphisms in *TLR3* seemed not to be associated with asthma.[57,65] Results for *TLR6* are conflicting. One study with a small sample size showed that *TLR6 Ser249Pro C>T* contributed to asthma susceptibility,[30] but a larger study on the same polymorphism reported no association in both children and adults.[59] However, other studies reported that polymorphisms in *TLR6* were significantly associated with asthma phenotypes.[44,45,56,73] Similarly, some polymorphisms in *TLR7/8* have been shown to be associated with asthma disease,[29,33] whereas other polymorphisms showed no association.[29] For *TLR10*, some polymorphisms showed significant association with asthma[23,33,45]; however, other polymorphisms showed no significant association.[27,45] Genetic association studies on *TLR1* and *TLR5* remain scarce or are lacking. Further investigations on *TLR* polymorphisms, including haplotype analysis along with gene-environment interaction, are needed.

SNPs, which occur at every 1.9 kb in the genome on average,[73] could result in reduced or increased gene expression when they occur in promoter.[74] This meta-analysis did not control for multiple known risk factors for asthma, such as age, gender, and ethnicity. These potential confounding factors may interact with *TLR* polymorphisms to moderate asthma susceptibility. Genetic associations were shown to be significantly influenced by age[75-78] likely because some genes are expressed in adults and not expressed in children and *vice versa*. Observational studies showed that the prevalence of asthma has increased during the last decade due to environmental factors.[3] Therefore, it is important to investigate other polymorphisms in *TLRs*, as well as gene-gene and gene-environment interactions. A possible gene-environment interaction has been reported,[79] in which the SNPs act as modifiers of asthma risk in individuals with various degrees of environmental endotoxin exposure. Future studies should include haplotype analysis and stratification of the study population according to related environmental risk factors.

Asthma is a complex disease involving several genes that might be more relevant than *TLR* genes. Although statistically significant, the effect of the *TLRs* on asthma was modest. This result may reflect the important role of other confirmed candidate genes in asthma disease. It has been reported that many genes are involved and make small contributions to the overall asthma phenotype.

The findings of our meta-analyses are consistent with experimental studies demonstrating that *TLR* polymorphisms are important factors in asthma disease, and support the need for further research into the mechanisms underlying asthma susceptibility. The significant association between *TLR* polymorphisms and asthma risk may be of clinical and public health importance. Further meta-analyses and studies on the expression of related genes, environmental factors, and gene-gene and gene-environment interactions would provide useful information for the prevention and treatment of asthma.

The recent discovery of TLRs, with their role as initiators of the innate immune response and inflammation, suggests that modulating these receptors may be beneficial in the treatment of allergic disorders.[7] TLRs are currently being exploited as possible targets for drug development.[80] Recently, several patents aimed to modulate the innate immune reaction occurring in asthma through the use of novel synthetic TLR2 ligands[8,11]; these open new therapeutic perspectives for the prevention of these pathologies. Greater knowledge of risk factors will translate into improved diagnosis, prevention and therapeutic strategies for this chronic disease.

ACKNOWLEDGMENTS

This work was supported by a grant from the Ministry of Higher Education, Tunisia.

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