

The I/D polymorphism of *angiotensin-converting enzyme* gene and asthma risk: a meta-analysis

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Keywords

angiotensin-converting enzyme; asthma; meta-analysis; polymorphism.

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Abstract

Background: The insertion/deletion (I/D) polymorphism of *angiotensin-converting enzyme* (*ACE*) gene has been implicated in susceptibility to asthma, but a large number of studies have reported inconclusive results. The aim of this study is to investigate the association between the I/D polymorphism of *ACE* gene and asthma risk by meta-analysis.

Methods: We searched Medline (Ovid), Pubmed, CNKI, Wanfang, and Weipu database, covering all papers until March 12, 2010. Statistical analysis was performed by using the software REVMAN 4.2 (The Cochrane Collaboration, www.cochrane.org) and STATA 10.0 (StataCorp, College Station, TX, USA, www.stata.com).

Results: A total of 1946 cases and 2152 controls in 18 case-control studies were included in this meta-analysis. The results indicated that the DD homozygote carriers had a 59% increased risk of asthma, when compared with the homozygotes II and heterozygote DI [odds ratio (OR) = 1.59, 95% confidence interval (CI): 1.16–2.18]. In the subgroup analysis by ethnicity, significant elevated risks were associated with DD homozygote carriers in Asians (OR = 2.02 and 95% CI: 1.29–3.16 for DD vs DI+II) but not in Caucasians (OR = 1.14 and 95% CI: 0.76–1.72 for DD vs DI+II). In the subgroup analysis by age, significant elevated risks were associated with DD homozygote carriers in children (OR = 2.44 and 95% CI: 1.36–4.38 for DD vs II+DI) but not in adults (OR = 1.54 and 95% CI: 0.94–2.51 for DD vs II+DI).

Conclusions: This meta-analysis suggested that the I/D polymorphism of *ACE* gene would be a risk factor of asthma. To further evaluate gene-to-gene and gene-to-environment interactions between polymorphisms of *ACE* gene and asthma risk, more studies with large groups of patients are required.

Asthma is an inflammatory lung disease that affects both adults and children. Its major characteristics include reversible airflow obstruction, airway inflammation, bronchial hyperresponsiveness, epithelial damage, and airway smooth-muscle hypertrophy (1). It is widely accepted that asthma is a complex disease, and both environmental factors (viruses, allergens, and occupational exposures) and genetic factors contribute to its inception and evolution (1). Numerous studies have published focusing on the topic of genetic factors to asthma susceptibility (2–4). Many genes that involve in the inception and evolution of asthma have

been identified as asthma-susceptible genes (4), among them *angiotensin-converting enzyme* (*ACE*) gene was one of the most extensively studied (5–20).

Angiotensin-converting enzyme, a key enzyme of the renin-angiotensin system, is mainly expressed in the lung and plays an important role in the pathogenesis of asthma (6). *Angiotensin-converting enzyme* converts angiotensin I to angiotensin II, which may be involved in the etiology of asthma by interaction with bronchial muscle (15). *Angiotensin-converting enzyme* also inactivates a wide range of inflammatory peptides, including bradykinin and substance P, which are

produced in the lungs of patients with asthma (8). The human *ACE* gene is located on chromosome 17q23, where an insertion/deletion polymorphism (I/D, dbSNP rs4646994) in intron 16 has been identified (8). This polymorphism is based on the presence (insertion, I) or absence (deletion, D) of a 287-bp nonsense DNA fragment. It affects the serum ACE level and has been investigated as a potential susceptibility factor for asthma (12). A large number of studies have reported the association between the I/D polymorphism of *ACE* gene and the risk of asthma (5–20), but the results were inconclusive. This may be partly because of the possible small sample size in each of the published studies and different populations. To derive a more precise conclusion, we performed a meta-analysis of all eligible studies. This is, to our knowledge, the first genetic meta-analysis conducted with respect to the association between the *ACE* polymorphism and asthma risk.

Materials and methods

Publication search

A search of the literature was made by using the electronic database Medline (Ovid), Pubmed, CNKI, Wanfang, and Weipu database to identify articles that evaluated the association between polymorphism of *ACE* gene and asthma risk (Last search was updated on March 12, 2010). The search terms were used as follows: asthma or asthmatic and *angiotensin-converting enzyme* or *ACE* in combination with polymorphism, mutation or variant. All languages were included. Studies included in our meta-analysis had to meet the following inclusion criteria: (i) the study should evaluate the I/D polymorphism of *ACE* gene and asthma risk, (ii) they should be case-control studies, (iii) genotype distributions in both cases and controls were available for estimating an odds ratio (OR) with 95% confidence interval (CI), (iv) genotype distributions of control population must be consistent with Hardy-Weinberg equilibrium (HWE). Accordingly, the following exclusion criteria were also used: (i) abstracts and reviews, (ii) genotype frequency not reported, and (iii) Repeat or overlapping publications.

Data extraction

Two reviewers independently checked all potentially relevant studies and reached a consensus on all items. In case of disagreement, a third author would assess these articles. The following data were collected from each study: first author, year of publication, ethnicity, definition of cases, source of control, genotyping methods, total number of cases and controls, and genotype distributions in cases and controls.

Statistical analysis

For each study, we first examined whether the genotype distribution in controls was according to Hardy-Weinberg equilibrium by using an Internet-based program (<http://ihg2.helmholtz-muenchen.de/cgi-bin/hw/hwa1.pl>).

The strength of the association between the I/D polymorphism and asthma risk was measured by ORs and 95% CI. The statistical significance of summary OR was determined with Z-test. We first estimated with the risk of recessive model (DD vs II+DI) and dominant model (DD+DI vs II) and then evaluated variant genotype DD and compared with the wild-type II homozygote. We also estimated the risks of D vs I and DI vs II.

Heterogeneity was evaluated by a X^2 -based Q statistic and was considered statistical significant at P -value <0.10 . When the P -value is >0.10 , the pooled OR of each study was calculated by the fixed-effects model; otherwise, a random-effects model was used. The significance of the pooled OR was determined by the Z-test, and $P < 0.05$ was considered as statistically significant. To evaluate the ethnicity-specific and age-specific effects, subgroup analyses were performed by ethnic group and age. Sensitivity analysis was performed through sequentially excluded individual studies to assess the stability of the results. Publication bias was analyzed by several methods. Visual inspection of asymmetry in funnel plots was carried out. The Begg's test and Egger's test were also used to statistically assess publication bias (21). All statistical tests were performed by using the REVMAN 4.2 software and STATA 10.0.

Results

Study inclusion and characteristics

Medline (Ovid), Pubmed, CNKI, Wanfang, and Weipu database were comprehensively searched. There were 114 records relevant to our searching strategy. After reading the titles and abstracts, 88 were excluded for being not relevant to asthma risk and *ACE* gene. The remaining 26 articles were identified for full-text view (5–20, 22–24) (25–31). Then, an additional six articles were excluded [three were repeat or overlapping studies (23–25), two were not case-control studies (22, 27), and one did not report usable data (26)]; thus, a total of 20 articles were left for data extraction. Two articles reported two cohorts (5, 8) each, and each cohort was considered as a separate case-control study. Thus, a total of 22 case-control studies were identified. Additionally, the genotypes in controls for four case-control studies were not consistent with Hardy-Weinberg equilibrium, and these four were excluded too (28–31). Finally, a total of 18 case-control studies in 16 articles were identified met our inclusion criteria (5–20), including 1946 cases and 2152 controls. The characteristics of each case-control are listed in Table 1. Genotype and allele distributions for each case-control are shown in Table 2. There were 12 case-controls of Asians (5, 6, 8, 12–20), six of Caucasians (5, 7–11). Nine studies were performed in adults (6, 9–13, 16, 17, 20), while six were in children (8, 14, 15, 18, 19), and three did not present the ages of patients (5, 7).

Quantitative data synthesis

All studies

As shown in Fig. 1, we analyzed the heterogeneity of DD vs II+DI for all eighteen studies, and the value of X^2 was

Table 1 Characteristics of the 18 case-control studies included in meta-analysis

First author	Year	Country	Ethnicity	Case age (year)	Cases number (n)	Control number (n)	Asthma definition	Genotyping method
Eryuksel (13)	2009	Turkey	Asian	39 ± 13	97	96	ATS diagnosis criteria	PCR
Gao (20)	1999	China	Asian	39 (16–69)	50	50	Chinese asthma diagnosis criteria (1993)	PCR
Gao (5)	1998	Japan	Asian	–	300	100	Doctor's diagnosis	Not mentioned
Guo (18)	2006	China	Asian	–	52	72	Chinese traditional medicine	PCR
Lee (6)	2000	South Korea	Asian	50.6 ± 20.1	310	121	ATS diagnosis criteria	PCR
Lu (17)	2004	China	Asian	37 (18–52)	18	15	Chinese asthma diagnosis criteria (1993)	PCR
Lue (15)	2006	China	Asian	9.91 ± 1.62	105	102	ATS diagnosis criteria	PCR
Nakahama (12)	1999	Japan	Asian	51.2 ± 17.1	119	208	ATS diagnosis criteria	PCR
Qin (19)	2000	China	Asian	6.9 ± 2.7	52	40	Chinese asthma diagnosis criteria for children (1998)	PCR
Song (14)	2001	China	Asian	7 (1–14)	108	56	Chinese asthma diagnosis criteria (1993)	PCR
Tomita (16)	1998	Japan	Asian	52.0 ± 2.0	71	142	ATS diagnosis criteria	PCR
Winchester (8)	2000	S. Asians	Asian	–	6	275	Questionnaire	PCR
Benessiano (10)	1997	France	Caucasian	–	54	79	ATS diagnosis criteria	PCR
Chagani (7)	1999	Ireland	Caucasian	–	231	43	Doctor's diagnosis	PCR
Gao (5)	1998	British	Caucasian	–	150	150	Doctor's diagnosis	Not mentioned
Holla (9)	1999	Czech	Caucasian	–	161	141	ATS diagnosis criteria	PCR
Winchester (8)	2000	UK/Irish	Caucasian	–	20	416	Questionnaire	PCR
Yildiz (11)	2004	Turkey	Caucasian	–	42	46	ATS diagnosis criteria	PCR

Gao, P S1: Japanese; Gao, P S2: British. Winchester, E C1: UK/Irish; Winchester, E C2: S. Asians.

48.38 with 17 degrees of freedom and $P < 0.0001$ in a random-effects model. Additionally, I -square value is another index of the test of heterogeneity. In Fig. 1, the I -square was 64.9%, suggesting a moderate heterogeneity. Thus, we chose the random-effects model to synthesize the data. Overall, OR was 1.59 (95% CI = 1.16–2.18), and the test for overall effect Z -value was 2.88 ($P = 0.004$) for DD vs DI+II model. The results suggested that individuals who carry DD homozygote may have a 59% increased asthma risk compared with the homozygote II and heterozygote DI. Summary results of other genetic comparisons are listed in Table 3.

Subgroup analyses

In the subgroup analysis by ethnicity, significantly increased risks were found among Asian (OR, 2.02; 95% CI, 1.29–3.16; $P = 0.002$) but not in Caucasian (OR, 1.14; 95% CI, 0.76–1.72; $P = 0.53$) subjects in a recessive model (DD vs DI+II) (Fig. 2). Thus, the Asian DD carriers may have higher risk of asthma than Caucasian DD carriers. In the subgroup analysis by age, significantly increased risks were found among children (OR, 2.14; 95% CI, 1.36–4.38; $P = 0.003$) but not in adults (OR, 1.54; 95% CI, 0.94–2.51; $P = 0.09$) (Fig. 3). Summary results of other genetic comparisons are listed in Table 3.

Publication bias

Publication bias was assessed by Begg's funnel plot and Egger's test. The shape of the funnel plots seemed symmetrical in the DD vs II+DI comparison genetic model, suggesting the absence of publication bias (Fig. S1). Then, the Egger's test was performed to provide statistical evidence of funnel plot asymmetry. The results indicated a lack of publication bias of the current meta-analysis ($t = 1.49$, $P = 0.155$).

Sensitivity analysis

To assess the stability of the results of the current meta-analysis, we performed sensitivity analysis by sequentially excluding each study. Statistically similar results were obtained after sequentially excluding each study, suggesting the stability of our meta-analysis.

Discussion

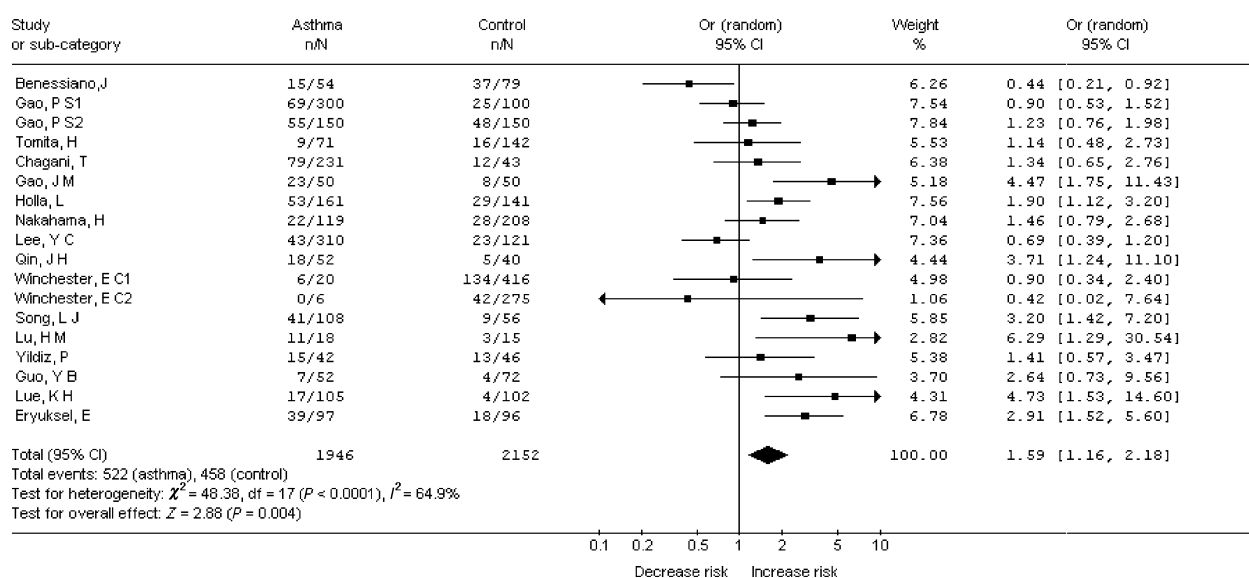
It is well recognized that asthma is a complex disease that demonstrates multistep, multigene, and gene-environment involvement (32). There is individual susceptibility to asthma even with the same environmental exposure. Host genetic factors, including variants of genes involved in path-

Table 2 Distribution of *angiotensin-converting enzyme* genotype among patients with asthma and controls included in the meta-analysis

Studies	Asthma			Control			Hardy–Weinberg equilibrium
	II	DI	DD	II	DI	DD	
Guo (18)	27	18	7	36	32	4	Yes
Lu (17)	3	4	11	7	5	3	Yes
Song (14)	45	22	41	29	18	9	Yes
Gao (20)	12	15	23	16	26	8	Yes
Qin (19)	24	10	18	21	14	5	Yes
Lue (15)	48	40	17	56	42	4	Yes
Lee (6)	109	158	43	48	50	23	Yes
Chagani (7)	52	100	79	7	24	12	Yes
Gao (5)	103	128	69	27	48	25	Yes
Gao (5)	31	64	55	31	71	48	Yes
Eryuksel (13)	14	44	39	30	48	18	Yes
Winchester (8)	3	11	6	89	193	134	Yes
Winchester (8)	4	2	0	105	128	42	Yes
Benessiano (10)	8	31	47	12	30	37	Yes
Holla (9)	23	85	53	37	75	29	Yes
Nakahama (12)	47	50	22	84	96	28	Yes
Yildiz (11)	10	17	15	13	20	13	Yes
Tomita (16)	25	37	9	57	69	16	Yes

Gao, P S1: Japanese; Gao, P S2: British. Winchester, E C1: UK/Irish; Winchester, E C2: S. Asians.

Review: The I/D polymorphism of ACE gene and asthma risk
 Comparison: 01 ACE I/D polymorphism
 Outcome: 01 DD vs II+DI

**Figure 1** Meta-analysis with a random-effects model for the association between asthma risk and the *angiotensin-converting enzyme* insertion/deletion polymorphism (DD vs II+DI).

ogenesis, may have affected this difference. Therefore, in recent years, interest in the genetic susceptibility to asthma has led to a growing attention to the study of polymorphisms of genes involved in the pathogenesis of asthma. The I/D polymorphism of the *ACE* gene in the etiology of

asthma was one of the most studied polymorphisms. A growing number of studies have suggested that this polymorphism was associated with an increased risk of asthma. To date, the results found on the association of ACE I/D polymorphism with asthma are inconclusive, and there is

Table 3 Summary of different comparative results

Genetic model	Overall or subgroup (ethnicity/age)	Study number (n)	Participant (n)	OR (95% CI)	Z	P	I (%)	P _{het}	Effect model
DD+DI vs II	All	18	4098	1.21 (1.04, 1.41)	2.43	0.02	24.1	0.17	F
	Asian	12	2565	1.20 (1.00, 1.44)	1.99	0.05	32.10	0.13	F
	Caucasian	6	1533	1.24 (0.92, 1.69)	1.40	0.16	18.7	0.29	F
	Adults	9	1820	1.41 (1.14, 1.75)	3.19	0.001	19.3	0.27	F
	Children	6	1304	1.25 (0.92, 1.71)	1.40	0.16	0	0.56	F
	Age not mentioned	3	974	0.80 (0.57, 1.12)	1.32	0.19	0	0.60	F
DD vs DI+II	All	18	4098	1.59 (1.16, 2.18)	2.88	0.004	64.9	<0.0001	R
	Asian	12	2565	2.02 (1.29, 3.16)	3.06	0.002	68.50	0.0003	R
	Caucasian	6	1533	1.14 (0.76, 1.72)	0.63	0.53	52.9	0.06	R
	Adults	9	1820	1.54 (0.94, 2.51)	1.71	0.09	74.6	0.0001	R
	Children	6	1304	2.44 (1.36, 4.38)	2.98	0.003	35.10	0.17	R
	Age not mentioned	3	974	1.12 (0.81, 1.53)	0.67	0.50	0.00	0.58	R
DD vs II	All	18	2273	1.71 (1.21, 2.41)	3.05	0.002	57.5	0.001	R
	Asian	12	1461	2.02 (1.26, 3.25)	2.91	0.004	64.90	0.0010	R
	Caucasian	6	812	1.32 (0.82, 2.13)	1.15	0.25	37.2	0.16	R
	Adults	9	960	1.85 (1.12, 3.05)	2.39	0.02	64.5	0.004	R
	Children	6	774	2.69 (1.64, 4.42)	3.90	<0.0001	0.00	0.48	R
	Age not mentioned	3	539	0.90 (0.60, 1.36)	0.49	0.63	0.00	0.60	R
DI vs II	All	18	3118	1.05 (0.89, 1.24)	0.57	0.57	1.7	0.43	F
	Asian	12	2081	1.01 (0.83, 1.23)	0.10	0.92	0.00	0.46	F
	Caucasian	6	1037	1.17 (0.84, 1.61)	0.94	0.35	15.5	0.31	F
	Adults	9	1415	1.30 (1.04, 1.64)	2.27	0.02	0	0.68	F
	Children	6	1017	0.89 (0.63, 1.25)	0.69	0.49	0.00	0.69	F
	Age not mentioned	3	686	0.74 (0.51, 1.06)	1.64	0.10	0.00	0.66	F
D vs I	All	18	4098	1.29 (1.08, 1.54)	2.79	0.005	62.8	0.0002	R
	Asian	12	2565	1.41 (1.10, 1.80)	2.73	0.006	68.90	0.0002	R
	Caucasian	6	1533	1.12 (0.87, 1.44)	0.89	0.37	46.9	0.09	R
	Adults	8	1820	1.38 (1.05, 1.81)	2.33	0.02	70.8	0.0006	R
	Children	6	1304	1.45 (1.08, 1.96)	2.44	0.01	34.90	0.17	R
	Age not mentioned	3	974	0.96 (0.78, 1.18)	0.36	0.72	0.00	0.47	R

still no meta-analysis study that has successfully established the relationship of *ACE* I/D polymorphism with asthma risk; thus, we performed this meta-analysis to estimate the association specifically. At the same time, because the same polymorphism seemed to have different functions in asthma susceptibility among different age and ethnic groups, subgroup analyses on the basis of age and ethnicity were conducted.

The current meta-analysis, including 1946 cases and 2152 controls from 18 case-control studies, explored the association between one potentially functional polymorphism within the *ACE* gene and asthma risk. Our results indicated that the DD homozygote carriers had a nearly 59% increased risk of asthma. This risk appeared to be more evident in Asians but not in Europeans, suggesting a possible role of ethnic differences in genetic backgrounds and the environment they lived in (33). In addition, there is no reported study using African population for this polymorphism. So it is also likely that the observed ethnic differences may be attributable to chance because studies with small sample size may have insufficient

statistical power to detect a slight effect. Therefore, additional studies are warranted to further validate ethnic difference in the effect of this polymorphism on asthma risk, especially in Africans.

In our meta-analysis, we also carried out subgroup analysis for age. The results showed that the DD homozygote carriers may have more increased risks of asthma in children than that in adults in a recessive model, in addition, in other comparison genetic models except for the DI vs II, results also showed that there was more increased risk for asthma in children than that in adults. These results strongly indicated that this polymorphism was significantly associated with increased asthma risk in children. Based on our study, it is worth noting that, even the same variant in the same gene, such as the I/D polymorphism of the *ACE* gene, may have a different effect on the pathogenesis and occurrence of disease in humans.

It is worth mentioning recent findings from genome-wide association (GWA) studies on patients with asthma, which found associations with some polymorphisms in some genes,

Review: The I/D polymorphism of ACE gene and asthma risk
 Comparison: 01 ACE I/D polymorphism
 Outcome: 03 Subgroup analysis by ethnicity

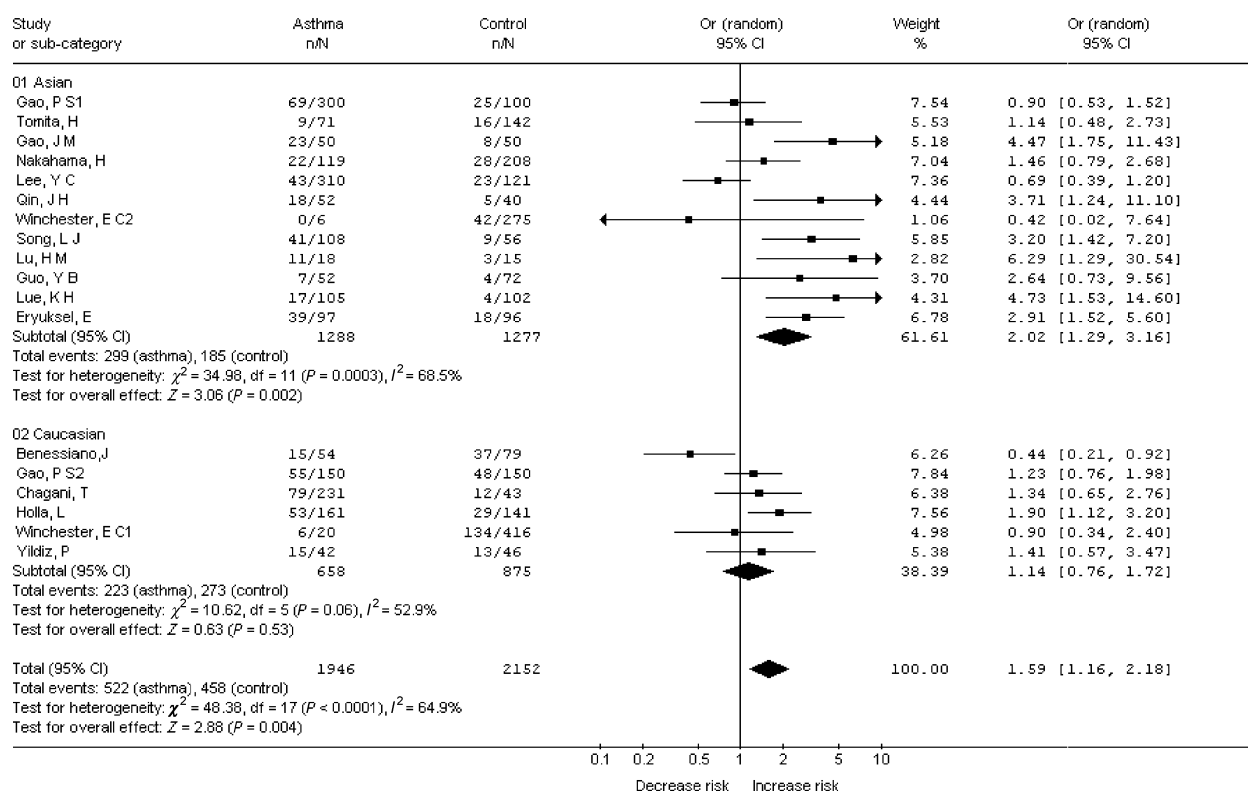


Figure 2 Meta-analysis with a random-effects model for the association between asthma risk and *angiotensin-converting enzyme*

insertion/deletion polymorphism (DD vs II+ DI), subgroup analysis by ethnicity.

such as *ORMDL3*, *CHI3LI*, *PDE4D*, *TLE4*, *WDR36*, *IL33*, *IL1RL1*, *MYB*, and *DENND1B* (34, 35). These genes were all thought as asthma risk factors. However, the current meta-analysis does not consider findings from these studies. The *ACE* I/D polymorphism was not directly assayed on the genotyping microarrays employed in these studies, precluding our consideration. It is noteworthy, however, that all but two of the published studies used Illumina genotyping arrays that include SNP rs4343 (36, 37), which is a good proxy for the I/D variant ($r^2 > 0.80$) (38). To our knowledge, none of the published GWAS studies for asthma report association with rs4343. Future meta-analysis of these datasets could help resolve whether the *ACE* locus shows consistent association in these population as well.

We also have to mention the importance of heterogeneity. Heterogeneity is an important issue when interpreting the results of meta-analysis. Significant heterogeneity existed in overall comparisons in the recessive model. After subgroup analyses by ethnicity or age, the heterogeneity was effectively decreased or removed in Caucasians and children. Possible explanations may be that differences in genetic backgrounds and environmental exposures existed among different ethnicities, and differences in etiology may exist in difference age

groups. Another important factor contributing to heterogeneity was that homogeneity in either the case or control groups was uncertain. Ideally, all cases and controls should be matched for age, sex, and environmental exposures. In this meta-analysis, these issues could not all be explained precisely because of insufficient information for individual person. In addition, because this polymorphism is based on the presence (insertion, I) or absence (deletion, D) of a 287-bp DNA fragment, every study expect for Gao P S's (Gao's study is published as a letter, the genotyping method was not mentioned) used the PCR method to detection this polymorphism. So the similarity of these studies might be very good, despite those studies were conducted in different populations.

Some limitations of this meta-analysis should be acknowledged when explaining our results. Firstly, only published studies that were included by the selected electronic databases were identified in this study, and it is possible that some relevant published studies or unpublished studies that had null results were missed, which might bias the results, while our statistical test may not have totally shown it. Second, all case-controls were from Asians and Caucasians; thus, our results may be applicable to these two ethnic groups only. Thirdly, data were not stratified by other factors such as

Review: The I/D polymorphism of ACE gene and asthma risk
 Comparison: 01 ACE I/D polymorphism
 Outcome: 02 Subgroup analysis by age

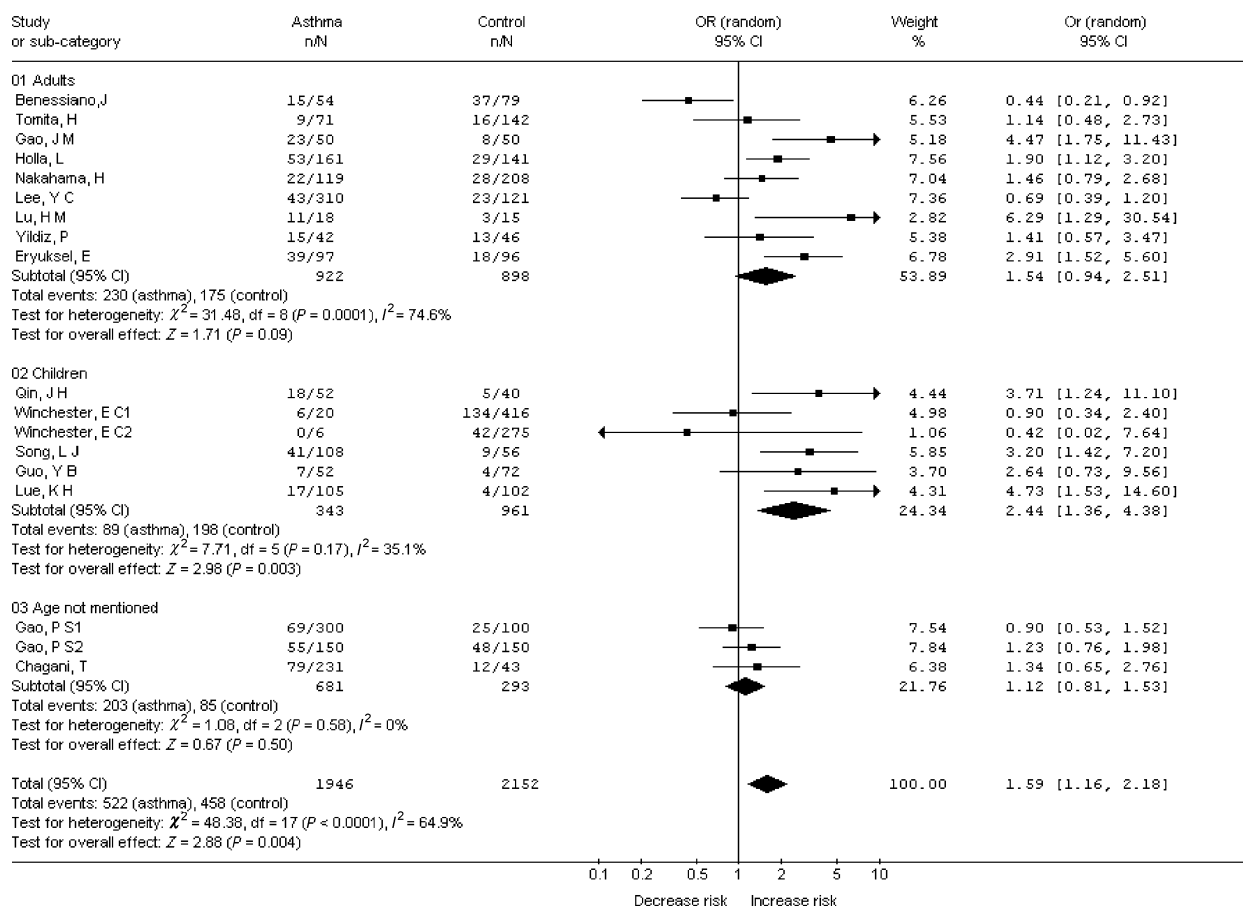


Figure 3 Meta-analysis with a random-effects model for the association between asthma risk and *angiotensin-converting enzyme*

insertion/deletion polymorphism (DD vs II + DI), subgroup analysis by age.

atopic status because sufficient information could not be extracted from the primary publication. Fourthly, some of the individual studies have a small number of cases, this may affect statistical power of the publication bias, thus, it should be caution when explained our results. Anyway, we minimized the likelihood of bias by creating a detailed protocol before initiating our study, by performing a meticulous search for publications, and by using explicit methods for publications selection, data extraction, and data analysis.

To our knowledge, this study is the first comprehensive meta-analysis to date to have assessed the relationship between the *ACE* I/D polymorphism and asthma susceptibility. It provided evidence of the association between *ACE* I/D polymorphism and asthma risk, supporting the hypothesis that the *ACE* I/D polymorphism may be a susceptibility marker for asthma. However, additional large case-control studies are warranted to validate our findings. Future analyses should be conducted in large-scale cohorts and should study the potential effect modification by age in different populations.

Acknowledgments

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Conflict of interest

The authors declare that they have no conflict of interest.

Supporting Information

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

Figure S1. Begg's funnel plot with pseudo 95% confidence limits.

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Begg's funnel plot with pseudo 95% confidence limits

