Meta Analysis

Ethnic differences in the association between angiotensin-converting enzyme gene insertion/deletion polymorphism and peripheral vascular disease: A meta-analysis

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Abstract

Background: Several studies have investigated the association of angiotensin-converting enzyme (*ACE*) gene insertion/deletion (I/D) polymorphism with peripheral vascular disease (PVD); however, the results remain controversial. Therefore, we conducted the current meta-analysis to evaluate this relationship in the general population of different ethnicities.

Methods: We searched PubMed, Embase, Web of Science, Wanfang Database, and CNKI to identify eligible studies. Random-effect models were applied to estimate the pooled odds ratio (*OR*) with a 95% confidence interval (*CI*), regardless of between-study heterogeneity.

Results: A total of 13 studies with 1966 cases and 6129 controls were included in this meta-analysis. The pooled *OR*s for the association between *ACE* I/D polymorphism and PVD risk were not statistically significant in the overall population under all genetic models. In further ethnicity-stratified analyses, we found a statistically significant association of *ACE* I/D polymorphism with PVD susceptibility in Asians under most models. However, the association among Caucasians did not reach statistical significance.

Conclusion: *ACE* I/D polymorphism might be associated with susceptibility to PVD in the Asian population, but there was no clear evidence indicating a similar significant relationship among Caucasians.

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Keywords: Peripheral vascular disease; Angiotensin-converting enzyme; Insertion/deletion polymorphism; Meta-analysis

Introduction

Peripheral vascular disease (PVD) is a common manifestation of systemic atherosclerosis, which encompasses numerous noncoronary arterial syndromes. It is associated with an increased risk of cardiovascular events,[1] affecting approximately 20% of adults aged 55 years or older and an estimated 27 million persons in North America and Europe.[2] Apart from traditional

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Peer review under responsibility of Chinese Medical Association.

cardiovascular risk factors, several novel biologic mediators and genetic predisposing factors have been implicated in the atherogenic process leading to PVD.[3–5] PVD is a complex trait influenced by multiple environmental triggers and genetic factors as well as their interactions, which are not yet completely defined.

Many studies have investigated the relevance of polymorphisms in renin angiotensin system (RAS) genes with PVD but reported conflicting results. Among the polymorphisms in RAS candidate genes, the angiotensin-converting enzyme (ACE) insertion/deletion (I/D) polymorphism has attracted special attention owing to its functional roles. The ACE gene is located on the long arm of chromosome 17 (17q23) in humans and contains 26 exons and 25 introns. ACE I/D polymorphism (dbSNP rs1799752, chromosome position 63,488,529 GRCh37) results from the presence or absence of a 287-bp Alu repeat in intron 16 of the ACE gene.[6] The population minor allele frequency (MAF) of this SNP is lower than 0.01. The ACE I/D polymorphism is known to be responsible for nearly half of the total phenotypic variance of circulating, intracellular, and tissue ACE in Caucasians, with higher serum ACE level and activity in D allele carriers.[7,8]

ACE, a key component of the RAS system, hydrolyzes angiotensin I to the potent vasoconstrictor and aldosterone-stimulating peptide angiotensin II, and inactivates the vasodilator bradykinin. This biological process results in decreased tissue perfusion, vascular smooth muscle cell growth, and stimulation of plasminogen-activator inhibitor type I.[9,10] Chronic exposure to high levels of circulating and tissue ACE may also lead to vascular wall thickening and atherosclerosis.[3] Thus, those who carry a susceptible gene polymorphism of ACE may experience chronically unbalanced vasoconstriction and vasorelaxation. The unbalanced vascular tones subsequently increase arterial stiffness, ultimately predisposing them to lower extremity arterial disease (LEAD) or other vascular diseases.[5]

There is mounting evidence proving the vital role of the D allele of the ACE gene in various cardiovascular diseases among different populations. Many studies supported the hypothesis that the ACE D allele confers an increased risk of vasculitis, especially in Behcet's disease and Henoch-Schönlein purpura.[11,12] The DD genotype and D allele were also found to be strongly associated with hypertension in different populations.[13,14] Moreover, cumulative evidence has confirmed the effect of ACE I/D polymorphism on the onset of type 2 diabetes mellitus (T2DM).[15,16] Given the importance of the ACE gene in the pathogenesis of hypertension, T2DM, vasculitis, and other predisposing causes to PVD,[17] it is biologically plausible that these variants modulate the risk of PVD. Moreover, PVD per se is an atherosclerotic process.[18] Thus, the ACE gene may be a good candidate gene for PVD study.

Although multiple studies have attempted to link ACE I/D polymorphism to PVD, the results remain controversial.[4,5,19–28] This lack of reproducibility might stem from methodological limitations of the available studies, including insufficient sample size, different definitions of PVD, ethnic heterogeneity, conceivable selection bias, environmental factors, as well as true variability between populations.[29] Therefore, we conducted a meta-analysis to derive a more precise estimation of the association between ACE I/D polymorphism and PVD risk.

Methods

Search strategy

The PubMed, Embase, Web of Science, Wanfang Database, and CNKI were searched for relevant studies published before July 2016 using the following searching strategy: "peripheral artery disease" OR "peripheral arterial disease" OR "peripheral vascular disease" OR "peripheral vascular occlusive disease" OR "peripheral arterial occlusive disease" OR "lower extremity arterial disease" OR "intermittent claudication" OR "limb ischemia" OR "atherosclerotic vascular disease" OR "PVD" OR "PAD" OR "PVOD" OR "PAOD" OR "LEAD") AND ("ACE" OR "angiotensin converting enzyme") AND ("gene" OR "genotype" OR "gene variant" OR "polymorphism" OR "gene polymorphism" OR "SNP"). Bibliographies in the published articles provided further references. Following this search, we also searched the reference list to identify potentially relevant articles. Studies with the most complete data were included when there were multiple publications based on overlapping data.

Inclusion and exclusion criteria

The titles, abstracts, and full texts were reviewed. In this article, we mainly focused on lower-extremity peripheral artery disease (PAD), a chronic occlusive disease of aortic, iliac, and lower-limb arteries.[30] Thus, cases were considered to be patients suffering from lower-extremity PAD, with the diagnosis based on both noninvasive and invasive diagnostic tools. Eligible studies fulfilled the following inclusion criteria: 1)

evaluation of the association of *ACE* I/D polymorphism with susceptibility to PVD; 2) involvement of human cases with clinically diagnosed PVD and healthy controls who were free of PVD; 3) complete information on genotype frequency or risk estimate; 4) retrospective case-control studies using either a hospital-based or population-based design; 5) original data; 6) genotyping performed using validated methods.

The exclusion criteria were: 1) duplicate studies, reviews, editorial comments, case reports, meta-analyses, meeting abstracts; 2) family-based studies or case-only studies; 3) studies investigating progression, severity, phenotype modification, response to treatment, or survival; 4) association between other gene polymorphism and PVD risks.

Data extraction

We collected the following information from each eligible study: first author, year of publication, ethnicity of subject, country of origin, demographic data, sample size, genotyping method, source of controls, and genotype distribution. Additionally, information from Hardy–Weinberg equilibrium (HWE) tests was also tracked or calculated manually if unavailable. Two investigators calculated and tabulated the data independently using a standard extraction formula. Finally, the discrepancies were addressed in a discussion and a consensus was reached.

Quality assessment

The Newcastle–Ottawa scale (NOS), which was used to assess the quality of the eligible studies by two investigators independently, consisted of three perspectives: selection, comparability, and exposure. The NOS scores varied from 0 to 9, and studies with a NOS score ≥ 7 were regarded as high-quality ones.

Statistical analysis

The association between *ACE* I/D polymorphism and PVD was assessed by calculating pooled odds ratios (*OR*s) at a 95% confidence interval (*CI*). Deviation from HWE for distribution of genotypes was tested by χ^2 tests in control groups. Since no available evidence favored any genetic models of inheritance for the polymorphism under study, we tested a co-dominant model (DD *vs.* II, ID *vs.* II), a dominant model (DD + ID *vs.* II), a recessive model (DD *vs.* ID + II), and an allele model (D *vs.* I). Heterogeneity

across the involved studies was assessed using Cochran's *Q*-test and I^2 statistics with the level of significance set at $P < 0.10$. In this study, we only used the random-effect model to combine the individual effect-size estimates, considering the ubiquitous nature of heterogeneity between studies. The significance of the pooled *OR*s was determined by the *Z*-test.

In addition, meta-regression was conducted to explore the possible source of heterogeneity. We further performed a cumulative meta-analysis to track evidence over time and measure the extent of genetic effects as evidence accumulated. A stratified analysis according to races was conducted to analyze different genetic architecture across races. In order to explore the assumed effect of study design on heterogeneity, we also conducted a stratified analysis according to the source of the controls. To test the robustness of the results, we conducted sensitivity analyses by removing an individual study each time or studies with similar features, as well as substituting unadjusted *OR*s with adjusted ones if available. Finally, Begg's test with a funnel plot[31] was used to assess the publication bias among the involved studies. All analyses were conducted using STATA version 11.2 (Stata Corp LP, College Station, TX). A value of $P < 0.05$ was defined as statistically significant.

Results

Literature search

A total of 2779 abstracts were initially retrieved from PubMed, Embase, Web of Science, Wanfang Database, and CNKI with the relative keywords (230 from PubMed, 2383 from Embase, 107 from Web of science, 57 from Wanfang Database, and 2 from CNKI). Consequently, 2613 studies remained after removing duplications. A majority of these references that did not match our research criteria were excluded after reviewing the abstracts or titles, including reviews, commentaries, meta-analyses, meeting abstracts, and the obviously irrelevant ones. Finally, we identified 18 articles potentially related to the association between *ACE* gene polymorphism and PVD, among which two studies were based on the same subjects recruited from the staff of the University of Florence[4,32]; only the more elaborate one was included.[4] Furthermore, three studies[33–35] focused on atherosclerotic progression, comorbidities, or outcome in the development of PVD and one[36] without detailed genotype data between groups were also excluded.

Additionally, two studies reported about this issue based on overlapping data by the same institution[26,37]; only the latest one was included.[26] We also inspected the references of all studies to identify further studies. In total, 12 original articles with 13 study populations with a total of 1966 cases and 6129 controls were included in our meta-analysis.[4,5,19–28] Among them, the article by Li et al[5] was used as two separate studies owing to different races. A flow chart showing our selection is presented in Fig. 1.

Study characteristics

The main characteristics and genotype distribution of the 13 involved studies, published between 1998 and 2014, are presented in Tables 1 and 2. The sample size of these studies ranged from 104 to 1257, of which 4 were conducted in a population-based design[5,19,21] and

the remaining in a hospital-based design.[4,20,22–28] The ethnicities of the study populations were Caucasian (n = 7), Asian (n = 5), and African–American (n = 1). The frequency of the *ACE* D allele in patients with PVD ranged from 29.1% to 65.8%, and in controls ranged from 26.2% to 61.9%. The genotype distribution in controls was consistent with HWE, except for one by Taute et al[19] and another by Tosic et al,[27] which deviated from HWE. The PCR or PCR-RFLP method was used for genotyping in all eligible studies, whereas the diagnostic standard of PVD varied among these studies. Ankle-brachial index (ABI), or as it is sometimes called, ankle-arm index (AAI), is a measure widely utilized for PVD. ABI or AAI was clearly reported as the diagnosis index in 11 studies,[4,5,20–26,28] even though the threshold values differed slightly. Most studies involved were of high quality with a NOS score no less than 7 points.
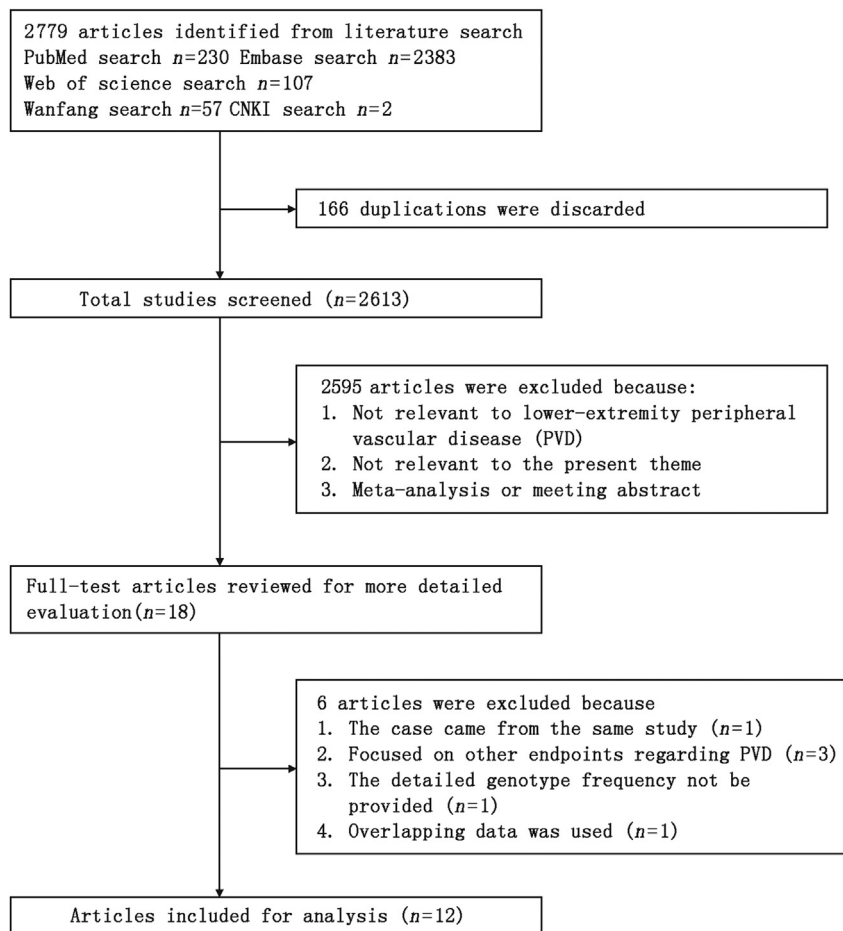

Fig. 1. Flow chart of the study selection process.

Table 1
Major characteristics of the 13 studies involved.

Study	Year	Country	Ethnicity	Design	Sample size		Age (Mean ± SD, year)		Gender (%, Male)		Comorbidity	Diagnosis index	Genotyping methods	NOS score
					Cases	Controls	Cases	Controls	Cases	Controls				
Taute et al.	1998	Germany	Caucasian	PB	98	240	60.46 ± NA	NA	78.6	NA	–	–	PCR	6
Chen et al.	2002	China (mainland)	Asian	HB	100	276	66.3 ± 4.8	65.9 ± 4.7	41.0	48.6	HP	ABI ≤ 0.9	PCR	6
Renner et al.	2002	Austria	Caucasian	PB	522	522	64.9 ± 11.9	64.9 ± 11.9	58.4	58.4	–	ABI < 1.0	PCR	7
Thomas et al.	2003	China (Hong Kong)	Asian	HB	194	1046	65.5 ± 10.5	66.5 ± 6.4	47.9	44.0	T2DM	ABI < 0.9	PCR–RFLP	8
Karagiannis et al.	2004	Greece	Caucasian	HB	100	100	66.7 ± 7.7	66.6 ± 7.9	78.0	45.0	–	ABI < 0.8	PCR	7
Jeong et al.	2004	Korea	Asian	HB	92	280	65.9 (48–82)	61.3 (20–90)	100	74.5	–	ABI < 0.9	PCR	6
Basar et al.	2007	Turkey	Caucasian	HB	78	73	54.91 ± 11.0	48.76 ± 10.6	76.9	NA	–	ABI < 0.9	PCR	5
Li et al. A	2007	United states	Caucasian	PB	124	1133	74.5 ± NA	73.6 ± NA	70.2	69.5	–	ABI < 0.9	PCR	8
Li et al. B	2007	United states	African–American	PB	234	872	74.1 ± NA	73.2 ± NA	42.7	42.8	–	ABI < 0.9	PCR	8
Fatini et al.	2009	Italy	Caucasian	HB	281	485	72 (30–93)	71 (24–95)	78.0	74.0	–	ABI < 0.9	PCR	8
Pan et al.	2010	China (mainland)	Asian	HB	43	61	74.5 ± 7.3	72.9 ± 6.1	57.1	59.5	T2DM	ABI < 0.9	PCR	8
Tseng et al.	2012	China (Taiwanese)	Asian	HB	81	864	72.9 ± 7.3	62.6 ± 11.3	38.3	49.0	T2DM	ABI < 0.9	PCR	7
Tosic et al.	2014	Yugoslavia (Serbia)	Caucasian	HB	19	177	62.3 ± 11.4 (all)		56.6 (all)		ESRD	–	PCR	6

T2DM: type 2 diabetes mellitus; HP: hypertension; ESRD: end-stage renal disease; ABI: ankle-Brachial index; HB: hospital-based; PB: population-based; NA: not available; SD: standard deviation; −: none; PCR: polymerase chain reaction; PCR-RFLP: polymerase chain reaction-restriction fragment length polymorphism; NOS: Newcastle–Ottawa scale.

Pooled prevalence of ACE deletion polymorphism in the controls

The pooled frequency of the *ACE* D allele was 47.5% (95% *CI*: 39.7%−55.3%) in the overall controls under the random-effect model with obvious heterogeneity ($P = 0.000$, $I^2 = 97.4\%$). The pooled D allele frequency was 56.2% (95% *CI*: 53.0%−59.3%), 31.8% (95% *CI*: 28.4%−35.2%), and 59.3% (95% *CI*: 56.1%−62.6%) in Caucasian, Asian, and African−American controls, respectively. Significant heterogeneity was detected among Caucasian controls ($P = 0.038$, $I^2 = 55.1\%$) and Asian controls ($P = 0.029$, $I^2 = 63.1\%$).

ACE I/D polymorphism and the risk of PVD occurrence

The association between *ACE* I/D polymorphism and susceptibility to PVD was detected in each study under all genetic models [co-dominant model (DD *vs.* II, ID *vs.* II); dominant model (DD + ID *vs.* II); recessive model (DD *vs.* ID + II); allele model (D *vs.* I)]. Overall, except for 5 articles suggesting the protective effect of the D allele,[5,19,23−25] the studies consistently favored the risk effect, especially the more recent studies.[4,26−28] The main results of this meta-analysis are shown in Table 3 and Fig. 2. As a whole, the pooled *OR*s of all eligible studies indicated that there were no statistically significant associations between *ACE* I/D polymorphism and PVD risk in all genetic models for DD *vs.* II (*OR* = 1.195, 95% *CI*: 0.909−1.569), ID *vs.* II (*OR* = 1.190, 95% *CI*: 0.970−1.460), DD + ID *vs.* II (*OR* = 1.190, 95% *CI*: 0.968−1.462), DD *vs.* ID + II (*OR* = 1.069, 95% *CI*: 0.874−1.308), and D *vs.* I (*OR* = 1.097, 95% *CI*: 0.956−1.258) with significant between-study heterogeneity.

Stratified analysis

To further explore the source of heterogeneity, we performed stratified analyses across several key characteristics including population ethnicity (Caucasian, Asian, and African−American) and research design (hospital- or population-based). However, in the stratified analysis by ethnicity, significant associations were observed in Asian populations under almost every genetic model [co-dominant model (DD *vs.* II: *OR* = 1.620, 95% *CI*: 1.077−2.436; ID *vs.* II: *OR* = 1.390, 95% *CI*: 1.075−1.798); dominant model: *OR* = 1.455, 95% *CI*: 1.164−1.818; D *vs.* I:

Table 2
Distributions of *ACE* I/D genotypes and alleles in PVD patients and controls.

Study	Year	Distribution of genotype						Distribution of allele				HWE P	MAF in controls
		PVD patients			Controls			PVD patients		Controls			
		DD	ID	II	DD	ID	II	D	I	D	I		
Taute et al.	1998	33	46	19	92	100	48	112	84	284	196	0.033[a]	0.592
Chen et al.	2002	23	43	34	29	127	120	89	111	185	367	0.589	0.335
Renner et al.	2002	166	234	122	146	252	124	566	478	544	500	0.454	0.521
Thomas et al.	2003	22	104	68	102	420	524	148	240	624	1468	0.200	0.298
Karagiannis et al.	2004	29	58	13	35	48	17	116	84	118	82	0.937	0.590
Jeong et al.	2004	12	46	34	43	133	104	70	114	219	341	0.964	0.391
Basar et al.	2007	22	42	14	31	27	15	86	70	89	57	0.057	0.610
Li et al. A	2007	37	58	29	365	560	208	132	116	1290	976	0.791	0.569
Li et al. B	2007	73	111	50	309	417	146	257	211	1035	709	0.792	0.593
Fatini et al.	2009	94	146	41	127	239	119	334	228	493	477	0.755	0.508
Pan et al.	2010	4	17	22	7	18	36	25	61	32	90	0.064	0.262
Tseng et al.	2012	12	34	35	76	358	430	58	104	510	1218	0.904	0.295
Tosic et al.	2014	8	9	2	60	99	18	25	13	219	135	0.014[a]	0.619

ACE: angiotensin-converting enzyme; PVD: peripheral vascular disease; HWE: Hardy−Weinberg equilibrium; MAFs: minor allele frequencies.
[a] Deviated from HWE.

OR = 1.319, 95% CI: 1.107−1.571], while the effect estimates remained insignificant among the Caucasian and African−American populations (all *P* > 0.05) (Table 3 and Fig. 2a). Furthermore, among Asian populations, this trend was potentiated after comparing homozygotes of the D allele with I allele (DD *vs.* II) with a 62.0% increased risk. In contrast, a comparison between ID genotype and II genotype carriers only

Table 3
Meta-analysis of *ACE* I/D polymorphism with PVD risk in the overall population and subgroups.

Total and subgroups	Genetic model	No. of studies	Sample size		Test of heterogeneity			Test of association			
			Patients	Controls	Q	P	I^2 (%)	OR	95% CI	Z	P
Total	DD + ID *vs.* II	13	1966	6129	27.43	0.007	56.3	1.190	0.968−1.462	1.65	0.098
	DD *vs.* ID + II				25.20	0.014	52.4	1.069	0.874−1.308	0.65	0.514
	DD *vs.* II				29.39	0.003	59.2	1.195	0.909−1.569	1.28	0.202
	ID *vs.* II				23.98	0.020	50.0	1.190	0.970−1.460	1.67	0.095
	D *vs.* I				31.98	0.001	62.5	1.097	0.956−1.258	1.32	0.186
Ethnicity											
Caucasian	DD + ID *vs.* II	7	1222	2730	11.31	0.079	46.9	1.14	0.859−1.513	0.91	0.365
	DD *vs.* ID + II				11.28	0.080	46.8	1.001	0.789−1.269	0.00	0.996
	DD *vs.* II				11.94	0.063	49.8	1.115	0.800−1.554	0.64	0.522
	ID *vs.* II				10.54	0.104	43.1	1.163	0.871−1.554	1.02	0.306
	D *vs.* I				12.39	0.054	51.6	1.036	0.877−1.224	0.42	0.675
Asian	DD + ID *vs.* II	5	510	2527	4.69	0.321	14.7	1.455	1.164−1.818	2.69	0.001
	DD *vs.* ID + II				7.80	0.099	48.7	1.385	0.906−2.118	1.51	0.132
	DD *vs.* II				6.31	0.177	36.6	1.620	1.077−2.436	2.32	0.021
	ID *vs.* II				5.39	0.249	25.8	1.390	1.075−1.798	2.51	0.012
	D *vs.* I				5.47	0.242	26.9	1.319	1.107−1.571	3.10	0.002
Study design											
Hospital-based	DD + ID *vs.* II	9	988	3362	6.82	0.557	0.0	1.521	1.284−1.801	4.86	0.000
	DD *vs.* ID + II				17.88	0.022	55.3	1.169	0.857−1.593	0.99	0.324
	DD *vs.* II				11.47	0.177	30.2	1.546	1.148−2.081	2.87	0.004
	ID *vs.* II				6.82	0.556	0.0	1.499	1.254−1.792	4.44	0.000
	D *vs.* I				13.09	0.109	38.9	1.236	1.064−1.435	2.77	0.006
Population-based	DD + ID *vs.* II	4	978	2767	2.86	0.413	0.0	0.882	0.730−1.065	1.31	0.192
	DD *vs.* ID + II				4.14	0.247	27.5	0.959	0.781−1.177	0.40	0.686
	DD *vs.* II				4.42	0.220	32.1	0.874	0.663−1.152	0.96	0.339
	ID *vs.* II				1.83	0.609	0.0	0.874	0.714−1.070	1.30	0.192
	D *vs.* I				4.50	0.212	33.4	0.936	0.813−1.079	0.91	0.363

ACE: angiotensin-converting enzyme; PVD: peripheral vascular disease; No.: number; OR: odds ratio; CI: confidence interval.

(a)

Study ID	Weight(%)	OR(95% CI)	Random Effect Analysis
Caucasian			
Taute et al 1998	7.54	0.92 (0.66, 1.29)	
Renner et al 2002	11.32	1.09 (0.92, 1.29)	
Karagiannis et al 2004	6.38	0.96 (0.64, 1.43)	
Basar et al 2007	5.43	0.79 (0.50, 1.24)	
Li et al A 2007	9.15	0.86 (0.66, 1.12)	
Fatini et al 2009	10.41	1.42 (1.15, 1.75)	
Tosic et al 2014	2.98	1.19 (0.59, 2.40)	
Subtotal (I^2=51.6%, P=0.054)	53.20	1.04 (0.88, 1.22)	
Asian			
Chen et al 2002	7.66	1.59 (1.14, 2.21)	
Thomas et al 2003	10.04	1.45 (1.16, 1.82)	
Jeong et al 2004	7.41	0.96 (0.68, 1.35)	
Pan et al 2010	3.65	1.15 (0.62, 2.13)	
Tseng et al 2012	7.51	1.33 (0.95, 1.87)	
Subtotal (I^2=26.9%, I^2=0.242)	36.28	1.32 (1.11, 1.57)	
African-American			
Li et al B 2007	10.52	0.83 (0.68, 1.02)	
Subtotal (I^2=., P=.)	10.52	0.83 (0.68, 1.02)	
Overall (I^2=62.5%, P=0.001)	100.00	1.10 (0.96, 1.26)	

Note: Weights are from random effects analysis

0.8 1.0 1.4 1.8 2.4 3.0

(b)

Study ID	Weight(%)	OR(95% CI)	Random Effect Analysis
Population-based			
Taute et al 1998	7.54	0.92 (0.66, 1.29)	
Renner et al 2002	11.32	1.09 (0.92, 1.29)	
Li et al A 2007	9.15	0.86 (0.66, 1.12)	
Li et al B 2007	10.52	0.83 (0.68, 1.02)	
Subtotal (I^2=33.4%, P=0.212)	38.52	0.94 (0.81, 1.08)	
Hospital-based			
Chen et al 2002	7.66	1.59 (1.14, 2.21)	
Thomas et al 2003	10.04	1.45 (1.16, 1.82)	
Karagiannis et al 2004	6.38	0.96 (0.64, 1.43)	
Jeong et al 2004	7.41	0.96 (0.68, 1.35)	
Basar et al 2007	5.43	0.79 (0.50, 1.24)	
Fatini et al 2009	10.41	1.42 (1.15, 1.75)	
Pan et al 2010	3.65	1.15 (0.62, 2.13)	
Tseng et al 2012	7.51	1.33 (0.95, 1.87)	
Tosic et al 2014	2.98	1.19 (0.59, 2.40)	
Subtotal (I^2=38.9%, P=0.109)	61.48	1.24 (1.06, 1.44)	
Overall (I^2=62.5%, P=0.001)	100.00	1.10 (0.96, 1.26)	

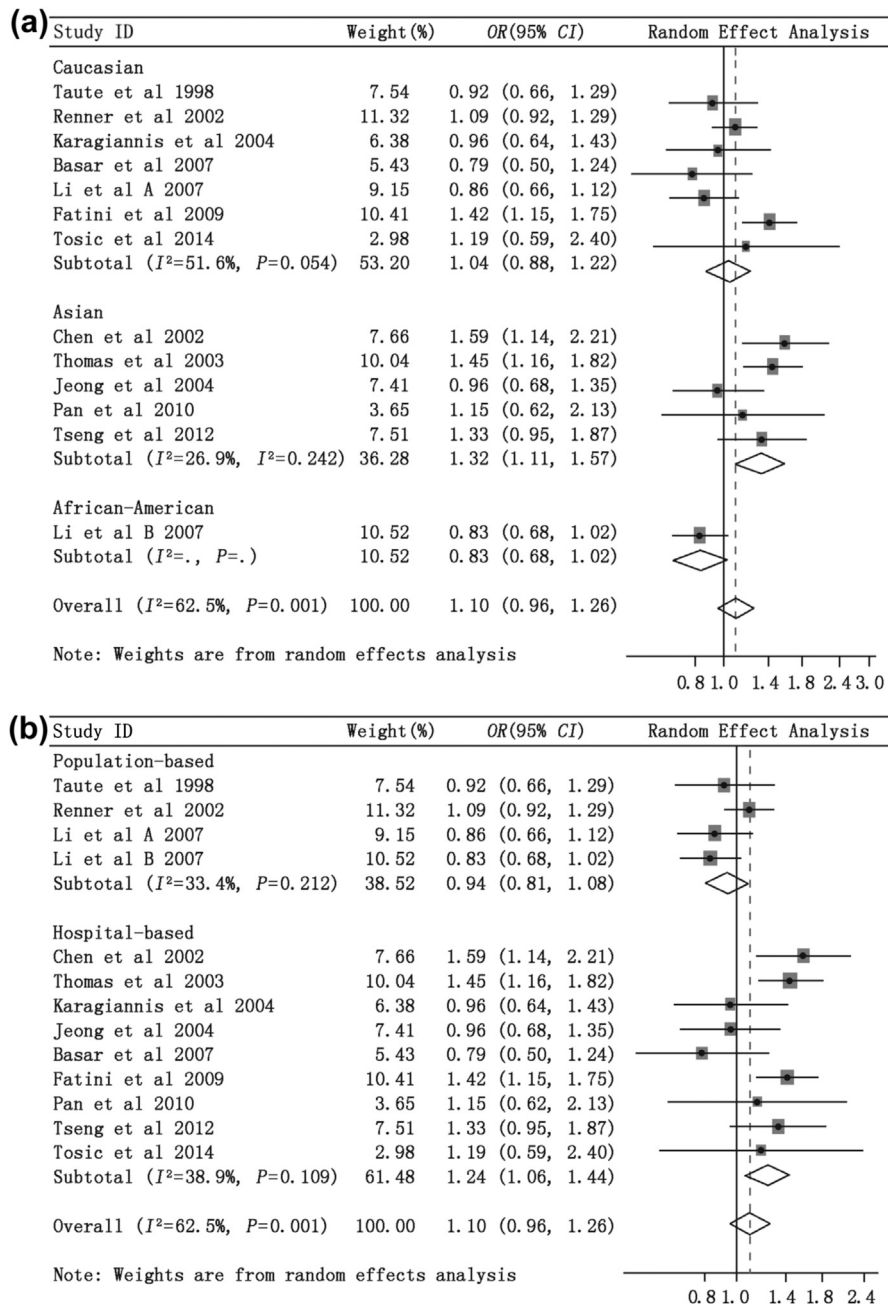Note: Weights are from random effects analysis

0.8 1.0 1.4 1.8 2.4

Fig. 2. Forest plot of the association between angiotensin-converting enzyme (*ACE*) I/D polymorphism and peripheral vascular disease (PVD) in the overall population stratified by ethnicities (a) and study design (b) (allele model: D *vs.* I).

yielded a marginally significant 39.0% increased risk for PVD.

Among the 13 studies, there were 4 population-based studies[5,19,21] and 9 hospital-based studies.[4,20,22–28] After classifying all studies according to study design, it was interesting to note that a significant association was consistently observed for hospital-based studies but not population-based studies (Table 3 and Fig. 2b). For example, under the homozygous model of inheritance, comparison of the *ACE* DD genotype carriers with II genotype carriers generated no significant results among population-based studies (*OR* = 0.874, 95% *CI*: 0.663–1.152); however, the strength of association was nearly doubled among hospital-based studies (*OR* = 1.546, 95% *CI*: 1.148–2.081).

Heterogeneity analysis and cumulative analysis

As is shown in Table 2, significant heterogeneity was observed in the overall comparisons. Thus, meta-regression was performed to detect source of heterogeneity in general variables. It showed that the source of the controls (population- or hospital-based) was the major source of heterogeneity [co-dominant model (DD *vs.* II $P = 0.021$, ID *vs.* II $P = 0.003$); dominant model: $P = 0.003$; recessive model: $P = 0.316$; D *vs.* I: $P = 0.026$]. Population ethnicity, whether participants had comorbidity, and whether cases and controls were matched for age and gender could also partially explain the heterogeneity in some genetic models. However, the year of publication, sample size, status of HWE, and diagnosis standard was not statistically correlated with heterogeneity (all $P > 0.05$).

Cumulative meta-analysis for the allele model (D *vs.* I) in the overall population revealed clear evidence of a random-effect *OR* not significantly larger than 1 (Fig. 3). This trend hardly varied after 2009 when it was around 1.07, indicating the stability of the association between *ACE* I/D polymorphism and risk of PVD. From 2009 to the present, the point estimates of the pooled effect gradually stabilized with narrowed *CI*s among other models.

Sensitivity analysis and publication bias

To evaluate the stability of the results, we further conducted sensitivity analysis by omitting one study at a time and recalculating the pooled *OR*s. The corresponding pooled *OR*s (D *vs.* I) were not substantially altered in the overall population (Fig. 4a) or Asian population (Fig. 4b), suggesting that the results were statistically robust. Similar results were identified when we excluded two studies in which genotype distribution in the controls slightly deviated from HWE,[19,27] two studies without a clear diagnosis threshold of ABI,[19,27] five studies with a NOS score ≤6,[19,20,23,25,27] and five studies in which participants had comorbidities.[20,22,26–28] Considering the effect of confounding factors, we also used substituted adjusted *OR*s for unadjusted ones if available[4,26]; however, no prominent changes were observed in pooled results (data not shown).

Symmetry was apparent in the funnel plots (Fig. 5), and statistical evidence from the Begg's test suggested that there was no publication bias among the overall population ($Z = -0.06$, $P = 1.000$) or Asian population ($Z = 1.22$, $P = 0.221$).

Discussion

To the best of our knowledge, this is the first meta-analysis of comprehensive assessment for the

Study ID	Number	OR (95% CI)
Taute et al 1998	338	0.92 (0.66, 1.29)
Chen et al 2002	376	1.21 (0.71, 2.07)
Renner et al 2002	1044	1.16 (0.89, 1.52)
Thomas et al 2003	1240	1.23 (0.99, 1.54)
Karagiannis et al 2004	200	1.19 (0.97, 1.45)
Jeong et al 2004	372	1.15 (0.96, 1.38)
Basar et al 2007	151	1.11 (0.93, 1.32)
Li et al A 2007	1257	1.07 (0.91, 1.27)
Li et al B 2007	1106	1.03 (0.88, 1.21)
Fatini et al 2009	766	1.07 (0.92, 1.25)
Pan et al 2010	104	1.08 (0.93, 1.25)
Tseng et al 2012	945	1.09 (0.95, 1.26)
Tosic et al 2014	196	1.10 (0.96, 1.26)

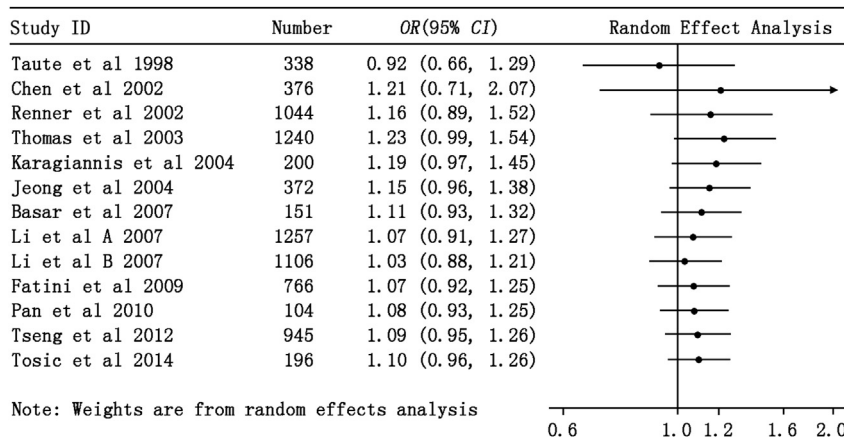Note: Weights are from random effects analysis

Fig. 3. Cumulative meta-analysis in the overall population (allele model: D *vs.* I).

relationship between *ACE* I/D polymorphism and risk of PVD. Our meta-analysis of 13 studies, including 1966 cases and 6129 controls, failed to show significant associations in overall comparisons. However, in subgroup analyses organized by race and study-design, we found that PVD cases had a significantly lower frequency of II genotype in Asians and hospital-based studies.

In the present study, significant association was remarkably found among Asians but not among Caucasians or African−Americans, suggesting possible ethnic differences in genetic backgrounds and the living environment. Indeed, an ethnic difference of *ACE* I/D polymorphism is present in healthy population studies, with D allele frequency ranging from 0.50 to 0.63 in Caucasians and 0.27 to 0.40 in Japanese individuals.[38,39] In this contribution, the pooled D allele frequency in controls was exceedingly higher among Caucasians (53.0%−59.3%) than among Asians (28.4%−35.2%). Ethnic factors also affect the genotype−phenotype relationship, as seen in the weaker correlation between *ACE* I/D genotype and circulating *ACE* levels in African−Americans than in

Caucasians.[40] In addition, the impact of this polymorphism might be diluted or masked by other as-yet unidentified causal genes involved in PVD development among Caucasians. Future studies should investigate I/D adjacent markers to confirm whether the association is causal or due to linkage disequilibrium (LD) which varied between populations because of regional variability, genetic drift, and mating patterns.[41] Other inherent factors such as selection bias, different matching criteria, or relatively small sample size may also play a role and lead to ethnic heterogeneity. Moreover, we cannot exclude the possibility that *ACE* I/D polymorphism may have pleiotropic effects on the pathogenesis of PVD among different ethnic groups in view of the high prevalence of the D allele among all studies. Since only one eligible study aimed at an African−American population, additional studies are warranted to further validate the ethnic impact on the association, especially among African−Americans.

No significant association between variant genotypes and PVD risk was observed in the subgroup analysis among population-based studies. The nonsignificant result might be ascribed to the limited number
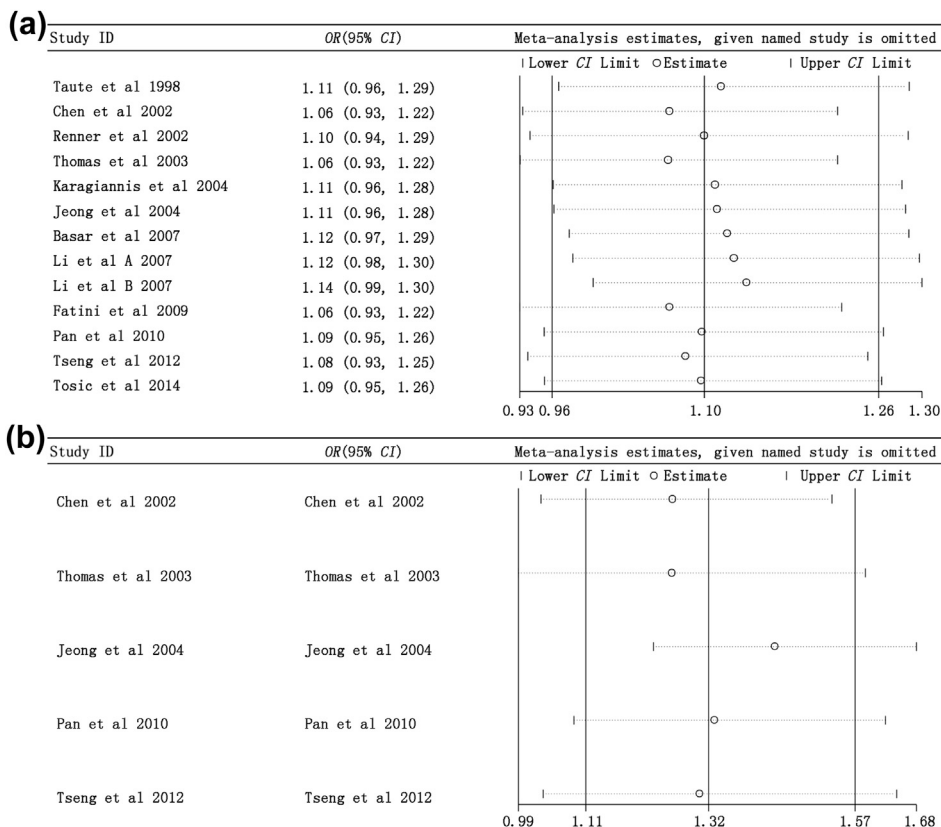

Fig. 4. Sensitivity analysis in the overall (a) and Asian (b) populations pooled with the random-effects model (allele model: D *vs.* I).

of studies with available data, which lacked sufficient statistical power to detect a slight effect or tended to generate a fluctuated risk estimate. With respect to this research, we only involved 4 population-based studies, among which two were based on the same Health ABC study[5] and accounted for nearly 50% weight in this subgroup. It is thus speculated that if omitted, the magnitude of estimated *OR* might fluctuate or even reverse, which was the case for the present meta-analysis (the pooled *OR* switched to 1.051, 95% *CI*: 0.901–1.225). Based on this, the results of the hospital-based studies might be considered statistically valid in this study.

Considering the fact that all the included studies were conducted among elderly adults, the issue of selective survival should not be neglected. It is likely that those who were genetically susceptible or environmentally prone to CVD either may not have survived to be enrolled or failed to meet the eligibility criteria. For instance, the criteria for accepting patients into the study conducted by Taute et al[19] included the ability to walk 100 meters without suffering pain and the absence of symptomatic or ECG-detectable coronary heart

disease. Individuals diagnosed with ischemic heart failure had to be ruled out of the Health ABC study,[5] failing to meet the criteria of having no difficulty walking one quarter of a mile, climbing 10 steps, or performing basic activities of daily living. Thus, we may be left with a relatively healthier crowd with less susceptibility to PVD, as is the case with another study,[42] which reported that the *ACE* D allele is associated with coronary artery disease in subjects younger than 61.7 years old but not in those aged 61.7 years and older.

Our meta-analysis had some notable advantages. First, a comprehensive searching strategy based on computer-assisted and manual searching allowed extensive access to all eligible studies. Second, sensitivity analyses and no publication bias contributed to the robustness of our results. Third, our analysis not only focused on papers published in English, but also included papers written in other languages such as Chinese and Korean, which might minimize the possibility of an English language bias.

Some limitations of our meta-analysis should also be mentioned. One limitation is that the included studies had relatively small sample sizes and were not uniformly designed, displaying obvious heterogeneity in our study. The discrepancy in the definition of controls (healthy people or PVD-free patients) might contribute to selection bias, considering that the control groups had different susceptibility to PVD. Second, the evidence of a significant association between *ACE* I/D polymorphism and PVD susceptibility among Asian populations may be weak, with only 5 studies reviewed. Thus, further large-sampled studies are warranted. Third, the lack of individual patient data hindered our ability to perform a thorough adjusted estimate for potential confounding factors (e.g. gender, obesity, smoking, and other lifestyles), as well as evaluate potential gene–gene interaction and gene–environment interaction.

Conclusions

In conclusion, the present meta-analysis provided evidence of an association between *ACE* I/D polymorphism and risk of PVD among Asian populations. However, currently, there is insufficient evidence to conclude that the association is consistent among Caucasian populations. In light of the overall limited existing data, the possible effect of study design on risk assessment is unclear. To confirm the hypothesis of the contribution of the D allele to genetic predisposition to PVD, additional longitudinal studies examining
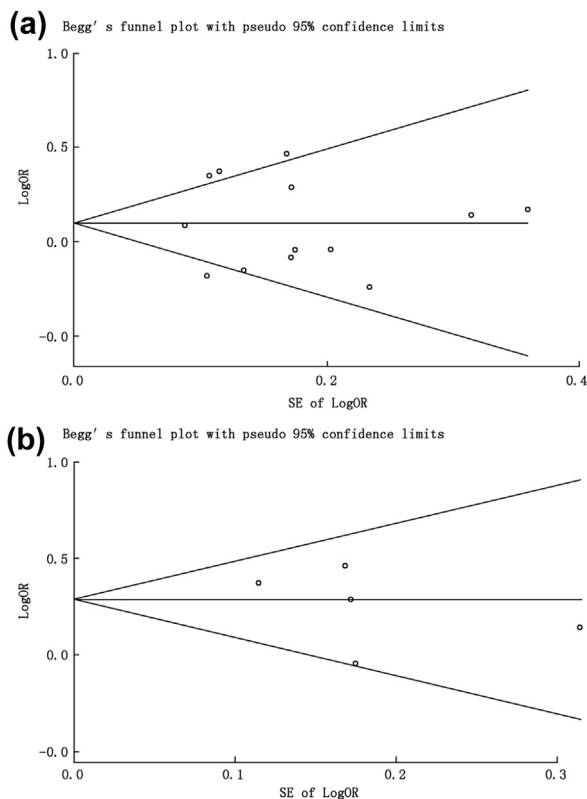

Fig. 5. Begg's funnel plot to explore the publication bias in the overall (a) and Asian population (b) (allele model: D *vs.* I).

gene–gene or gene-environmental interactions, as well as studies seeking to provide biological mechanisms or clinical confirmation of our results, are needed. Future studies should recruit homogeneous patients and well-matched controls from multi-ethnic populations. Such cohorts representative of the general population could enable a more conclusive evaluation of the role of *ACE* I/D polymorphism in the etiology of PVD.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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Edited by Jing-ling Bao