

Meta analysis

Association between the polymorphisms of interleukin-4, the interleukin-4 receptor gene and asthma

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Keywords: interleukin-4; interleukin-4 receptor; asthma; gene polymorphism; case-controlled study; meta-analysis

Background Interleukin-4 (IL4) is one of the most important cytokines involved in a variety of allergic disorders, particularly, asthma. A number of genetic epidemiological studies have identified an association between the gene polymorphisms of IL4 and interleukin-4 receptor (IL4R) and asthma in different populations. However, these studies have been inconsistent and inconclusive. The aim of this study was to investigate the association between the single nucleotide polymorphism (SNP) of IL-4, IL-4R and asthma risk in case-controlled studies using meta-analysis.

Method A genetic model-free approach was used to perform the meta-analysis. Asthma (atopy status nondefined), nonatopic and atopic asthma subgroups were separately analyzed. Next, the ethnic subgroup was analyzed. Heterogeneity and publication bias were also explored.

Results Only two polymorphisms of IL4 (rs2243250 and rs2070874) and four polymorphisms of IL4R (rs1801275, rs1805011, rs1805010, and rs1805015) were included in the meta-analysis. Polymorphisms rs2243250 and rs2070874 of IL-4 and rs1801275 and rs1805011 of IL4R were associated with asthma. The overall odds ratio (OR) of rs2243250 in the CC versus TT+TC genotypes was 0.84 (95% CI: 0.75–0.94), and the Z-test for the overall effect was 3.0 ($P=0.003$). We obtained significant results from this polymorphism in the Caucasian ethnicity and adult groups. However, the overall OR of rs1801275 for the GG+AG versus AA genotype was 1.16 (95% CI: 1.00–1.35), and the Z-test for the overall effect was 1.87 ($P=0.06$). Moreover, significant results were only obtained from the sub-group analysis in Asians ($P=0.02$). In the rs1805011 polymorphism of IL4R, the overall OR for the CC +AC versus AA genotypes was 0.39 (95% CI: 0.16–0.95), and the Z-test for the overall effect was 2.08 ($P=0.04$).

Conclusions Both the IL4 and IL4R polymorphisms were associated with asthma. The rs2243250 polymorphism of IL4 was more important in the white and adult groups. Individuals who carried the C allele for rs2070874 of the IL4 gene demonstrated increased asthma risk compared to TT homozygotes. An individual with an AA genotype in rs1805011 of the IL4R gene was less likely to suffer from asthma compared to the other two genotypes.

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Asthma affects over 14 million people in the United States.¹ The prevalence of asthma in the United States has increased by 74.9% from 1980 to 1996.¹ Asthma exhibits a complex etiology, resulting from interactions between multiple genes and environmental factors. For reasons that are still unclear, specific ethnic groups disproportionately exhibit asthma morbidity.²⁻⁴

Through activation of its receptor, interleukin-4 (IL4) stimulates the production of serum IgE. The loci of IL4 and the IL4 receptor (IL4R) are located on genomic regions that are linked to asthma phenotypes (5q31 for IL4 and 16p12 for IL4R). A functional single-nucleotide polymorphism (SNP) in the promoter of IL4 has been associated with total serum IgE levels,⁵⁻⁷ asthma,⁶⁻¹⁰ and other asthma-related phenotypes.^{8,11-13} SNPs in the exons of IL4R have also been associated with asthma,¹⁴⁻¹⁸ total serum IgE levels^{15,19-23} and other asthma-related phenotypes.^{24,25}

Burchard and colleagues¹² have investigated the association between a polymorphism (C-589T) in the promoter region of IL4 and two asthma-related phenotypes (low FEV₁ and high total serum IgE levels) in 772 patients with asthma. Among white asthmatic individuals, the T allele was

associated with FEV₁ with <50% prediction ($P=0.01$), although this was not observed in the total serum IgE levels. However, among African-American asthmatic individuals, there was no association between the T allele and a reduced FEV₁ or a decrease in the IgE levels of total serum. Furthermore, Ober et al¹⁸ showed that polymorphisms in IL4R differed across three ethnic groups (Hutterites, African Americans, and whites). Although several SNPs in IL4R were associated with asthma and/or atopy in Hutterites, there was no significant association between any of the five SNPs examined and asthma/atopy in 56 African-American families. Moreover, a number of studies on genetic epidemiology have assessed the association of

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IL4 and IL4R gene polymorphisms and asthma in different populations. However, these studies were inconsistent and inconclusive.

Population-based genetic epidemiology often seeks to identify relatively small effects against a complex etiological background. Thus, most genetic association studies tend to be statistically underpowered. Meta-analysis enables the use of all of the available published data to increase the statistical power and to identify causative genes with greater sensitivity. There have also been new developments in the methodology of meta-analysis in genetic studies.²⁶⁻²⁹ A genetic model-free approach has been suggested for use in the meta-analysis of genetic association studies.^{27,28}

Thus, we used the genetic model-free approach to perform a meta-analysis of the association of SNPs in IL4, IL4R and asthma with the following objectives: to determine if there is an effect in these polymorphisms on asthma susceptibility and if so, to estimate the magnitude of that effect and the potential mode of inheritance in case-controlled studies.

METHODS

Search strategy

We conducted a literature search using the electronic databases Medline (Ovid), Pubmed, Embase, ScienceDirect, Springer, CNKI, Wanfang, Weipu and CBM to identify articles that evaluated the association between IL4 and IL4R genetic variants and the risk of asthma. (The last search was updated on August 31, 2012). The search strategy used was the following: (1) asthma (keyword); (2) interleukin-4 or interleukin-4 receptor (keyword); (3) genotype or allele or mutation or variant polymorphism (text word); (4) (1) and (2) and (3). The search was performed in duplicate by two independent reviewers (He J and Xia JW), and the results were limited to English and Chinese language articles.

Inclusion criteria

Human studies, regardless of the sample size, were included if they meet the following criteria: (1) IL4 and IL4 receptor polymorphisms were determined; (2) The studies were case-controlled in design (retrospective or nested case-control). The outcome was asthma (incidence or prevalence), and there were at least two comparison groups, for example, asthma vs. control (non-asthma) groups. However, the participants could be of any age. Studies on the basis of family or sibling pairs were excluded; (3) Each genotype frequency was reported, and there was sufficient information for data extraction.

Data extraction

The data were independently extracted and in duplicate by two reviewers (Zhu N and Dong L) who used a standardized data extraction protocol. Genotypes, authors, journals, years of publication, demographics, selection/ characteristics of cases and controls, and racial descent

were extracted from each study. Any disagreement was resolved by discussion and consensus.

Quality score assessment

The quality of the studies was also independently assessed by two reviewers (Long F and Zhang J), who used the quality assessment scores for the molecular association studies of asthma recommended by Thakkestian et al.²⁸ These scores were generated on the basis of both traditional epidemiological considerations and genetic issues.²⁶ The total scores ranged from 0 (worst) to 15 (best). Any disagreement was adjudicated by a third author (Chen XD). Low quality studies with a quality score of less than four were excluded.

Statistical analysis

First, all of the asthma groups were combined for the meta-analysis. Second, the subgroups were analyzed on the basis of the characteristics of their asthma: asthma (atopic status not defined); atopic asthma; and non-atopic asthma. Genetic association, magnitude and genetic model were tested using a genetic model-free approach as previously described by Thakkestian et al.^{27,28} For each case-controlled study, we first examined whether the genotype distribution in the control group followed the Hardy-Weinberg equilibrium using Pearson's χ^2 test. Any polymorphism that had been studied in at least three case-controlled studies was included in the meta-analysis. The strength of the associations between asthma risk and genetic variants were estimated using odds ratios (ORs) and 95% confidence intervals (CIs). The statistical significance of the summary ORs was assessed using the Z-test. The evaluated genetic models for each study were based mostly on the models used in primary studies. Heterogeneity was evaluated using an χ^2 -based Q statistic and was considered statistically significant at a *P* value <0.10. *I*² was used to measure the percentage of variability in the point estimate due to

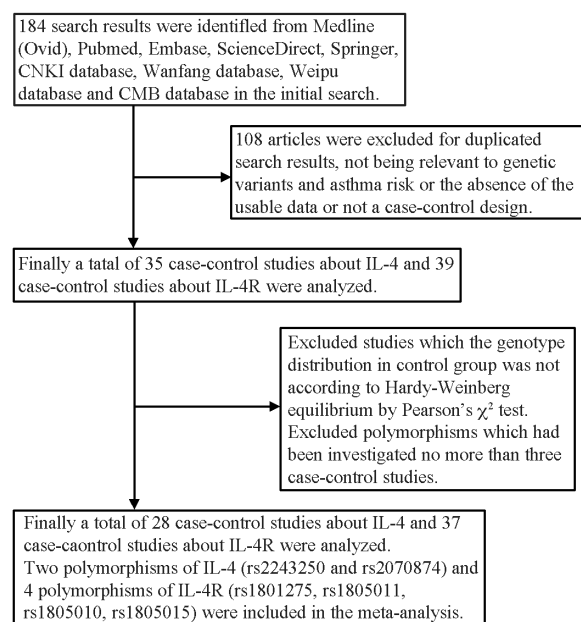


Figure 1. Flow diagram of included/excluded studies

heterogeneity rather than sampling error. When the P value was >0.10 , the pooled OR was calculated using the fixed-effects model; otherwise, a random-effects model was used. Publication bias was examined using funnel plots, Begg's test and Egger's test.³⁰ A funnel plot is asymmetrical when there is evidence of publication bias. Data analyses were performed using the Cochrane systematic review software, Review Manager version 5.0 (Cochrane Collaboration, Denmark) and STATA11.0 (StataCorp, USA).

RESULTS

Candidate SNPs in the IL4 and IL4R genes associated with asthma. The selection process is described in a flow diagram of included and excluded studies (Figure 1). Briefly, 184 search results were identified from Medline (Ovid), Pubmed, Embase, ScienceDirect, Springer, CNKI database, Wanfang database, Weipu database and the CBM database in the initial search. After reading the articles, 108 articles were excluded for duplicate search results, irrelevance to genetic variants and asthma risk, or the absence of usable data or because they were not designed as case-controlled studies. Finally, a total of 28 case-controlled studies on IL4^{9-11,14,31-54} and 37 case-controlled studies on IL4R^{10,11,14,15,17,21,22,24,33,35,36,38,40,44,46,47,50,54-72} were analyzed. In the meta-analysis, the small study number weakened the conclusions. Thus, only polymorphisms that had been investigated in at least three case-controlled studies were included. Thus, only two polymorphisms of IL4 (rs2243250 and rs2070874) and four polymorphisms of IL4R (rs1801275, rs1805011, rs1805010, and rs1805015) were included in the meta-analysis. Other polymorphisms of IL4 (rs2243290 and rs2243251) and IL4R (rs1805012, rs2234900 and rs1805016) were excluded due to an insufficient number of studies on each polymorphism. The models selected to synthesize the data for each polymorphism are shown in Table 1.

Quantitative Syntheses of Data

Two IL4 Polymorphisms (rs2243250 and rs2070874)

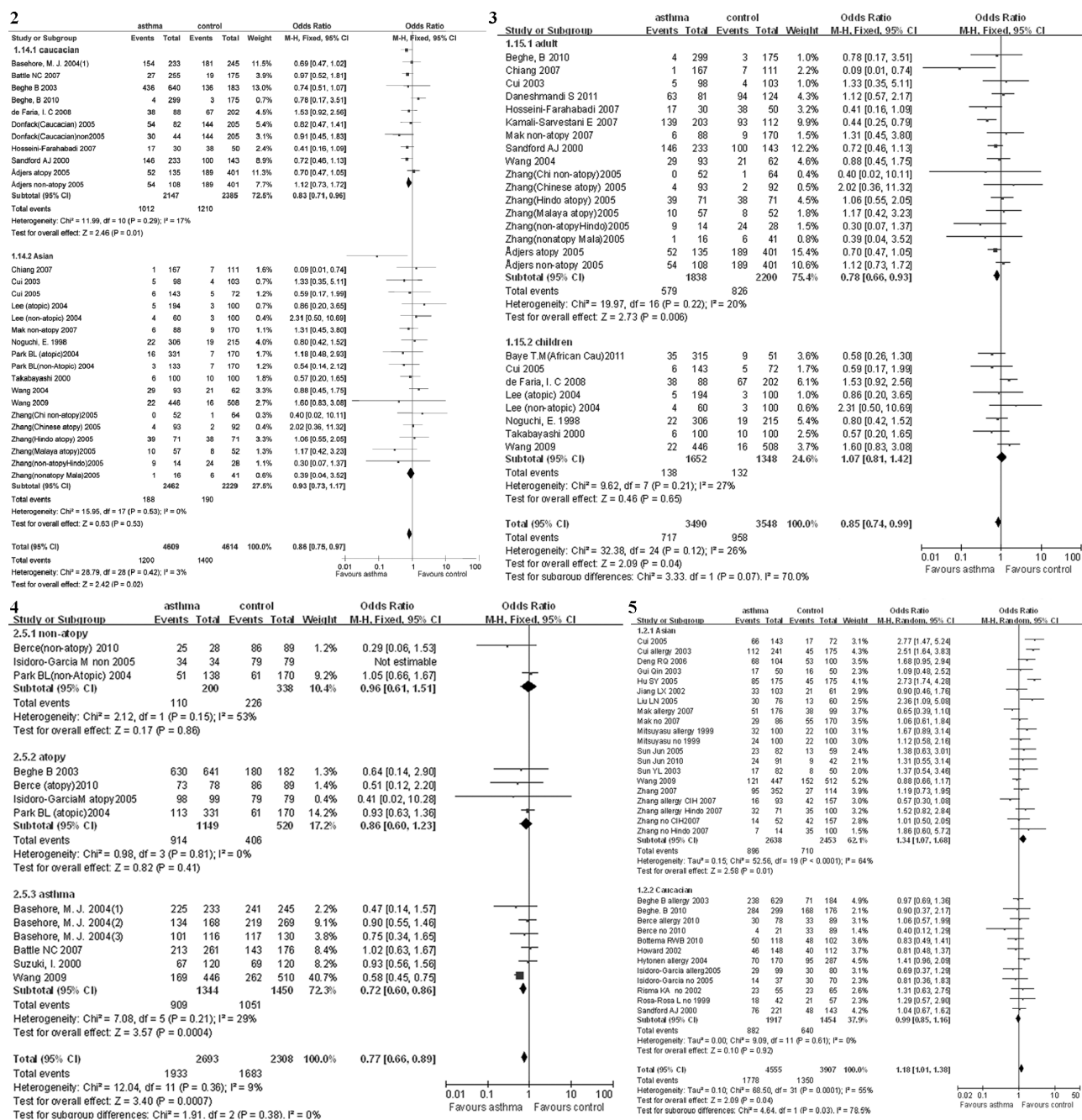
Twenty-five case-controlled studies on the relationship between the rs2243250 polymorphism and the risk

of asthma were included in the meta-analysis. The heterogeneity of CC/CT+TT was analyzed in all of the studies, χ^2 was 37.77 with 37 degrees of freedom, and $P=0.41$ in a fixed-effect model. In addition, the I^2 value, another index of heterogeneity, was 3%, suggesting low heterogeneity. Thus, the fixed-effect model was used to synthesize the data. The overall OR for the CC genotypes versus TT+TC genotypes was 0.84 (95% CI : 0.75–0.94), and the Z -test for the overall effect was 3.0 ($P=0.003$). These results suggested that individuals who were CC homozygotes had an increased asthma risk compared to T allele carriers. The funnel plots were nearly symmetrical, and neither Egger's test nor Begg's test (data not shown) indicated significant publication bias (all $P > 0.05$). To evaluate the ethnic-specific effects, subgroup analyses were performed using ethnic groups for polymorphisms that were investigated in a sufficient number of studies (i.e., data were available from at least three case-controlled studies for at least one subgroup). Two subgroups were used: Caucasian and Asian. We obtained significant results from this polymorphism in the Caucasian group (Figure 2). After subgroup analysis by age, rs2243250 in IL-4 gene polymorphisms were found to be significantly associated with asthma risk in adults but not in children (Figure 3).

Seven case-controlled studies on the relationship between the rs2070874 polymorphism and the risk of asthma were included in the meta-analysis. As shown in Figure 4, the heterogeneity of CC+CT/TT was analyzed for all of the studies, χ^2 was 12.24 with 12 degrees of freedom, and $P=0.36$ in a fixed-effect model. In addition, the I^2 value was 9%, suggesting a low heterogeneity (Figure 4). Thus, the fixed-effect model was used to synthesize the data. The overall OR for the CC+CT genotypes versus TT genotypes was 0.77 (95% CI : 0.66–0.89), and the Z -test for the overall effect was 3.4 ($P=0.0007$). These results suggested that individuals who carried the C allele had an increased asthma risk compared to TT homozygotes. The funnel plots were nearly symmetrical, and neither Egger's test nor Begg's test (data not shown) indicated significant

Table 1. Summary of comparative results of IL4 and IL4R genes

Gene	SNP	Outcome	Studies	Participant	Statistical method	Effect estimate
IL-4	rs2243250	CC/CT+TT	38	11546	OR (M-H, fixed, 95% CI)	0.84 (0.75, 0.94)
		CC+CT/TT	38	11546	OR (M-H, random, 95% CI)	0.91 (0.81, 1.02)
		C/T	38	23092	OR (M-H, random, 95% CI)	0.83 (0.73, 0.94)
	rs2070874	CC+CT/TT	13	5001	OR (M-H, random, 95% CI)	0.78 (0.66, 0.93)
		CC/CT+TT	13	5001	OR (M-H, random, 95% CI)	0.88 (0.75, 1.06)
		C/T	13	10002	OR (M-H, fixed, 95% CI)	0.91 (0.83, 1.00)
IL-4R	rs1801275	GG+AG/AA	34	8813	OR (M-H, random, 95% CI)	1.15 (1.01, 1.37)
		GG/AG+AA	34	8813	OR (M-H, random, 95% CI)	1.21 (0.92, 1.61)
		A/G	34	17626	OR (M-H, random, 95% CI)	0.86 (0.73, 1.00)
	rs1805011	CC+AC/AA	7	2519	OR (M-H, random, 95% CI)	0.39 (0.16, 0.95)
		CC/AC+AA	7	2519	OR (M-H, random, 95% CI)	0.10 (0.01, 1.97)
		A/C	7	5038	OR (M-H, random, 95% CI)	1.22 (0.79, 1.89)
	rs180510	AA+AG/GG	23	6850	OR (M-H, random, 95% CI)	0.97 (0.78, 1.18)
		AA/AG+GG	23	6850	OR (M-H, random, 95% CI)	0.93 (0.80, 1.09)
		A/G	23	13700	OR (M-H, random, 95% CI)	0.95 (0.84, 1.07)
	rs1805015	CC+CT/TT	6	2522	OR (M-H, random, 95% CI)	1.01 (0.68, 1.49)
		CC/CT+TT	6	2522	OR (M-H, random, 95% CI)	0.81 (0.50, 1.32)
		C/T	6	5044	OR (M-H, random, 95% CI)	0.99 (0.7, 1.42)



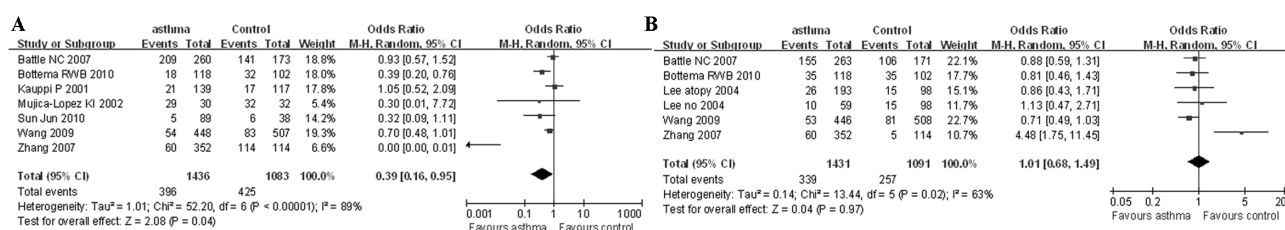


Figure 6. 6A: Meta-analysis of studies on the association between asthma risk and the rs1805011 polymorphism in the IL4R gene, using a random-effect model (CC+AC/AA); 6B: Meta-analysis of studies on the association between asthma risk and the rs1805015 polymorphism in the IL4R gene, using a random-effect model (CC+CT/TT).

three polymorphisms were nearly symmetrical, and neither Egger's test nor Begg's test (data not shown) indicated significant publication bias (all $P > 0.05$).

Twenty-three case-controlled studies on the relationship between the rs1805010 polymorphism and the risk of asthma were included in the meta-analysis. The overall OR for the AA+AG genotype versus GG genotype was 0.97 (95% CI: 0.79–1.18) in a random-effects model (Figure 7). Neither the Asian nor the Caucasian groups differed between asthma patients and control subjects in the subgroup analysis. After analyzing the subgroups by age, neither the adults nor the children differed between asthma patients and control subjects. Only in the atopic asthma group did individuals who carried an A allele display protection against asthma compared to the G homozygotes ($P=0.01$).

DISCUSSION

It has been well recognized that there is individual susceptibility to asthma even when individuals are exposed to the same environment. Host factors, including polymorphisms of genes involved in asthma, may account for this difference. Thus, genetic susceptibility to asthma has been an attractive research focus in the scientific community. SNPs in candidate genes have become standard in association tests and have been used to characterize candidate genes or specific genomic regions on a whole-genome scale. Recently, variants of the IL4 and IL4 receptor gene have been studied in association with asthma risk, although a number of studies have reported inconsistent results, which is partly due to an inadequate sample size. Therefore, a combined meta-analysis with a case-controlled study may avoid or reduce such a bias. Our meta-analysis aimed to combine evidence that can assess the association between the IL4 and IL4 receptor polymorphisms and asthma risk. This meta-analysis included only the extensively investigated SNPs (rs2243250 and rs2070874 in the IL4 gene, and rs1801275, rs1805011, rs1805010, and rs1805015 in the IL4R gene) because the meta-analysis included a small number of studies that could weaken the conclusions. Thus, any polymorphism that had been studied in at least three case-controlled studies was included in the meta-analysis.

The present results indicated that SNPs in IL4 and IL4R were associated with asthma. Our meta-analysis included

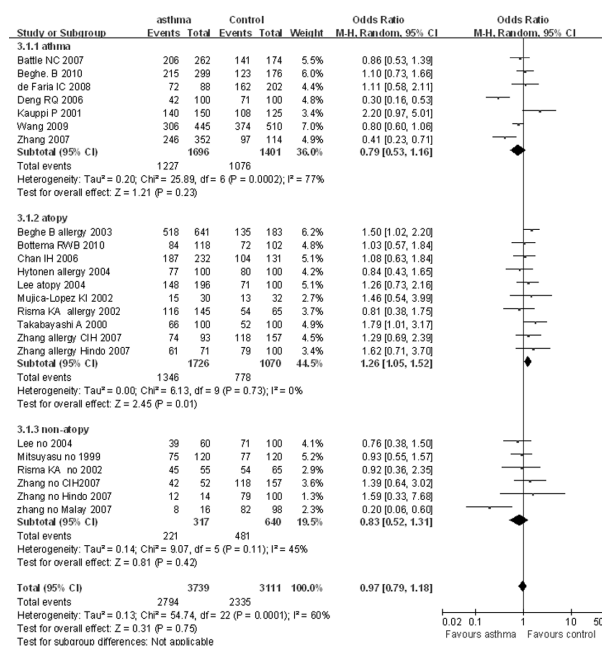


Figure 7. Meta-analysis of studies on the association between asthma risk and the rs1805010 polymorphism in the IL4R gene, using a random-effect model (AA+AG/GG)

5780 patients and 5766 controls and confirmed the IL-4 promoter C-589T substitution as a risk factor for developing asthma. Our results indicated a significant association and recessive genetics of C-589T polymorphisms of IL4 in atopic asthma. The CC genotype was approximately 21% less likely to have atopic asthma compared to the CT and TT genotypes. However, we did not find an association in the C-589T polymorphisms that was significantly associated with non-atopic asthma. C-589T in the IL4 gene is located in the IL4 promoter region, where the T-allele is more convenient for binding to a transcription factor.^{6,73} Because IL4 plays an important role in converting an IgM to an IgE, higher total serum IgE levels were observed in IL4-589T allele carriers.^{32,74} However, there was no evidence indicating the production of IgE at the other SNPs in IL4. Thus, we can speculate that the T allele upregulated the expression of IL4, thereby increasing serum IgE levels. Therefore, IL4 C-589T SNPs affect atopic asthma risk. In addition, we analyzed the association between the rs2070874 polymorphism and asthma in a meta-analysis. We found that individuals who carried the C allele had an increased asthma risk compared with TT homozygotes in rs2070874. Thus, IL4 was thought to be a direct participant in the generation of high serum levels of

IgE, which were observed in atopic individuals. However, the exact mechanisms in which these IL4 polymorphisms affect atopic asthma risk at the molecular level remain speculative.

Because IL4R plays an important role in regulating Th2 cell development and IgE production via its response to IL4 or IL13, it is a good candidate to be an asthma susceptibility gene. Polymorphisms in both IL4 and IL4R α have been associated with an exacerbating phenotype of severe asthma.^{11,75} The single nucleotide polymorphisms in IL4R α were also associated with an inflammatory pattern identified by increased airway mast cells and IgE (+) cells.⁷⁶ The IL4 receptor gene is located on chromosome 16 p12.1-p11.2. The rs1801275, rs1805011, and rs1805015 SNPs are located on exon 10, while the rs1805010 SNP is located on exon 4. Several polymorphisms have been identified as risk factors for asthma, such as rs1801275 (-1902G/A) and rs1805010 (-223G/A). Our results suggested that individuals who carried a G allele in rs1801275 are protected from asthma compared with CC homozygotes ($P=0.03$). In addition, individuals who carried the C allele in rs1805011 were more vulnerable to asthma. Franjkovic et al⁷⁷ analyzed the functional and association relevance of common IL4R-coding SNPs. They found that common coding SNPs in the IL4R α gene were unlikely to significantly contribute to increased IgE levels. This partially explained why we did not find any clear significance in the atopy group in rs1801275 in the IL4R gene polymorphism. Most likely, the rs1801275 SNP was one of the minor genes. Single allele mutation may not be sufficient to exert a significant effect on elevating IgE levels. Moreover, our results showed that the rs1805010 polymorphism was associated with asthma risk in the atopic asthma group. Individuals who carried the A allele were most likely protected from asthma compared to G homozygotes ($P=0.01$). A previous study showed that the Ile50 variant of IL4RA significantly upregulated the receptor response to IL-4, resulting in increased activation of Stat6 and increased IgE production.^{15,25,78} In addition, this may have resulted in higher total serum IgE levels in the Ile50 variant of the IL4RA allele carriers. Therefore, the Ile50 variant of the IL4RA SNP affected atopic asthma risk.

The heterogeneity of the clinical information among these studies should be noted. Heterogeneity is an important issue when interpreting the results of a meta-analysis. Significant heterogeneity existed in the overall comparisons in a few meta-analyses, such as IL4 C-589T and rs1801275 in IL4RA. After analyzing the subgroups by age, the amount of heterogeneity was effectively decreased or removed in children with IL4 C-589T. A potential explanation may be that differences in the etiology may exist in different age groups. Another important factor that contributed to heterogeneity was racial homogeneity in either the case or the control groups. An association between the C-589T polymorphism was found in the Caucasian groups. Individuals who carried a G allele in rs1801275 were protected against asthma compared to CC homozygotes in

Asians ($P=0.01$). Ideally, all of the cases and controls in this meta-analysis should be matched for age, sex, atopic status and environmental exposures. However, we could not simultaneously analyze multiple factors (age, ethnicity and asthma type) because the sample size was small for stratified analyses. In addition, it was also likely that the observed differences in the ethnicity and asthma type were due to chance because studies with a small sample size may have insufficient statistical power to detect a slight effect or may have generated a fluctuated risk estimate.⁷⁹ Considering the relatively limited number of studies and population numbers included in this meta-analysis, our results should be interpreted with caution.

In conclusion, both IL4 and IL4R polymorphisms are associated with asthma. The CC genotype in the rs2243250 SNP of the IL4 gene was less likely to develop asthma compared to the CT and TT genotypes, particularly in the Caucasian and adult groups. Individuals who carried the C allele in rs2070874 of the IL4 gene had an increased asthma risk compared with TT homozygotes. The AA genotype in rs1805011 of the IL4R gene was less likely to suffer from asthma compared to the CC and AC genotypes. The rs1801275 polymorphism of IL4R was likely significant in the Asian group, while the rs1805010 polymorphism of IL4R was more significant in the atopic group.

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