Systematic Review and Meta-Analysis

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# Association of single-nucleotide polymorphisms in *toll-like receptor 2* gene with asthma susceptibility

## A meta-analysis

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**Abstract**

**Background:** An increasing number of studies have been carried out on the relationship between polymorphisms in toll-like receptor 2 (*TLR2*) gene and asthma risk. However, the results were controversial. With the purpose of yielding a more reliable estimation of the association, we conducted the present meta-analysis.

**Methods:** Multiple electronic databases up to August 22, 2016 were searched for literature retrieval. The association between the asthma susceptibility and the rs5743708 polymorphism, rs3804099 polymorphism, rs3804100 polymorphism, and rs4696480 polymorphism in *TLR2* gene was appraised. The odds ratios (ORs) with 95% confidence intervals (CIs) under different genetic models were calculated.

**Results:** A total of 13 studies were eligible in our meta-analysis according to the predefined inclusion and exclusion criteria. There was no significant association between asthma risk and rs5743708, rs3804099, and rs3804100 polymorphisms in *TLR2* gene under any genetic model. With respect to the TLR2 rs4696480 polymorphism, significant association was detected between asthma susceptibility and TLR2 rs4696480 polymorphism under dominant model (OR = 2.455, 95% CI = 1.235–4.88, $P = .01$) and codominant 3 model (OR = 2.776, 95% CI = 1.199–6.427, = 0.017).

**Conclusions:** Our meta-analysis reveals that the TLR2 rs4696480 polymorphism is significantly associated with asthma susceptibility, and the TLR2 rs4696480 polymorphism is a risk factor for asthma.

**Abbreviations:** CIs = confidence intervals, ORs = odds ratios, PAMPs = pathogen-associated molecular patterns, PRRs = pattern recognition receptors, SNPs = single-nucleotide polymorphisms, TLR2 = toll-like receptor 2, TLRs = toll-like receptors.

**Keywords:** asthma risk, meta-analysis, polymorphisms in TLR2 gene

## 1. Introduction

Asthma is a heterogeneous and chronic inflammatory airway disease, which is starting in childhood and persisting to adulthood among many sufferers.[1] It has been reported that there are >300 million individuals affected by asthma across the world, which makes asthma to become one of the most common chronic diseases.[2,3] The disease, with recurrent wheezing, shortness of breath, and coughing as the clinical manifestations, is resulted from the interaction and collaboration of the environmental and genetic factors that cooperatively lead to the immune responses induced by the infiltration of inflammatory cells into the airway.[4–6] Toll-like receptors (TLRs), a subgroup of pattern recognition receptors (PRRs) that are parts of the innate immune system, have been reported to be correlated with the inflammation in asthma.[6,7]

TLRs, located on the plasma membrane or endosomal vesicles of immune cells, play important roles in the recognition and responses to microbes and microbial components, which are described as pathogen-associated molecular patterns (PAMPs).[8,9] The family of TLRs, consisting of 10 members identified in humans currently, belongs to innate immunity and can build a connection between autoimmunity and innate immunity.[10] Among TLRs, TLR2, which has been documented to be expressed by several cell types, for instance, macrophages, can recognize various PAMPs including the membrane components of Gram-negative/positive bacterial cell wall, parasites, and so on.[11] The mutation in TLR2 is believed to be associated with increased susceptibility to infectious diseases.[12]

TLR2 is encoded by a DNA sequence comprising 2352 bases, and the genetic variation in TLR2 is considered as a main determinant of susceptibility to asthma.[13,14] Several polymorphisms have been identified in *TLR2* gene, such as rs3804099 polymorphism, rs3804100 polymorphism, and rs4696480 polymorphism. A case-control study, published in 2016, appraised the association between single-nucleotide polymorphisms (SNPs) in

*Editor: Jian Liu.*

*The authors report no conflicts of interest.*

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*Medicine (2017) 96:20(e6822)*

*Received: 16 January 2017 / Received in final form: 23 March 2017 / Accepted: 17 April 2017*

*http://dx.doi.org/10.1097/MD.0000000000006822*

TLR2 and the asthma susceptibility among the Puerto Rican asthmatic population, and the results revealed that the rs3804099 polymorphism in *TLR2* gene was a potential protective locus for asthma susceptibility, whereas the rs4696480 polymorphism in *TLR2* gene had null association with asthma susceptibility.[15] However, a nested case-control study, published in 2007[16] and another case-control study, performed by Noguchi et al,[17] indicated conflicting results. Herein, we conducted the present meta-analysis to drive a more reliable and precise assessment of the association between the asthma susceptibility and the SNPs in *TLR2* gene including rs5743708, rs3804099, rs3804100, and rs4696480 polymorphisms.

## 2. Materials and Methods

### 2.1. Literature research

Two authors independently carried out the electronic literature search of several databases to identify literatures about the relationship between the asthma susceptibility and the SNPs in *TLR2* gene. The databases included the web of science, PubMed, and Embase up to August 22, 2016. The following keywords and search terms were used for the search: "TLR2" or "TLR-2" or "TLR 2" or "Toll-like receptor 2" or "TL receptor 2" or "Toll like receptor 2" and ("asthma" or "asthmatic") and ("Variants" or "SNP" or "polymorphisms" or "mutation" or "allele" or "genotype" or "Susceptibility" or "Variant" or "SNPs" or "polymorphism" or "mutations" or "alleles" or "genotypes" or "Susceptibilities").

### 2.2. Inclusion and exclusion criteria

We only recruited publications meeting the following inclusion criteria: case-control studies evaluating the association between the asthma susceptibility and the rs5743708 polymorphism, rs3804099 polymorphism, rs3804100 polymorphism, or rs4696480 polymorphism in TLR2 gene; studies providing the number of individual genotypes of asthmatics and controls, respectively; studies that were published in English. Studies were excluded if: their designs were not case-control study; the raw data were unavailable for examining an odds ratio (OR) with 95% confidence interval (CI); they were in the forms of conference abstract, case report, and letters to the editors.

### 2.3. Data extraction

Two authors separately estimated the eligibility of each full-text original study and extracted relevant data as follows: author name, year of publication, country or territory, study population, number of cases, and controls, type of cases and controls, diagnostic criteria, the polymorphism(s) of *TLR2* gene and the genotyping methods. Discrepancies or inconsistencies between the 2 authors were settled by consensus before being standardized into a unified dataset. This meta-analysis only dealt with the statistical data meta-analysis and involved no patient's personal data, so the ethical approval and patient consent were not applicable in the current meta-analysis.

### 2.4. Data synthesis and statistical analysis

The relationship between the asthma susceptibility and the SNPs in *TLR2* gene was assessed by ORs with 95% CIs on forest plots under allelic model, homozygote model, recessive model, dominant model, and 3 codominant models. The heterogeneity between studies was checked by using the $\chi^2$-based Cochran Q test. The effect of heterogeneity was quantified by adopting the $I^2$ metric ranged from 0% to 100%. $I^2$ <50% or $P>.1$ was regarded as the absence of significant heterogeneity across incorporated studies, and the Mantel-Haenszel fixed-effects model was chosen to combine the data. Otherwise, significant heterogeneity was considered in the included studies, and the DerSimonian-Laird random-effects model was utilized to combine the data. Both Begg test and Egger test were selected to examine the possible publication bias. The STATA 12 software (STATA Corp LP, College Station, TX) was used to finish the statistical manipulations, and a 2-sided $P<.05$ was deemed to be statistically significant.

The related data in controls were considered as reference to generate the pooled OR with 95% CI for each analysis. An OR >1 indicated that the SNP in *TLR2* gene was correlated with the asthma risk and the genotype and allele frequencies in asthmatics were higher than those in controls.

## 3. Results

### 3.1. Study selection and characteristics of studies

The flow diagram of selection process and results of literature search were displayed in Figure 1. The comprehensive search of publications retrieved 307 literatures, among which 53 articles were from PubMed, 175 articles were from Embase, and 79 articles were from the web of science. A total of 214 literatures were included after removing the duplications. After reading the titles and abstracts of the remaining publications, 175 articles were eliminated. Then a full-text review was taken for the rest of 39 potential literatures, and 26 articles were excluded because of the irrelevant diseases, unavailable raw data or overlapped data. Finally, 13 case-control studies were included in our meta-analysis.[15–27] The characteristics of the 13 eligible studies were summarized in Table 1. All the included studies were published between 2004 and 2016. The study population involved both children and adults. The detailed information of the 4 SNPs in *TLR2* gene was exhibited in Table 2.

### 3.2. Association of TLR2 rs5743708 polymorphism with asthma susceptibility

Data from 6 eligible studies were incorporated to explore the relationship between the TLR2 rs5743708 polymorphism and asthma susceptibility under allelic model, homozygote model, and recessive model. Considering the absence of significant heterogeneity under the 3 models (allelic model: $I^2=30.60\%$, $P=.206$; Homozygote model: $I^2<0.01\%$, $P=.518$; Recessive model: $I^2=5.10\%$, $P=.384$), the fixed-effects model was selected for the generation of the pooled OR with corresponding 95% CI, and the results were shown in Table 3. The values of OR were 0.951 (95% CI=0.573–1.578, $P=.846$, Fig. 2A) for the allelic model, 1.128 (95% CI=0.651–1.955, $P=.688$, Fig. 2B) for the homozygote model, and 1.003 (95% CI=0.591–1.704, $P=.99$, Fig. 2C) for the recessive model, which indicated that no significant correlation was detected between the TLR2 rs5743708 polymorphism and asthma susceptibility in the three genetic comparisons.

### 3.3. Association of TLR2 rs3804099 polymorphism with asthma susceptibility

A total of 4 studies were included to investigate the relationship between the TLR2 rs3804099 polymorphism and asthma susceptibility under allelic model, homozygote model, recessive

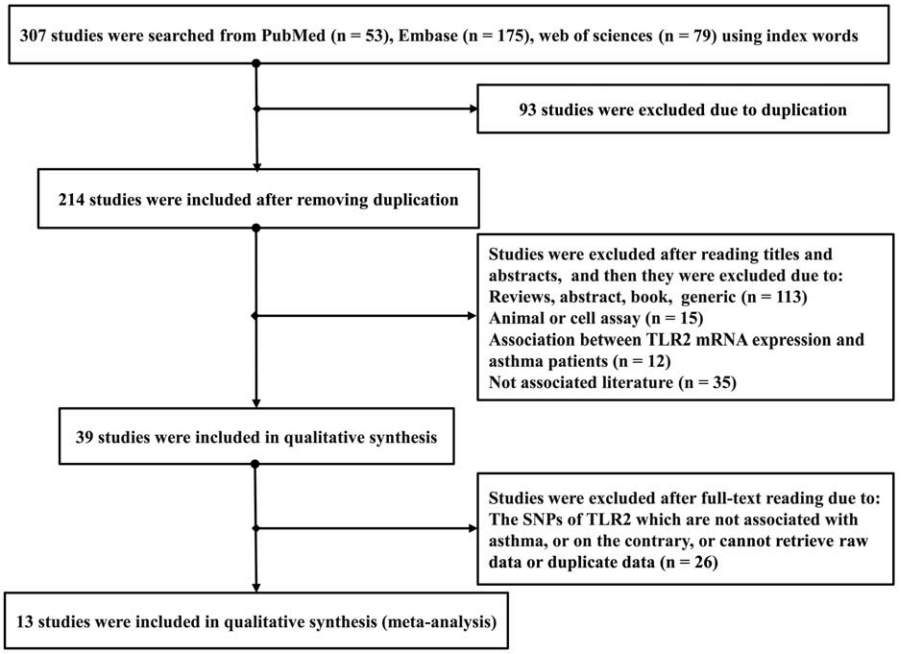


**Figure 1.** Flow diagram of selection of literatures.

model, dominant model, and the 3 codominant models. The fixed-effects or random-effects model was adopted to yield the pooled ORs with corresponding 95% CIs based on the heterogeneity (allelic model: $I^2=22.80\%$, $P=.274$; homozygote model: $I^2$ 0.01%, $P=.443$; recessive model: $I^2=68.40\%$, $P=.013$; dominant model: $I^2<0.01\%$, $P=.708$; Codominant model contains 3 types in the part, 1=TT vs.TC, 2=TT vs.CC, 3=TC vs.CC, codominant[1] model (TT vs. TC): $I^2=48.80\%$, $P=.419$; codominant[2] model (TT vs. CC): $I^2=32.50\%$, $P=.217$; codominant[3] model (TC vs. CC): $I^2<0.01\%$, $P=.869$), and the results were summarized in Table 3. The values of OR were 0.978 (95% CI=0.839–1.14, $P=.755$, Fig. 3A) for the allelic model, 0.923 (95% CI=0.745–1.142, $P=.46$, Fig. 3B) for the homozygote model, 0.786 (95% CI=0.557–1.109, $P=.171$, Fig. 3C) for the recessive model, 1.032 (95% CI=0.782–1.362, $P=.824$, Fig. 3D) for the dominant model, 0.909 (95% CI=0.71–1.163, $P=.447$, Fig. 3E) for the codominant[1] model, 0.975 (95% CI=0.703–1.352, $P=.879$, Fig. 3F) for the codominant[2] model, and 1.07 (95% CI=0.798–1.433, $P=.652$, Fig. 3G) for the codominant[3] model, which signified that no association of TLR2 rs3804099 polymorphism and asthma susceptibility was observed in the 5 genetic comparisons.

### 3.4. Association of TLR2 rs3804100 polymorphism with asthma susceptibility

There were 4 eligible studies for the analysis of the relationship between the TLR2 rs3804100 polymorphism and asthma susceptibility under allelic model, homozygote model, recessive model, dominant model, and the 3 codominant models. The values of $I^2$ were all <50% under the 7 models (allelic model: $I^2<0.01\%$; homozygote model: $I^2<0.01\%$; recessive model: $I^2<0.01\%$; dominant model: $I^2=10.20\%$; codominant[1] model (TT vs. CC): $I^2<0.01\%$; codominant[2] model (TT vs. TC): $I^2=1.40\%$; codominant[3] model (TC vs. CC): $I^2=20.30\%$), and the values of $P$ were all >0.1 under the 7 models (allelic model: $P=.91$; homozygote model: $P=.743$; recessive model: $P=0.925$; dominant model: $P=.342$; codominant[1] model (TT vs. CC): $P=.759$; codominant[2] model (TT vs. TC): $P=.385$; codominant[3] model (TC vs. CC): $P=.288$],which suggested that the heterogeneity between the incorporated studies was not significant, so the fixed-effects model was selected for the calculation of pooled ORs with corresponding 95% CIs. The results were exhibited in Table 3. The values of OR were 0.866 (95% CI=0.682–1.099, $P=.237$, Fig. 4A) for the allelic model, 0.855 (95% CI=0.643–1.136, $P=.279$, Fig. 4B) for the homozygote model, 0.842 (95% CI=0.638–1.111, $P=.224$, Fig. 4C) for the recessive model, 0.863 (95% CI 0.447–1.664, $P=.66$, Fig. 4D) for the dominant model, 0.853 (95% CI=0.639–1.138, $P=.28$, Fig. 4E) for the codominant[1] model, 0.799 (95% CI=0.408–1.566, $P=.513$, Fig. 4F) for the codominant[2] model, and 0.96 (95% CI=0.485–1.901, $P=.907$, Fig. 4G) for the codominant[3] model, which demonstrated that the TLR2 rs3804100 polymorphism was not associated with asthma susceptibility under the 7 genetic comparisons.

### 3.5. Association of TLR2 rs4696480 polymorphism with asthma susceptibility

Relevant data from 4 studies were pooled to investigate the relationship between TLR2 rs4696480 polymorphism and asthma susceptibility under allelic model, homozygote model, recessive model, dominant model, and the 3 codominant models. The random-effects model was used to compute the pooled ORs with corresponding 95% CIs because of the presence of significant heterogeneity (allelic model: $I^2=79.50\%$, $P=.002$; homozygote model: $I^2=78.90\%$, $P=.003$; recessive model: $I^2=84.30\%$, $P<.01$; dominant model: $I^2=64.10\%$, $P=.056$; codominant[1] model (AA vs. TT): $I^2=75.10\%$, $P=.007$; codominant[2] model (AA vs. AT): $I^2=84.70\%$, $P<.01$;

**Table 1**

**The characteristics of the included studies.**

| Study | Country | Study population | No.of cases | Type of cases | Diagnostic criteria | No. of controls | Type of controls | Hardy-Weinberg | SNP | Genotyping methods |
|---|---|---|---|---|---|---|---|---|---|---|
| Bahrami et al (2015)[18] | Iranian | Adult | 99 | Asthma patients | GINA | 120 | Healthy | Yes | rs5743708 | PCR-RFLP |
| Bjornvold et al (2009)[19] | Norway | Children | 108 | Allergic asthma patients | Having 2 of the 3 following criteria in the presence of at least 1 positive skin prick test against a standard panel of food and inhalant allergens: symptoms, medication, a doctor's diagnosis by the age of 10 | 494 | Healthy | Yes | rs3804099 rs3804100 | TaqMan genotyping system |
| Carvalho et al (2008)[20] | Italy | Adult | 14 | Severe asthma phenotype associated with fungal sensitization | The diagnosis of SAFS was made on the basis of the following recently proposed criteria: severe asthma, total IgE levels <1000 IU/mL, and positive skin-prick test and/or raised specific IgE to *A fumigatus* | 80 | Healthy | Yes | rs5743708 | Bi-PASA |
| Eder et al (2004)[21] | Austria and Bavaria | Children | 50 | Asthma patients | Physician's diagnosis | 574 | No asthma | — | rs4696480 rs3804099 rs3804100 | TaqMan genotyping system |
| Hussein et al (2012)[22] | Egypt | Children | 500 | Asthma patients | A history of chest tightness and wheezing during the previous 12 months, a >12% reversibility of forced expiratory volume (FEV) spontaneously or after b2-agonist inhalation, and/or a methacholine provocation test result with a PC20 <16 mg/mL | 300 | No asthma | — | rs5743708 | ARMS–PCR |
| Koponen et al (2014)[23] | African white Caucasian | Infants | 17 | Asthma patients | Physician's diagnosis | 53 | Allergic rhinitis | — | rs5743708 | Pyrosequencing assay |
| Lachheb et al (2008)[24] | African white Caucasian | Children | 210 | Asthma patients | GINA | 224 | No asthma | — | rs5743708 | PCR-RFLP |
| Noguchi et al (2004)[17] | Japanese | Children and adult | 113 | Asthma patients | Dermatophagoidesfarinae RAST scores ≥3 | 190 | Healthy | | rs3804099 rs3804100 | Direct sequencing |
| Ortiz-Martínez et al (2016)[15] | Puerto Rican | adult | 62 | Asthma patients | Physician's diagnosis | 61 | No asthma | — | rs4696480 rs3804099 rs3804100 rs13150331 | Taqman real-time PCR assay |
| Potaczek et al (2011)[25] | Polish | Adult | 36 | Asthma patients | GINA | 127 | No asthma | — | rs3804099 | LightCycler 480 platform |
| Smit et al (2007)[16] | Danish | Adult | 106 | Asthma patients | Based on self-reported answers to the 4 questions "Have you ever had attacks of breathlessness at rest with wheezing?," "Have you ever had asthma attacks?," "Was this diagnosis confirmed by a physician?," and "Have you had an asthma attack in the last 12 months?," or a positive response to at least 2 questions and a positive expertise of their medical record | 102 | No asthma | — | rs4696480 rs5743708 | SSP-PCR |
| Smit et al (2009)[26] | French | Children and adult | 105 | Asthma patients | Based on self-reported answers to the 4 questions "Have you ever had attacks of breathlessness at rest with wheezing?," "Have you ever had asthma attacks?," "Was this diagnosis confirmed by a physician?," and | 254 | No asthma | — | rs3804099 rs13150331 rs3804100 | SSP-PCR |

| Study | Country | Study population | No.of cases | Type of cases | Diagnostic criteria | No. of controls | Type of controls | Hardy-Weinberg | SNP | Genotyping methods |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | "Have you had an asthma attack in the last12 months?," or a positive response to at least 2 questions and a positive expertise of their medical record | | | | | |
| Smit et al (2011)[27] | French | Adult | 524 | Asthma patients | Based on self-reported answers to the 4 questions "Have you ever had attacks of breathlessness at rest with wheezing?," "Have you ever had asthma attacks?," "Was this diagnosis confirmed by a physician?," and "Have you had an asthma attack in the last 12 months?," or a positive response to at least 2 questions and a positive expertise of their medical record | 719 | No asthma | — | rs3804099 | SSP-PCR |

"—" = not mentioned, ARMS–PCR = tetraprimer amplification refractory mutation system-polymerase chain reaction, Bi-PASA = bidirectional PCR amplification of specific alleles, FEV = forced expiratory volume, GINA = Global Initiative for Asthma, no-asthma = all those controls did not suffer from any chronic disease and did not have any allergic disease, RAST = radioallergosorbent test, SNP = single-nucleotide polymorphisms, SSP-PCR = sequence-specific primer and PCR.

codominant$^3$ model (AT vs. TT): $I^2 = 68.40\%$, $P = .023$), and the results were displayed in Table 3. The values of OR were 1.64 (95% CI = 0.967–2.781, $P = .066$, Fig. 5A) for the allelic model, 0.612 (95% CI = 0.291–1.29, $P = .197$, Fig. 5B) for the homozygote model, 1.415 (95% CI = 0.563–3.558, $P = .461$, Fig. 5C) for the recessive model, 2.455 (95% CI = 1.235–4.88, $P = .01$, Fig. 5D) for the dominant model, 2.576 (95% CI = 0.949–6.997, $P = .063$, Fig. 5E) for the codominant$^1$ model, 0.98 (95% CI = 0.354–2.713, $P = .969$, Fig. 5F) for the codominant$^2$ model, and 2.776 (95% CI = 1.199–6.427, $P = .017$, Fig. 5G) for the codominant$^3$ model, which inferred that the TLR2 rs4696480 polymorphism was significantly associated with asthma susceptibility under the dominant and codominant$^3$models, and individuals carrying A allele of the TLR2 rs4696480 polymorphism were more susceptible to develop asthma.

### 3.6. Publication bias

According to the results of Begg and Egger tests (Table 3), all the values of $P$ were $>0.05$, which revealed that there was no significant evidence of publication bias in all of the genetic models.

## 4. Discussion

Several related studies have been performed to estimate the association between the asthma risk and the rs5743708 polymorphism, rs3804099 polymorphism, rs3804100 polymorphism, and rs4696480 polymorphism in *TLR2* gene, and the results are still under debate. The current study combines all relevant data together and conducts the meta-analysis to have a comprehensive appraisal. Our results demonstrate that the TLR2 rs4696480 polymorphism is significantly correlated with asthma risk, whereas no significant relationship is observed between the asthma risk and rs5743708, rs3804099, and rs3804100 polymorphisms in *TLR2* gene.

Asthma is the most common chronic disease of the lower airways with the mucus hyperproduction, airway hyperresponsiveness and eosinophilic inflammation as the main characteristics.[2] According to the World Health Organization, >7 million children younger than 18 years are affected by asthma in the United States, which causes a heavy economic burden that is assessed to exceed that of the tuberculosis and AIDS combined.[28] The incidence of Asthma remains increasing in both the developing and developed countries.[29] Although its pathogenesis is complicated and incompletely understood, multiple genes, various genetic background, and environmental factors have been believed to be associated with asthma.[30] And a number of researches have inferred that the pathogenesis of asthma has a strong genetic component.[30]

TLRs, as transmembrane receptors, can regulate the immune system via the recognition of both PAMPs and damage-associated molecular patterns and the stimulation of the downstream signaling pathways resulting in the release of inflammatory cytokines and chemokines.[10,31] And TLRs play crucial roles in the interactions between the host and the invading pathogens and innate immunity.[32] TLR2, capable of recognizing a wide spectrum of microbes, is a member of TLRs family.[32] Our meta-analysis shows that the TLR2 rs4696480 polymorphism was associated with asthma susceptibility and the genotype of TLR2 rs4696480 polymorphism was significantly different between asthmatics and control subjects.

**Table 2**

**The detailed information of the 4 SNPs in *TLR2* gene.**

| SNP | Allele change | Function | Protein residue change | MAF |
|---|---|---|---|---|
| rs5743708 | CGG⇒CAG | Missense | R [Arg]⇒Q [Gln] | A = 0.0173/2091 (ExAC); A = 0.0068/34 (1000 genomes); A = 0.0222/289 (GO-ESP) |
| rs3804099 | AAT⇒AAC | Synonymous codon | N [Asn]⇒N [Asn] | C = 0.4113/49886 (ExAC); C = 0.4147/2077 (1000 Genomes); C = 0.4947/6434 (GO-ESP) |
| rs3804100 | AGT⇒AGC | Synonymous codon | S [Ser]⇒S [Ser] | C = 0.0914/11082 (ExAC); C = 0.1084/543 (1000 genomes); C = 0.0638/830 (GO-ESP) |
| rs4696480 | T⇒A | Intron variant | Hpy188III | A = 0.4223/2115 (1000 genomes) |

ExAC = exome aggregation consortium, GO-ESP = "Grand Opportunity" Exome Sequencing Project, MAF = minor allele count, SNP = single-nucleotide polymorphisms, TLR2 = toll-like receptor 2.

A previous meta-analysis,[30] published in 2015, evaluated the association of TLR2 rs5743708 polymorphism, TLR4 rs4986790 polymorphism, TLR4 rs4986791 polymorphism, and TLR9 rs187084 polymorphism with asthma susceptibility, and found that the asthma susceptibility was significantly related to TLR2 rs5743708 polymorphism, TLR4 rs4986790 polymorphism and TLR9 rs187084 polymorphism, which was inconsistent with our results that there was no significant association between the asthma risk and TLR2 rs5743708 polymorphism. With regard to the number of incorporated studies evaluating the association between the TLR2 rs5743708 polymorphism and asthma risk, the previous meta-analysis included 4 eligible studies up to 2012, whereas our meta-analysis incorporated 6 eligible studies up to 2016, and with larger sample size, results of the present meta-analysis were more reliable. Despite that different databases were selected between 2 analyses, we proposed that the statistical analysis was an important reason for the difference between 2 studies. We used the STATA 12 software for the statistical analysis of under allelic model, homozygote model, recessive model. It is indicated that different analysts might try to reduce the heterogeneity by limiting the meta-analysis to a smaller more homogeneous group of studies. However, this limits the scope of the meta-analysis and essentially throws away useful information. Models that incorporate and evaluate sources of heterogeneity are available. In comparison, the study of Tizaoui et al[30] showed less description of statistical analysis with no details on analysis of different models, which may lead to the difference in statistical results. In addition, the magnitude of the association of polymorphisms with asthma varies depending on factors including genetics, ethnic groups, methodologies of data synthesis, and statistical analysis. The databases in our research included the web of science, PubMed, and Embase, with the search terms containing "Susceptibility" or "Susceptibilities," whereas Tizaoui et al performed the literature search by using the Medline and Embase databases and no "Susceptibility" or "Susceptibilities" was involved in search terms, which may explain the reason that other 7 studies were included in our manuscript while not in the previous meta-analysis. Inclusion criteria give us a set of inclusive standards to screen potential participants. For instance, inclusion criteria may include general factors such as age and sex, but many other health-related factors compromise a full list of inclusion criteria. We set inclusion criteria in recruited publications, such as 4 specific polymorphisms, number of individual genotypes, along with exclusion criteria including no case-control study, raw data unavailable for examining OR with 95% CI, conference abstract, case report,

**Table 3**

**Meta-analysis of the association between asthma susceptibility and the 4 SNPs in *TLR2* gene.**

| SNP ID | Model | | OR | Lower limit | Upper limit | *P* (OR) | $I^2$ | *P* (heterogeneity) | *P* (Begg test) | *P* (Egger test) |
|---|---|---|---|---|---|---|---|---|---|---|
| rs5743708 | Allele | G vs. A | 0.951 | 0.573 | 1.578 | .846 | 30.60% | .206 | 1 | .984 |
| | Homozygous | GG + AA vs. GA | 1.128 | 0.651 | 1.955 | .688 | < 0.01% | .518 | 1 | .465 |
| | Recessive | GG vs. GA + AA | 1.003 | 0.591 | 1.704 | .99 | 5.10% | .384 | 1 | .824 |
| rs3804099 | Allele | T vs. C | 0.978 | 0.839 | 1.14 | .755 | 22.80% | .274 | .308 | .4 |
| | Homozygous | TT + CC vs. TC | 0.923 | 0.745 | 1.142 | .46 | < 0.01% | .443 | .089 | .074 |
| | Recessive | TT vs. TC + CC | 0.786 | 0.557 | 1.109 | .171 | 68.40% | .013 | .806 | .889 |
| | Dominant | TT + TC vs. CC | 1.032 | 0.782 | 1.362 | .824 | < 0.01% | .708 | 1 | .877 |
| | Codominant 1 | TT vs. TC | 0.909 | 0.71 | 1.163 | .447 | 48.80% | .419 | .73 | .119 |
| | Codominant 2 | TT vs. CC | 0.975 | 0.703 | 1.352 | .879 | 32.50% | .217 | .308 | .394 |
| | Codominant 3 | TC vs. CC | 1.07 | 0.798 | 1.433 | .652 | < 0.01% | .869 | .734 | .28 |
| rs3804100 | Allele | T vs. C | 0.866 | 0.682 | 1.099 | .237 | < 0.01% | .91 | .734 | .964 |
| | Homozygous | TT + CC vs. TC | 0.855 | 0.643 | 1.136 | .279 | < 0.01% | .743 | .734 | .827 |
| | Recessive | TT vs. TC + CC | 0.842 | 0.638 | 1.111 | .224 | < 0.01% | .925 | 1 | .965 |
| | Dominant | TT + TC vs. CC | 0.863 | 0.447 | 1.664 | .66 | 10.20% | .342 | 1 | .654 |
| | Codominant 1 | TT vs. CC | 0.853 | 0.639 | 1.138 | .28 | < 0.01% | .759 | .734 | .835 |
| | Codominant 2 | TT vs. TC | 0.799 | 0.408 | 1.566 | .513 | 1.40% | .385 | 1 | .688 |
| | Codominant 3 | TC vs. CC | 0.96 | 0.485 | 1.901 | .907 | 20.30% | .288 | .734 | .734 |
| rs4696480 | Allele | A vs. T | 1.64 | 0.967 | 2.781 | .066 | 79.50% | .002 | .308 | .276 |
| | Homozygous | AA + TT vs. AT | 0.612 | 0.291 | 1.29 | .197 | 78.90% | .003 | .308 | .289 |
| | Recessive | AA vs. AT + TT | 1.415 | 0.563 | 3.558 | .461 | 84.30% | 0 | .734 | .765 |
| | Dominant | AA + AT vs. TT | 2.455 | 1.235 | 4.88 | .01 | 64.10% | .056 | .308 | .075 |
| | Codominant 1 | AA vs. TT | 2.576 | 0.949 | 6.997 | .063 | 75.10% | .007 | .308 | .086 |
| | Codominant 2 | AA vs. AT | 0.98 | 0.354 | 2.713 | .969 | 84.70% | 0 | 1 | .707 |
| | Codominant 3 | AT vs. TT | 2.776 | 1.199 | 6.427 | .017 | 68.40% | .023 | .308 | .291 |

OR = odds ratio, SNP = single-nucleotide polymorphisms, TLR2 = toll-like receptor 2.

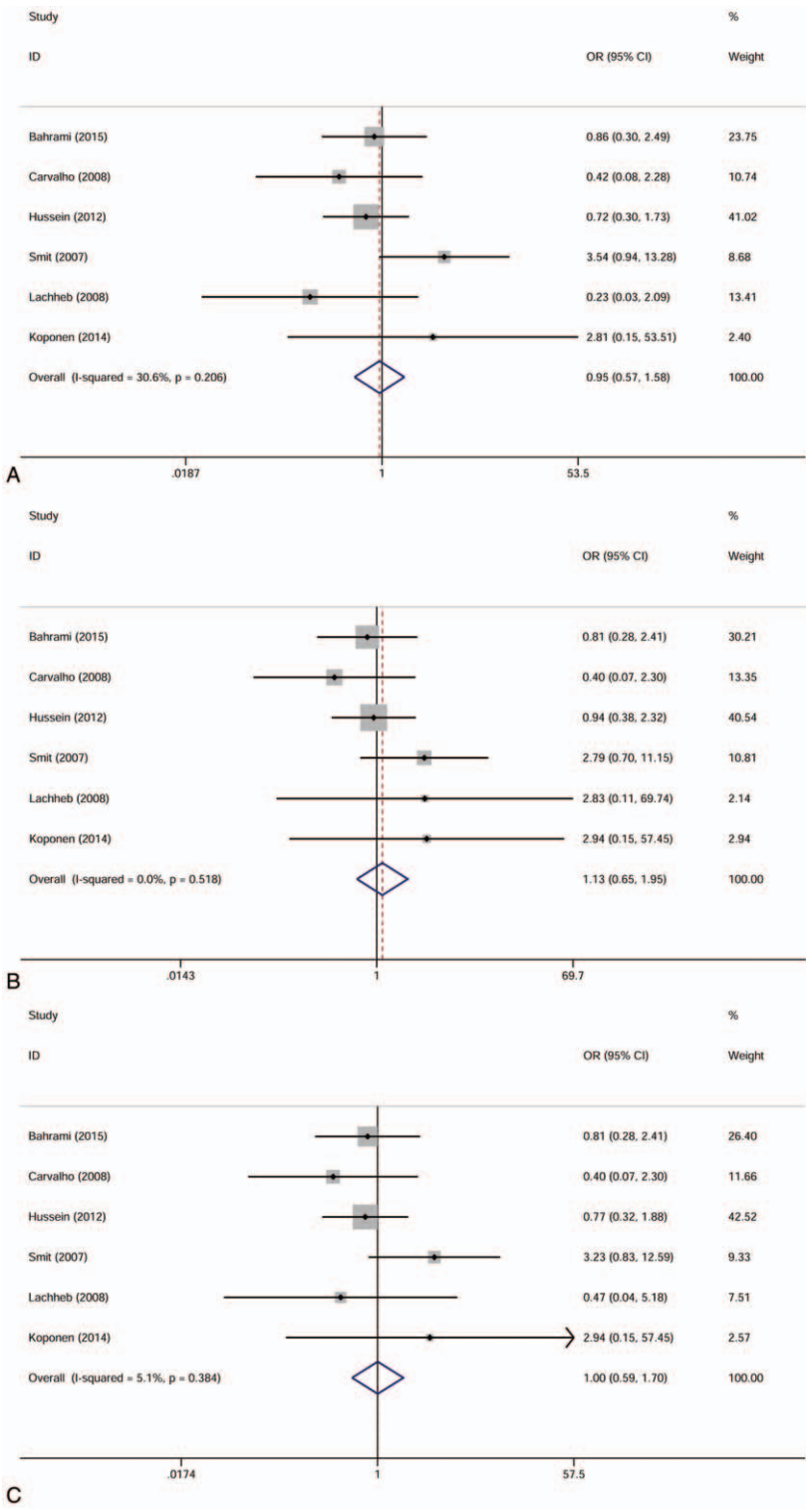


**Figure 2.** Forest plots of studies estimating the relationship between the TLR2 rs5743708 polymorphism and asthma susceptibility under allelic model (A), homozygote model (B), and recessive model (C).

and letters to the editors. This was partially different with the study of Tizaoui et al[30] in which no specific polymorphisms and no unavailable raw data were particularly demonstrated. Moreover, in 2 meta-analyses, although 2 authors were required in the data extraction, discrepancies or inconsistencies between the 2 authors were settled by consensus before being standardized into a unified dataset. Therefore, we cannot absolutely exclude the influence of artificial factors. So the association of TLR2 rs5743708 polymorphism with asthma risk needs to be further validated based on a great number of experimental data.

Additionally, polymorphisms in *TLR2* gene have also been reported to be associated with other diseases. A systematic

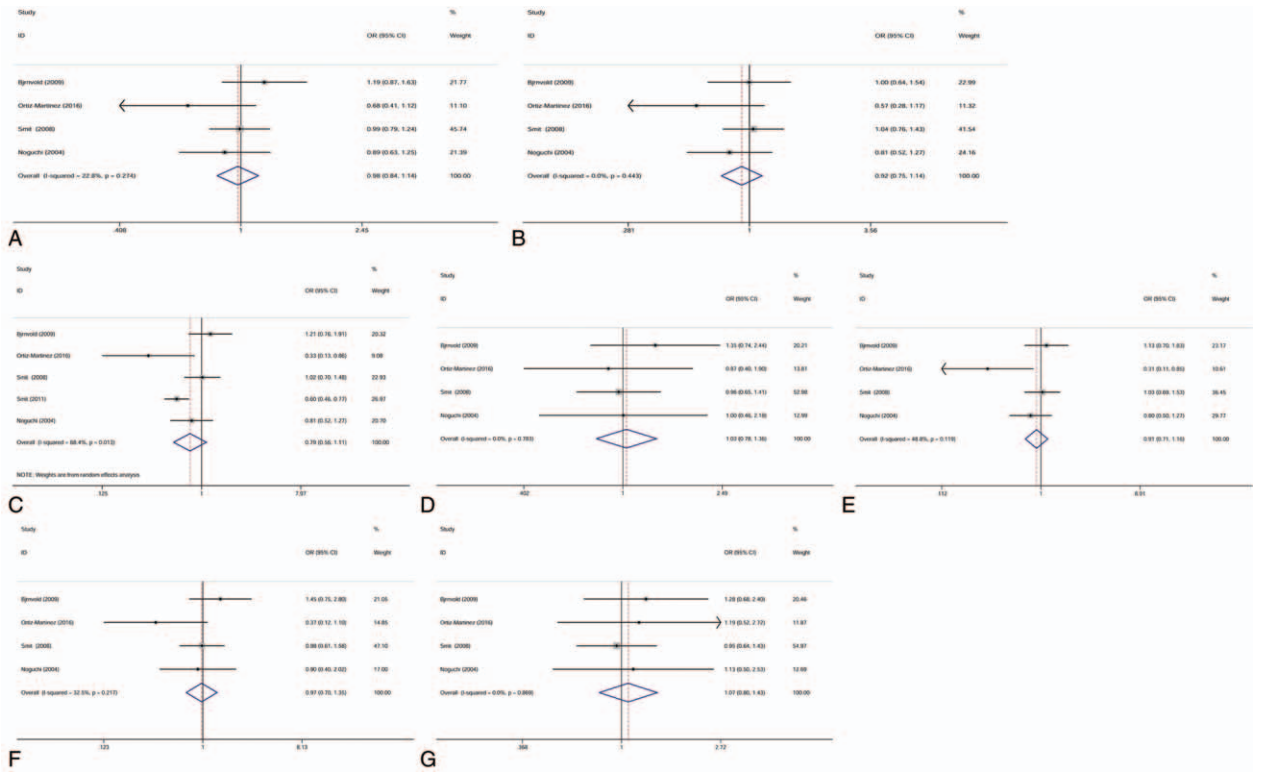


**Figure 3.** Forest plots of studies assessing the relationship between the TLR2 rs3804099 polymorphism and asthma susceptibility under allelic model (A), homozygote model (B), recessive model (C),dominant model (D), codominant[1] model (E), codominant[2] model (F), and codominant[3] model (G).

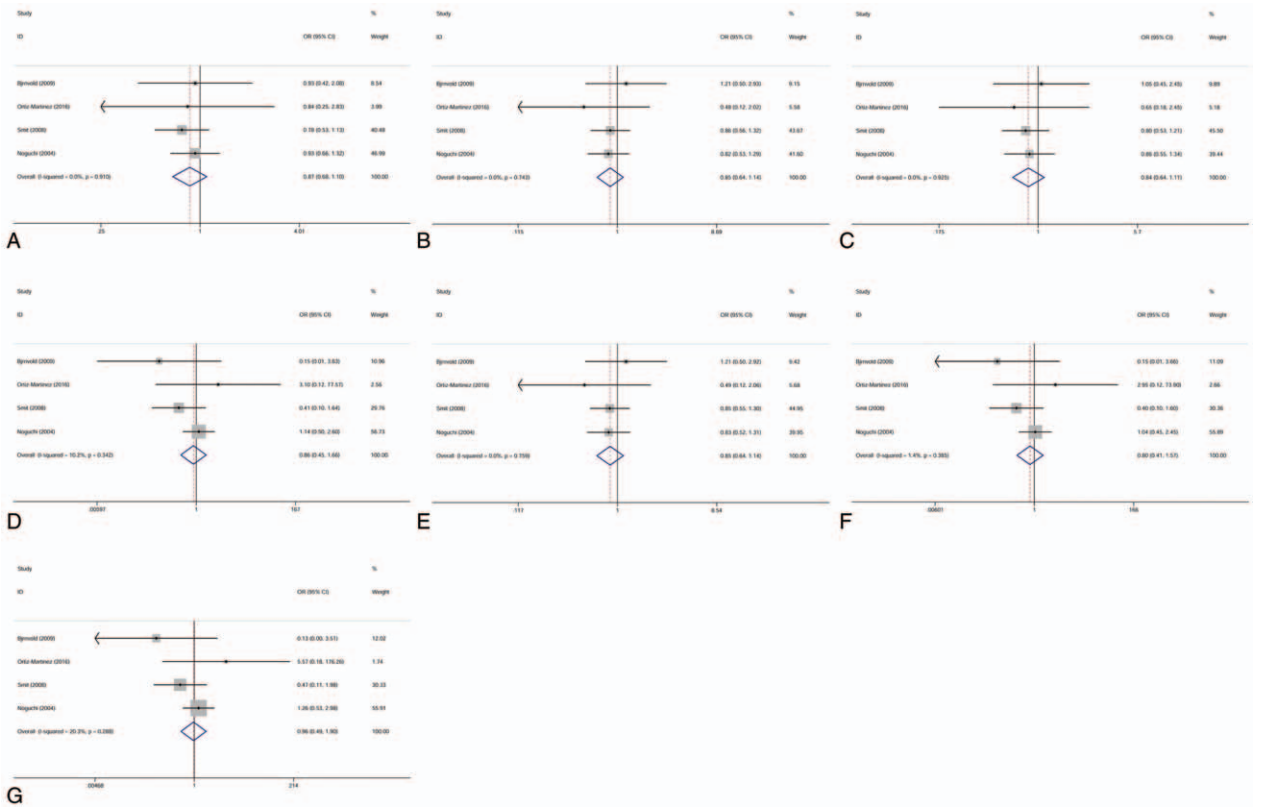


**Figure 4.** Forest plots of studies appraising the relationship between the TLR2 rs3804100 polymorphism and asthma susceptibility under allelic model (A), homozygote model (B), recessive model (C), dominant model (D), codominant[1] model (E), codominant[2] model (F), and codominant[3] model (G).

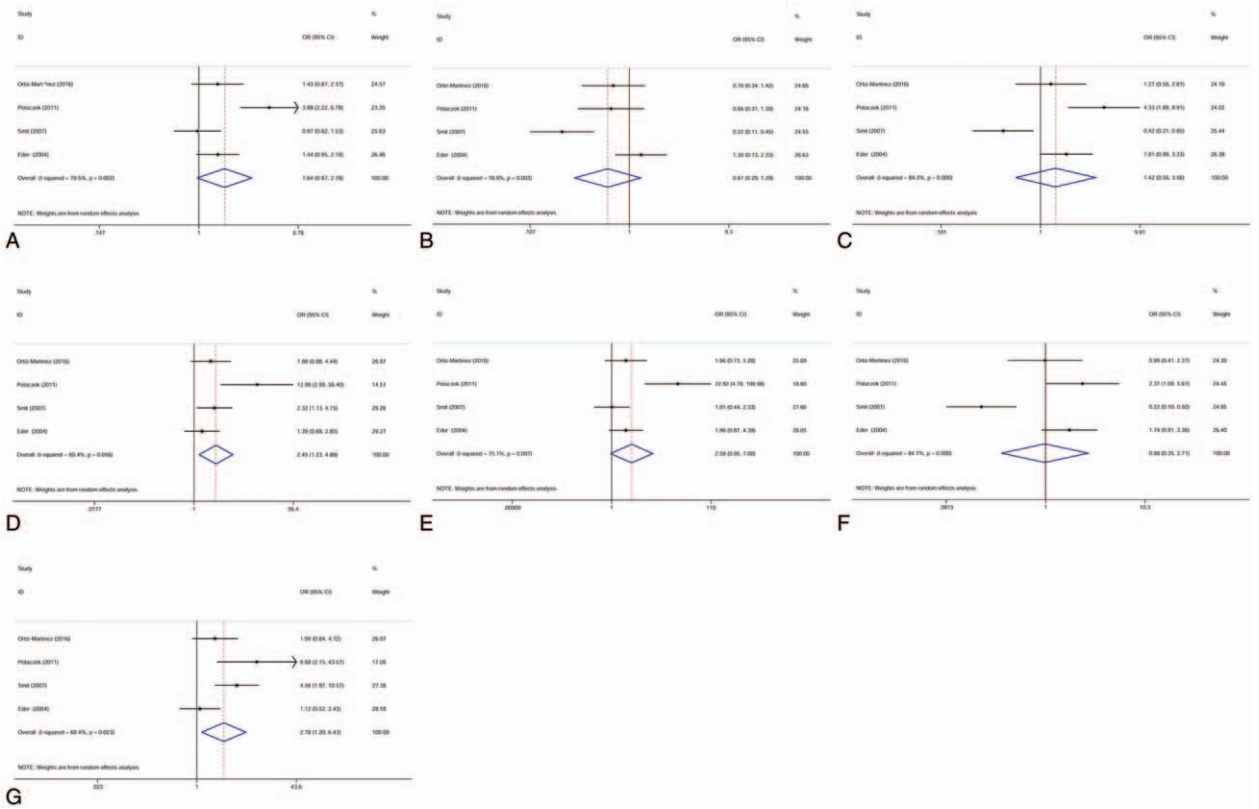


**Figure 5.** Forest plot of study evaluating the relationship between the TLR2 rs4696480polymorphism and asthma susceptibility under allelic model (A), homozygote model (B), recessive model (C), dominant model (D), codominant[1] model (E), codominant[2] model (F), and codominant[3] model (G).

research,[33] published in 2015, showed that the TLR2 rs5743708 polymorphism was correlated with the tuberculosis risk. A case-control association study,[34] performed on Korean population, revealed that the rs3804099 and rs3804100 polymorphisms in *TLR2* gene were related to the clinicopathologic features of papillary thyroid cancer. And the rs3804099 and rs3804100 polymorphisms in *TLR2* gene have also been recorded to be associated with severe ischemic stroke.[35]

However, the results described in the present study do have limitations. Firstly, we failed to make the subgroup analysis stratified by different genetic backgrounds because of insufficient data. Second, the diagnostic criteria for asthma were not exactly the same in all the included studies, which might lead to some bias for our results. Additionally, we only incorporated studies published in English.

Conclusively, despite the limitations mentioned above, our meta-analysis suggests that the rs5743708, rs3804099, and rs3804100 polymorphisms in *TLR2* gene are not associated with asthma risk, whereas significant association has been observed between the TLR2 rs4696480 polymorphism and asthma susceptibility, and TLR2 rs4696480 polymorphism is a risk factor for asthma. And understanding the risk factors of asthma will be beneficial for the early diagnosis, prevention, and therapeutic strategies for this chronic inflammatory disease.

## References

[1] Kabesch M. Early origins of asthma (and allergy). Mol Cell Pediatr 2016;3:31.
[2] van Rijt L, von Richthofen H, van Ree R. Type 2 innate lymphoid cells: at the cross-roads in allergic asthma. Semin Immunopathol 2016;38:483–96.
[3] Masoli M, Fabian D, Holt S, et al. The global burden of asthma: executive summary of the GINA Dissemination Committee report. Allergy 2004;59:469–78.
[4] Wenzel SE. Asthma phenotypes: the evolution from clinical to molecular approaches. Nat Med 2012;18:716–25.
[5] Nieto-Fontarigo JJ, Gonzalez-Barcala FJ, San Jose E, et al. CD26 and Asthma: a Comprehensive Review. Clin Rev Allergy Immunol 2016.
[6] Dong Z, Xiong L, Zhang W, et al. Holding the inflammatory system in check: TLRs and their targeted therapy in asthma 2016;2016:2180417.
[7] Phipps S, Lam CE, Foster PS, et al. The contribution of toll-like receptors to the pathogenesis of asthma. Immunol Cell Biol 2007;85:463–70.
[8] Zeyer F, Mothes B, Will C, et al. mRNA-mediated gene supplementation of toll-like receptors as treatment strategy for asthma in vivo. PLoS One 2016;11:e0154001.
[9] Hussein WM, Liu TY, Skwarczynski M, et al. Toll-like receptor agonists: a patent review (2011-2013). Expert Opin Ther Pat 2014;24:453–70.
[10] Liu Y, Yin H, Zhao M, et al. TLR2 and TLR4 in autoimmune diseases: a comprehensive review. Clin Rev Allergy Immunol 2014;47:136–47.
[11] Kanagaratham C, Camateros P, Flaczyk A, et al. Polymorphisms in TOLL-like receptor genes and their roles in allergic asthma and atopy. Recent Pat Inflamm Allergy Drug Discov 2011;5:45–56.
[12] Lorenz E. TLR2 and TLR4 expression during bacterial infections. Curr Pharm Des 2006;12:4185–93.
[13] Palsson-McDermott EM, O'Neill LA. The potential of targeting Toll-like receptor 2 in autoimmune and inflammatory diseases. Ir J Med Sci 2007;176:253–60.
[14] Rock FL, Hardiman G, Timans JC, et al. A family of human receptors structurally related to Drosophila Toll. Proc Natl Acad Sci U S A 1998;95:588–93.
[15] Ortiz-Martínez MG, Frías-Belén O, Nazario-Jiménez S, et al. A case–control study of innate immunity pathway gene polymorphisms in Puerto Ricans reveals association of toll-like receptor 2+ 596 variant with asthma. BMC Pulm Med 2016;16:112.

[16] Smit LA, Bongers SI, Ruven HJ, et al. Atopy and new-onset asthma in young Danish farmers and CD14, TLR2, and TLR4 genetic polymorphisms: a nested case-control study. Clin Exp Allergy 2007;37:1602–8.
[17] Noguchi E, Nishimura F, Fukai H, et al. An association study of asthma and total serum immunoglobin E levels for Toll-like receptor polymorphisms in a Japanese population. Clin Exp Allergy 2004;34:177–83.
[18] Bahrami H, Daneshmandi S, Heidarnazhad H, et al. Lack of association between toll like receptor-2 and toll like receptor-4 gene polymorphisms and other feature in iranian asthmatics patients. Iran J Allergy Asthma Immunol 2015;14:48–54.
[19] Bjornvold M, Munthe-Kaas MC, Egeland T, et al. A TLR2 polymorphism is associated with type 1 diabetes and allergic asthma. Genes Immun 2009;10:181–7.
[20] Carvalho A, Pasqualotto AC, Pitzurra L, et al. Polymorphisms in toll-like receptor genes and susceptibility to pulmonary aspergillosis. J Infect Dis 2008;197:618–21.
[21] Eder W, Klimecki W, Yu L, et al. Toll-like receptor 2 as a major gene for asthma in children of European farmers. J Allergy Clin Immunol 2004;113:482–8.
[22] Hussein YM, Awad HA, Shalaby SM, et al. Toll-like receptor 2 and Toll-like receptor 4 polymorphisms and susceptibility to asthma and allergic rhinitis: a case-control analysis. Cell Immunol 2012;274:34–8.
[23] Koponen P, Vuononvirta J, Nuolivirta K, et al. The association of genetic variants in toll-like receptor 2 subfamily with allergy and asthma after hospitalization for bronchiolitis in infancy. Pediatr Infect Dis J 2014;33:463–6.
[24] Lachheb J, Dhifallah IB, Chelbi H, et al. Toll-like receptors and CD14 genes polymorphisms and susceptibility to asthma in Tunisian children. Tissue Antigens 2008;71:417–25.
[25] Potaczek DP, Nastalek M, Okumura K, et al. An association of TLR2-16934A >T polymorphism and severity/phenotype of atopic dermatitis. J Eur Acad Dermatol Venereol 2011;25:715–21.
[26] Smit LA, Siroux V, Bouzigon E, et al. CD14 and toll-like receptor gene polymorphisms, country living, and asthma in adults. Am J Respir Crit Care Med 2009;179:363–8.
[27] Smit LA, Bouzigon E, Bousquet J, et al. Mold allergen sensitization in adult asthma according to integrin beta3 polymorphisms and Toll-like receptor 2/+596 genotype. J Allergy Clin Immunol 2011;128:185–91. e187.
[28] Vercelli D. Does epigenetics play a role in human asthma? Allergol Int 2016;65:123–6.
[29] Ho SM. Environmental epigenetics of asthma: an update. J Allergy Clin Immunol 2010;126:453–65.
[30] Tizaoui K, Kaabachi W, Hamzaoui K, et al. Association of single nucleotide polymorphisms in toll-like receptor genes with asthma risk: a systematic review and meta-analysis. Allergy Asthma Immunol Res 2015;7:130–40.
[31] Arslan F, Keogh B, McGuirk P, et al. TLR2 and TLR4 in ischemia reperfusion injury. Mediators Inflamm 2010;2010:704202.
[32] van Bergenhenegouwen J, Plantinga TS, Joosten LA, et al. TLR2 & Co: a critical analysis of the complex interactions between TLR2 and coreceptors. J Leukoc Biol 2013;94:885–902.
[33] Guo XG, Xia Y. The rs5743708 gene polymorphism in the TLR2 gene contributes to the risk of tuberculosis disease. Int J Clin Exp Pathol 2015;8:11921–8.
[34] Kim MK, Park SW, Kim SK, et al. Association of Toll-like receptor 2 polymorphisms with papillary thyroid cancer and clinicopathologic features in a Korean population. J Korean Med Sci 2012;27:1333–8.
[35] Park HJ, Kim SK, Yun DH, et al. Association of Toll-like receptor 2 polymorphisms with National Institute of Health Stroke Scale scores of ischemic stroke patients. J Mol Neurosci 2012;46:536–40.