

ORIGINAL ARTICLE

The +252A/G polymorphism in the *Lymphotoxin- α* gene increases the risk of asthma: A meta-analysis

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ABSTRACT

Background and Objective: A number of studies have shown that the +252A/G polymorphism (rs909253) in the *lymphotoxin- α* (*LT- α*) gene is implicated in susceptibility to asthma. However, the findings have been inconclusive. The aim of this study was to investigate the association between the +252A/G polymorphism in the *LT- α* gene and the risk of asthma by performing a meta-analysis.

Methods: The Pubmed and Embase databases were searched for all studies relating to this polymorphism and the risk of asthma. Statistical analyses were performed using the Revman4.2 and STATA 10.0 software.

Results: Thirteen case-control studies that included a total of 2220 cases and 6428 controls were included in the meta-analysis. There was no significant association between this polymorphism and the risk of asthma in the all-combined analysis (odds ratio (OR) 1.14, 95% confidence interval (CI): 0.89–1.45 for GG+GA vs AA). In a subgroup analysis by ethnicity, no significant association with asthma risk was identified in Asians (OR 1.31, 95% CI: 0.97–1.77) or Europeans (OR 1.08, 95% CI: 0.77–1.53). In a subgroup analysis by age, a significantly increased risk was identified among adults (OR 1.25, 95% CI: 1.03–1.50) but not children (OR 1.04, 95% CI: 0.28–3.89). In a subgroup analysis by atopic status, a significantly elevated risk was identified among atopic (OR 1.55, 95% CI: 1.28–1.87) but not non-atopic individuals (OR 0.94, 95% CI: 0.53–1.68).

Conclusions: This meta-analysis suggested that the +252A/G polymorphism in the *LT- α* gene is a risk factor for asthma in adults and atopic populations.

SUMMARY AT A GLANCE

A meta-analysis was performed to investigate the association between the +252A/G polymorphism in the *LT- α* gene and the risk of asthma. This polymorphism was shown to be associated with an increased risk of asthma among adults and atopic populations.

Key words: asthma, lymphotoxin- α , meta-analysis, polymorphism.

INTRODUCTION

Asthma is a heterogeneous and multi-factorial inflammatory disease that currently affects about 300 million people worldwide.¹ The major features of asthma include episodes of wheezing, coughing and shortness of breath due to chronic airway inflammation and remodelling.^{2–4} It is well known that asthma is a complex genetic disorder, and both environmental and genetic factors may contribute to the initiation, as well as the evolution of the disease.^{5,6} There is strong evidence that genes involved in the pathogenesis of asthma, such as the cytokine genes involved in bronchial inflammation, are implicated in susceptibility to the disease.^{7–10} Recently, a number of cytokine genes have been consistently reported to be asthma susceptibility genes.⁹ Among these, the lymphotoxin- α (*LT- α*) gene has been one of the most intensively studied.^{8,11–20}

LT- α , also referred to as tumour necrosis factor- β , is a potent pro-inflammatory cytokine that participates in the airway inflammatory response in patients with atopic asthma.¹² It is a member of the tumour necrosis factor superfamily and plays an important role in the pathogenesis of asthma.²¹ The human *LT- α* gene, which encodes the *LT- α* protein, is located in the class III major histocompatibility complex region of

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chromosome 6q21.3,²² a region that has been previously linked with asthma in various genome-wide screens.²¹ Many polymorphisms have been identified in this gene, and the +252A/G polymorphism (rs909253) is one of the most intensively studied. The +252A/G polymorphism, also known as the *NocI* polymorphism, is located in the first intron of the *LT- α* gene, appears to be associated with high *LT- α* expression²³ and has been investigated as a potential susceptibility factor for asthma.¹¹ Numerous studies have been performed to investigate this association. However, the results have been inconclusive due to limited sample sizes and differences in the study populations. Meta-analysis is a useful method for investigating associations between genetic factors and diseases, because a quantitative approach is used to combine the results from different studies on the same topic, thereby providing more reliable conclusions.²⁴ Therefore, we performed a meta-analysis, which to our knowledge is the most comprehensive genetic meta-analysis of the association between *LT- α* polymorphisms and the risk of asthma.

METHODS

Search for publications

Electronic databases (Pubmed and Embase) were searched in order to identify articles that evaluated the association between the polymorphism in the *LT- α* gene and asthma risk (the last search was updated on Oct. 28th, 2010). The search terms used were: 'asthma or asthmatic' and 'tumor necrosis factor or lymphotoxin or TNF-beta or LT-alpha or lymphotoxin-alpha or tumor necrosis factor-beta' in combination with 'polymorphism or mutation or variant'. There was no restriction on languages. The following inclusion criteria were used: (i) the study should have evaluated the association between the +252G/A polymorphism in the *LT- α* gene and asthma risk; (ii) the study should have had a case-control design based on unrelated individuals; (iii) sufficient data should have been provided in order to calculate odds ratios (OR) and 95% confidence intervals (CI); and (iv) the distribution of genotypes in the control groups should have conformed to Hardy-Weinberg equilibrium (HWE). Studies were excluded if any of the following conditions applied: (i) only abstracts or reviews were available, without sufficient data; (ii) genotype frequencies were not reported; (iii) studies were repeated or publications overlapped; and (iv) the study was based on individuals who were members of the same family.

Data extraction

Two reviewers checked the data independently and reached a consensus on all items. If there was disagreement, a third reviewer was asked to assess the data. The following information was extracted from each publication: first author, year of publication, ethnicity of the study population, definition of cases,

source of controls, genotyping methods, total number of cases and controls, and genotype distribution among cases and controls.

Statistical analysis

For each case-control study, conformity with HWE of the control group genotypes was assessed using the Internet-based program (<http://ihg2.helmholtz-muenchen.de/cgi-bin/hw/hwa1.pl>). OR with 95% CI were used to assess the strength of the association between the +252A/G polymorphism and asthma risk. The OR and associated 95% CI were calculated by comparing the frequencies of rare alleles with that of the wild type homozygous alleles, that is GG+GA vs AA.

Heterogeneity among studies was assessed using the chi-square based *Q*- and *I*²-statistics. Heterogeneity was considered to be significant for *P* values <0.10. Fixed-effects or random-effects models were used to pool the data according to heterogeneity. When the *P* value for heterogeneity was >0.10, the fixed-effects model was used, otherwise, the random-effects model was used, as it is more appropriate when there is heterogeneity. The significance of the pooled OR was determined by the *Z*-test, and a *P* value <0.05 was considered statistically significant. To evaluate the ethnicity-specific, age-specific and atopy-specific effects, subgroup analyses were performed by ethnicity, age and atopic status, respectively. For the subgroup analysis by ethnicity, the study populations were stratified into three groups: Asian, European and mixed; for the subgroup analysis by age, study populations were also stratified into adults (>18 years of age), children (<18 years of age) and mixed; for the subgroup analysis according to atopic status, study populations were stratified into three groups: atopic (atopic asthma patients), non-atopic (non-atopic asthma patients) and mixed (both atopic and non-atopic asthma patients included and the data could not be extracted separately or atopic status was not documented). Other comparative genetic models were also used to assess the association between the polymorphism and the risk of asthma (GG vs GA+AA, GG vs AA, GA vs AA and G vs A).^{24,25}

Publication bias in the literature was investigated using several methods, including visual inspection of asymmetry in funnel plots. Begg's test and Egger's test were also used.²⁵ Analyses were performed using Revman4.2 and STATA 10.0 software.

RESULTS

Characteristics of the studies included

The initial search identified 520 studies from the selected electronic databases (Fig. 1). After reading the titles and abstracts, 30 potential articles and one abstract were included in the full-text review. After reading the full texts, four studies were excluded because they were not relevant to asthma risk and the +252A/G polymorphism in the *LT- α* gene. Therefore,

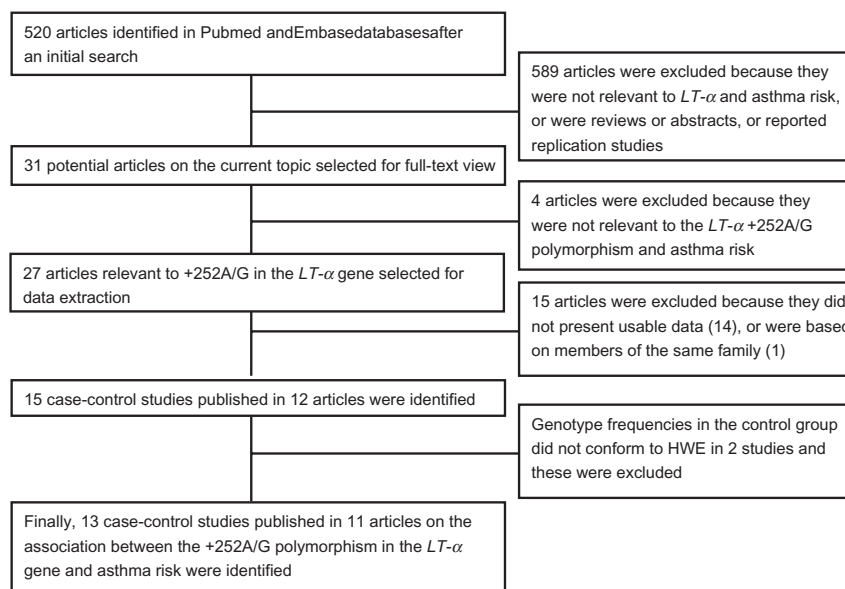


Figure 1 Schematic representation of the criteria used for inclusion/exclusion of studies before the meta-analysis. LT- α , lymphotoxin- α ; HWE, Hardy-Weinberg equilibrium.

27 articles remained for data extraction. Fourteen articles did not report usable data, and one study was based on individuals who were members of the same family; these studies were all excluded. Three articles each reported on two cohorts, and each cohort was considered as a separate case-control study.^{8,14,18} Therefore, a total of 15 case-control studies published in 12 articles were identified. However, the control group genotypes in two case-control studies were not consistent with HWE, and these studies were excluded. Thus, a final total of 13 case-control studies published in 11 articles met the criteria for inclusion in the meta-analysis.^{8,11–20}

Six case-control studies were performed in Asian populations;^{8,12–14,16} three were performed in European populations,^{11,18,20} two were performed in populations of mixed ethnicity^{15,19} and one study did not provide information on ethnicity.¹⁷ Six case-control studies were performed in adults,^{11,13,14,18,19} whereas three were performed in children,^{12,15,20} one in a mixed population of adults and children¹⁶ and one did not provide information on the age of the population.⁸ Five case-control studies were performed specifically among atopic asthma patients,^{12,14,18,20} two included both atopic and non-atopic asthma patients^{13,16} (data for atopic asthma patients and non-atopic asthma patients could be separately extracted from only one article¹³) and six did not report the atopic status of the patients.^{8,11,15,17,19} The characteristics of each case-control study and the genotype and allele distributions in each case-control study are presented in Table 1.

Quantitative data synthesis

All studies

A total of 2220 cases and 6428 controls were included in the meta-analysis. As shown in Figure 2, the hetero-

geneity of GG+GA vs AA was assessed for all 13 studies, and the chi-square value was 37.06 with 12 degrees of freedom and $P = 0.0002$ in a random-effects model. The I -square statistic, which is another index of heterogeneity, was 67.6%, suggesting moderate heterogeneity. Therefore, a random-effects model was used for synthesis of the data. The overall OR was 1.14 (95% CI: 0.89–1.45), and the Z -test value for overall effect was 1.05 ($P = 0.30$) for the GG+GA vs AA model. These results did not suggest a significant association between the +252A/G polymorphism and overall risk of asthma. Summary results for all comparisons are presented in Table 2.

Subgroup analyses

Subgroup analyses by ethnicity, age and atopic status were performed. For ethnicity, the populations were stratified into three groups: Asian (1444 cases and 1259 controls), European (407 cases and 4565 controls) and mixed (369 cases and 604 controls). There were no significant associations with asthma risk in any of these populations: Asian: OR 1.31, 95% CI: 0.97–1.77, $P = 0.08$; European: OR 1.08, 95% CI: 0.77–1.53, $P = 0.64$; mixed: OR 0.75, 95% CI: 0.32–1.75, $P = 0.50$ (Fig. S1 in the online supporting information). For the subgroup analysis by age, the populations were stratified into three groups: adults (1286 cases and 5553 controls), children (339 cases and 305 controls) and mixed or age not documented (595 cases and 570 controls). A significantly increased risk of asthma was identified among adults (OR 1.25, 95% CI: 1.03–1.50, $P = 0.02$) but not among children (OR 1.04, 95% CI: 0.28–3.89, $P = 0.95$) or the mixed group (OR 1.02, 95% CI: 0.63–1.64, $P = 0.95$) (Fig. S2 in the online supporting information). In the subgroup analysis by atopic status, a significantly increased risk was identified among atopic patients (1052 cases and 1003 controls: OR 1.55, 95% CI: 1.28–1.87, $P < 0.00001$)

Table 1 Characteristics of the studies that were included in the meta-analysis

First author	Year	Country	Ethnicity	Age	Atopic status	Case			Control			Case/control (n)
						AA	AG	GG	AA	AG	GG	
Albuquerque RV ²⁰	1998	Australia	European	Children	Atopic	9	40	75	7	19	24	124/50
Bilolifar H ¹⁵	2005	UK	Mixed [†]	Children	NM	39	29	33	16	51	33	101/100
Buckova D ¹⁸	2002	Czech	European	Adult	Atopic	78	63	10	87	54	14	151/155
Castro-Giner F ^{11†}	2009	Switzerland	European	Adult	NM	67	59	6	2089	1832	439	132/4360
El Bahlawan L ¹⁷	2003	USA	NM	NM	NM	11	21	6	71	110	50	38/231
Huang SC ¹²	2008	China	Asian	Children	Atopic	20	69	25	45	69	41	114/155
Mak JC ¹³	2007	China	Asian	Adult	Mixed	70	146	69	79	134	76	285/289
Sharma S-N ¹⁴	2006	India	Asian	Adult	Atopic	116	115	17	147	93	12	248/252
Sharma S-W ¹⁴	2006	India	Asian	Adult	Atopic	110	113	17	129	79	16	240/224
Shin H D ¹⁶	2004	Korea	Asian	Mixed	Mixed	156	199	98	38	80	33	453/151
Tan EC-C ⁸	1999	Singapore	Asian	NM	NM	11	20	7	16	15	4	38/35
Tan EC-M ⁸	1999	Singapore	Asian	NM	NM	15	38	13	39	84	30	66/153
Witte JS ¹⁹	2002	USA	Mixed [§]	Adult	NM	98	101	31	128	117	28	230/273

[†] The population-based Swiss Cohort Study on Air Pollution and Lung and Heart Disease in Adults (SAPALDIA); [‡] Caucasian and other ethnicity in London; [§] European-American, Hispanic-American, African-American and Asian-American; NM, not mentioned; Sharma S-N: Northern India; Sharma S-W: Western India; Tan EC-M: Malays; Tan EC-C: Chinese.

but not among non-atopic patients (87 cases and 167 controls: OR 0.94, 95% CI: 0.53–1.68, $P = 0.84$) or populations with mixed or unknown atopic status (1058 cases and 5303 controls: OR 0.88, 95% CI: 0.62–1.26, $P = 0.48$) (Fig. S3 in the online supporting information). Summary results from the other comparisons are presented in Table 2.

Publication bias

Publication bias was assessed using Begg's funnel plots and Egger's test. The shape of the Begg's funnel plots appeared symmetrical for the GG+GA vs AA model (Fig. 3). Egger's test was performed to provide statistical evidence of funnel plot asymmetry ($t = 0.03$, $P = 0.98$). These data indicated that there was no significant evidence of publication bias.

DISCUSSION

It is well known that there is individual variation in susceptibility to the same disease, even given the same environmental exposures. Genetic variants implicated in the pathogenesis of disease may account for these differences. Asthma is a common, heterogeneous inflammatory disease, and many cytokines play important roles in the pathogenesis of asthma. It is clear from epidemiological studies that asthma is a complex disease with multiple determinants, including genetic and environmental factors and gene–environment interactions.²⁶ Several candidate genes in the asthma pathogenesis pathway have been identified as asthma susceptibility genes, one of which is the *LT-α* gene. A growing number of studies have suggested that the +252A/G polymorphism in intron 1 of the *LT-α* gene is a potential risk factor for asthma.^{8,11–14,16,18,20,27} Because the first study reported an association between this polymorphism and asthma risk in an Australian population,²⁷ several studies have been performed to replicate this finding.^{8,11–14,16,18,20} However, the results have been inconsistent and inconclusive. Therefore, we performed a meta-analysis specifically to assess this association. In addition, because the candidate polymorphism may have different roles in asthma susceptibility among different ethnic and age groups, and among individuals with differing atopic status, subgroup analyses were also performed according to ethnicity, age and atopic status.

This meta-analysis of 13 case-control studies, which included 2220 asthma patients and 6428 controls, investigated the association between one polymorphism in the *LT-α* gene and the risk of asthma. There was no evidence of statistically significant overall effects on asthma risk from any of the genetic comparisons (Table 2). Subgroup analyses according to age, ethnicity and atopic status were also performed. The subgroup analysis by ethnicity showed no significant effect of the polymorphism. It is likely that there is no effect of the polymorphism on the studied phenotype or that the subgroup analysis did not have sufficient power to detect a significant asso-

Review: The +252A/G polymorphism in *LT- α* gene and asthma risk
 Comparison: 01 +252A/G
 Outcome: 01 GG+GA vs. AA

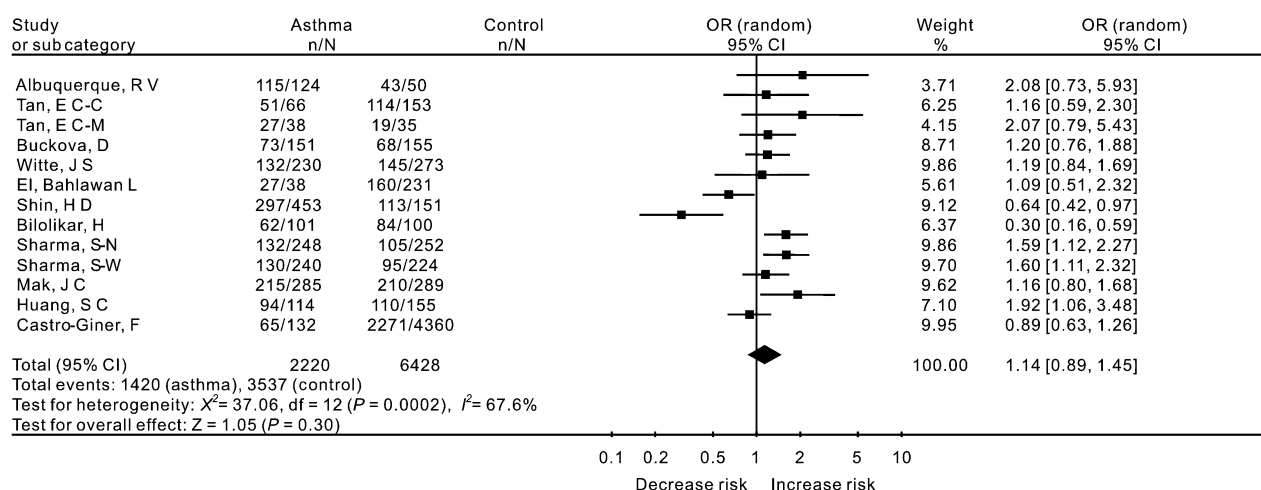


Figure 2 Meta-analysis of the association between asthma risk and the lymphotoxin (LT)- α +252A/G polymorphism (GG+AG vs AA), using a random-effects model. CI, confidence interval; OR, odds ratio.

ciation. In the subgroup analysis by age, a significantly increased risk was identified among adults (OR 1.25, 95% CI: 1.03–1.50, $P = 0.02$ for GG+AG vs AA; OR 1.29, 95% CI: 1.10–1.52, $P = 0.002$ for AG vs AA). These results strongly indicated that this polymorphism is significantly associated with increased asthma risk among adults. Based on these results, it is worth noting that even the same variant of the same gene, such as the +252A/G polymorphism in the *LT- α* gene, may have different effects on the pathogenesis and occurrence of disease in different populations.

The results of the subgroup analysis by atopic status showed that atopic carriers of the G allele had almost a 55% increased risk of asthma, suggesting a possible role of atopic status in the pathogenesis of asthma. However, a non-atopic population was investigated in only one case-control study. Therefore, it is likely that the observed differences in relation to atopy may be due to chance, because the small sample size of studies that included non-atopic individuals may have resulted in insufficient statistical power to detect a small effect. In addition, it is worth noting that atopic status was unknown in half of the 13 original studies, which may weaken the conclusions. Therefore, additional studies are warranted to further validate the association between atopic status and the effect of this polymorphism on asthma risk, especially in non-atopic patients.

The aim of performing a meta-analysis is to integrate the results from comparable studies on the same topic, in order to increase sample size and statistical power, and to draw more valid conclusions. However, there are several confounding factors that may influence the results of a meta-analysis, including publication bias and quality of the analysis. Therefore, strict inclusion and exclusion criteria were used in order to reduce selection bias. Only those studies published as articles were included. Two case-control

studies that were published as a comment⁸ and as an abstract¹⁷ should be mentioned. As the genotype frequencies could be extracted, these case-control studies were included. Funnel plots, Begg's test and Egger's test were all used to assess publication bias,²⁵ and analyses of HWE were also performed. All these analyses ensured the reliability of the meta-analysis.

The importance of heterogeneity should also be mentioned, as it is an important issue when interpreting the results of meta-analyses. There was significant heterogeneity in the overall comparisons, which may weaken the corresponding conclusions. When subgroup analyses by age, ethnicity and atopic status were performed, the heterogeneity among adults, atopic asthma patients and Asian and European populations was effectively reduced or removed. Possible explanations may be the differences in genetic backgrounds and environmental exposures among populations of different ethnicities and differences in aetiology among different age groups and groups with differing atopic status. Another important factor that contributes to heterogeneity is conformity with HWE among the control groups. In this study, conformity with HWE was a criterion for inclusion or exclusion of case-control studies, and this also resulted in more reliable conclusions.

In the past two decades, an increasing number of studies have focused on the genetics of asthma, and many variants have been suggested as potential asthma risk factors. With the advent of the HapMap, genome-wide association studies have become an important focus of research into susceptibility to asthma. To date, several variants have been identified as potential risk factors for asthma,^{28–33} and these results have been replicated in some populations.^{34–37} However, none of these studies have reported on the +252A/G (rs909253) polymorphism in the *LT- α* gene. This may be partly explained by the small effect of this

Table 2 Summary of comparisons based on different genotypes

	<i>n</i>	Cases/controls	GG+GA vs AA		GG vs AA+AG		GG vs AA		AG vs AA		G vs A	
			OR (95% CI)	<i>P</i>	OR (95% CI)	<i>P</i>	OR (95% CI)	<i>P</i>	OR (95% CI)	<i>P</i>	OR (95% CI)	<i>P</i>
Total	13	2220/6428	1.14 (0.89, 1.45)	0.30	0.96 (0.81, 1.14)	0.67	1.02 (0.77, 1.34)	0.91	1.15 (0.89, 1.49)	0.29	1.06 (0.93, 1.22)	0.38
Subgroup analysis by ethnicity												
Asian	7	1444/1259	1.31 (0.97, 1.77)	0.08	0.97 (0.78, 1.21)	0.80	1.10 (0.86, 1.42)	0.44	1.34 (0.96, 1.86)	0.08	1.12 (0.95, 1.31)	0.19
European	3	407/4565	1.08 (0.77, 1.53)	0.64	0.82 (0.54, 1.25)	0.36	0.89 (0.35, 2.29)	0.81	1.13 (0.86, 1.49)	0.39	1.04 (0.62, 1.73)	0.89
Mixed	3	369/604	0.75 (0.32, 1.75)	0.50	1.07 (0.74, 1.54)	0.72	0.79 (0.35, 1.82)	0.59	0.70 (0.26, 1.87)	0.48	0.97 (0.76, 1.23)	0.80
Subgroup analysis by age												
Adults	6	1286/5553	1.25 (1.03, 1.50)	0.02	0.93 (0.73, 1.17)	0.52	1.06 (0.75, 1.51)	0.73	1.29 (1.10, 1.52)	0.002	1.13 (0.96, 1.34)	0.14
Children	3	339/305	1.04 (0.28, 3.89)	0.95	1.04 (0.74, 1.47)	0.82	1.06 (0.39, 2.90)	0.91	0.94 (0.20, 4.38)	0.94	1.06 (0.74, 1.51)	0.75
Mixed	4	595/570	1.02 (0.63, 1.64)	0.95	0.96 (0.70, 1.37)	0.88	0.88 (0.59, 1.32)	0.54	1.02 (0.61, 1.71)	0.94	0.90 (0.60, 1.34)	0.60
Subgroup analysis by atopic status												
Atopic	6	1052/1003	1.55 (1.28, 1.87)	<0.00001	1.04 (0.80, 1.35)	0.77	1.38 (1.01, 1.88)	0.04	1.61 (1.32, 1.96)	<0.00001	1.26 (1.10, 1.44)	0.0006
Non-atopic	1	87/167	0.94 (0.53, 1.68)	0.84	0.78 (0.42, 1.45)	0.43	0.79 (0.38, 1.65)	0.53	1.02 (0.56, 1.89)	0.94	0.89 (0.62, 1.29)	0.55
Mixed	7	1058/5305	0.88 (0.62, 1.26)	0.48	0.94 (0.74, 1.20)	0.62	0.82 (0.53, 1.26)	0.37	0.87 (0.59, 1.29)	0.49	0.98 (0.77, 1.12)	0.45

Bold typeface indicates significant results.
OR, odds ratio; *P*, *P* value for the Z-test.

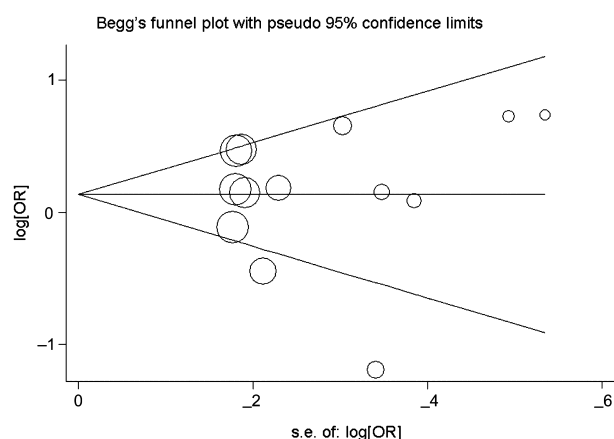


Figure 3 Begg's funnel plot for evaluation of publication bias in the selection of studies on the association between asthma risk and the lymphotoxin- α +252A/G polymorphism (GG+GA vs AA). OR, odds ratio; s.e., standard error.

polymorphism on susceptibility to asthma. In addition, no genome-wide association studies have been performed to specifically investigate atopic asthma patients. Future genome-wide association studies of atopic asthma patients may help to resolve the question of whether the +252A/G polymorphism shows a consistent association with asthma risk.

There are several limitations to this study. First, only published studies that were included in the selected electronic databases were identified; it is possible that some relevant published or unpublished studies that supported the null hypothesis may have been missed, which may have biased the results, and this may not have been detected by the statistical tests that were performed. Second, no study has been performed in an African population; therefore, the findings may not be applicable to African populations. Third, there was heterogeneity among the studies, possibly due to differences in age, ethnicity, atopic status and disease severity among the populations. Fourth, some of the studies were based on small numbers of cases and controls, which may have affected the statistical power to detect publication bias; therefore, caution should be exercised when interpreting the results. Fifth, due to the lack of original data, the level of environmental exposures, such as smoking, among cases and controls were not analysed in this study.

In conclusion, the results of this meta-analysis show that the +252A/G polymorphism in LT- α gene is significantly associated with an increased risk of asthma among adults and atopic populations. The findings suggest that this polymorphism may be a low-penetrance marker for susceptibility to atopic asthma among adults. Future studies should investigate larger cohorts, who are more completely phenotyped with respect to atopic status and environmental exposures, in order to further analyse these associations.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

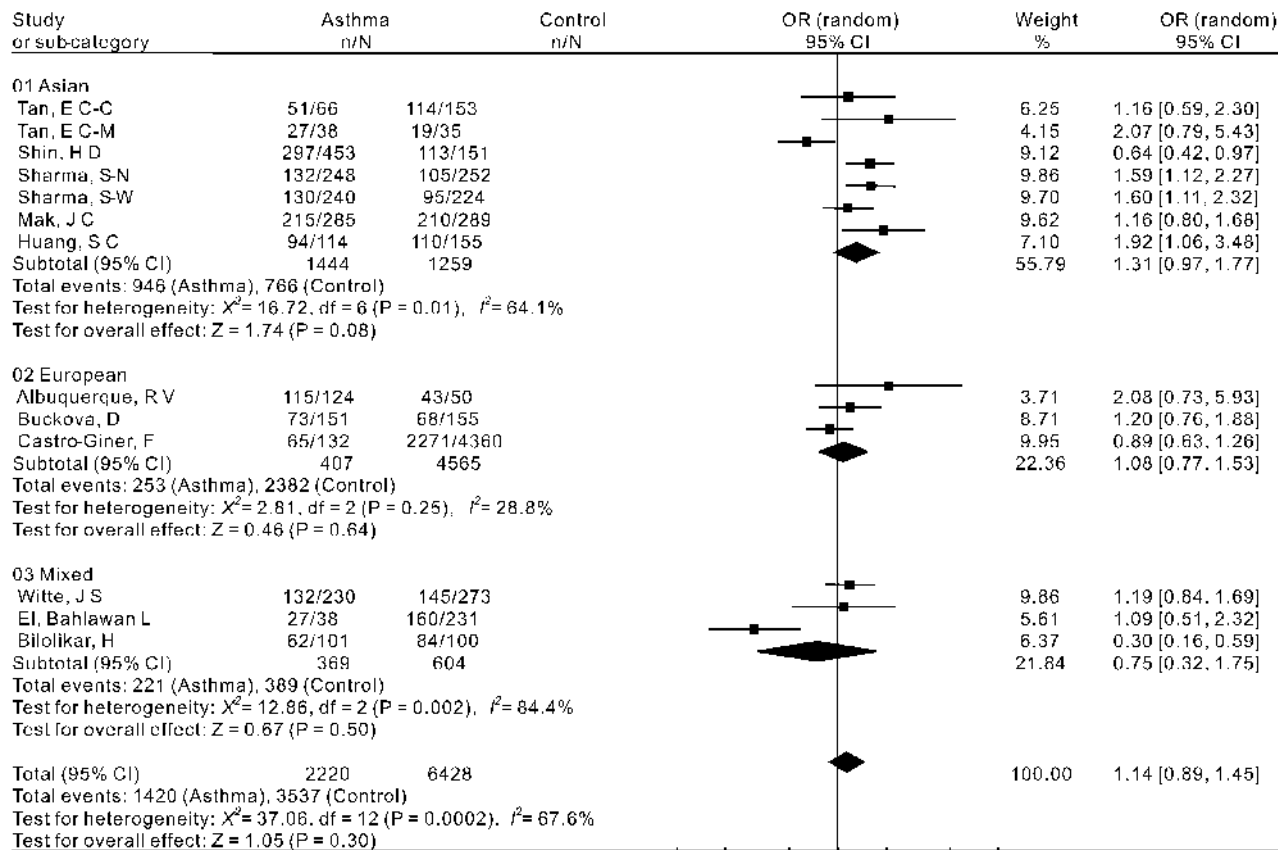
Figure S1 Meta-analysis with a random-effects model for the association between asthma risk and the LT- α +252A/G polymorphism (GG+AG vs AA): subgroup analysis by ethnicity.

Figure S2 Meta-analysis with a random-effects model for the association between asthma risk and the LT- α +252A/G polymorphism (GG+AG vs AA): subgroup analysis by age.

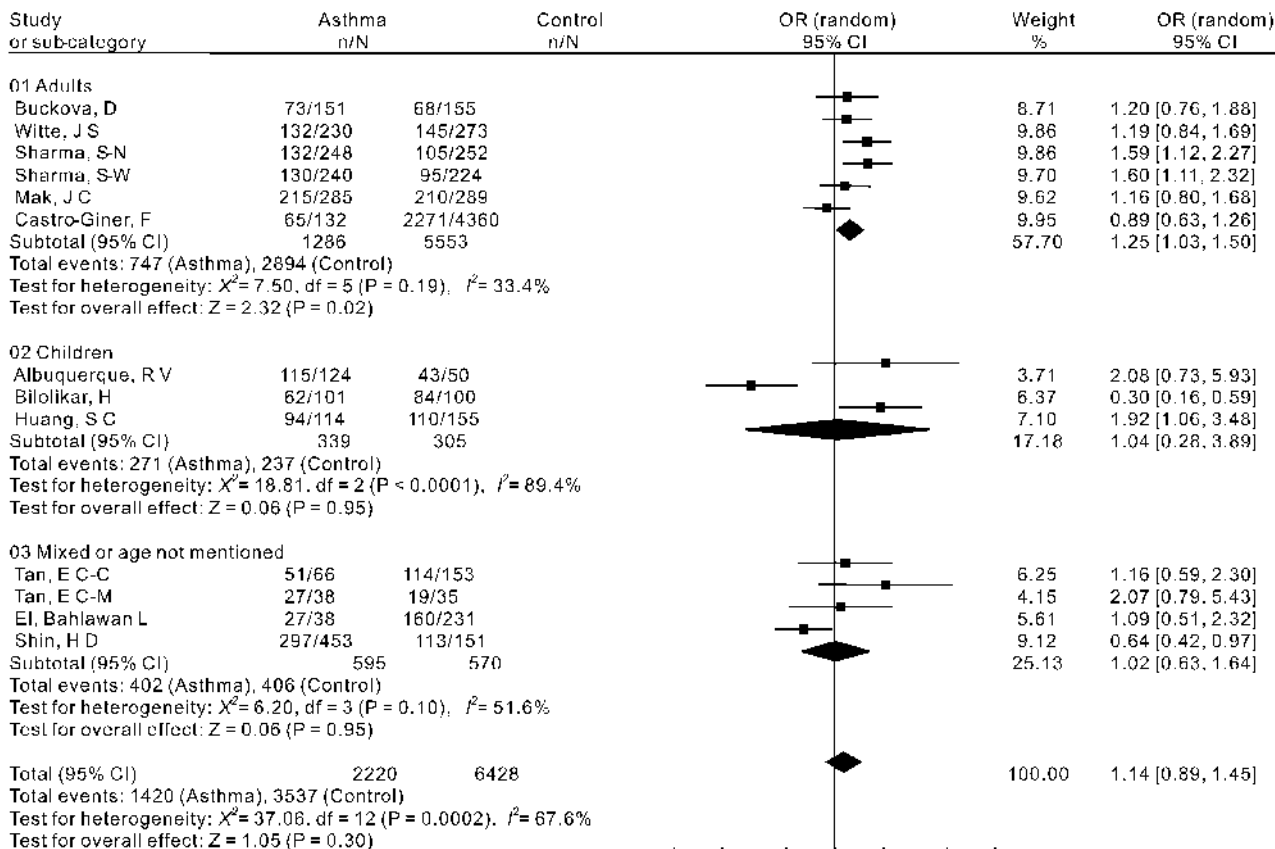
Figure S3 Meta-analysis with a random-effects model for the association between asthma risk and the LT- α +252A/G polymorphism (GG+AG vs AA): subgroup analysis by atopic status.

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Review: The +52A/G polymorphism in LI-alpha gene and asthma risk
 Comparison: 01 +252A/G
 Outcome: 02 Subgroup analysis by ethnicity



Review: The +252A/G polymorphism in LI-alpha gene and asthma risk
 Comparison: 01 +252A/G
 Outcome: 03 Subgroup analysis by age



Review: The +252A/G polymorphism in LI-alpha gene and asthma risk
 Comparison: 01 +252A/G
 Outcome: 04 Subgroup analysis by atopic status

