Association between angiotensin-converting enzyme insertion/deletion polymorphisms and intracranial aneurysm susceptibility: A meta-analysis

YAN-PING CUN[1*], CHENG-JIE XIONG[2*], BO DIAO[3], YU YANG[4], LI PAN[3] and LIAN-TING MA[1,3]

[1]Postdoctoral Research Station of Neurosurgery and Departments of [2]Orthopaedics, [3]Neurosurgery and [4]Healthcare, Wuhan General Hospital of Guangzhou Military Command, Wuhan, Hubei 430070, P.R. China

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Abstract. Various studies have evaluated the association between polymorphisms of angiotensin-converting enzyme (ACE) and intracranial aneurysm (IA) risk; however, the results remain inconsistent. The PubMed, Embase, and Wanfang Data databases were systematically searched until January 6th 2016. Case-control studies investigating the association between the ACE polymorphism and IA risk were included. Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated with the fixed or random-effects model assuming allele, homozygote comparison of codominant, heterozygote comparison of codominant, dominant, and recessive models. Seven studies including 1,074 cases and 1,500 controls were included in the current meta-analysis. The results of the analysis indicated that the ACE polymorphism significantly increased IA risk in the allele, homozygote comparison of codominant and dominant models. According to the further stratified analysis by ethnicity, source of control and sample sizes, a significant association was identified between the ACE variant and IA risk in Asian individuals, hospital-based, or large (>300) subgroups in all of the genetic models, not including the recessive model. Furthermore, no significantly increased risk was indicated in Caucasian individuals, population-based, or small (<300) subgroups in the heterozygote comparison of codominant, dominant and recessive models. The available evidence indicates that the ACE polymorphism is associated with an increased risk of IA, particularly in Asian individuals. However, other factors may impact this association. Further large, well-designed multicenter studies are required to validate the findings from the present study.

Correspondence to: Professor Lian-Ting Ma, Postdoctoral Research Station of Neurosurgery, Wuhan General Hospital of Guangzhou Military Command, 627 Wuluo Road, Wuhan, Hubei 430070, P.R. China
E-mail: mlt1937@163.com

*Contributed equally

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Introduction

The incidence of intracranial aneurysm (IA) is high at approximately 2-3% in the general population worldwide (1). IA rupture is the most common reason for life-threatening subarachnoid hemorrhages (SAH), which accounts for ~85% of overall SAH (2). Despite advances in diagnosis and treatment, the rates of mortality and morbidity of SAH associated with a ruptured IA remain high (3). Therefore, it is important to understand the molecular pathogenesis of IA to reduce the occurrence of SAH.

There is evidence to indicate that environmental and genetic factors are associated with the pathogenesis of IAs (4,5). The modifiable factors, such as smoking, hypertension, and excessive alcohol consumption have been reported to lead to IA formation (6). In addition, accumulating evidence indicates that genetics are significant in the pathogenesis of IA. Familial IA is not rare, accounting for 7-20% of patients with aneurysmal SAH (7). The risk of experiencing unruptured IAs is significantly higher (~4 times) in familial IA families than in the general population (8). Furthermore, certain genetic factors have been investigated and identified to be associated with IA (9-13), including angiotensin-converting enzyme (ACE).

ACE is the key enzyme in the renin-angiotensin-aldosterone system (RAAS), as an important modulator of cerebrovascular disease (CVD) (14). The *ACE* gene, which consists of 26 exons and 25 introns is located on chromosome 17q23 (14). The *ACE* gene contains functional insertion/deletion (I/D) polymorphism of a 287-bp Alu sequence within intron 16 (15). Individuals with the DD genotype exhibit increased serum ACE levels and activity when compared with individual with the ID and II genotypes (15,16). In addition, increased serum ACE levels may contribute to vascular injury, which is hypothesized to confer increased risk of IA.

Certain previous studies investigated the influence of *ACE* I/D gene polymorphisms on IA susceptibility (17-23); however, the results of these studies remain inconsistent. As a single study might not be powered to demonstrate the overall effects,

a meta-analysis using currently available data was performed in the present study to detect the potential association of ACE I/D gene polymorphisms on IA risk.

Materials and methods

Publication search. The search was performed using PubMed (https://www.ncbi.nlm.nih.gov/pubmed), Embase (www.embase.com), and Wanfang databases (updated to January 6th, 2016; http://www.wanfangdata.com.cn) with the following terms: 'Polymorphism', 'genotype', 'allele', 'mutation', 'variant', in combination with 'cerebral aneurysm', 'brain aneurysm', 'intracranial aneurysm', 'SAH', 'subarachnoid hemorrhage', in combination with 'ACE', 'angiotensin converting enzyme', and 'insertion/deletion'. The search was conducted without a limitation on language. Reference lists of eligible studies were also retrieved to find additional articles.

Inclusion criteria. Two reviewers independently screened the literature for relevance and disagreements were resolved by consensus. Studies included in the current meta-analysis met the following criteria: The studies i) evaluated the *ACE* gene polymorphisms and IA risk; ii) were case-control studies; iii) contained available genotype frequencies for calculating odds ratios (ORs) with their 95% confidence interval (CI). The exclusion criteria were as follows: i) Duplicated studies; ii) limited sample size; iii) reviews, editorials or comments.

Data extraction. Information was carefully extracted from all eligible publications independently by two authors of the present paper. For conflicting evaluations, agreement was reached by discussion. For each study, the following characteristics were considered: First author surname, year of publication, country of origin, ethnicity of study subjects, source of control groups (population- or hospital-based controls), total number of cases and controls, genotyping method, evidence of Hardy-Weinberg equilibrium (HWE) and numbers of cases and controls with the II, ID, and DD genotypes for ACE. Subjects were categorized as East Asian and Caucasian.

Statistical analysis. Deviation from HWE was examined by χ^2 test and P<0.05 was considered to indicate a statistically significant difference. The strength of associations between *ACE* gene polymorphisms and IA risk were measured by OR with 95%CI. The pooled ORs were performed for I allele contrast (I vs. D), homozygote comparison of codominant, homozygote comparison of codominant (II vs. DD) and heterozygote comparison of codominant (ID vs. DD), dominant model (II+ID vs. DD), and recessive model (II vs. ID+DD), respectively. Heterogeneity assumption was verified by χ^2-based Q-test and quantified using the I^2 value. If the studies lacked heterogeneity (P_h>0.1 and I^2<50%), the fixed-effects model (the Mantel-Haenszel method) was adopted to calculate the overall ORs value (24). Otherwise, the random-effects model (the DerSimonian and Laird method) were used (25). To assess the stability of the results, sensitivity analyses were performed by removing each study individually, and recalculating the OR and the 95% CI. Publication bias was assessed using Begg's funnel plots and Egger's linear regression test, and P<0.05 was considered to indicate a statistically significant difference (26). All statistical analyses were performed with Stata software (version 12.0; StataCorp LP, College Station, TX, USA), using two-sided P-values.

Results

Study characteristics. Our initial search identified 43 studies according to the search terms. After removing replicates and screening of titles and abstracts, 13 articles remained for further detailed evaluation (Fig. 1). Finally, a total of seven case-control studies were included in our meta-analysis, involving 1,074 IA cases and 1,500 control subjects. The main study characteristics are presented in Table I (19-23). Three studies involving 390 cases and 432 control subjects were from East Asian populations and four involved 684 cases and 1,068 control participants from Caucasian populations. In terms of the source of controls, threes studies included population-based studies, and four studies included hospital-based studies. Four studies were conducted in large samples and three in small samples. The genotype distributions among the controls of all studies were consistent with HWE except one study by Yu *et al* (23).

Meta-analysis. Table II summarizes the key results of the meta-analysis. In the overall analysis, a significant strong association between the ACE polymorphism and an increased risk of IA was identified in the allele (I vs. D; OR=1.27, 95% CI=1.13-1.42; P_{OR}=0.000), homozygote comparison of codominant (II vs. DD; OR=1.64, 95% CI=1.30-2.07; P_{OR}=0.000) and dominant (II+ID vs. DD; OR=1.38, 95% CI=1.05-1.80; P_{OR}=0.021) models. By contrast, no statistically significant association was detected under the heterozygote comparison of the codominant model (ID vs. DD; OR=1.25, 95% CI=0.79-1.96; P_{OR}=0.343) and the recessive model (II vs. ID+DD; OR=1.35, 95% CI=0.98-1.87; P_{OR}=0.064).

When stratified by ethnicity, the ACE polymorphism showed a significant contribution to IA risk in the Asian population in all genetic models except in the recessive model (II vs. ID+DD; OR=1.15, 95% CI=0.71-1.87; P_{OR}=0.561). In addition, a significant strong association was detected under the allele model (I vs. D; OR=1.21, 95% CI=1.06-1.40; P_{OR}=0.006) and homozygote comparison of codominant model (II vs. DD; OR=1.44, 95% CI=1.09-1.90; P_{OR}=0.011) in the Caucasian population (Table II; Fig. 2A-D).

In the subgroup analysis for the HB group, a significantly increased risk in all genetic models was observed except for in the recessive model (II vs. ID+DD; OR=1.15, 95% CI=0.83-1.59; P_{OR}=0.401). Similar findings were revealed in the allele model (I vs. D; OR=1.28, 95% CI=1.08-1.52; P_{OR}=0.004) and homozygote comparison of codominant model (II vs. DD; OR=1.56, 95% CI=1.12-2.17; P_{OR}=0.009) in the PB subgroup (Table II).

When stratified by sample size, a statistically significant association was found in the large group in all of the genetic models. However, only the allele and homozygote comparison of codominant models showed significant associations in the small subgroup (OR=1.33, 95% CI=1.05-1.68; P_{OR}=0.019 and OR=1.93, 95% CI=1.19-3.13; P_{OR}=0.007, respectively) (Table II).

Heterogeneity analysis. Significant heterogeneity in the ACE polymorphism was observed in the heterozygote comparison

Table I. Characteristics of studies included in the current metaanalysis.

Author, year	Ethnicity	Country	Sample size (case/control)	SC	Genotyping methods	HWE	Case			Control			(Refs.)
							II	ID	DD	II	ID	DD	
Liu, 2013	East Asian	China	220/220	HB	PCR-RFLP	Yes	64	106	50	44	99	77	(17)
Staalsø, 2011	Caucasian	Denmark	174/498	HB	TaqMan	Yes	39	96	39	104	270	124	(18)
Pannu, 2005	Caucasian	USA	162/143	PB	ASO-PCR	Yes	33	85	44	27	73	43	(19)
Slowik, 2004	Caucasian	Poland	90/128	PB	ASO-PCR	Yes	47	14	29	30	65	33	(20)
Keramatipour, 2000	Caucasian	UK	258/299	PB	Triple-primer PCR	Yes	78	126	54	71	146	82	(21)
Takenaka, 1998	East Asian	Japan	83/104	HB	ASO-PCR	Yes	38	40	5	43	45	16	(22)
Yu, 2005	East Asian	China	87/108	HB	Quantitative PCR, Sequencing	No	31	47	9	47	38	23	(23)

SC, source of control; HB, hospital-based study; PB, population-based study; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; ASO, allele-specific oligonucleotide; HWE, Hardy-Weinberg equilibrium.

Figure 1. Flow chart presenting the detailed study selection process.

of codominant model (I^2=76.7%; P_h=0.000), dominant model (I^2=44.5%; P_h=0.094) and recessive model (I^2=66.1%; P_h=0.007). To investigate the potential sources of heterogeneity across studies, the pooled ORs under all comparisons were assessed via subgroup and sensitivity analyses. In the subgroup analysis by ethnicity, the heterogeneity of ACE was significant in the Caucasian studies in the heterozygote comparison of the codominant and recessive models (I^2=80.2%; P_h=0.002 and I^2=74.9%; P_h=0.008, respectively). When stratified by source of control, the heterogeneity of ACE in the population-based studies was significant in the heterozygote comparison of codominant and recessive models (I^2=86.5% and P_h=0.001; I^2=79% and P_h=0.009, respectively). When stratified by sample size, the heterogeneity of ACE in the small subgroup studies was significant in the heterozygote comparison of the codominant, dominant and recessive models (I^2=91.4% and P_h=0.000; I^2=74% and P_h=0.021; and I^2=86.9% and P_h=0.000, respectively) (Table II).

Notably, when the Caucasian population-based control study with <300 cases by Slowik *et al* (20) was excluded, the heterogeneity significantly decreased in the heterozygote comparison of codominant and recessive models (Table III). Therefore, the study by Slowik *et al* (20) may have contributed to the substantial heterogeneity of the ACE polymorphism.

Sensitivity analyses. Sensitivity analyses were performed by sequential deletion of single studies for all subjects to determine the influence of its individual data sets to the pooled OR, and the corresponding pooled ORs were not materially altered in the allele and homozygote comparison of the codominant models. The pooled ORs ranged from 1.11 to 1.42 in the heterozygote comparison of codominant model, 1.27 to 1.47 in the dominant model, and 1.21 to 1.48 in the recessive model (Table III). When excluding the study by Slowik *et al* (20), a significantly increased risk was identified with the ACE polymorphism in the heterozygous comparison of the codominant (OR=1.42, 95% CI=1.15-1.76; P_{OR}=0.001) and recessive (OR=1.21, 95% CI=1.00-1.47; P_{OR}=0.046) models (Table III; Fig. 3A and B). In addition, a significantly increased risk was detected after excluding the study by Yu *et al* (23) in the recessive model (OR=1.48, 95% CI=1.08-2.03; P_{OR}=0.01)

Table II. Meta-analysis results for the angiotensin-converting enzyme polymorphisms and IA risk.

							Codominant model															
			Allele I vs. D				Homozygote II vs. DD				Heterozygote ID vs. DD				Dominant model (II+ID) vs. DD				Recessive model II vs. (ID+DD)			
Variables	Cases, n	Case/ control	I^2 (%)	P_h	OR (95%CI)	P_{OR}	I^2 (%)	P_h	OR (95%CI)	P_{OR}	I^2 (%)	P_h	OR (95%CI)	P_{OR}	I^2 (%)	P_h	OR (95%CI)	P_{OR}	I^2 (%)	P_h	OR (95%CI)	P_{OR}
Total	7	1074/ 1500	6.8	0.376	1.27 (1.13, 1.42)	0	0	0.577	1.64 (1.30, 2.07)	0	76.7	0	1.25 (0.79, 1.96)	0.343	44.5	0.094	1.38 (1.05, 1.80)	0.021	66.1	0.007	1.35 (0.98, 1.87)	0.064
Ethnicity																						
East Asian	3	390/ 432	6.2	0.344	1.38 (1.13, 1.68)	0.002	0	0.761	2.18 (1.44, 3.31)	0	6.8	0.342	2.02 (1.35, 3.03)	0.001	0	0.691	2.01 (1.41, 2.86)	0	59.5	0.085	1.15 (0.71, 1.87)	0.561
Caucasian	4	684/ 1068	8.3	0.352	1.21 (1.06, 1.40)	0.006	0	0.663	1.44 (1.09, 1.90)	0.011	80.2	0.002	0.86 (0.49, 1.53)	0.615	11.8	0.334	1.15 (0.90, 1.47)	0.264	74.9	0.008	1.53 (0.95, 2.45)	0.08
Source of control																						
HB	4	564/ 930	31.1	0.226	1.25 (1.07, 1.46)	0.004	19.3	0.293	1.72 (1.25, 2.38)	0.001	48.8	0.119	1.72 (1.11, 2.68)	0.015	38.4	0.181	1.68 (1.15, 2.44)	0.007	41	0.166	1.15 (0.83, 1.59)	0.401
PB	3	510/ 570	2.1	0.36	1.28 (1.08, 1.52)	0.004	0	0.652	1.56 (1.12, 2.17)	0.009	86.5	0.001	0.76 (0.32, 1.80)	0.526	41.2	0.183	1.12 (0.78, 1.62)	0.543	79	0.009	1.73 (0.92, 3.27)	0.089
Sample size																						
>300	4	814/ 1160	31.9	0.221	1.25 (1.09, 1.42)	0.001	16.4	0.309	1.56 (1.20, 2.03)	0.001	0	0.629	1.30 (1.04, 1.63)	0.023	1.5	0.384	1.38 (1.11, 1.71)	0.003	0	0.534	1.31 (1.05, 1.63)	0.015
<300	3	260/ 340	0	0.4	1.33 (1.05, 1.68)	0.019	0	0.738	1.93 (1.19, 3.13)	0.007	91.4	0	1.27 (0.22, 7.48)	0.79	74	0.021	1.58 (0.63, 3.97)	0.325	86.9	0	1.45 (0.58, 3.67)	0.429
HWE																						
Yes	6	987/ 1392	12.6	0.334	1.28 (1.14, 1.45)	0	0	0.449	1.64 (1.29, 2.08)	0	76.4	0	1.11 (0.70, 1.75)	0.669	44.9	0.106	1.31 (1.00, 1.73)	0.054	60.5	0.027	1.48 (1.08, 2.03)	0.014
No	1	87/ 108			1.07 (0.71, 1.61)	0.757			1.69 (0.69, 4.12)	0.252			3.16 (1.31, 7.63)	0.01			2.35 (1.02, 5.38)	0.044			0.72 (0.40, 1.28)	0.264

P_h, P-value for heterogeneity; P_{OR}, P-value for OR. P_{OR}<0.05 was considered to indicate a statistically significant difference. OR, odds ratio; CI, confidence interval; HB, hospital-based study; PB, population-based study.

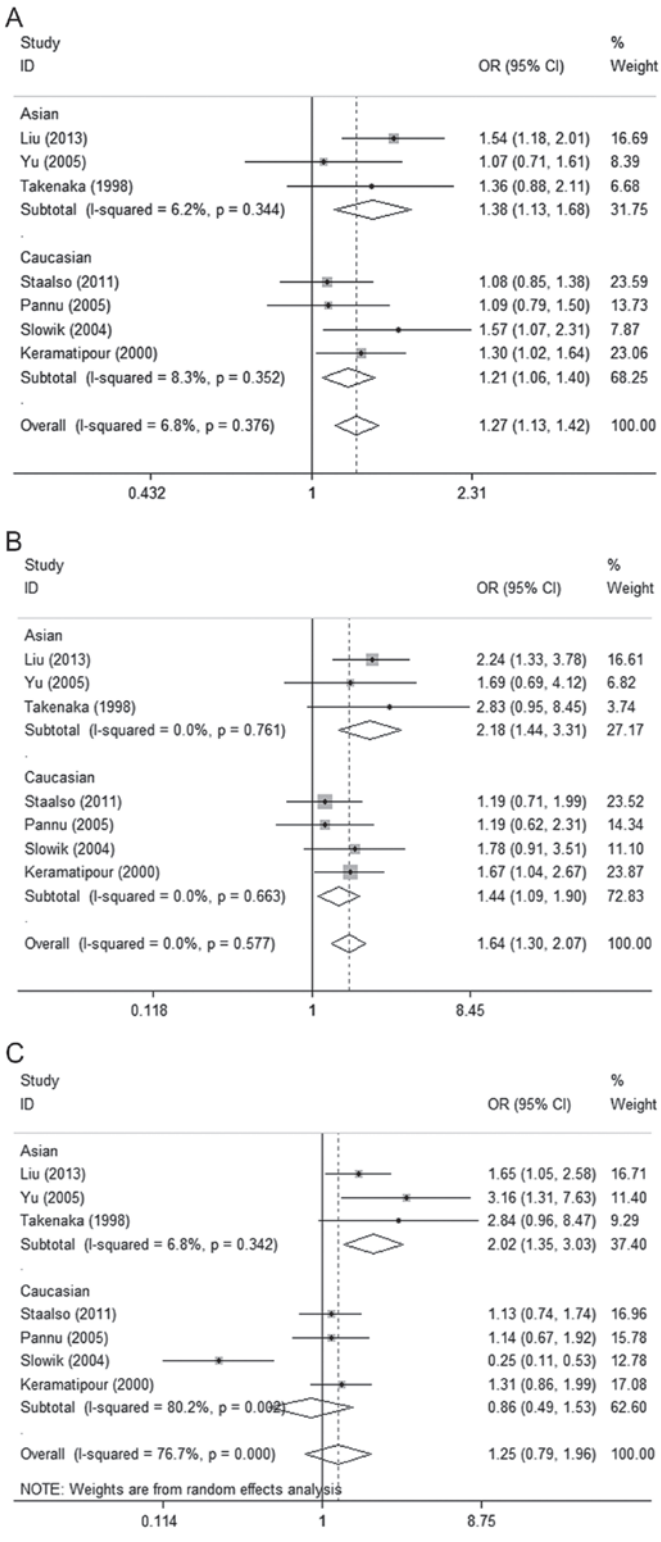

Figure 2. Forest plot of the association between the angiotensin-converting enzyme polymorphism and intracranial aneurysm susceptibility stratified by ethnicity. (A) Allele model (I vs. D); (B) homozygote comparison of codominant model (II vs. DD); (C) heterozygote comparison of codominant model (ID vs. DD).

(Table III and Fig. 3B). Furthermore, a significantly decreased risk was observed after excluding the study by Keramatipour *et al* (21) in the dominant model (OR=1.38, 95% CI=0.98-1.94; P_{OR}=0.067) (Table III and Fig. 3C). Thus,

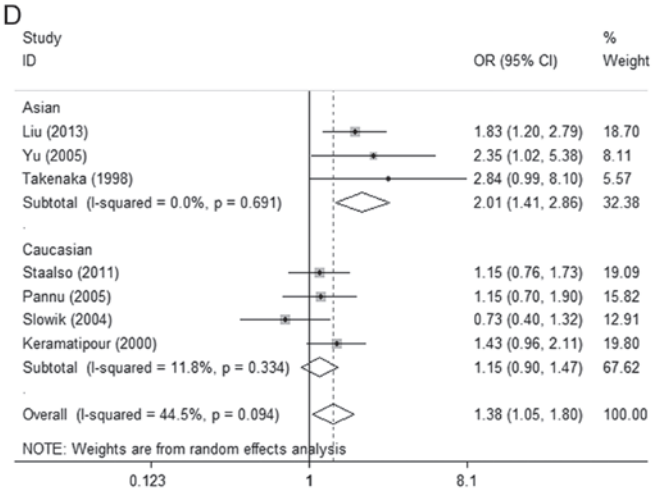

Figure 2. Continued. (D) Dominant model (II+ID vs. DD). CI, confidence interval; OR, odds ratio.

the overall association between the ACE polymorphism and IA risk was significantly influenced by these three studies.

Publication bias. Begg's funnel plot and Egger's test were performed to access the potential publication bias of the literature. The funnel plot shapes of these polymorphisms were symmetrical in each genetic model (Fig. 4 for the allele model). Subsequently, the Egger's test was used to provide statistical evidence of funnel plot symmetry. The results indicated that the current meta-analysis demonstrated a lack of publication bias in all of the genetic models (I vs. D: P=0.865; II vs. DD: P=0.542; ID vs. DD: P=0.945; II+ID vs. DD: P=0.520; II vs. ID+DD: P=0.956. data not shown). The calculation results are consistent with the shape of the Begg's funnel plot.

Discussion

IA is a growing problem and leads to devastating consequences, such as SAH, hemiplegia and epilepsy. Rupture of IA is the most common cause of SAH, which is associated with particularly high mortality and morbidity rates (3). Thus, it is important to identify the characteristics of individuals who are at high risk of IA and focus on regularly screening for IA. Previous studies indicate that genetics contribute to the development of IA (27-29). Furthermore, it is of great value to investigate the genetic architecture and establish the underlying molecular mechanism of IA. Until now, various polymorphisms in candidate genes have been considered as an important risk contributor to the development of IA.

Hypertension, which is a polygenic and multifactorial disorder, has been demonstrated to be a risk factor for IA (30). The RAAS plays a critical role in the development of hypertension and CVD (31). ACE is a key circulating enzyme in the RAAS, which catalyzes the conversion of angiotensin I to angiotensin II, and further degrades bradykinin (14). The D allele of the I/D polymorphism of a 287-bp Alu sequence within intron 16 of the *ACE* gene has been reported to be correlated with increased circulating ACE levels in humans (15,16). Furthermore, the ACE I/D polymorphisms

Table III. Sensitivity analysis of the angiotensin-converting enzyme polymorphism on IA risk.

					Codominant model																
	Allele I vs. D				Homozygote II vs. DD				Heterozygote ID vs. DD				Dominant model (II+ID) vs. DD				Recessive model II vs. (ID+DD)				
Author, year	I^2 (%)	P_h	ORs (95%CIs)	P_{OR}	I^2 (%)	P_h	ORs (95%CIs)	P_{OR}	I^2 (%)	P_h	ORs (95%CIs)	P_{OR}	I^2 (%)	P_h	ORs (95%CIs)	P_{OR}	I^2 (%)	P_h	ORs (95%CIs)	P_{OR}	(Refs.)
Liu, 2013	0	0.561	1.21 (1.07, 1.37)	0.003	0	0.697	1.52 (1.18, 1.97)	0.001	79	0.000	1.18 (0.69, 2.04)	0.542	40.5	0.135	1.27 (1.03, 1.57)	0.028	70.2	0.005	1.31 (0.90, 1.91)	0.163	(17)
Staalsø, 2011	0	0.486	1.32 (1.16, 1.50)	0.000	0	0.713	1.78 (1.37, 2.31)	0.000	80.4	0.000	1.28 (0.72, 2.26)	0.398	49.9	0.076	1.44 (1.04, 2.00)	0.028	69.7	0.006	1.41 (0.97, 2.06)	0.075	(18)
Pannu, 2005	9.2	0.357	1.29 (1.14, 1.46)	0.000	0	0.587	1.72 (1.34, 2.20)	0.000	80.5	0.000	1.27 (0.74, 2.20)	0.387	51.5	0.067	1.43 (1.04, 1.97)	0.028	70.8	0.004	1.40 (0.97, 2.02)	0.072	(19)
Slowik, 2004	2.1	0.403	1.24 (1.10, 1.40)	0.000	0	0.457	1.62 (1.27, 2.08)	0.000	29.2	0.216	1.42 (1.15, 1.76)	0.001	18.2	0.296	1.47 (1.20, 1.80)	0.000	13.8	0.326	1.21 (1.00, 1.47)	0.046	(20)
Keramatipour, 2000	21.7	0.271	1.26 (1.10, 1.43)	0.001	0	0.449	1.63 (1.25, 2.13)	0.000	80.5	0.000	1.24 (0.70, 2.20)	0.459	53.5	0.057	1.38 (0.98, 1.94)	0.067	71.7	0.003	1.35 (0.90. 2.01)	0.142	(21)
Takenaka, 1998	20.8	0.277	1.26 (1.12, 1.42)	0.000	0	0.588	1.59 (1.26, 2.02)	0.000	78.7	0.000	1.14 (0.71, 1.84)	0.576	43.6	0.114	1.33 (1.10, 1.62)	0.004	71.4	0.004	1.38 (0.95, 1.99)	0.086	(22)
Yu, 2005	12.6	0.334	1.28 (1.14, 1.45)	0.000	0	0.449	1.64 (1.29, 2.08)	0.000	76.4	0.001	1.11 (0.70, 1.75)	0.669	44.9	0.106	1.33 (1.09, 1.61)	0.005	60.5	0.027	1.48 (1.08, 2.03)	0.014	(23)

P_h, P-value for heterogeneity; P_{OR}, P-value for OR. P_{OR}<0.05 was considered to indicate a statistically significant difference. OR, odds ratio; CI, confidence interval.

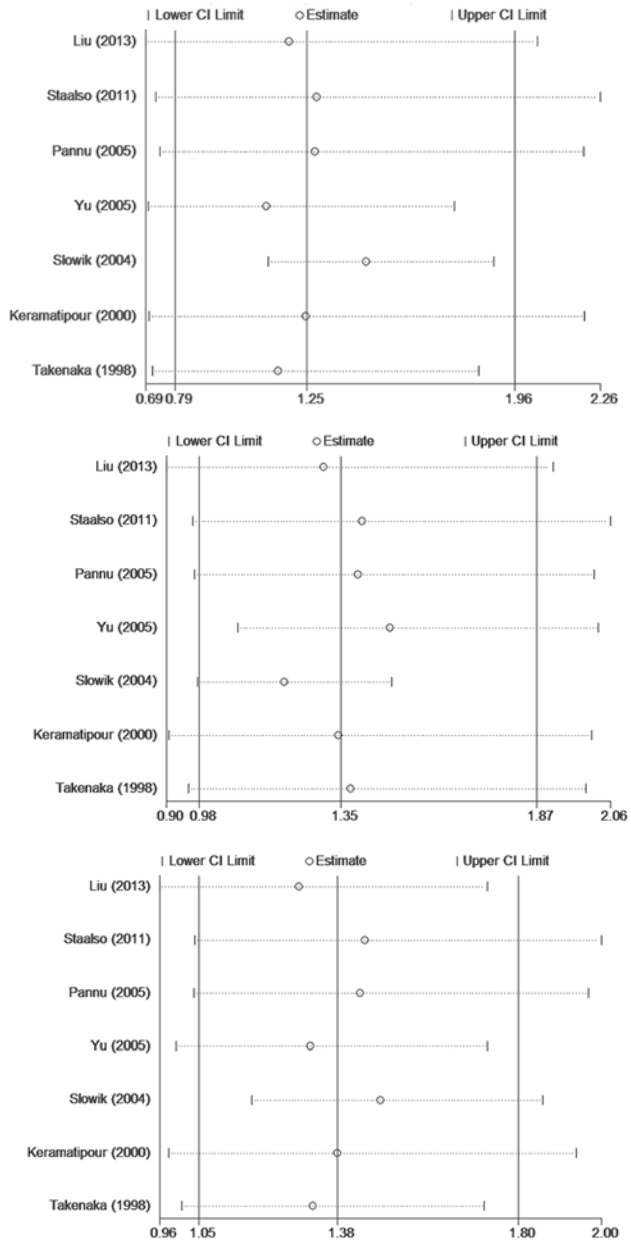

Figure 3. Sensitivity analysis of the angiotensin-converting enzyme polymorphism. Pooled odds ratio and 95% CI of the six remaining studies, subsequent to exclusion of each individual study. (A) Heterozygote comparison of codominant model (ID vs. DD); (B) Recessive model (II vs. ID+DD); (C) Dominant model (II+ID vs. DD). CI, confidence interval.

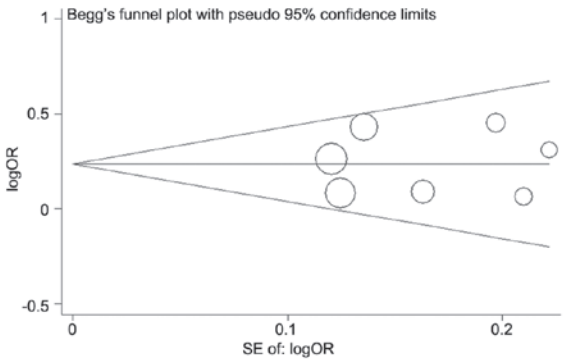

Figure 4. Begg's funnel plot to assess the publication bias of the angiotensin-converting enzyme polymorphism under the allele model (I vs. D). Each point corresponds to a separate study. OR, odds ratio.

have been extensively evaluated in numerous types of vascular disease (32). Therefore, ACE may present as a candidate gene for IA development.

A meta-analysis of two case-control studies by Keramatipour *et al* (21) showed that the I allele of the *ACE* gene maybe a risk factor for IA. Subsequently, various studies with conflicting opinions have been reported (18,19,23). A previous meta-analysis failed to detect an association between the ACE I/D polymorphism and IA susceptibility in the dominant (OR=1.23, 95% CI=0.82-1.85; P_{OR}=0.31) or the recessive (OR=1.58, 95% CI=0.98-2.57; P_{OR}=0.06) models (11). By contrast, another meta-analysis demonstrated a close association between the ACE I/D polymorphism and IA risk. In the current study, a comprehensive meta-analysis was conducted with a markedly larger sample size to derive a more precise estimation of the association.

By combining data from seven case-control studies, including 1,074 IA patients and 1,500 control subjects, the present meta-analysis evaluated the association between the I/D polymorphisms of the *ACE* gene and IA susceptibility. The pooled result showed a significant association between the I allele of the *ACE* gene and IA risk (OR=1.27, 95% CI=1.13-1.42; P_{OR}=0.000). The II genotype was associated with an increased risk for IA when compared with the DD genotype (OR=1.64, 95% CI=1.30-2.07; P_{OR}=0.000). The ACEII + ID genotype was correlated with a significantly increased risk for IA (OR=1.38, 95% CI=1.05-1.80; P_{OR}=0.021).

In the subgroup analysis by ethnicity, an increased risk between ACE and IA was identified among Asian individuals (ID vs. DD: OR=2.02, 95%CI=1.35-3.03, P_{OR}=0.001; II+ID vs. DD: OR=2.01, 95%CI=1.41-2.86, P_{OR}=0.000), although not in Caucasian individuals (ID vs. DD: OR=0.86, 95%CI=0.49-1.53, P_{OR}=0.615; II+ID vs. DD: OR=1.15, 95%CI=0.90-1.47, P_{OR}=0.264). A number of factors may also be involved in the underlying mechanism for this difference between ethnic groups. In addition to the genetic backgrounds and the living environments, other factors, such as selection bias and different matching criteria may account for the different genetic effects. Thus, additional studies using different populations are warranted to further validate ethnic differences on the impact of the ACE polymorphism on IA risk.

Subgroup analysis by source of controls revealed a significantly increased risk among studies using hospital-based controls, but not population-based controls in the heterozygote comparison of codominant model and the dominant model, suggesting that the controls in the hospital-based studies may be sufficient to represent the general population. Thus, the use of proper and representative cancer-free control subjects is important in reducing bias in such genotype association studies.

Subgroup analysis by sample size revealed a significantly increased risk among studies using large samples in each genetic model, although no significance was found in the small sample subgroup in the heterozygote comparison of codominant, dominant and recessive models, suggesting that studies with large samples are required.

The statistical significance of genotype distributions was also detected in male and female groups (males: OR=3.56,

95% CI=1.43-8.86; P_{OR}=0.0006; females: OR=3.86, 95% CI=1.75-8.51; P_{OR}=0.0005) in the study by Slowik *et al* (20). In addition, Liu *et al* (17) reported that no statistically significant differences were identified between genotypes in patients with IA, when stratified by the site, shape, size and Fisher Grade of aneurysms (17). In future studies, greater focus on clinical characteristics, such as gender, alcohol consumption, smoking, family history, and site, shape, size and Fisher Grade of IA should be taken into consideration to provide a more powerful analytical framework.

There were certain limitations of the current meta-analysis. First, the number of eligible studies and subjects of studies were relatively small, particularly for the subgroup analyses, which may result in insufficient power to detect a slight, although real effect of the ACE polymorphisms on IA risk. In addition, the study by Yu *et al* (23) whose genotype distribution in the control group was not consistent with HWE may contribute to the bias of the meta-analysis, as the results were affected after excluding this study in the sensitivity analysis. Finally, the results were based on single-factor estimates without adjustments for other risk factors.

In conclusion, this meta-analysis identified that the ACE polymorphism is associated with an increased risk of IA. However, large, well-designed multicenter studies are required to verify the present findings. In addition, further evaluation of the ACE polymorphism on IA risk should focus on the effect of gene-to-gene and gene-to-environment interactions.

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