ORIGINAL ARTICLE

The -33C/T Polymorphism in the Interleukin 4 Gene Is Associated With Asthma Risk: A Meta-Analysis

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Abstract

Objective: The association between the *interleukin 4 (IL-4)* gene -33C/T polymorphism and asthma risk is a subject of debate. We conducted a meta-analysis to evaluate the association between this polymorphism and asthma susceptibility.
Materials and Methods: A systematic search of electronic databases (Pubmed, EMBASE, Wanfang, China National Knowledge Infrastructure, and Weipu) was performed, and 18 studies involving 5523 cases and 5618 controls were identified. ORs with 95% CIs were used to assess the strength of association.
Results: A significant association between the -33C/T polymorphism and asthma susceptibility was observed for TT vs CT + CC (OR, 1.16; 95% CI, 1.05-1.28; *P*=.005). In the subgroup analysis by race, a significant association was found among whites (OR, 1.71; 95% CI, 1.14-2.57; *P*=.01) and Asians (OR, 1.14; 95% CI, 1.01-1.28; *P*=.04) but not among African Americans. In the subgroup analysis by atopic status, no significant association was found among atopic asthma patients (OR, 1.05; 95% CI, 0.89-1.24; *P*=.54) or nonatopic asthma patients (OR, 1.16; 95% CI, 0.81-1.67; *P*=.42). In the age-stratified analysis, an increased asthma risk was found in children (OR, 1.28; 95% CI, 1.01-1.63; *P*=.04) but not in adults.
Conclusions: The results of this meta-analysis suggest that the *IL-4* -33C/T polymorphism is a risk factor for asthma.

Key words: Interleukin-4. Asthma. Polymorphism. Meta-analysis.

Resumen

Introducción: La asociación entre el polimorfismo -33C/T del gen de la *interleucina-4 (IL-4)* y el riesgo de presentar asma bronquial es un tema conflictivo. En este trabajo se realiza un meta-análisis con el fin de evaluar dicha asociación.
Material y métodos: Se realizó una búsqueda sistemática en bases de datos electrónicas (Pubmed, EMBASE, Wanfang Database, China National Knowledge Infrastructure [CNKI] and Weipu Database) identificando 18 estudios que agrupan 5523 casos y 5618 controles. Se analizaron las Odds ratios (ORs) con un intervalo de confianza (CIs) del 95% para cuantificar la fuerza de la asociación.
Resultados: En cuanto a los resultados obtenidos se observa una asociación significativa entre el polimorfismo -33C/T y la susceptibilidad de presentar asma para TT vs. CT + CC (OR = 1.16, 95% CI 1.05 – 1.28, *P* = 0.005). En el análisis de la etnicidad se observó una asociación significativa en caucásicos (OR = 1.71, 95% CI 1.14 – 2.57, *P* = 0.01) y asiáticos (OR = 1.14, 95% CI 1.01 – 1.28, *P* = 0.04) pero no en africanos americanos. En el análisis del estatus atópico no se encontró asociación significativa en pacientes con asma atópica (OR = 1.05, 95% CI 0.89 – 1.24, *P* = 0.54) ni en pacientes con asma no atópica (OR = 1.16, 95% CI 0.81 – 1.67, *P* = 0.42). En el análisis realizado según la edad se encontró un aumento de riesgo de padecer asma en los niños (OR = 1.28, 95% CI 1.01 – 1.63, *P* = 0.04) pero no en adultos.
Conclusión: Este meta-análisis sugiere que el polimorfismo -33C/T del gen de la *IL-4* constituye un factor de riesgo para padecer asma.

Palabras clave: Interleucina-4. Asma. Polimorfismo. Meta-análisis.

Introduction

Asthma is a complex, chronic disease in which allergen-induced inflammatory processes in the airways contribute to the development of symptoms, such as wheezing, cough, dyspnea, and breathlessness. Masoli et al [1] suggested that asthma has affected an estimated 300 million people worldwide. Predictive markers to identify high-risk populations are urgently needed for early detection and preventive care. Genetic susceptibility to asthma is one of the research focuses in the scientific community. Many studies have focused on the association between genetic variants and asthma risk, and the interleukin 4 (*IL-4*) gene has been extensively studied [2,3].

IL-4 plays a crucial role in type 2 T-helper (T_H2) responses and isotype class switching of B cells to IgE synthesis, and it is also involved in mast cell recruitment [4]. It has thus been suggested that *IL-4* may have an important role in the pathogenesis of asthma. The human *IL-4* gene is located on chromosome 5q31. The -33C/T polymorphism (rs2070874), which is defined relative to its position in the *IL-4* gene sequence upstream of the transcription initiation site, has been linked to the risk of asthma in numerous studies [5-22]. However, the relationship remains uncertain and inconclusive. This may be because the small effect size of the polymorphism and the small samples of patients analyzed in these studies did not provide sufficient statistical power to detect significant associations.

We performed a meta-analysis to derive a more precise estimation of the association between the *IL-4* -33C/T polymorphism and asthma risk. This is, to our knowledge, the most comprehensive meta-analysis of the association between this polymorphism and asthma susceptibility.

Methods

Publication Search

We searched PubMed, EMBASE, Wanfang Database, China National Knowledge Infrastructure, and Weipu Database using the terms (asthma or asthmatic) and (interleukin-4 or interleukin 4 or *IL-4* or IL 4) and (polymorphism or mutation or variant). No publication date or language restrictions were imposed and the last search was updated in August, 2012. Other studies were identified by searching the references of identified studies or reviews on the same topic.

Inclusion and Exclusion Criteria

Studies meeting the following selection criteria were included in this meta-analysis: *1*) evaluation of the *IL-4* -33C/T polymorphism and asthma risk, *2*) a case-control design, *3*) sufficient data for estimating an OR with a 95% CI, and *4*) genotype distribution of the control population in Hardy-Weinberg equilibrium (HWE). Studies were excluded if one or more of the following criteria were present: *1*) lack of relevance to *IL-4* or asthma risk, *2*) design based on family or sibling pairs, *3*) nonclinical study, *4*) failure to report genotype frequencies or number, and *5*) inappropriate article type (review or abstract). For overlapping studies, we included the study with the largest sample.

Data Extraction

Data were extracted independently by 2 investigators (Liu and Zhou) using predesigned data collection forms. We verified the accuracy of data by comparing the completed forms. The following data were collected from each study: first author's name, year of publication, country of origin, race, age group, atopic status, sample size, genotyping method, and genotype numbers in cases and controls. Discrepancies were resolved by discussion or additional assessment by a third author (Li). Authors were contacted by e-mail when additional study data were needed.

Statistical Analysis

ORs and 95% CIs were used to assess the strength of association between the -33C/T polymorphism and asthma risk, with estimation of the following: TT vs CC (OR1), CT vs CC (OR2), and TT vs CT (OR3). These pairwise differences were used to indicate the most appropriate genetic model as follows: if $OR1 = OR3 \neq 1$ and $OR2 = 1$, a recessive model was suggested; if $OR1 = OR2 \neq 1$ and $OR3 = 1$, a dominant model was suggested; if $OR2 = 1/OR3$ and $OR1 = 1$, a complete overdominant model was suggested; and if $OR1 > OR2 > 1$ and $OR1 > OR3 > 1$ (or $OR1 < OR2 < 1$ and $OR1 < OR3 < 1$), a codominant model was suggested [23-25]. Once the best genetic model had been identified, it was used to collapse the 3 genotypes into 2 groups (except in the case of a codominant model) and to pool the results again. A random-effects model (Mantel-Haenszel method) was used to calculate the pooled ORs. The statistical significance of the OR was determined by the Z test.

HWE in the control group was assessed via the χ^2 test. The Q statistic and the I^2 statistic were employed to assess the degree of heterogeneity among the studies included in the meta-analysis. Sensitivity analysis was performed by omitting each study in turn to evaluate the stability of the results. Subgroup analyses were performed by race, atopic status, and age. Potential publication bias was evaluated using Begg's funnel plot [26], and Egger's test [27].

All statistical tests were performed using STATA 11.0 software (Stata Corporation). A *P* value of less than .05 was considered statistically significant, except for the test of heterogeneity where a level of .10 was used. All tests were 2-sided.

Results

Study Characteristics

Our comprehensive search retrieved 645 articles. After removing 115 duplications and reading the abstracts, a further 459 articles were excluded. Following examination of the full texts of the remaining 71 articles, 53 were excluded. Of the remaining 18 articles, 2 reported 2 cohorts [9,17] and 1 reported 3 cohorts [7]; each cohort was considered a separate case-control study. Ultimately, 22 case-control studies in 18 articles with 5523 cases and 5618 controls were eligible for inclusion in our meta-analysis [5-22]. The literature search and study selection procedures are shown in Figure 1. There were 10 studies of Asians [5,8,12,15,17,18,20-22], 7 studies of whites [6,7,9,10,13,16,19], 4 studies of African

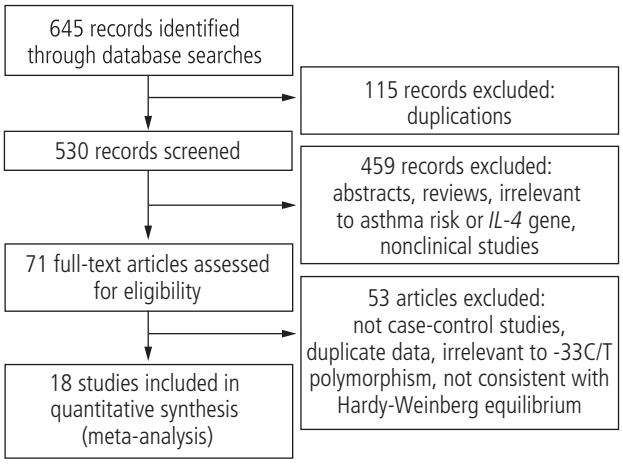

Figure 1. Flow diagram showing study identification, inclusion, and exclusion.

Americans [7,9,11,14], and 1 study of Hispanics [7]. Two studies included atopic asthma patients only [17], 7 studies included both atopic and nonatopic patients [8-10,16,19,21], and 13 studies did not offer detailed information [5-7,11-15,18,20,22]. Ten studies were performed in adults [5-7,10,12,14,17,21], 7 in children [15-20,22], and 3 in both adults and children [8,11,13]. The remaining 2 studies did not provide this information [9]. The characteristics of each study are presented in Table 1. Genotype numbers and HWE examination results are listed in Table 2.

Quantitative Data Synthesis

For the -33C/T polymorphism, OR1, OR2, and OR3 were 1.24 (P=.01), 1.11 (P=.08), and 1.15 (P=.01), respectively. These estimates suggested a recessive genetic model, and therefore CT and CC were combined and compared with TT. As shown in Figure 2, the pooled OR was 1.16 (95% CI, 1.05-1.28; P=.005). There was no significant heterogeneity ($P_{heterogeneity}$=.64 and I^2=0%).

Table 1. Characteristics of Case-Control Studies Included in Meta-Analysis

First author	Year	Country	Race	Age Group	Atopic Status	No. of Cases	No. of Controls	Genotyping Method
Suzuki[5]	2000	Japan	Asian	Adult	NA	120	120	PCR-RFLP
Beghe[6]	2003	UK	White	Adult	NA	187	182	PCR-RFLP
Basehore a[7]	2004	USA	White	Adult	NA	233	245	PCR
Basehore b[7]	2004	USA	African American	Adult	NA	168	269	PCR
Basehore c[7]	2004	USA	Hispanic	Adult	NA	116	130	PCR
Park[8]	2004	Korea	Asian	Mixed	Mixed[a]	532	170	SNaPshot
Donfack a[9]	2005	NA	White	NA	Mixed[a]	126	205	LAS
Donfack b[9]	2005	NA	African American	NA	Mixed[a]	205	183	LAS
Isidoro-García[10]	2005	Spain	White	Adult	Mixed[a]	133	79	TaqMan
Battle[11]	2007	USA	African American	Mixed	NA	261	176	PCR-RFLP
Jiang[12]	2009	China	Asian	Adult	NA	24	24	PCR-RFLP
Daley[13]	2009	Australia	White	Mixed	NA	644	751	Illumina Bead Array System
Haller[14]	2009	USA	African American	Adult	NA	72	70	PCR-RFLP
Wang JY[15]	2009	China	Asian	Children	NA	446	510	TaqMan
Berce[16]	2010	Slovenia	White	Children	Mixed[a]	106	89	PCR-RFLP
Undarmaa a[17]	2010	Japan	Asian	Children	Atopic	325	336	TaqMan-ASA
Undarmaa b[17]	2010	Japan	Asian	Adult	Atopic	367	676	TaqMan-ASA
Wu[18]	2010	China	Asian	Children	NA	252	227	PCR-RFLP
Michel[19]	2010	Germany	White	Children	Mixed	703	658	Illumina Sentrix HumanHap300 BeadChip
Huang HR[20]	2011	China	Asian	Children	NA	100	122	PCR-RFLP
Yang[21]	2011	China	Asian	Adult	Mixed[a]	202	205	MALDI-TOF
Chen[22]	2011	China	Asian	Children	NA	202	191	MALDI-TOF

Abbreviations: PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; SNaPshot, single nucleotide primer extension using SNaPshot Multiplex Kit; LAS, multiplex PCR and an immobilized linear array system; SSP, single-specific primer; TaqMan-ASA, TaqMan allele-specific amplification method; MALDI-TOF, matrix-assisted laser desorption ionization-time of flight mass spectrometry platform; NA, not available.
[a]It was possible to extract data for atopic and nonatopic asthma patients separately.

Table 2. Distribution of Interleukin 4 Gene -33C/T Genotypes Among Asthma Patients and Controls

Study (First Author)	Patients TT	CT	CC	Controls TT	CT	CC	HWE
Suzuki[5]	53	56	11	51	59	10	Yes
Beghe[6]	6	41	140	2	48	132	Yes
Basehore a[7]	8	72	153	4	56	185	Yes
Basehore b[7]	34	83	51	50	132	87	Yes
Basehore c[7]	15	53	48	13	57	60	Yes
Park[8]	349	164	19	106	57	7	Yes
Donfack a[9]	6	37	83	5	50	150	Yes
Donfack b[9]	30	107	68	27	86	70	Yes
Isidoro-García[10]	1	39	93	0	15	64	Yes
Battle[11]	48	128	85	32	87	57	Yes
Jiang[12]	15	9	0	12	10	2	Yes
Daley[13]	12	150	481	15	181	555	Yes
Haller[14]	15	36	21	10	33	27	Yes
Wang JY[15]	277	147	22	308	186	16	Yes
Berce[16]	8	31	67	3	35	51	Yes
Undarmaa a[17]	155	142	27	155	144	37	Yes
Undarmaa b[17]	185	154	28	326	286	64	Yes
Wu[18]	163	83	6	129	87	11	Yes
Michel[19]	35	210	458	11	173	474	Yes
Huang HR[20]	76	23	1	70	49	3	Yes
Yang[21]	132	56	14	131	67	7	Yes
Chen[22]	124	72	6	123	62	6	Yes

Abbreviation: HWE, Hardy-Weinberg equilibrium.

In the subgroup analysis by race, significant associations were found among whites (OR, 1.71; 95% CI, 1.14-2.57; *P*=.01) and Asians (OR, 1.14; 95% CI, 1.01-1.28; *P*=.04) but not among African Americans (OR, 1.09; 95% CI, 0.82-1.44; *P*=.56). Subgroup analyses were also performed by atopic status. A significant increased risk of asthma was not found among atopic asthma patients (OR, 1.05; 95% CI, 0.89-1.24; *P*=.54) or nonatopic asthma patients (OR, 1.16; 95% CI, 0.81-1.67; *P*=.42). In the stratified analysis by age, the *IL-4* -33C/T polymorphism was associated with a higher risk of asthma development among children (OR, 1.28; 95% CI, 1.01-1.63; *P*=.04). No significant association was found in adults (OR, 1.15; 95% CI, 0.97-1.37; *P*=.10). In the subgroup of white children, there was also a significant association between the *IL-4* -33C/T polymorphism and asthma risk (OR, 2.13; 95% CI, 1.13-4.04; *P*=.02). The results of the other genetic comparisons are summarized in Table 3.

Sensitivity Analysis and Cumulative Meta-Analysis

In order to assess the stability of the results of the meta-analysis, we performed a sensitivity analysis by omitting 1 study at a time. As shown in Figure 3, the results were not materially altered. Cumulative meta-analysis was also carried out via the sorting of studies by publication time. Figure 4 shows the results of the cumulative meta-analysis. Inclinations toward significant associations were evident with each accumulation of data over time.

Publication Bias

Publication bias was assessed by Begg's funnel plot. The shape of the plot was asymmetric, suggesting potential bias (Figure 5). Egger's test was conducted to provide statistical evidence of funnel plot asymmetry. The result showed evidence of publication bias (*P*=.006).

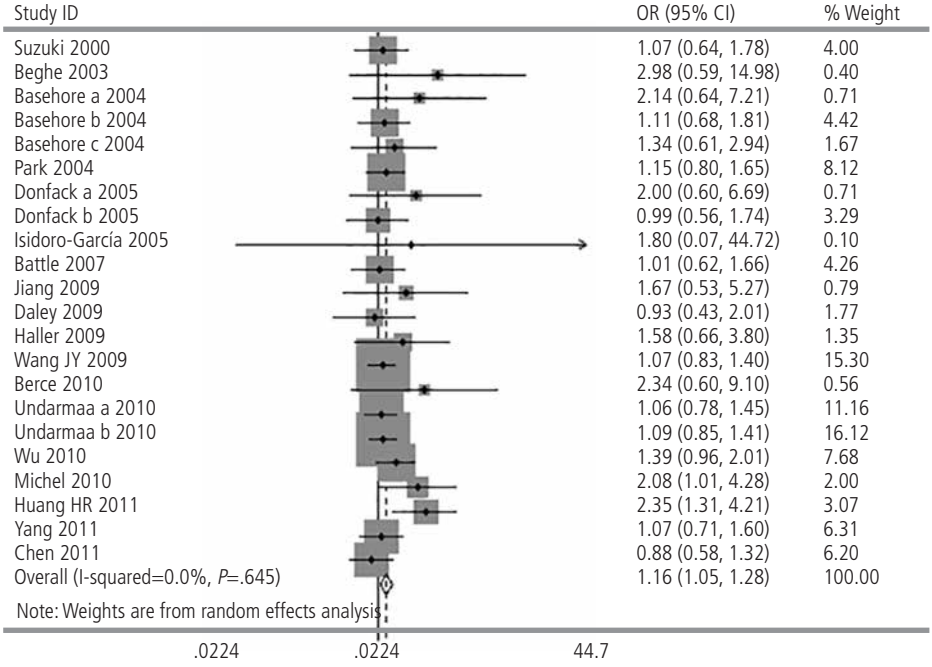

Figure 2. Meta-analysis for the association between asthma risk and the IL-4 -33C/T polymorphism (TT vs CT + CC).

Table 3. Determination of the Genetic Effect of the Interleukin 4 Gene -33C/T Polymorphism on Asthma and Subgroup Analyses

Comparison	Study	Sample Size		No. of Studies	Test of Association		*P* Value	Model	Heterogeneity		
		Cases	Controls		OR (95% CI)	Z			χ^2	*P* Value	I^2 (%)
TT vs CC	Overall	3630	3658	22	1.24 (1.04-1.48)	2.43	0.01	R	18.88	.59	0.0
CT vs CC	Overall	3786	4035	22	1.11 (0.99-1.24)	1.76	0.08	R	22.53	.37	7.0
TT vs CT	Overall	3629	3543	22	1.15 (1.03-1.28)	2.52	0.01	R	15.54	.80	0.0
TT vs CT+CC	Overall	5523	5618	22	1.16 (1.05-1.28)	2.83	0.005	R	18.07	.64	0.0
TT vs CT+CC	Asian	2570	2581	10	1.14 (1.01-1.28)	2.06	0.04	R	9.69	.38	7.0
TT vs CT+CC	White	2131	2209	7	1.71 (1.14-2.57)	2.57	0.01	R	3.54	.74	0.0
TT vs CT+CC	African American	706	698	4	1.09 (0.82-1.44)	0.58	0.56	R	0.88	.83	0.0
TT vs CT+CC	Atopic	1522	1855	8	1.05 (0.89-1.24)	0.62	0.54	R	4.43	.73	0.0
TT vs CT+CC	Nonatopic	361	849	6	1.16 (0.81-1.67)	0.80	0.42	R	3.59	.46	0.0
TT vs CT+CC	Children	2134	2133	7	1.28 (1.01-1.63)	2.01	0.04	R	12.39	.05	52.0
TT vs CT+CC	Adults	1622	2000	10	1.15 (0.97-1.37)	1.63	0.10	R	3.86	.92	0.0
TT vs CT+CC	Asian children	1325	1386	5	1.20 (0.94-1.53)	1.44	0.15	R	8.98	.06	55.0
TT vs CT+CC	White children	809	747	2	2.13 (1.13-4.04)	2.33	0.02	R	0.02	.88	0.0
TT vs CT+CC	Asian adults	713	1025	4	1.10 (0.90-1.33)	0.91	0.36	R	0.54	.91	0.0
TT vs CT+CC	White adults	553	506	3	2.36 (0.93-5.96)	1.81	0.07	R	0.13	.94	0.0

Abbreviation: R, random-effects model.

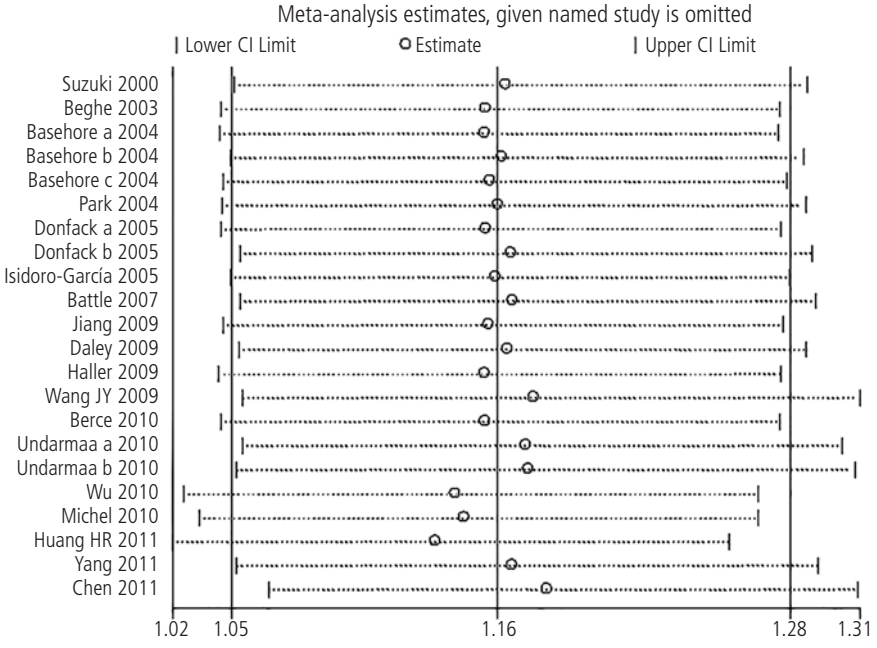

Figure 3. Sensitivity analysis for the IL-4 -33C/T polymorphism with asthma risk (TT vs CT + CC).

Discussion

IL-4, which is expressed by T_H2 cells, is a critical cytokine in the pathogenesis of atopy and asthma. Rankin et al [28] reported that the selective expression of *IL-4* within the lung elicited an inflammatory response characterized by epithelial cell hypertrophy and the accumulation of macrophages, lymphocytes, eosinophils, and neutrophils. Moreover, IL-13-independent airway hyperresponsiveness and goblet-cell hyperplasia have been induced by *IL-4* in

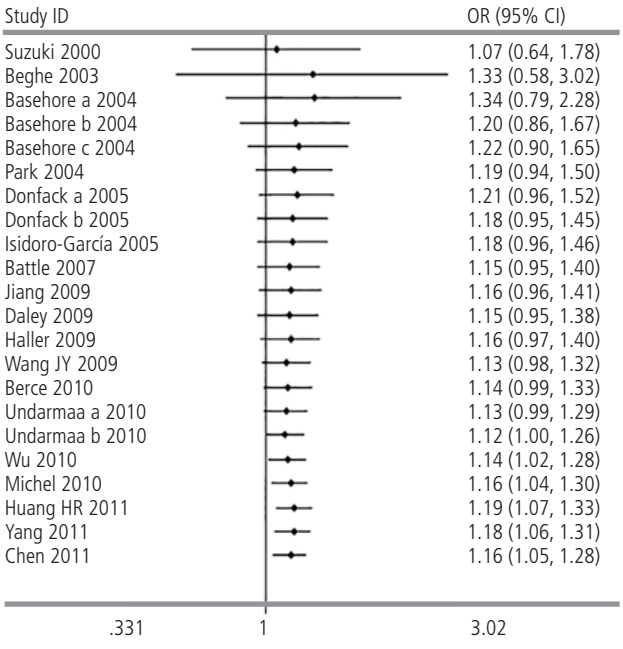

Figure 4. Cumulative meta-analysis of associations between the IL-4 -33C/T polymorphism and asthma risk (TT vs CT + CC).

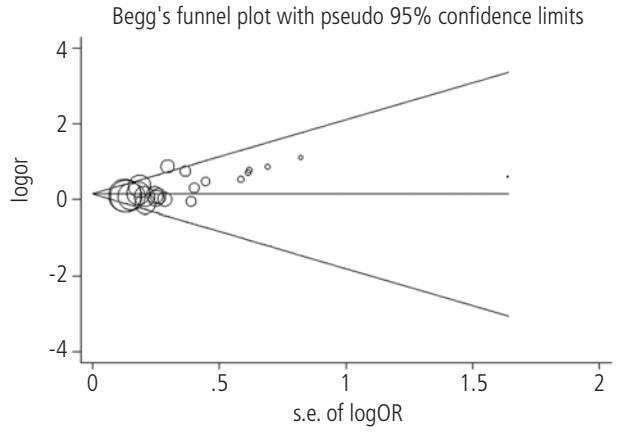

Figure 5. Funnel plot for publication bias in selection of studies on the IL-4 -33C/T polymorphism (TT vs CT + CC). s.e. of logOR indicates standard error of log odds ratio.

a mouse model of asthma [29], and *IL-4* has been seen to upregulate expression of vascular cell adhesion molecule-1 on the endothelium, promoting selective egress of eosinophils from the bloodstream [30]. At the systemic level, circulating leukocytes from mild, moderate, and refractory asthmatics have been found to produce more *IL-4* when compared to levels in healthy individuals [31]. In addition, Lama and coworkers [32] showed that the average serum level of *IL-4* in steroid-naïve asthmatic children was 52.25 pg/mL, compared with just 32.81 pg/mL in healthy controls (P<.0001) These data suggest that *IL-4* may play a key role in the development of asthma. It has been shown that transcription factor Oct-1 overexpression decreases *IL-4* promoter activity [33]. Specifically, Gervaziev et al [34] found that Oct-1 bound predominantly to the -33 C allele rather than the -33 T allele. Furthermore, previous studies have shown that patients with asthma carrying the -33 T allele had higher levels of serum *IL-4* than those carrying the -33 C allele [35]. Taken together, these results led us to speculate that the -33C/T polymorphism might influence asthma risk by affecting the level of *IL-4* expression.

This meta-analysis of 18 studies including 5523 cases and 5618 controls evaluated the association between the -33C/T polymorphism in the *IL-4* gene and asthma susceptibility. The results suggest that this polymorphism is indeed a risk factor for developing asthma in the overall study populations and that individuals carrying the TT genotype may have an increased risk compared with CC or CT genotypes carriers.

In the subgroup analysis by race, no significant association was found in African Americans, but asthma risk was increased in whites and Asians. Van Dyke et al [36] showed that -33T allele frequency in African Americans was significant lower than in whites. This might explain racial differences in asthma risk. It should be noted that only 4 studies involving African Americans were included in our meta-analysis. A positive association between African Americans and asthma risk cannot be ruled out, however, because studies with small sample sizes may have insufficient statistical power to detect a slight effect.

In our subgroup analysis by atopic status, we did not observe a significantly increased risk of asthma in atopic patients, suggesting that the *IL-4* -33C/T polymorphism does not play a role in the pathogenesis of atopic asthma. However, asthma is a complex pulmonary disorder which results from interactions between multiple environmental factors and susceptibility genes. Thus, the effect of *IL-4* -33C/T-environment interactions on atopic asthma risk cannot be ruled out. More studies should be designed to analyze these associations.

We also performed subgroup analysis by age. The results showed that TT homozygote carriers may have an increased risk of asthma in childhood compared with adulthood. In other words, the polymorphism may be significantly associated with an elevated risk of child-onset asthma. However, the exact mechanisms by which this polymorphism affects pediatric asthma risk at the molecular level were unclear. This is an area that needs further study. We also considered the effect of age-race interaction on the association between the *IL-4* -33C/T polymorphism and asthma risk. Interestingly, we found that white children who carried the TT genotype had an increased risk. No statistically significant results were observed in the other subgroups (Asian children, Asian adults, and white adults), possibly due to insufficient statistical power. More case-control studies should be conducted in these populations.

Publication bias and heterogeneity, which can influence the results of meta-analyses, should be considered, and in our case, Egger's test showed evidence of significant publication bias. Thus, our results should be interpreted with caution. Future studies are needed to validate our findings. Heterogeneity does not seem to have influenced our results, as significant heterogeneity was detected in only a few comparisons. We also carried out a sensitivity analysis. The omission of a single study each time did not alter our results significantly, suggesting that they are reliable. Cumulative meta-analysis showed that the evidence was consistent over time and our results can therefore be considered robust and stable.

This meta-analysis had some limitations. First, the overall result was based on unadjusted estimates; a more precise evaluation should be performed when other covariates (eg, obesity, sex, and lifestyle) are available. Second, only 4 studies were conducted in African Americans. Third, we were unable to address gene-gene and gene-environment interactions due to a lack of extractable data.

In summary, our meta-analysis found an association between the *IL-4* -33C/T polymorphism and asthma risk. More well-designed studies with larger samples are needed to estimate the association between this polymorphism and asthma risk in various racial populations. Moreover, gene-environment and gene-gene interactions should also be considered in future studies.

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Conflicts of Interest

The authors declare no conflicts of interest.

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