Association of Angiotensin-Converting Enzyme Insertion/Deletion Polymorphism with the Risk of Atherosclerosis

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Aims: The objective of this study was to perform a meta-analysis to evaluate the association between angiotensin-converting enzyme (*ACE*) gene insertion/deletion (I/D) polymorphism and susceptibility to atherosclerosis (AS). *Methods:* MEDLINE, EMBASE, and the ISI Web of Science were searched for all eligible published studies concerning the relationship of *ACE* gene polymorphism with AS without language restrictions. Pooled odds ratios (ORs) with 95% confidence intervals (CIs) were calculated to evaluate this relationship under different genetic models using meta-analytic methods. *Results:* A total of 15 articles (16 studies) were involved in this meta-analysis. The D allele of the *ACE* gene had a nonsignificant increase in the risk of AS (D versus I: OR = 1.23, 95% CI, .98-1.53, $P = .07$; $I^2 = 87.2\%$, $P_{\text{heterogeneity}} < .01$). Compared with the II genotype, the DI (relative risk [RR]: 1.35, 95% CI: 1.09, 1.67, $P < .01$; $I^2 = 47.8\%$, $P_{\text{heterogeneity}} = .017$) and (DD + DI) (RR = 1.38, 95% CI: 1.04, 1.82, $P = .02$; $I^2 = 73.3\%$, $P_{\text{heterogeneity}} < .01$) genotype of *ACE* was associated with higher risk of AS, respectively. Subjects with the DD genotype showed a statistically nonsignificant trend toward greater risk of AS (RR = 1.53, 95% CI: .97, 2.43, $P = .07$; $I^2 = 88.6\%$, $P_{\text{heterogeneity}} < .01$). Further subgroup analyses showed that significant relationships were only found in Europeans under different gene polymorphism or different genotype models rather than Asians. *Conclusions:* The present meta-analysis indicated that the D allele in the *ACE* gene was associated with the risk of AS, especially in Europeans. Furthermore, increased copy number of D allele was significantly associated with increased AS risk in a dose-dependent manner.

Key Words: *ACE*—insertion/deletion polymorphism—atherosclerosis—meta-analysis

Introduction

Atherosclerosis (AS) is one of the most common leading causes of morbidity and mortality among cardiovascular disease around the world.[1] AS is deemed as a series of vascular alterations ranging from endothelial dysfunction to overt carotid, coronary, cerebral, or peripheral arterial disease, eventually leading to myocardial ischemia and infarction.[2] As a complex multifactorial disease, the pathophysiological process of AS involves a large number of biological pathways such as lipid metabolism, endothelial

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dysfunction, and inflammation.[3,4] Although the precise etiology of AS remains unclear, this complex disease has been generally recognized to correlate significantly with genetic factors.[5,6] Genome-wide association studies have found that single nucleotide polymorphisms in several genes, such as renin-angiotensin-aldosterone system (RAAS) and apolipoprotein (apo)E-CI-CII cluster gene, are closely implicated to be associated with AS risk or the marker of AS.[7,8]

Belonging to the crucial component of the *RAAS*, angiotensin I-converting enzyme *(ACE)* plays a central role in the production of aldosterone-stimulating peptide angiotensin II (Ang II) in endothelial cells of normal vessels.[9,10] Circulating ACE levels are demonstrated to correlate with polymorphism of the *ACE* gene.[11] As a most common functional polymorphism of this gene, the insertion/deletion (I/D) polymorphism would influence the level of *ACE*, subsequently resulting in the alteration of the Ang II plasma level.[12] Overproduction of Ang II due to the mutation of D allele may lead to the remodeling of the vascular tissue and augmentation of the atherosclerotic process.[13] Recently, several studies have shown the close linkage between *ACE* I/D polymorphism and AS; however, the results are inconsistent and inconclusive.[14-16] Furthermore, most of those studies have also revealed that the copy number of the D allele varied among different populations; but to date, whether such ethnic or racial discrepancies alter this association between allelic *ACE* gene and AS is still unclear.[16,17]

Therefore, we conducted a meta-analysis of the currently available studies to evaluate the association between *ACE* I/D polymorphism and AS risk. Moreover, subgroup analyses were performed to explore associations with discrepancies in different populations.

Methods

Search Strategy

We performed a systematic search of the MEDLINE, EMBASE, and ISI Web of Science databases up to March 2018 using the following keywords: ("angiotensin converting enzyme" or "*ACE*" or "peptidyl-dipeptidase A" or "genetic polymorphism" or genetic variation") and ("atherosis" or atherosclerosis" or "arteriosclerosis" or "atherosclerotic plague" or "atheroma" or "intima-media thickness" or "AS" or "atherogenesis"or "atherosclerotic plague" or "artery disease" or "arterial lipoidosis"). Language restrictions were not imposed. We also additionally hand searched the references of the retrieved articles that met our prespecified inclusion and exclusion criteria for any additional potentially eligible studies. We also contacted the author and experts of ongoing research in the field to obtain additional information.

Study Selection

We firstly examined any potentially relevant studies by scanning the identified title and abstract, and then assessed their eligibility by full-text review. Articles that fulfilled all of the following specific criteria were included: (1) the study design was cohort, case-control, or cross-sectional studies; (2) the study objective was to evaluate the relationship between *ACE* polymorphism and AS; (3) the primary outcome was clearly defined as AS, including coronary AS, carotid AS cerebral AS, peripheral AS or renal AS; and (4) relative risk (RR) or odds ratio (OR) estimates and their 95% confidence intervals (CIs) were provided or could be calculated. When there were multiple publications reported on the same or overlapping data, the most updated publication or the publication with the largest sample size was selected for the meta-analysis. Two reviewers performed a literature search and screening independently in a standardized manner, and all discrepancies were resolved by consensus.

Data Extraction and Quality Assessment

We extracted the standard information from eligible full-text articles, and the available data were as follows: the first author name, year of publication, study design, country, number of subjects, average age, sex distribution, diagnostic method, study end point, D allele frequency, and *P* values for Hardy-Weinberg equilibrium (HWE) in controls for all enrolled studies. If an original study provided several risk estimates, the maximal adjustment was extracted for the meta-analysis. For studies that involved subcategories of AS disease status, gradings were merged into a single AS group.

The study quality of the included cohort studies and case-control studies were assessed according to the Newcastle-Ottawa Quality Assessment Scale.[18] Criteria for the assessment of study quality were based on 3 broad aspects: selection of study groups (4 points: case definition, representativeness of the cases, selection of controls, and definition of controls), comparability of study groups (2 points: comparability of cases and controls on the basis of the design or analysis [1 point: whether the study controlled for the most important factor; 1 point: whether the study controlled for any additional factor]), and appraisement of the outcome (3 points: ascertainment of exposure, same method of ascertainment for cases and controls, and comparison of nonresponse rate between cases and controls).[18] A maximum of one star can be given for each numbered criterion within the selection and outcome categories, and a maximum of 2 stars can be given for comparability. According to this effective scale with a star system ranging from 0 (worst) to a maximum of 9 (best), each included study in our meta-analysis that met 5 or more of the recognized Newcastle-Ottawa Quality Assessment Scale criteria were categorized as high quality, whereas scores 4 or lower were assigned low quality. Additionally, eligible cross-sectional studies were assessed for their quality with the use of the Agency for Healthcare Research and Quality (USA) standards.[19] Two

investigators conducted eligibility assessment and data extraction independently. If disagreements or uncertainties existed, consensus was achieved by a third author.

Statistical Analysis and Risk of Bias Assessment

The HWE of the distribution of genotypes in the controls for each study was initially tested via the chi-square test (cutoff point: $P < .05$). Individual study ORs with corresponding 95% CIs were extracted or calculated from each raw article before data pooling. Heterogeneity across studies was evaluated by the Cochran Q and I^2 test statistical tests, a P value of less than .05 was considered significant. If heterogeneity was observed between studies, the DerSimonian and Laird method for random-effects model was used to calculate the pooled OR and 95% CI. Otherwise, the Mantel-Haenszel method for fixed-effects model was adopted for the meta-analysis.[20,21] The following 4 pooled ORs and their 95% CIs were calculated in each study: OR for DI versus II, OR for (DD + DI) versus II, OR for DD versus II, and OR for D versus I. To explore the potential sources of between-study heterogeneity, stratified analyses were applied to assess whether there were some possible differences in age, race/ethnicity, study design, the position of AS and HWE, or the sample size. Additionally, we conducted sensitivity analyses to estimate the stability of the pooled results by systematically removing one study, and then re-evaluated the important significance of analysis results for the remaining data sets. Potential publication bias was determined with the Egger's regression test and represented graphically with Begg's funnel plots of the natural log of the OR.[22,23] All statistical analyses were performed by STATA software, version 12.0 (StataCorp, College Station, TX).

Results

Study Selection and Characteristics

In total, we preliminarily identified 1444 citations from the initial database search based on our criteria, and 1307 remained after removal of duplicate references. Through screening of titles and abstracts, 98 articles were estimated as potentially relevant. Finally, 15 articles including 16 studies with a total of 2808 AS cases and 3316 controls were included in the present meta-analysis (Fig 1).[14-17,24-34]

The baseline characteristics of all qualified studies are presented in Table 1. Of the eligible studies, 11 studies were conducted in Europe,[14,15,24-26,28-30,32-34] and 5 in Asia.[16,17,27,31] Among these studies, there were 1 cohort study,[14] 1 cross-sectional study,[28] and 14 case-control studies.[15-17,24-27,29-34] The mean age of subjects varied widely from 44.6 to 70.5 years in our included studies. The study subjects in the control group consisted of men and women in all studies. In the case group, 14 of 16 studies[14-17,24-29,32-34] included both men and women, and the other 2 studies[30,31] consisted of only men. The frequencies

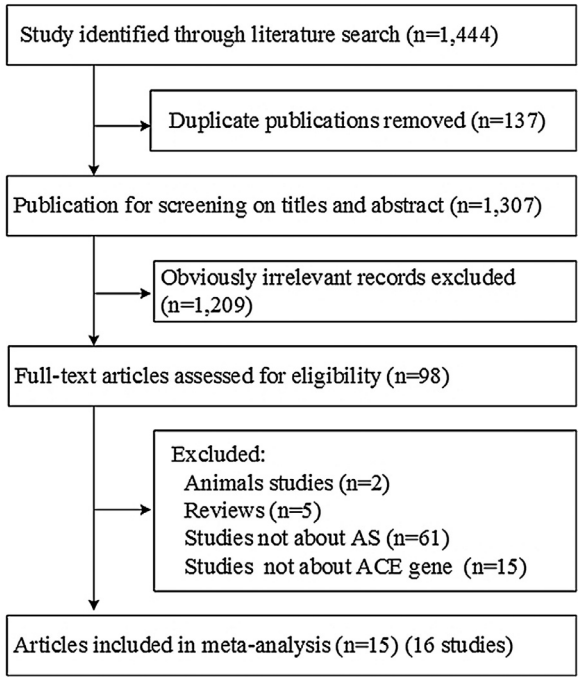

Figure 1. *Flow diagram of literature search and study selection.*

of the D-allele ranged from .49 to .72 for Europeans, and from .31 to .48 for Asians in patients. Moreover, those studies demonstrated no deviation from the HWE except for 2 studies.[16] The polymorphism of the *ACE* gene was detected by polymerase chain reaction technology in all eligible studies. Furthermore, the methodologic quality of the included studies was rated high (see Tables S1 and S2, Supplementary Material).

Allele of ACE *Gene and AS*

Among the included studies, most of the studies showed an increased AS risk with this gene, and 7 established a significant association.[14,15,25,26,29,33,34] Significant heterogeneity was detected across the data sets ($I^2 = 87.2\%$, $P_{\text{heterogeneity}} < .01$) under the allelic (D versus I) model, and the pooled result from the random-effects model showed that the allele (D) of the *ACE* gene had a 23% increase in the risk of AS, although this association did not achieve statistical significance (D versus I: OR = 1.23, 95% CI, .98-1.53, $P = .07$; Fig 2). When subgroup analysis was carried out for ethnicity, it was demonstrated that the *ACE* gene had a stronger and more significant effect for this disease in European populations in the allele D versus I (OR = 1.42, 95% CI, 1.15-1.76, $P < .01$), whereas no significant relationship was found in Asian populations (OR = .88, 95% CI, .59-1.30, $P = .51$). In parallel, the frequencies of allele of the *ACE* gene among normal subjects in Europeans were noted to be much greater than those in Asians (53 % versus 41%). Stratified analyses using other participant and study characteristics did not substantially alter the shape of association

Table 1. *Characteristics of studies included in this meta-analysis of ACE gene polymorphism and AS*

Author	Country	Study design	Participants (% Male)	Mean age (y)	No. of total AS cases	Endpoint	Atherosclerosis diagnosis	*P* value for HWE	DAF	Study Quality
Arbustini et al, 1995[14]	Italian	Cohort	388 (72.4)	60.7	255	Coronary AS	Angiography	.93	.71	High*
Dessi-Fulgheri et al, 1995[24]	Italian	Case-control	184 (56.0)	52.4	137	Carotid AS	B-Mode Ultrasonography	.47	.65	High*
Missouris et al, 1996[25]	UK	Case-control	130 (58.9)	65.0	56	Renal AS	Angiography	.35	.63	High*
Sertic et al, 1996[26]	Croatia	Case-control	75 (53.3)	57.4	50	Cerebral AS	Angiography	.69	.72	High*
Watanabe et al, 1997[27]	Japan	Case-control	169 (50.8)	59.2	43	Carotid AS	B-Mode Ultrasonography	.10	.48	High*
Aalto-Setala et al, 1998[28]	Finland	Cross-sectional	234 (42.3)	≤2.	148	Cerebral AS	Angiography	.74	.57	High†
Olivieri et al, 1999[29]	Italian	Case-control	160 (76.3)	64.8	58	Renal AS	Angiography	.62	.70	High*
Spiridonova et al, 2001[30]	Russia	Case-control	216 (NR)	44.6	94	Coronary AS	Angiography	.97	.49	High*
Jeong et al, 2004[31]	Korea	Case-control	372 (80.6)	62.4	92	Peripheral AS	Angiography	.96	.38	High*
van et al, 2004[32]	Holland	Case-control	370 (56.5)	53.0	109	Renal AS	Angiography	.72	.53	High*
Szperl et al, 2008[33]	Poland	Case-control	151 (74.2)	60.3	40	Renal AS	Angiography	.31	.58	High*
Sticchi et al, 2011[34]	Italian	Case-control	1,668 (67.0)	70.5	821	Carotid AS	Angiography	.60	.63	High*
Yun et al, 2011[17]	Chinese	Case-control	536 (42.9)	61.9	260	Carotid AS	Angiography	>.05	NR	High*
Kolaković et al, 2012[15]	Serbia	Case-control	996 (54.8)	56.6	504	Carotid AS	Angiography	.36	.59	High*
Chutinet et al, 2012[16]	Thailand	Case-control	225 (63.1)	67.6	58	Cerebral AS	TCD/MRA	.01	.31	High*
Chutinet et al, 2012[16]	Thailand	Case-control	250 (60.0)	65.7	83	Cerebral AS	TCD/MRA	.01	.44	High*

Abbreviation: Carotid AS, carotid atherosclerosis; Cerebral AS, cerebral atherosclerosis; Coronary AS, coronary atherosclerosis; DAF, D allele frequency; HWE, Hardy-Weinberg equilibrium; MRA, magnetic resonance angiography; NR, not reported; Peripheral AS, peripheral atherosclerosis; Renal AS, renal atherosclerosis; TCD, transcranial Doppler ultrasonography.

*Study quality was judged based on Newcastle-Ottawa Scale.

†Study quality was judged based on Agency for Healthcare Research and Quality Assessment Scale.

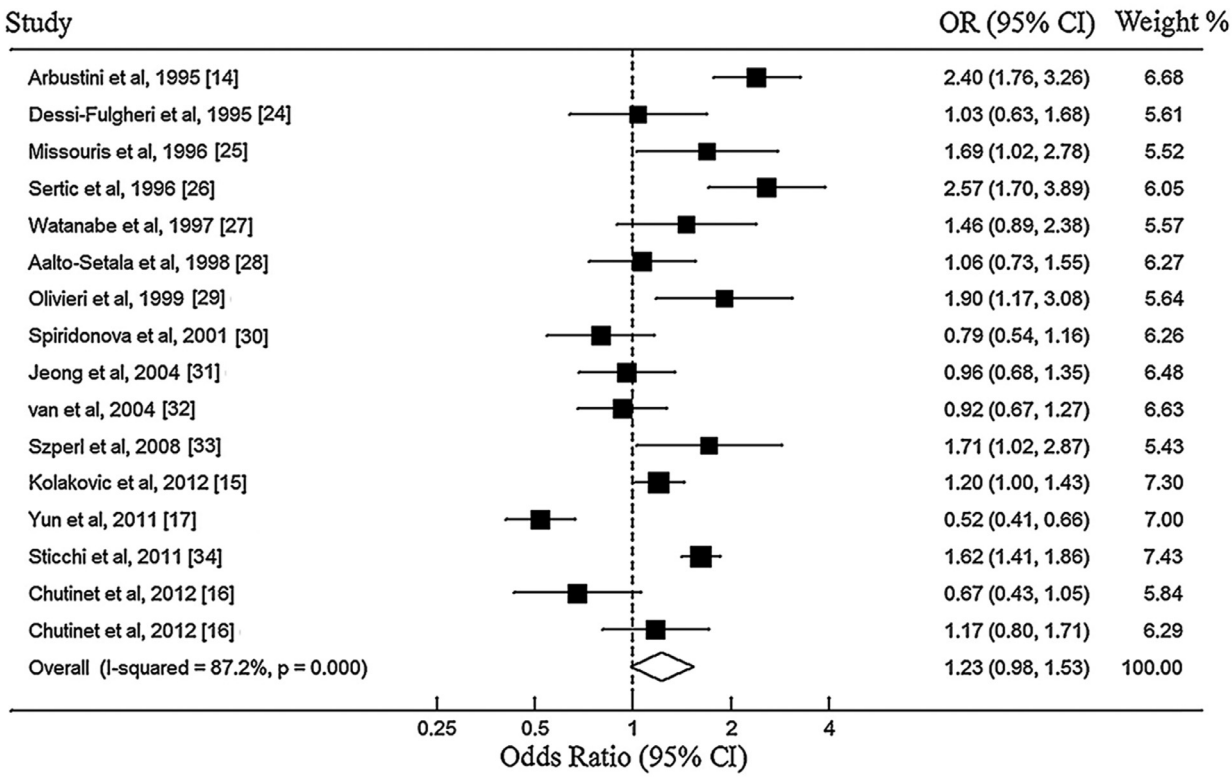

Figure 2. *Forest plot on the associations between* ACE *gene and AS risk under the allelic model (D versus I). For each study, the estimation of OR and its 95% CI are plotted with a box and a horizontal line. The pooled odds ratio is represented by a diamond. The area of the black squares reflects the weight of the study in the meta-analysis. ACE, angiotensin-converting enzyme; AS, atherosclerosis; CI, confidence interval; OR, odds ratio.*

between allele of the *ACE* gene and AS risk (Table 2). Furthermore, we performed stratified analyses across these characteristics for European studies. The results showed that these factors did not significantly alter the shape of the association between *ACE* I/D and AS risk (see Table S3, Supplementary Material). In addition, we conducted a sensitivity analysis by excluding each individual study one at a time and the pooled ORs did not yield a marked difference, indicating that the results were not influenced by any single study. Neither the Egger's test ($P = .94$) nor the Begg's test ($P = .44$) was suggestive of publication bias for the allelic (D versus I) genetic model. The funnel in all models also revealed no obvious publication bias, and Figure 3 showed the funnel plot of the (D versus I) model.

Genotype of ACE *Gene and AS*

Due to the significant heterogeneity between studies (all $P < .05$), in all comparisons of DI versus II, (DD + DI) versus II, and DD versus II, the random effects model was applied to calculate the pooled OR. The summary estimates showed that the risk of AS was significantly higher in subjects with the DI genotype than in those with the II genotype with an OR of 1.35 (95% CI: 1.09, 1.67; $I^2 = 47.8\%$, $P_{\text{heterogeneity}} = .02$; Fig 4). Similarly, compared with the II genotype, the DD + DI genotype of *ACE* was associated with a 1.38-fold increase in the odds of AS (RR = 1.38, 95% CI: 1.04, 1.82, $P = .02$; $I^2 = 73.3\%$, $P_{\text{heterogeneity}} < .01$; Fig 5). No significant difference was observed between subjects with the DD genotype and those with the II genotype (RR = 1.53, 95% CI: .97, 2.43, $P = .07$; $I^2 = 88.6\%$, $P_{\text{heterogeneity}} < .01$; Fig 6). To explore sources of heterogeneity in the association of different genotype and AS, several stratified analyses were done based on study and subject characteristics of the included studies. In the prespecified subgroup analyses, the pooled estimate of the associations varied significantly across the ethnicity strata (Table 2). Compared with the nonsignificant relationship in Asians (DI versus II: OR = 1.30, 95% CI, .92-1.85, $P = .14$; [DD + DI] versus II: OR = .99, 95% CI, .72-1.36, $P = .95$; and DD versus II: OR = .74, 95% CI, .37-1.49, $P = .41$; Table 2), the random-effects pooled OR in Europeans revealed that significant relationships were observed under a heterozygous model [DI versus II: OR = 1.38, 95% CI, 1.03-1.84, $P = .03$], the dominant model ([DD + DI] versus II: OR = 1.61, 95% CI, 1.15-2.27, $P < .01$), and the homozygous model (DD versus II: OR = 2.08, 95% CI, 1.33-3.26, $P < .01$). The result of the stratified analysis by study design, age, the position of AS and HWE, and sample size showed that these variables did not substantially alter the association between different genetic models and AS risk (Table 2). Moreover, in sensitivity

Table 2. *Stratified analysis of the association between the* ACE *gene polymorphism and AS*

Subgroup		Allele model				Genotype model											
		D versus I				DD versus II				DD + DI versus II				DI versus II			
	N	OR(95% CI)	I^2 (%)	P_z	P_h	OR(95% CI)	I^2 (%)	P_z	P_h	OR(95% CI)	I^2 (%)	P_z	P_h	OR(95% CI)	I^2 (%)	P_z	P_h
Ethnicity																	
European	11	1.42 (1.15-1.76)	79.20	<.01	.04	2.08 (1.33-3.26)	78.70	<.01	.04	1.61 (1.15-2.27)	70.30	<.01	.17	1.38 (1.03-1.84)	52.90	.02	.95
Asian	5	.88 (.59-1.30)	82.60	<.01		.74 (.37-1.49)	77.90	<.01		.99 (.72-1.36)	45.30	.12		1.30 (.92-1.85)	45.60	.12	
Study design																	
Cohort	1	2.40 (1.76-3.26)	-	<.01	.68	5.78 (2.97-11.27)	-	.69	.69	3.53 (1.94-6.42)	-	0	.57	2.39 (1.27-4.48)	-	<.01	.75
Cross-sectional	1	1.06 (.73-1.55)	-	.18		1.18 (.55-2.54)	-	.67		1.24 (.63-2.43)	-	.08		1.30 (1.03-1.63)	-	.49	
Case-control	14	1.18 (.93-1.49)	86.80	.75		1.41 (.87-2.28)	86.30	.67		1.28 (.97-1.70)	71.50	.52		1.29 (1.02-1.63)	49.20	.02	
Age																	
>60 years	9	1.26 (.87-1.81)	91.50	<.01	.85	1.57 (.76-3.25)	92.20	<.01	.94	1.38 (.94-2.03)	78.90	<.01	.99	1.39 (1.09-1.78)	36.10	.13	.69
≤60 years	7	1.19 (.92-1.53)	71.80	<.01		1.40 (.82-2.40)	69.50	<.01		1.38 (.89-2.14)	66.10	<.01		1.37 (.90-2.08)	59.10	.02	
Endpoint																	
Carotid	5	1.08 (.70-1.67)	93.70	<.01	.90	1.20 (.50-2.87)	93.50	<.01	.95	1.36 (.87-2.14)	81.80	<.01	.70	1.54 (1.20-1.97)	32.40	.02	.14
Coronary	2	1.39 (.47-4.10)	94.90	<.01		1.93 (.22-16.91)	94.60	<.01		1.60 (.33-7.64)	91.20	<.01		1.36 (.45-4.15)	82.60	.21	
Peripheral	1	.96 (.68-1.35)	NA	NA		.85 (.40-1.80)	NA	NA		1.01 (.62-1.64)	NA	NA		1.06 (.63-1.77)	NA	NA	
Renal	4	1.45 (.99-2.13)	66.40	.03		2.04 (.97-4.33)	61.80	.05		1.35 (.86-2.11)	20.70	.29		1.11 (.74-1.67)	0	.47	
Cerebral	4	1.21 (.72-2.04)	84.80	<.01		1.70 (.53-5.47)	84.60	<.01		1.54 (.69-3.44)	79.80	<.01		1.48 (.71-3.10)	72.30	.01	
HWE (P)																	
≥.05	14	1.28 (1.01-1.83)	88.10	<.01	. 31	1.69 (1.03-2.80)	87.7	<.01	.31	1.48 (1.09-2.01)	74.80	<.01	.30	.86 (.61-1.22)	74.10	<.01	.31
<.05	2	.90 (.52-1.55)	71.00	.06		.79 (.25-2.46)	73.2	.05		.92 (.61-1.37)	0	.34		.41 (.26-.67)	0	.70	
Sample																	
≥250	7	1.14 (.81-1.61)	93.00	<.01	.58	1.32 (.67-2.63)	92.7	<.01	.57	1.31 (.91-1.89)	82.10	<.01	.73	1.37 (1.09-1.73)	46.40	.08	.08
<250	9	1.31 (.97-1.76)	74.70	<.01		1.77 (.92-3.39)	73.7	.39		1.51 (.94-2.42)	65.10	<.01		1.38 (.89-2.14)	53.80	.03	

Abbreviation: CI, confidence interval; HWE, Hardy-Weinberg equilibrium; NA, not applicable; OR, odds ratio; P_h, *P* for between-study heterogeneity; P_z, *P* for Z test.

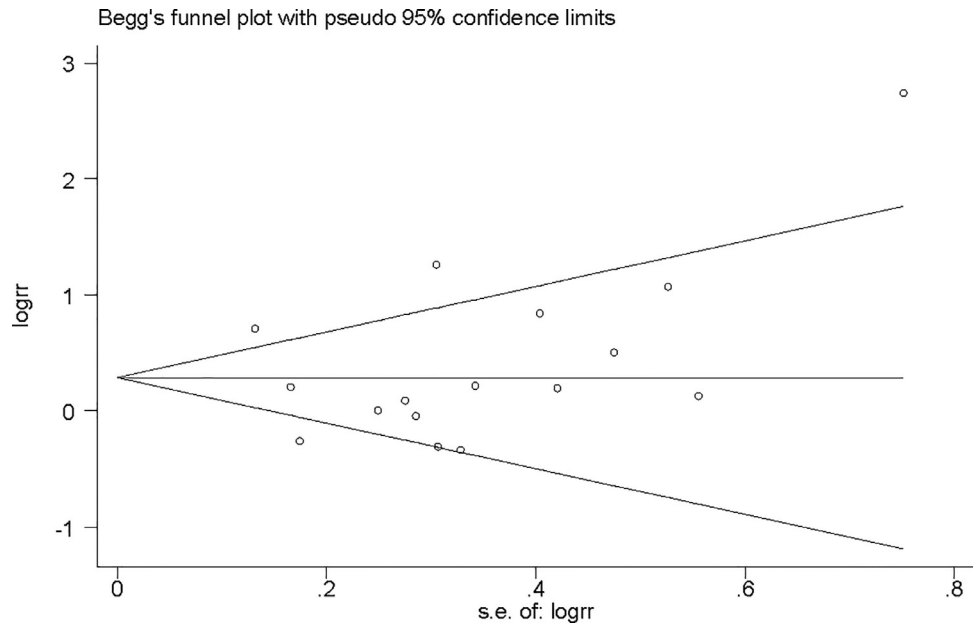

Figure 3. *The funnel plot on the associations between* ACE *gene and AS risk under the allelic model (D versus I). The funnel reveals no obvious publication bias. ACE, angiotensin-converting enzyme; AS, atherosclerosis.*

analyses excluding one study at a time, we showed that no individual study had a particular effect on the overall results under all the genotype models. Neither Begg's funnel plot nor Egger's regression test revealed obvious evidence for the presence of acknowledged publication bias in the qualified studies (both $P > .10$; Figs. 7-9).

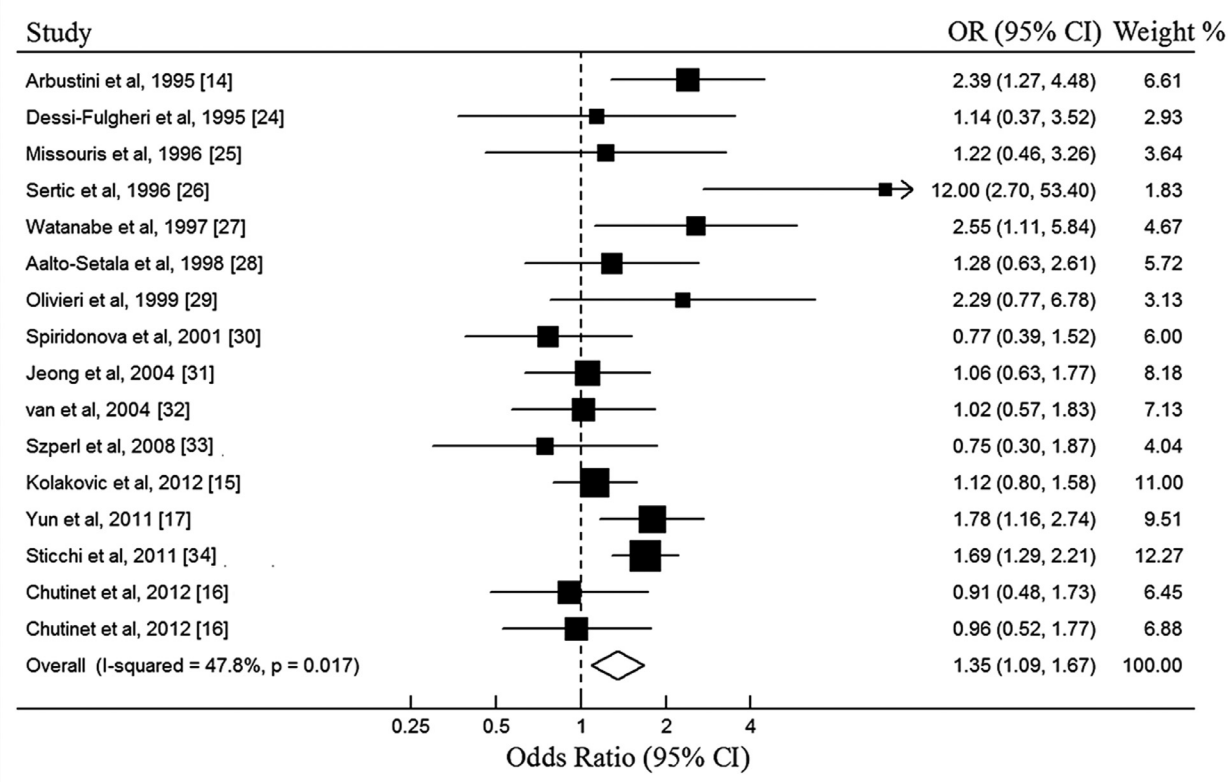

Figure 4. *Forest plot on the associations between* ACE *gene and AS risk under heterozygous genetic model (DI versus II). For each study, the estimation of OR and its 95% CI are plotted with a box and a horizontal line. The pooled odds ratio is represented by a diamond. The area of the black squares reflects the weight of the study in the meta-analysis. ACE, angiotensin-converting enzyme; AS, atherosclerosis; CI, confidence interval; OR, odds ratio.*

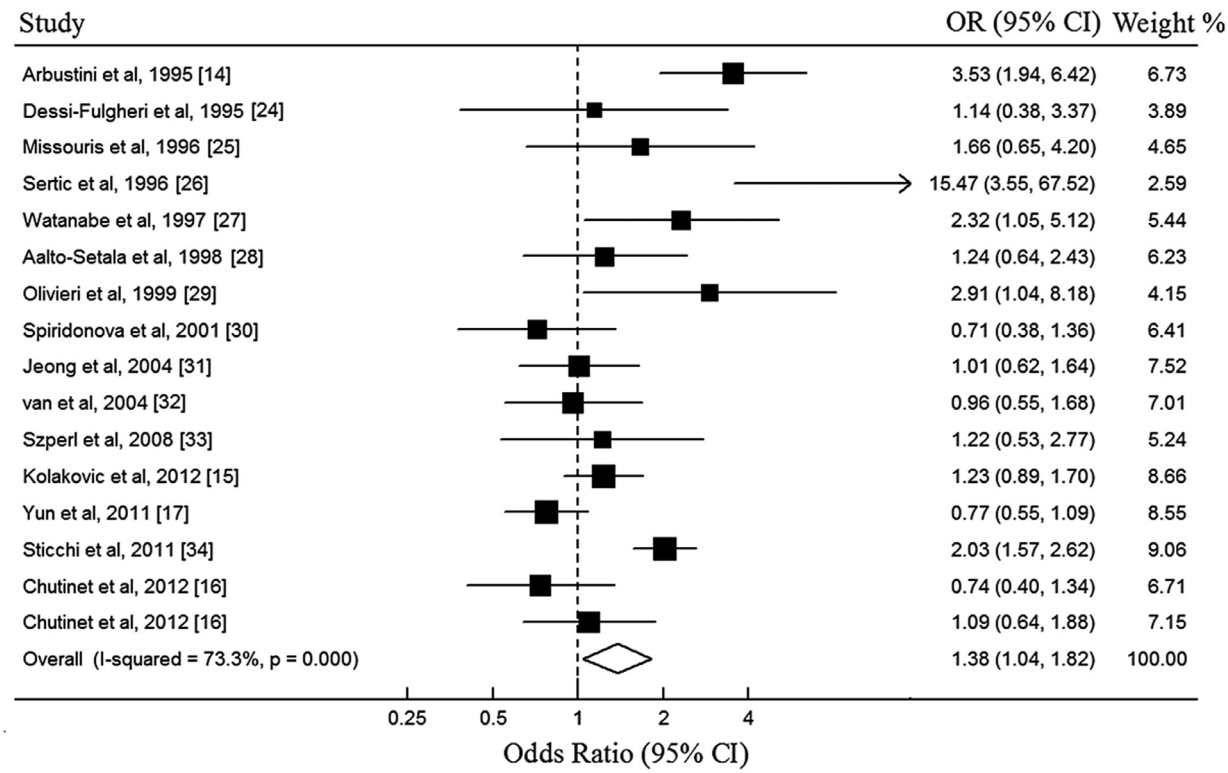

Figure 5. *Forest plot on the associations between* ACE *gene and AS risk under dominant genetic model (DD + DI versus II). For each study, the estimation of OR and its 95% CI are plotted with a box and a horizontal line. The pooled odds ratio is represented by a diamond. The area of the black squares reflects the weight of the study in the meta-analysis. ACE, angiotensin-converting enzyme; AS, atherosclerosis; CI, confidence interval; OR, odds ratio.*

Discussion

We conducted a meta-analysis to evaluate the potential association between *ACE* I/D polymorphism and AS based on available data from original published studies. The pooled result from this study showed that the allele of the *ACE* gene was associated with the increased risk of AS, especially in Europeans. Additionally, frequencies of this mutation in Europeans were higher than those in Asians, and individuals with homozygotes would be more susceptible to AS than heterozygous subjects, indicating that copying the number of D alleles might increase the risk of developing AS.

ACE is a vital enzyme in the RAAS that has long been regarded as a key part in the pathogenesis of various cardiovascular diseases.[35] This prominent enzyme could cleave angiotensin I (Ang I) to generate Ang II, which shifts in endothelial equilibrium though reduction of nitric oxide (NO) bioavailability.[36,37] Several previous studies have proved that ACE which is encoded by I/D polymorphism of the *ACE* gene would affect the level of Ang II.[38,39] Owing to the mutation of the deletion allele for the *ACE* gene, exorbitant carboxyterminal dipeptide has a growth-promoting effect on vascular smooth muscle cells of vascular walls, inducing vessel endothelial injury and cellular hyperplasia, which may be implicated in the development of atherosclerotic vascular disease.[40,41] Moreover, the *apoE e4* allele is known to be associated with high low density lipoprotein (LDL) cholesterol level and LDL oxidation. Under the effect of the *ACE* gene, Ang II could stimulate the uptake of oxidized LDL elicited by their endotheliocytes and macrophages via a proteoglycan-mediated pathway.[42] This enhanced activity is regulated by *apoE*, the expression of which is increased in atherosclerotic lesions.[43] Therefore, *ACE* gene may interact with *apoE* in affecting lipid metabolism and cellular repair mechanism, which might accelerate the progress of AS. Recently, several epidemiologic studies investigated the association between *ACE* gene I/D polymorphisms and AS risk, but inconsistent results were obtained. The results of the present study demonstrated that the D allele of *ACE* could increase the risk of AS. The mechanism for this observed relationship might be in part that the level of Ang II was mediated by polymorphism of *ACE* gene via influences on the ACE levels.[44,45] D allele of *ACE* gene could promote the formation from Ang I to the abundant active peptide hormone.[46] Excessive carboxyterminal dipeptide binds to angiotensin type 1 receptors on vascular smooth muscle cells and the binding initiates the activity of several intracellular secondary messenger systems including phospholipase C and protein kinase C, causing the subsequent release of free calcium from intracellular

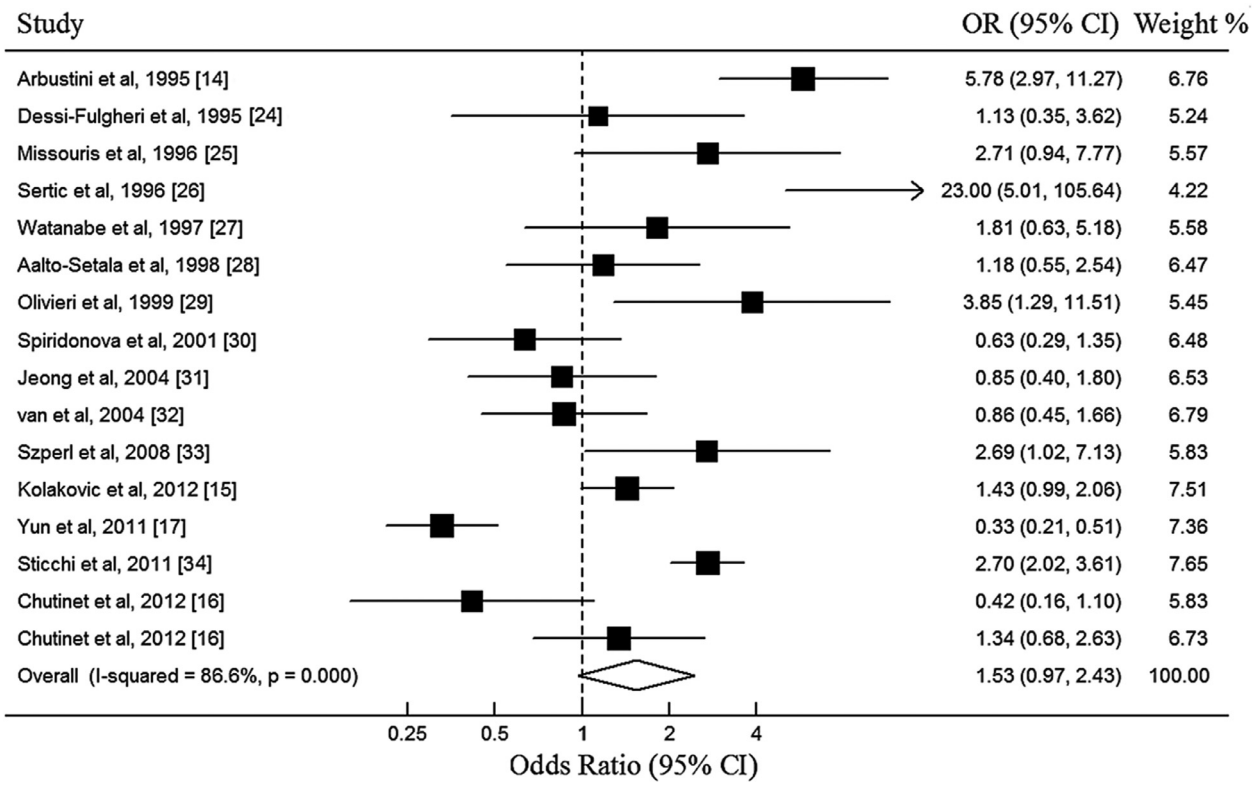

Figure 6. *Forest plot on the associations between* ACE *gene and AS risk under homozygous (DD versus II) genetic model. For each study, the estimation of OR and its 95% CI are plotted with a box and a horizontal line. The pooled odds ratio is represented by a diamond. The area of the black squares reflects the weight of the study in the meta-analysis. ACE, angiotensin-converting enzyme; AS, atherosclerosis; CI, confidence interval; OR, odds ratio.*

pools.[47,48] Through the calcium-sensitive mechanisms, plenty of autocrine-paracrine growth factors were produced including fibroblast growth factor and platelet-derived growth factor, both of which induced an increase in smooth muscle migration and proliferation.[49,50] On the other hand, by provoking activity of NADH/NADPH oxidase, active peptide hormone triggers the generation of intracellular superoxide anions that degrade NO, which

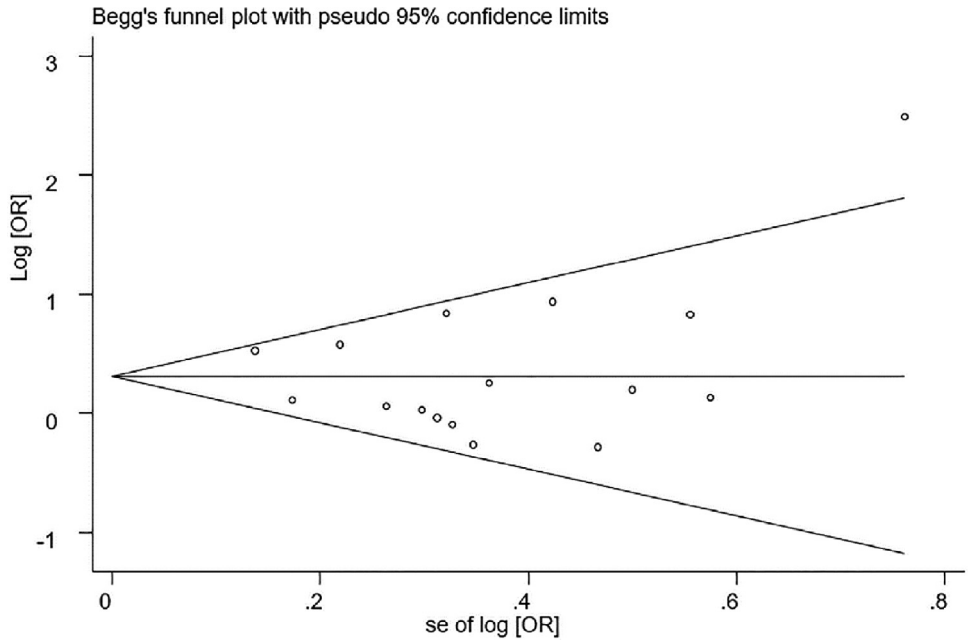

Figure 7. *The funnel plot on the associations between* ACE *gene and AS risk under the heterozygous genetic model (DI versus II). The funnel reveals no obvious publication bias. ACE, angiotensin-converting enzyme; AS, atherosclerosis.*

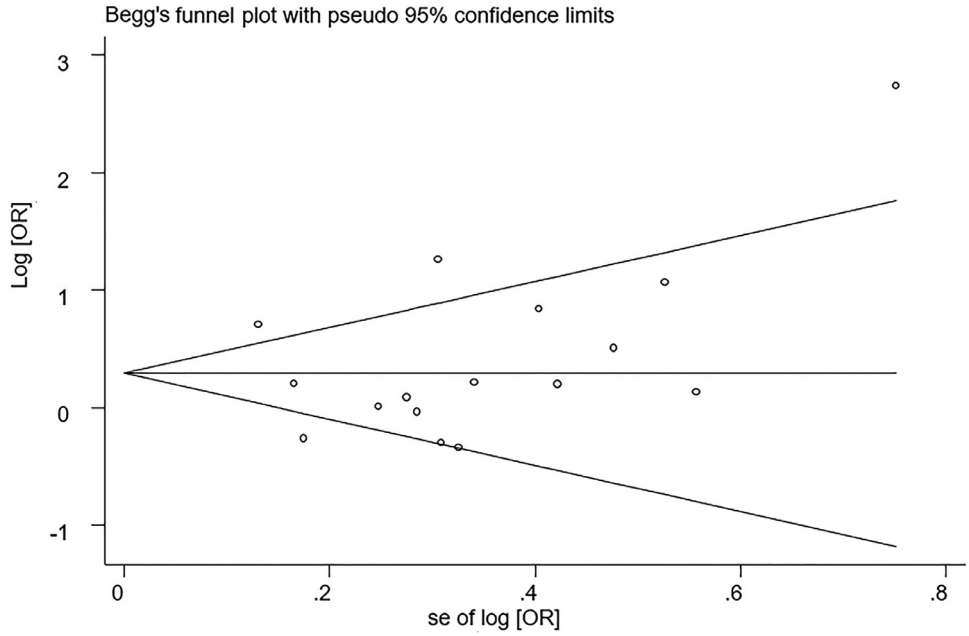

Figure 8. *The funnel plot on the associations between* ACE *gene and AS risk under the dominant genetic model (DD + DI versus II). The funnel reveals no obvious publication bias. ACE, angiotensin-converting enzyme; AS, atherosclerosis.*

conducts to the vascular restructure and neointimal hyperplasia.[51] Thus, such dysfunction could be involved in the pathogenesis of AS.

It should be noteworthy that such associations were stronger for Europeans, in contrast to the absent relationship in Asians. It seemed quite likely that a divergence in the frequency of this mutation between individuals in all included studies could partially interpret the different observed associations across the 2 studied populations. Meanwhile, the results of our study showed that the D allele frequency was significantly higher in Europeans compared with Asians, which is also supported by the hypothesis that Europeans were more likely than Asians to achieve a significant relationship. Furthermore, findings from this meta-analysis found a stronger linkage among DD homozygous subjects than heterozygous subjects. This finding was also in

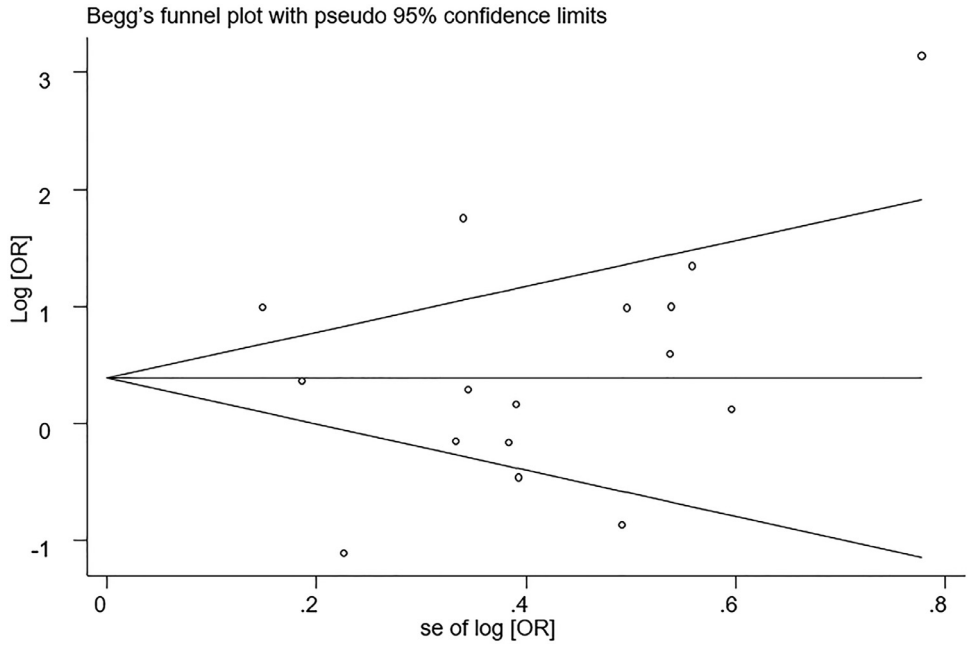

Figure 9. *The funnel plot on the associations between* ACE *gene and AS risk under homozygous (DD versus II) genetic model. The funnel reveals no obvious publication bias. ACE, angiotensin-converting enzyme; AS, atherosclerosis.*

accordance with the assumptions that D allele may have an inducing influence, resulting in AS with a dose-dependent effect, indicating that enhanced copy numbers of the D allele could increase the AS risk.

The present study also has several potential limitations that merit consideration when interpreting our results. First, this study was based on observational studies and might have the potential for bias due to selective reporting. Such bias, to some extent, would confound the results of our analysis. Second, the analysis was based primarily on unadjusted ORs without controlling for confounding components such as age, diet, and cultural status, which could not completely exclude the effect of mixed factors. Nevertheless, the qualities of most of our enrolled studies were high, indicating that the results of the study were credible. Third, this meta-analysis was not built on individual participant data of each qualified study and did not have enough information from the original article, limiting further gene-to-gene and gene-to-environment interaction analysis. Fourth, some other genes were also inclined to affect the risk of AS, which might have an impact on our results in partial. As our analysis was mainly on account of the data provided from the original articles that did not control for these confounding factors or report sufficient data to analyze such association adjusted for different genes. Hence, other genes might influence the AS risk interactively with lipid metabolism could not be entirely excluded in the current study. Further large research studies that allow for the adjustment by related genes should be carried out. Finally, potential publication bias also deserves attention. Despite no statistically significant publication bias being detected in our meta-analysis, it is still hard to fully rule out such potential bias.

In summary, this study indicated that the subjects with D allele of the *ACE* gene have a higher risk of AS, particularly for Europeans. Moreover, increased D allele copy numbers were significantly associated with increased AS risk in a dose-dependent fashion. It should also be noted that gene products and environmental factors may exert different influences on the development and progression of AS. Therefore, to reach a more definitive conclusion, further detailed and well-designed studies with a larger population and different ethnicities are greatly warranted to evaluate the possible effects of this variation on AS.

Conflict of Interest

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

Supplementary Materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.jstrokecerebrovasdis.2019.02.012.

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