Systematic Review and Meta-Analysis

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Association of polymorphisms in *TLR2* and *TLR4* with asthma risk

An update meta-analysis

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Abstract

Background: Several epidemiological studies have focused on the association between polymorphisms in toll-like receptors (*TLRs*) and asthma. However, the results remained inconclusive.

Methods: We systematically reviewed the database of PubMed, EMBASE, Web of Science, CNKI, and Google scholar for all related articles on *TLR* polymorphisms and asthma. We used the software STATA 12.0 to conduct the meta-analysis. The heterogeneity and publication bias were examined, respectively.

Results: Eighteen studies consisting of 3538 asthma cases and 4090 controls were selected into the meta-analysis. The pooled odds ratios (ORs) show that rs3804099 was associated with asthma in dominant model (OR = 1.51, 95% CI = 1.17–1.96, *P* = .002), and rs4986791 was associated with asthma in additive model (OR = 0.81, 95% CI = 0.64–1.02, *P* = .07) and dominant model (OR = 0.76, 95% CI = 0.60–0.97, *P* = .025).

Conclusion: The combined results show that rs3804099 in *TLR2* and rs4986791 in *TLR4* were significantly associated with asthma risk. Polymorphisms in *TLRs* play important roles in asthma.

Abbreviations: CI = confidence interval, HWE = Hardy–Weinberg equilibrium, OR = odds ratio, SNP = single nucleotide polymorphism, TLR = toll-like receptor.

Keywords: asthma, meta-analysis, polymorphism, TLR2, TLR4

1. Introduction

Asthma is a chronic inflammatory disorder of the airways, which is characterized by reversible airflow obstruction and airway hyper-responsiveness.[1] It has been one of the most common disease in the world.[2] Although the exact mechanisms in asthma have not been completely elucidated,[3] it is generally accepted that both genetic and environmental factors play important roles in this disease.[4] Currently, more than 200 genetic variations have been reported to be associated with asthma.[5]

Toll-like receptors (TLRs) are a class of proteins that belong to the family of transmembrane receptors. They are counted among the key molecules in pathogen recognition and activation of innate immunity.[6] The TLRs include 11 members in humans (TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, and TLR11).[6] TLR2 and TLR4 are the most important members of them. Several variants in them have been proved associated with asthma.[7–11] However, the results remained conflicting. Among the reported studies, most of them found that these polymorphisms were associated with asthma, while others reported contrary opinions. Meantime, most of these studies were conducted with small numbers of subjects. Only 1 meta-analysis was conducted to examine the relationship between *TLR* gene polymorphisms and asthma 2 years ago.[12] During the past years, several studies about *TLR* gene polymorphisms and asthma have been conducted.[13,14] So, we conducted the update meta-analysis expecting to give a more determinately conclusion.

2. Methods

2.1. Search strategy

We systematically searched the database of Pubmed, EMBASE, Web of Science (ISI), China National Knowledge Infrastructure (CNKI), and Google scholar for all related articles on TLR

Editor: Jesper Kers.

Authorship: Conceived and designed the study: XL, MX, and JX. Analyzed the data: JZ, HS, and XC. Contributed reagents/materials/analysis tools: XL. Wrote the paper: JZ, YH, XF, and SZ. All authors read and approved the final manuscript.

Funding/support: This study was supported by the National Natural Science Foundation of China (No. 81670048).

The authors have no conflicts of interest to disclose.

Supplemental Digital Content is available for this article.

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Medicine (2017) 96:35(e7909)

Received: 31 January 2017 / Received in final form: 16 July 2017 / Accepted: 3 August 2017

http://dx.doi.org/10.1097/MD.0000000000007909

polymorphisms and asthma. We used the following keywords: "TLR2" or "TLR4," "polymorphism," and "asthma." Both English and Chinese language articles were included in the analysis. The included studies were published from January 1, 2000 to September 30, 2016. In addition, the references and citations of the originally retrieved articles, which were not captured by our database searches, were identified through manual searching.

The study was approved by the institutional ethics committees of Tongji Hospital.

2.2. Inclusion criteria

Although several single nucleotide polymorphisms (SNPs) in *TLR2* and *TLR4* have been previously studied, only those most widely studied variants (reported in more than 3 studies) were analyzed in this analysis. Finally, 2 SNPs (rs5743708 and rs3804099) in *TLR2* and 2 SNPs (rs4986790 and rs4986791) in *TLR4* were included in the analysis. The title and abstract of the articles identified through electronic literature search were scanned. If the article could not be verified, the full text was further examined. The articles that met the following inclusion criteria were included in the analysis: case–control study; aims at the association between *TLR2* (rsrs5743708, rs3804099) or TLR4 (rs4986790, rs4986791) and asthma; and sufficient data to perform the meta-analysis. The case-only studies, reviews, case reports and studies without sufficient information were excluded. If the study subjects were reported in more than 1 publication, we selected the study with the largest sample size.

2.3. Data extraction

Two reviewers (JLZ and HHS) extracted the data separately. Characters extracted from the studies including: journal, first author, year of publication, ethnicity, genotyping methods, asthma diagnosis, source of controls, average age at baseline, male percentage, distribution of genotypes for each polymorphism among cases and controls, and the odds ratio (OR) with their 95% confidence intervals (CIs). Four polymorphisms were extracted respectively from selected studies. The results of items were compared and all of them reached consistencies in the end.

2.4. Statistical analysis

We examined the Hardy–Weinberg equilibrium (HWE) for each polymorphism by using the Chi-square test. The association between polymorphisms and asthma was estimated by means of OR and corresponding 95% CIs comparing cases to controls. Additive model (M vs N), dominant model (MM vs MN + NN), and recessive model (MM + MN vs NN) were used to estimate statistical significance between TLR polymorphisms and asthma risk, respectively. The heterogeneity represents the total percentage of variation across the selected studies. We used the Chi-square-based Q statistic and I^2 statistic to evaluate the heterogeneity. The percentages of I^2 around 25%, 50%, and 75% indicate low, medium, and high heterogeneity, respectively.[15] $P < .10$ was representative of significant heterogeneity.[16] When there was no significant heterogeneity ($P > .10$ and I^2 less than 50%), the fixed-effects model was used to calculate the pooled ORs; otherwise, the random-effects model was used.[17] In addition, we performed sensitivity analysis to investigate the influence of individual study on the overall meta-analysis. Finally, we evaluated publication bias of selected analysis by funnel plot and Egger tests. We performed the analyses by using the STATA 12.0 software (StataCorp, College Station, TX).[18] A $P < .05$ was considered statistically significant and all statistical testing were 2-sided.

3. Results

3.1. Search results

On the initial search, 342 papers were identified by the keywords mentioned in the search strategy. A total of 258 papers were excluded for the reason that they were not aiming at the association of *TLR2* or *TLR4* and asthma. When we reviewed abstracts of the remained papers, 64 articles were excluded. For the reason that these studies were case reports, gave insufficient data for subsequent analysis, or subjects of these studies had other diseases. Then we reviewed the full texts and excluded 2 articles. The first excluded from our meta-analysis was reported by Liang et al.[19] Neither the Asp299Gly nor Thr399Ile polymorphism was found by DNA sequencing in the study. So, we excluded this study in the meta-analysis. Another study conducted by Saçkesen et al was case-only study and was excluded as well.[20] Finally, 18 literatures were selected into the meta-analysis.[10,13,14,21–35]Figure 1 outlines our study selection process. The characteristics of studies included in the analysis were listed in Table 1. The raw data we got from the 18 studies consist of 3538 asthma cases and 4090 controls. The general characteristics of each study, genotype frequencies, and HWE examination results were presented in Table 2.

3.2. Association of the polymorphisms rs5743708 and rs3804099 in TLR2 with asthma

The polymorphism rs5743708 is also known as 2408G/A, which causes a nonsynonymous amino acid change (Arg753Gln) in this protein. Six studies analyzed the polymorphism rs5743708 and were selected in our study. They consisted of 968 asthma cases

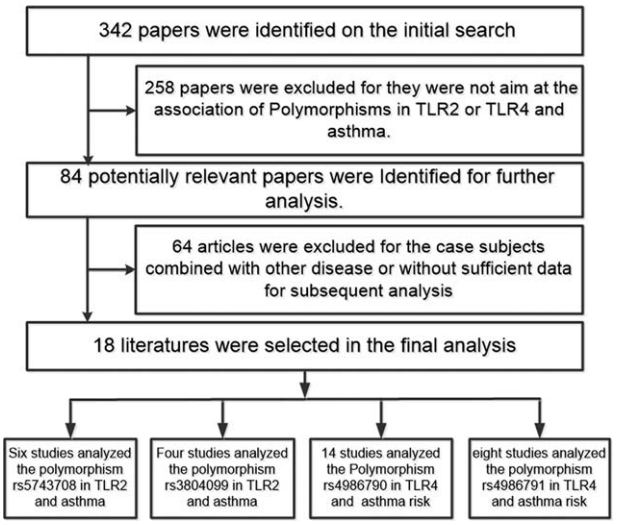

Figure 1. Flow chart for the process of selecting eligible publications.

Table 1

Study characteristics in the analysis of TLR polymorphisms and asthma.

First author	Year	Country	Case			Control			TLR2			TLR2		TLR4		TLR4	
			Number	Mean age	Male (n)	Number	Mean age	Male (n)	rs5743708	2408G/A	2258 G/A (Arg753Gln)	rs3804099	597 C/T	rs4986790	+896 A/G (Asp299Gly)	rs4986791	+1196 C/T (Asp399Gly)
Martinez et al	2016	USA	62	41.2	11	61	46	21				√			√		√
Bahrami et al	2015	Iran	99	44.15	28	120	46.6	37			√				√		
Sinha et al	2014	India	481	37.22	191	483	34.29	189							√		√
Sahin et al	2014	Turkish	131	36	35	75	43	25							√		√
Lyakhovskaya et al	2013	Ukraine	45	NA	NA	90	NA	NA		√							
Hussein et al	2012	Egypt	500	8.4	270	150	8.3	77		√					√		
Zaborowski et al	2011	Poland	106	49	32	159	41	96							√		
Voronko et al	2011	Russia	227	34.4/41.8*	136	283	38.5	103							√		
Smit et al	2009	France	239	46	123	586	47.1	293				√					√
Bjørnvold et al	2009	Norway	108	NA	NA	494	NA	NA				√					
Carvalho et al	2008	Portugal	14	NA	NA	80	NA	NA		√					√		
Lachheb et al	2008	Tunisian	210	10.5	50	224	8	107		√					√		√
Smit et al	2007	Danish	100	NA	85	87	NA	79		√					√		√
Schubert et al	2006	Germany	321	NA	NA	270	NA	NA							√		√
Liu et al	2005	Chinese	197	NA	97	156	NA	88							√		√
Adjers et al	2005	Finnish	245	59	0	405	60	0							√		
Yang et al	2004	Britain	320	NA	NA	179	NA	NA							√		
Noguchi et al	2004	Japanese	133	NA	NA	188	46.9	108					√				

√ represents the SNP was studied. NA = not available, SNP = single nucleotide polymorphism, TLR = toll-like receptor.
*The cases were divided into 2 groups: mild BA individuals (n = 131) and moderate or severe BA (n = 152).

and 751 healthy controls. When we pooled all eligible publications into the meta-analysis, no significant association was found between rs5743708 and asthma in additive model (OR = 1.40, 95% CI = 0.85–2.32, P = .186), as shown in Fig. 2. Among the 6 studies, the genotypes of 2 studies conducted by Hussein et al[23] and Lachheb et al[28] were not in HWE. Therefore, we excluded these 2 studies and the pooled OR was consistent in direction (OR = 1.23, 95% CI = 0.67–2.26, P = .496). Under recessive model and dominant model, no significant association was found between rs5743708 and asthma as well, as shown in Table 3.

Four studies analyzed the polymorphism rs3804099. The study conducted by Bjørnvold et al[26] only reported the frequency of each alleles of rs3804099, but did not report the frequency of each genotype. When we pooled all eligible publications into the meta-analysis, significant association was found between rs3804099 and asthma in dominant model (OR = 1.51, 95% CI = 1.17–1.96, P = .002), as shown in Fig. 3. No significant association was found in additive model (OR = 1.12, 95% CI = 0.97–1.31, P = .13) and recessive model (OR = 1.06, 95% CI = 0.77–1.46, P = .74).

3.3. Association of the TLR4 rs4986790 and rs4986791 polymorphisms with asthma

A total of 14 studies encompassing 3011 cases and 2728 controls investigated the association of the polymorphism rs4986790 with asthma risk. The study conducted by Ådjers et al[32] contains 92 male cases and 151 male controls, as well as 151 female cases and 250 female controls. Therefore, we extracted these data separately. When we pooled all data extracted from the eligible publications, no significant association was found between rs5743708 and asthma (additive model: OR = 0.95, 95% CI = 0.81–1.11, P = .51, as shown in Fig. 4; dominant model: OR = 0.89, 95% CI = 0.74–1.07, P = .22; and recessive model: OR = 1.27, 95% CI = 0.68–2.36, P = .453), as shown in Table 3. Low heterogeneity was observed among these studies (I^2 = 0%).

Eight studies analyzed the association of the variant rs4986791 with asthma risk. They contained 1741 asthma cases and 1952 healthy controls. When we pooled all selected studies together, significant association was found between rs4986791 and asthma in additive model (OR = 0.80, 95% CI = 0.64–0.99, P = .04). The genotypes of the study conducted by Liu et al[31] were not in HWE. When we excluded the study not in HWE, a barely significant association was found between rs4986791 and asthma in additive model (OR = 0.81, 95% CI = 0.64–1.02, P = .07), as shown in Fig. 5. Significant association was found between dominant model (OR = 0.76, 95% CI = 0.60–0.97, P = .025, as shown in Fig. 6), while not in recessive model (OR = 0.86, 95% CI = 0.35–2.14, P = .75).

3.4. Sensitivity analysis and publication bias

In the sensitivity test, we evaluated the sensitivity by exclusion of 1 study at a time, and the results indicated that the pooled ORs were stable, which confirmed the stability and reliability of the pooled results. The Begg funnel plot and Egger test were used to assess the publication bias of the selected studies. The results did not suggest any publication bias in our meta-analysis (as shown in Supplemental Figures, http://links.lww.com/MD/B846).

Table 2

Genotypes of the polymorphisms in selected studies.

ID						Case						Control					
Rs5743708 2258 G/A 2408 G/A (Arg753Gln) 753G>A	Author	Year	Country	Case	Control	MM GG	MN GA	NN AA	M G	N A	*Ph*[*] *P*	MM GG	MN GA	NN AA	M G	N A	*Ph*[*] *P*
	Bahrami et al	2015	Iran	99	120	92	7	0	191	7	.72	113	7	0	233	7	.74
	Lyakhovskaya et al	2013	Ukraine	45	90	40	5	0	85	5	.69	88	2	0	178	2	.91
	Hussein et al	2012	Egypt	500	150	484	13	3	968	19	<.001	243	7	0	493	7	.82
	Carvalho et al	2008	Portugal	14	80	12	2	0	26	2	.77	75	5	0	155	5	.77
	Lachheb et al	2008	Tunisian	210	224	208	0	2	416	4	<.001	223	1	0	447	1	.97
	Smit et al	2007	Danish	100	87	97	3	0	197	3	.88	80	7	0	167	7	.69
Rs3804099 597 C/T 199T>C						**TT**	**TC**	**CC**	**T**	**C**	***P***	**TT**	**TC**	**CC**	**T**	**C**	***P***
	Martínez et al	2016	USA	62	61	19	36	7	74	50	.1	17	27	17	61	61	.37
	Smit et al	2009	France	239	596	52	122	49	226	220	.16	207	259	99	673	457	.25
	Bjørnvold et al	2009	Norway	108	494	NA	NA	NA	118	78	NA	NA	NA	NA	515	405	NA
	Noguchi et al	2004	Japanese	133	188	59	62	12	180	86	.45	93	78	17	264	112	.91
Rs4986790 +896 A/G (Asp299Gly) 299A>G						**AA**	**AG**	**GG**	**A**	**G**	***P***	**AA**	**AG**	**GG**	**A**	**G**	***P***
	Martínez et al	2016	USA	62	61	54	7	1	115	9	.2	51	10	0	112	20	.49
	Bahrami et al	2015	Iran	99	120	85	14	0	184	14	.45	104	16	0	224	16	.43
	Sinha et al	2014	India	481	483	390	87	4	867	95	.72	381	95	7	857	109	.7
	Sahin et al	2014	Turkish	131	75	122	9	0	253	9	.68	71	4	0	146	4	.81
	Zaborowski et al	2011	Poland	106	159	94	12	0	200	12	.54	142	17	0	301	17	.48
	Hussein et al	2012	Egypt	500	150	223	27	1	473	29	.85	434	64	2	932	68	.83
	Voronko et al	2011	Russia	227	283	245	31	7	521	45	<.001	200	27	0	427	27	.34
	Carvalho et al	2008	Portugal	14	80	12	2	0	26	2	.77	70	10	0	150	10	.55
	Lachheb et al	2008	Tunisian	210	224	209	1	0	419	1	.97	223	0	1	446	2	<.001
	Smit et al	2007	Danish	100	87	91	8	1	190	10	.11	78	9	0	165	9	.61
	Schubert et al	2006	Germany	321	270	NA	NA	NA	629	13	NA	NA	NA	NA	527	13	NA
	Liu et al	2005	Chinese	197	156	161	29	7	351	43	<.001	128	23	5	279	33	.006
	Ådjers et al-male	2005	Finnish	245	405	77	15[†]	NA	NA	NA	122	29[†]	NA	NA	NA		
	Ådjers et al-female	2005	Finnish	245	405	125	26[†]	NA	NA	NA	212	38[†]	NA	NA	NA		
	Yang et al	2004	Britain	320	179	280	38	2	598	42	.57	159	19	1	337	21	.60
Rs4986791 +1196 C/T (Asp399Gly) 399C>T						**CC**	**CT**	**TT**	**C**	**T**	***P***	**CC**	**CT**	**TT**	**C**	**T**	***P***
	Martínez et al	2016	USA	62	61	55	7	0	117	7	.64	53	8	0	114	8	.58
	Sinha et al	2014	India	481	483	408	68	5	884	78	.26	384	92	7	860	106	.58
	Sahin et al	2014	Turkish	131	75	120	11	0	251	11	.62	71	4	0	146	4	.81
	Smit et al	2009	France	239	596	198	26	0	422	26	.36	492	76	0	1060	76	.09
	Lachheb et al	2008	Tunisian	210	224	209	1	0	419	1	.97	221	2	1	444	4	<.001
	Smit et al	2007	Danish	100	87	93	6	1	192	8	.03	77	10	0	164	10	.57
	Schubert et al	2006	Germany	321	270	NA	NA	NA	627	15	NA	NA	NA	NA	528	12	NA
	Liu et al	2005	Chinese	197	156	191	4	2	386	8	<.001	150	5	1	305	7	<.001

HWE = Hardy–Weinberg equilibrium, M = major allele, MM = wild type, MN = heterozygosity, N = minor allele, NA = not available, NN = homozygote.

[*] *Ph*: exact *P* value for HWE test.

[†] Represents the genotype of "GA + AA."

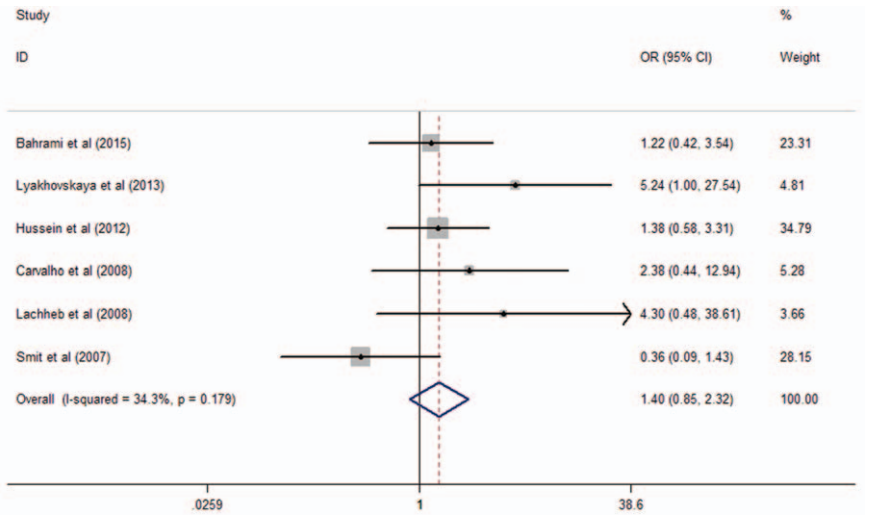

Figure 2. Meta-analysis of the association between *TLR2* rs5743708 and asthma in additive model. TLR = toll-like receptor.

Therefore, publications bias did not have significant influence on the pooled results.

4. Discussion

In the meta-analysis, 18 studies consisting of 3538 asthma cases and 4090 healthy controls were selected to evaluate the association between polymorphisms in *TLRs* and asthma. The combined results show that rs3804099 in *TLR2* and rs4986791 in *TLR4* were significantly associated with asthma risk.

Up to now, more than 200 variants have been reported to be associated with asthma risk, but only a few of them have been replicated.[36] Lack of reproducibility has become a big challenge in genetic association studies. Noguchi et al[33] firstly reported that the variant rs3804099 in *TLRs* was marginally associated with asthma risk. Since then, many subsequent replication studies have evaluated the association between variants in *TLRs* and asthma risk. However, the results were inconclusive and further assessment was needed. In current study, we conducted an extensive, up-to-date, and unbiased meta-analysis combining all together 18 studies consisting of 3538 asthma cases and 4090 healthy controls to evaluate the association between 4 polymorphisms (rs5743708 and rs3804099 in *TLR2*; rs4986790 and rs4986791 in *TLR4*) and asthma. Our study demonstrated that variants in *TLR2* and *TLR4* might influence the risk of asthma.

Genome-wide association study (GWAS) has been a useful methods in discovering new genetic variants associated with complex disease.[37] However, most complex diseases, such as asthma, are usually caused by accumulation of several genetic

Table 3

Pooled measures on the relation of TLR polymorphisms and asthma.

Group		No. of studies	Number		Inherited model	Pooled OR (95% CI)	I^2	*P*
			Case	Control				
rs5743708	Additive model	5	968	751	G vs A	1.40 (0.85, 2.32)	34.3%	.186
2408G/A	Additive model	4	377	258	G vs A excluded for not in HWE	1.23 (0.67, 2.26)	54%	.496
2258G/A	Recessive model	2	710	374	GG + GA vs AA	4.31 (0.51, 36.3)	0%	.18
(Arg753Gln)	Dominant model	5	968	751	GG vs GA + AA	1.25 (0.74, 2.10)	28.7%	.41
rs3804099	Additive model	4	542	1339	T vs C	1.12 (0.97, 1.31)	74.7%	.13
597 C/T	Recessive model	3	434	845	TT + TC vs CC	1.06 (0.77, 1.46)	71.3%	.741
	Dominant model	3	434	845	TT vs TC + CC	1.51 (1.17, 1.96)	54%	.002
rs4986790+896A/G (Asp299Gly)	Additive model	13	2768	2327	A vs G	0.95 (0.81, 1.11)	0%	.51
	Additive model	10	2134	1664	A vs G excluded for not in HWE	0.89 (0.74, 1.07)	0%	.22
	Recessive model	8	2097	1623	AA + AG vs GG	1.27 (0.68, 2.36)	0%	.453
	Dominant model	14	2937	2867	AA vs AG + GG	0.97 (0.82, 1.14)	0%	.70
rs4986791+1196C/T (Asp399Gly)	Additive model	7	1741	1952	C vs T	0.80 (0.64, 0.99)	0%	.04
	Additive model	5	1234	1485	C vs T excluded for not in HWE	0.81 (0.64, 1.02)	0%	.07
	Recessive model	4	877	1138	CC + CT vs TT	0.86 (0.35, 2.14)	0%	.75
	Dominant model	7	877	1138	CC vs CT + TT	0.76 (0.60, 0.97)	0%	.025

CI = confidence interval, HWE = Hardy–Weinberg equilibrium, OR = odds ratio, TLR = toll-like receptor.

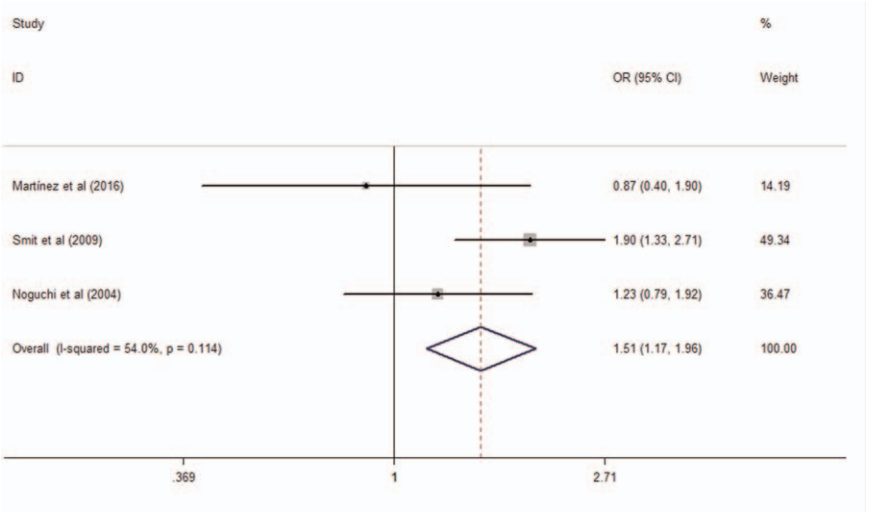

Figure 3. Meta-analysis of the association between *TLR2* rs3804099 in TLR2 and asthma in dominant model. TLR = toll-like receptor.

determinants and environmental factors.[5] Each of these variants gets a small effect, and therefore it is difficult to find out new susceptibility locus in small sample size analysis. Many variants look promising in the original GWAS while do not meet the levels of significance. Meanwhile, the results of independent cohorts were not conclusive, and meta-analysis gives more conclusive results. For these variants did not reach statistical significance in single analysis, we get the chance to detect novel variants by combining results from single analysis to meta-analysis.

In current meta-analysis, we extracted data from a combined sample of 7628 individuals and the results show that rs3804099 in *TLR2* and rs4986791 in *TLR4* contribute to the increased risk of asthma. During the past years, several studies have analyzed variants in *TLR2* and *TLR4* with asthma, while the results were discordance due to small sample size and some other reasons. By combining results from single analysis, we got more conclusive results. Furthermore, our results further confirmed the hypothesis that common genetic variants with modest or small effects in single study might contribute to the risk of complex disease and most of them were not identified. Our results highlight the power of meta-analysis in genetic association studies. However, further studies with larger sample size and prospective study are needed to confirm these findings, and biological study is needed to investigate the mechanisms underling these variants and asthma.

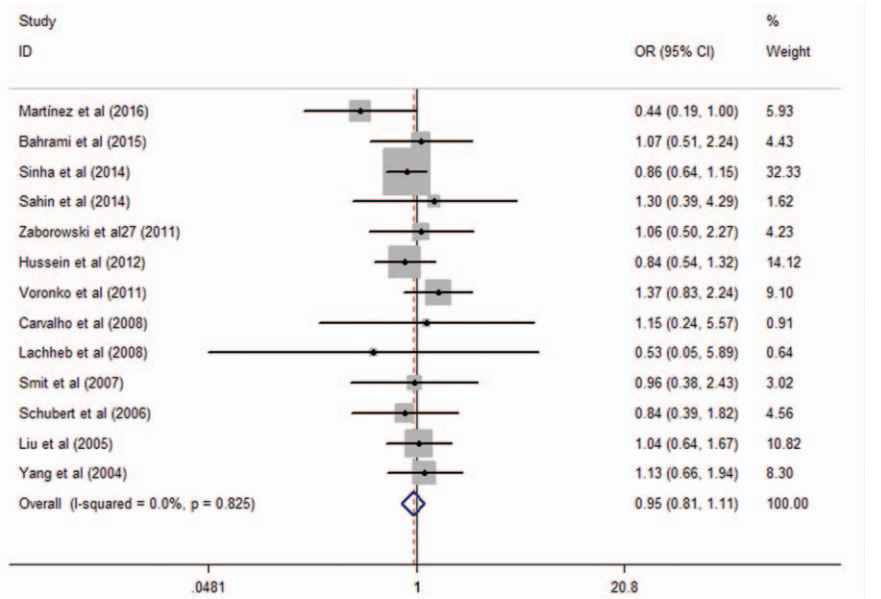

Figure 4. Meta-analysis of the association between *TLR4* rs4986790 in TLR4 and asthma in additive model. TLR = toll-like receptor.

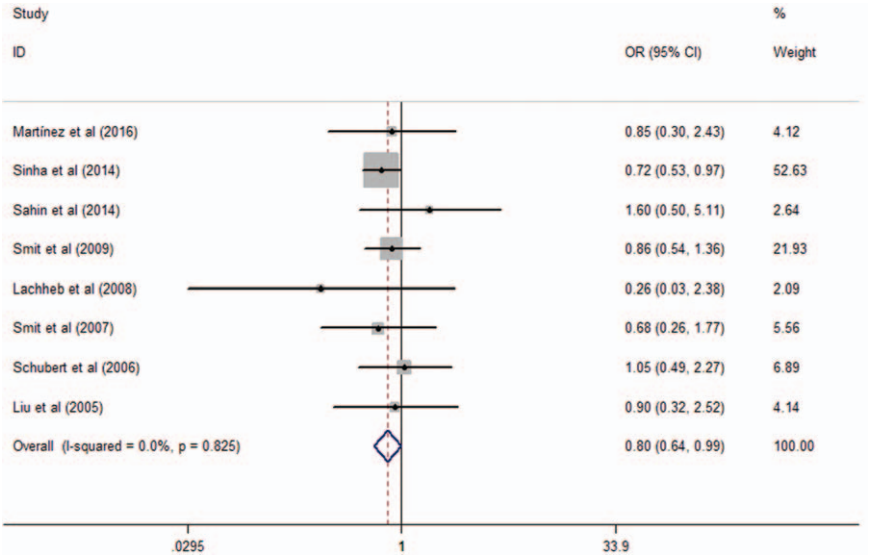

Figure 5. Meta-analysis of the association between *TLR4* rs4986791 in TLR4 and asthma in additive model. TLR = toll-like receptor.

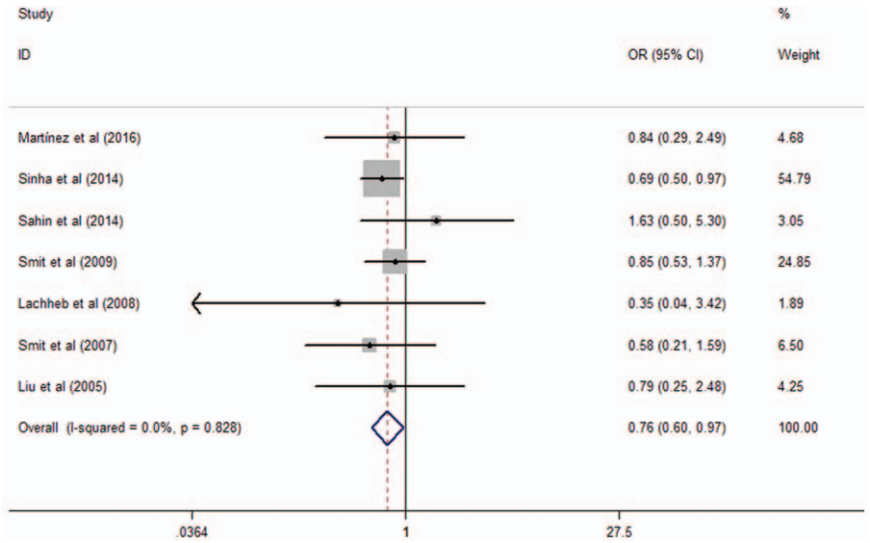

Figure 6. Meta-analysis of the association between *TLR4* rs4986791 in TLR4 and asthma in dominant model. TLR = toll-like receptor.

5. Conclusion

This meta-analysis provides the evidence that variations rs3804099 in TLR2 and rs4986791 in TLR4 were associated with asthma risk. However, further prospective studies with larger sample size are needed to clarify the association between other variation in TLRs and asthma.

Acknowledgments

The authors thank all the participants in this study.

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