

GENETICS

Association between the *TLR4* +896A>G (Asp299Gly) Polymorphism and Asthma: A Systematic Review and Meta-Analysis

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Objective. To determine whether there is an association between the toll-like receptor 4 (*TLR4*) +896A>G single nucleotide polymorphism and asthma by conducting a systematic review and meta-analysis. **Methods.** The review was conducted according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. A systematic search for relevant studies was performed using PubMed (MEDLINE), Scopus, and HuGE Literature Finder databases with additional consultation of the reference lists of included studies. Summary odds ratios (ORs) and corresponding 95% confidence intervals (CIs) were calculated for the allelic comparison (G vs. A) and the genotypic comparison assuming a dominant genetic model (AG + GG vs. AA). I^2 statistics were calculated to assess the presence of between-study heterogeneity and funnel plots were inspected for indication of publication bias. Sensitivity analysis was performed to evaluate the influence of individual studies on the overall effect estimates. **Results.** Meta-analysis of nine studies consisting of 1838 asthma cases and 1764 controls did not find a significant association between *TLR4* +896A>G and asthma (genotypic OR = 1.12, 95% CI: 0.91–1.39, $p = .27$). Between-study heterogeneity was not detected ($I^2 = 0\%$) and publication bias was not evident. Sensitivity analysis demonstrated the stability of the null association. **Conclusions.** The meta-analysis findings suggest a lack of direct association between the *TLR4* +896A>G polymorphism and asthma, but gene–environment and gene–gene interaction effects and other considerations involving this polymorphism may exist. Therefore, further study is necessary to fully elucidate the role of *TLR4* +896A>G in asthma.

Keywords endotoxin, gene–environment interaction, gene–gene interaction, lipopolysaccharide, single nucleotide polymorphism, toll-like receptor 4

INTRODUCTION

Asthma is a chronic inflammatory disease of the lung with a growing global burden and a complex etiology. According to 2010 estimates, asthma affected approximately 300 million people worldwide and caused 250,000 deaths annually (1). Atopic asthma is the most common asthma phenotype (2) with symptoms precipitated by environmental allergens such as bacterial endotoxin, or lipopolysaccharide (LPS), which is a cell wall component of Gram-negative bacteria that is ubiquitous in the environment (3). The exact etiology of asthma remains unknown; however, multiple genetic and environmental factors and their interactions have been implicated (4), including the gene encoding toll-like receptor 4 (*TLR4*).

TLR4 is a surface receptor expressed in macrophages and other cell types (5). The receptor detects LPS (6) and plays an important role in the LPS-mediated activation of the innate immune system (7). An A-to-G missense nucleotide substitution at position +896 in the *TLR4* gene (rs4986790; also reported as Asp299Gly) has been demonstrated to result in the reduction of cell surface expression of *TLR4*, thereby disrupting LPS-mediated signaling and altering host immune response to microbial agents (8). This genetically determined alteration in LPS responsiveness may be involved in the development and severity of immune-mediated diseases such as asthma. The *TLR4* +896A>G single nucleotide polymorphism

(SNP) has been shown to modify the association between asthma and LPS exposure; asthma risk was significantly increased with elevated LPS levels in 896A allele carriers, whereas the relationship was nonsignificant among 896G carriers (9). Increased atopy severity was also observed among asthmatics harboring the 896G allele (10). Several studies have reported the association between the *TLR4* +896A>G polymorphism and asthma risk, including an early case-only study reporting a significant association in children (11).

There has been continued interest in examining this potential gene–disease relationship, but no serious attempt has been made to review the existing literature. Therefore, to appraise and synthesize the accumulated evidence on the association between the *TLR4* +896A>G polymorphism and asthma, a systematic review and meta-analysis of the literature were conducted.

METHODS

Search Strategy

This review conformed to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (12). PubMed (MEDLINE) and Scopus databases were searched on 12 July 2012 using a combination of the following keywords: asthma; toll-like receptor 4 or *TLR4*; and mutation, polymorphism, or variant. Abstracts of studies listed under the asthma phenotype and *TLR4* gene in the HuGE Literature Finder were also retrieved. The reference lists of included studies were additionally consulted for

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relevant studies. No language or publication date restrictions were applied.

Studies identified from the databases were excluded from review if the following criteria were met: non-asthma phenotype, review article, conference abstract, basic science, or animal research. Full text of the remaining studies was abstracted and studies were further excluded from meta-analysis based on the following criteria: not a case-control study design, no reported allelic/genotypic frequencies, or multiple studies with shared subjects. In the latter case, the study with the largest sample size was included. Study identification and data extraction were conducted independently by me and my colleague. Discrepancies were resolved by discussion and consensus.

Statistical Analysis

Meta-analysis was performed using Review Manager 5.1 (Nordic Cochrane Centre, Cochrane Collaboration, Copenhagen, Sweden). Hardy-Weinberg equilibrium (HWE) was checked using the chi-square goodness-of-fit test in the controls of each study. As the +896GG genotype is rare (<1% in most populations (13)), only the allelic comparison (G vs. A) and the genotypic comparison assuming a dominant genetic model (AG + GG vs. AA) were performed, with the minor allele (+896G) considered the risk allele. A fixed-effect inverse variance analysis method was used to estimate the summary odds ratios (ORs) and corresponding 95% confidence intervals (CIs). Between-study heterogeneity for the comparisons was assessed using the I^2 statistics, where a value of <25% indicates an absence of significant heterogeneity (14). A sensitivity analysis was conducted to assess the relative influence of each study on the pooled estimates by omitting one study at a time. Similarly, the impact of HWE-deviated studies on the pooled estimates was evaluated by excluding the affected studies. Publication bias was assessed visually using funnel plots. Subgroup analysis by ethnicity was performed to evaluate its effect on the association.

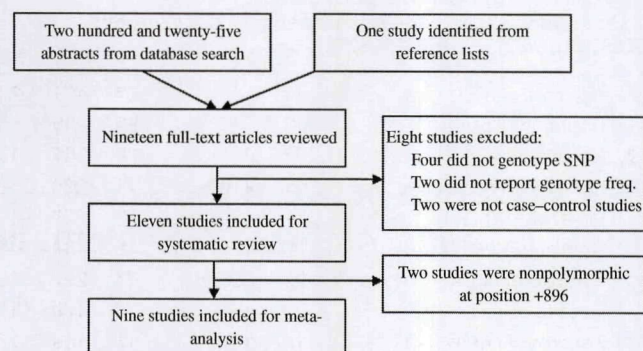


FIGURE 1.—Flow diagram of the systematic review and meta-analysis of study selection process.

RESULTS

The study selection process is shown in Figure 1. A total of 11 relevant studies (10, 15–24) investigating the *TLR4* +896A>G polymorphism and asthma for meta-analysis were identified. However, a Singaporean Chinese population (20) and a Japanese population (21) were monomorphic at site and were excluded from meta-analysis. Therefore, a total of nine case-control studies were included in the meta-analysis with a total of 3602 unique subjects ($n = 1838$ asthma cases and 1764 controls). Six of nine study populations were of the broadly categorized European ethnicity (10, 15, 16, 22–24). The remaining study populations were of Chinese (19), Egyptian (17), and Tunisian (18) ethnicities, considered as non-European in the analysis. Deviation from HWE was detected in the controls of two studies (18, 19). Characteristics of included studies are summarized in Supplemental Table S1 available online at [http://informahealthcare.com/doi/suppl/\[10.3109/02770903.2012.738270\]](http://informahealthcare.com/doi/suppl/[10.3109/02770903.2012.738270]) and the corresponding allelic and genotypic frequencies are presented in Table 1.

Meta-analysis found a nonsignificant association between the *TLR4* +896A>G polymorphism and asthma

TABLE 1.—Genotype frequencies of studies included in the meta-analysis of *TLR4* +896A>G and asthma.

Study	Country	Cases					Controls					
		N	MAF	Genotypes			N	MAF	Genotypes			HWE
				AA	AG	GG			AA	AG	GG	p-Value
Adjers et al. (15)	Finland	243	0.088	202	39	2	401	0.087	334	64	3	.973
Carvalho et al. (16)	The United Kingdom	14	0.071	12	2	0	80	0.063	70	10	0	.551
Hussein et al. (17)	Egypt	500	0.068	434	64	2	251	0.058	223	27	1	.851
Lachheb et al. (18)	Tunisia	210	0.002	209	1	0	224	0.004	223	0	1	<.001
Liu et al. (19)	China	197	0.109	161	29	7	156	0.106	128	23	5	.006
Smit et al. (22)	Denmark	100	0.050	91	8	1	88	0.052	78	9	0	.611
Voronko et al. (23)	Russia	283	0.080	245	31	7	227	0.059	200	27	0	.341
Yang et al. (10)	The United Kingdom	185	0.081	155	30	0	179	0.059	159	19	1	.603
Zaborowski et al. (24)	Poland	106	0.057	94	12	0	159	0.053	142	17	0	.476
Total		1838		1603	216	19	1764		1557	196	11	

Note: HWE, Hardy-Weinberg equilibrium; MAF, minor allele frequency; *N*, genotyped sample size.

Italic values represent the total frequencies for each genotype and genotype sample size for cases and controls and was applied to stylistically distinguish the total frequencies from individual study frequencies.

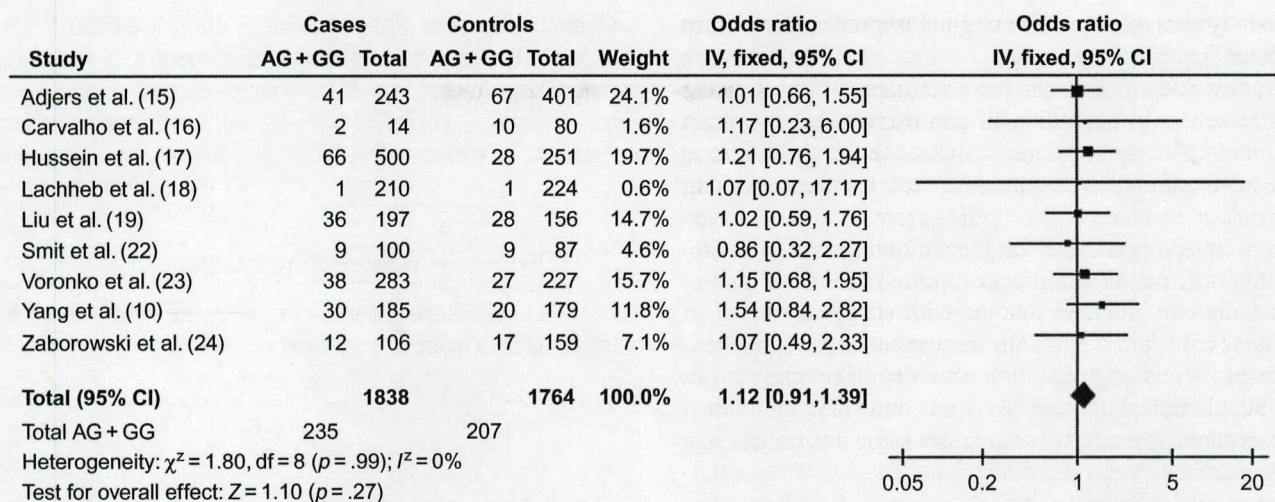


FIGURE 2.—Forest plot of *TLR4* +896 AG + GG versus AA genotypes for all studies included in the meta-analysis. Meta-analysis was conducted using an inverse variance (IV), fixed-effect model and the overall effect estimate is plotted as a diamond. The OR of each study is represented on the plot as a square with the area of each square proportional to the corresponding study's weight in the meta-analysis. Horizontal lines are the associated 95% CIs for the OR of each study. Bold values highlight the total frequency of cases and controls as well as the overall OR and 95% CI.

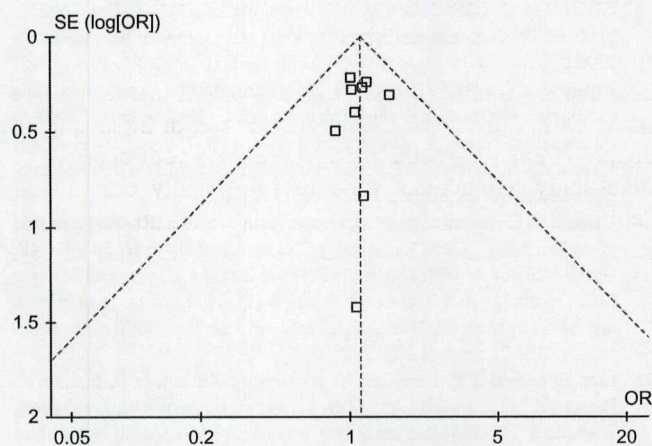


FIGURE 3.—Funnel plot of *TLR4* +896 AG + GG versus AA genotypes for all studies included in the meta-analysis. Standard error of the logarithm of the OR ($SE(\log[OR])$) was plotted against the OR for each study. The symmetric, inverted funnel shape implies an absence of publication bias.

for both the allelic comparison (OR = 1.14, 95% CI: 0.94–1.39, $p = .19$) and the genotypic comparison assuming a dominant model (OR = 1.12, 95% CI: 0.91–1.39, $p = .27$) (Figure 2). No significant between-study heterogeneity was detected for the comparisons ($I^2 = 0\%$). Sensitivity analysis confirmed the stability of the null association; exclusion of individual studies or the HWE-deviated studies did not meaningfully change the pooled estimates (data not shown). Inspection of the funnel plots (see Figure 3 for the genotypic comparison) did not reveal evidence of publication bias.

No meaningful differences were observed between the results of the subgroup analysis of the nine studies conducted in European populations ($n = 931$ cases and 1133 controls) and the overall estimates. The allelic (OR = 1.16, 95% CI: 0.91–1.49, $p = .22$) and genotypic (OR = 1.12,

95% CI: 0.87–1.46, $p = .38$) comparisons for the subgroup analysis were both not statistically significant. As with the overall analysis, overt between-study heterogeneity ($I^2 = 0\%$) and publication bias were not detected. Subgroup analysis of non-European studies found similar nonsignificant results (data not shown).

DISCUSSION

On the basis of nine case-control studies involving 1838 asthma cases and 1764 controls, the present meta-analysis did not find a significant association between the *TLR4* +896A>G polymorphism and asthma. Between-study heterogeneity was not significant and subgroup analysis detected no meaningful difference by ethnicity (European vs. non-European), suggesting consistent gene effects across individual studies and ethnicities, respectively. Sensitivity analysis further demonstrated the stability of the null association.

Certain limitations to this review need to be taken into consideration when interpreting the findings. First, although multiple sources of literature were consulted and obvious publication bias was not detected, the effects of language bias and file drawer problem may impede the completeness of evidence. Second, the asthma phenotype was not consistently defined across studies (see Supplemental Table S1 for a comparison of diagnostic criteria), which may undermine the validity of the meta-analysis. Likewise, the reference groups were not uniformly defined, with some studies recruiting population-based controls, while others recruiting hospital-based controls. Of additional concern, the controls for two studies included in this meta-analysis were not in HWE (18, 19). Studies with controls in violation of HWE have been shown to give significantly different results from studies with controls conforming to HWE (25). Although

sensitivity analysis showed marginal impact on the overall estimates in this meta-analysis, exclusion of studies with HWE-deviated controls can have substantial impact on the significance and magnitude of summary effect estimates and between-study heterogeneity (25). Third, age and gender are important determinants of the asthma phenotype that may influence susceptibility gene expression, gene-environment interaction, and epigenetic effects and, consequently, may act as modifiers of gene-disease association (26). Only one included study conducted gender-stratified analysis (15). Noncomparable proportions of the same gender in cases and controls were observed in several studies (see Supplemental Table S1). At least one study combined both children and adults for analysis (19). Insufficient age and gender data were reported to conduct informative subgroup analysis. Fourth, the *TLR4* +896G allele frequency differs across ethnic groups (13). Populations with relatively higher G allele frequencies, such as African-American, black African, and Indian, were not among the meta-analyzed studies, of which six were conducted in European populations. Therefore, the meta-analysis results may not generalize to non-European ethnicities. Fifth, population stratification may confound the gene-disease relationship in studies underlying the meta-analysis, creating spurious associations or masking true associations (27), threatening the validity of the individual genetic association study as well as the meta-analysis. None of the included studies reported an assessment of population stratification. However, deviation from HWE, as observed in studies by Lachheb et al. (18) and Liu et al. (19), may be indicative of population stratification and other issues (28).

Due to the complex nature of asthma, it is unlikely that genetic or environmental factors act alone in disease causation and susceptibility; thus, a single SNP such as *TLR4* +896A>G is unlikely to have a significant contribution to asthma risk without consideration of gene-environment interaction and epistatic effects. As discussed previously, the *TLR4* +896A>G polymorphism was shown to modify the association between LPS exposure and asthma (9). Female carriers of the G allele of *TLR4* +896A>G and the T allele of *IL4* -590C>T have been found to have a significantly increased risk for asthma, although the alleles did not separately affect asthma risk, showing an epistatic effect (15). Despite a nonsignificant direct relationship, these reports on the interaction effects of the *TLR4* polymorphism showed that important covariates involved in asthma, including relevant environmental exposures, other genetic variants, race/ethnicity, age, and gender, should be considered when studying a complex, heterogeneous disease such as asthma. In addition, although no direct association was observed, there may still be direct effects of the *TLR4* +896A>G SNP on asthma severity without consideration of gene-environment or gene-gene interactions.

CONCLUSIONS

The present meta-analysis found a nonsignificant association between the *TLR4* +896A>G polymorphism and

asthma. However, to fully explore the role of this SNP in asthma, the impact of gene-environment and gene-gene interactions, other important covariates, and methodological concerns, such as population stratification effects, needs to be evaluated in future studies.

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DECLARATION OF INTEREST

The author reports no conflicts of interest. The author alone is responsible for the content and writing of the paper.

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