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Association Between Three eNOS Polymorphisms and Intracranial Aneurysms Risk

A Meta-Analysis

Chao Yang, MM, Zhen-yu Qi, MD, PhD, Chuan Shao, MM, Wei-kang Xing, MM, and Zhong Wang, MD, PhD

Abstract: Endothelial nitric oxide synthase (eNOS) is the catalyst of endothelial nitric oxide (NO) synthesis. Polymorphisms in the eNOS gene may influence the risk of intracranial aneurysm (IA), but the results of existing researches are still inconsistent. Thus, we performed the present meta-analysis to derive a more precise estimation between eNOS polymorphisms (T786C, G894T, 27-bp-variable number of tandem repeat [VNTR]) and IA risk.

Case–control studies evaluating the association between the eNOS polymorphisms and IA risk were searched in PubMed, Ovid & Embase, Web of Science, and Chinese Wanfang datasets with the last search up to July 15, 2014. The pooled odds ratios (ORs) for the association between eNOS polymorphisms and IA and their corresponding 95% confidence intervals (CIs) were estimated using the random or fixed-effects model.

Finally, 10 studies for T786C polymorphism (1819 cases and 1893 controls), 9 studies for G894T polymorphism (1393 cases and 1508 controls), and 7 studies for 27-bp-VNTR polymorphism (1281 cases and 1406 controls) were included in the meta-analyses. In the overall analysis, no evidence of association between eNOS polymorphisms and susceptibility of IA was found. When subgrouped by race descent, significantly increased risk was detected among Asians for T786C polymorphism (heterozygous comparison of codominant model: OR = 1.294, 95% CI = 1.025–1.634; dominant model: OR = 1.277, 95% CI = 1.019–1.600), but not in Caucasians or the other 2 polymorphisms.

Our meta-analysis suggested that T786C polymorphism was associated with increased risk of IA among Asians, whereas G894T and 27-bp-VNTR polymorphisms might have no influence on the susceptibility of IA.

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Abbreviations: aSAH = aneurysmal subarachnoid hemorrhage, eNOS = endothelial nitric oxide synthase, IA = intracranial aneurysm, SAH = subarachnoid hemorrhage.

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From the Department of Neurosurgery (CY, ZQ, WX, ZW), The First Affiliated Hospital of Soochow University, Suzhou, Jiangsu; and Department of Neurosurgery (CS), The Second Clinical Medical College of North Sichuan Medical College, Nanchong, Sichuan, China.
Correspondence: Zhen-yu Qi, Department of Neurosurgery, The First Affiliated Hospital of Soochow University, 188 Shizi Street Suzhou, Jiangsu 215006, China (e-mail: qizhenyu@suda.edu.cn).
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INTRODUCTION

The incidence of intracranial aneurysm (IA) in general population has been reported to be approximately 2% and the annual risk of IA rupture causing subarachnoid hemorrhage (SAH) was 1.9%.[1,2] Aneurysmal subarachnoid hemorrhage (aSAH) constitutes about 85% of overall SAH, which accounts for 5% of all cases of stroke.[3] Despite the development of intensive care and neurosurgical therapy, mortality of aSAH remains 27% to 50%[3–5] and only around 60% of survival can be cured without disability.[6] The high morbidity and mortality of aSAH make it a major public health problem. Yet, the main cause of IA remains obscure. Previous epidemiological studies have revealed innate (heritable connective tissue disorders, familial predisposition, and female gender) and postnatal (smoking and hypertension) factors of IA formation.[7,8] At the same time, pathophysiology researches also showed that hemodynamic factors, inflammatory factors, and elevated arterial blood pressure played important roles in the pathogenesis of IAs.[8–10] In the past decades, with the improvement of genetics and molecular biology, gene factors of IA were intensively investigated and polymorphisms of eNOS were one of the focuses.

Nitric oxide (NO), also known as ''endothelial-derived relaxing factor,''[11] is mostly produced by the catalyzing action of the 3 nitric oxide synthase (NOS) family enzymes via the conversion of L-arginine.[12] It is a multifunctional molecule and participates in a large number of biological reactions, for example, active biological mediator in relaxing vascular smooth muscle in response to vasoactive substances and shear stress,[11] vasodilatation maintenance the structure of the vessel wall,[13] inhibiting vascular smooth muscle cell proliferation,[14] and platelet and monocyte adhesion.[15,16] A substantial part of NO functions may participate in the mechanism of aneurysms formation and downregulation of NO level has been reported to be associated with several vascular diseases.[17]

The 3 members of NOS family are neuronal (nNOS/NOS1), inducible (iNOS/NOS2), and endothelial (eNOS/NOS3).[18] eNOS that is found primarily in the endothelium continuously generating NO serves to maintain basal vascular tone and cerebral blood flow, and its dysregulation may participate in the early development of aneurysms.[19] eNOS is encoded by gene located on chromosome 7*q*35–36 that has 26 exons that span >21 kb of the genome (GenBank D26607).[20] There are many functional polymorphisms in different regions of eNOS gene and several studies have been done to elucidate the relationships between polymorphisms and susceptibility of IAs. The T786C (rs2070744) is an important point mutation of thymine to cytosine at coden-786 in the 5′-flanking region of the eNOS gene, which could significantly reduce eNOS gene promoter activity and serum NO level[21]; G894T (Glu298Asp, rs1799983) corresponds to a Glu-Asp change at nucleotide 298

in exon 7 that demonstrated a trend for a reduced eNOS enzyme activity,[22] and 27-bp-VNTR that is a variable number of tandem repeats (VNTRs, 27 bp) in intron 4 accounts influencing basal plasma NO generation.[23] T786C, G894T, and 27-bp-VNTR were the 3 most clinically relevant IA-associated polymorphisms in the eNOS gene that have been reported. From 2003, these 3 polymorphisms have been reported to be associated with IA risk in a number of case–control studies with conflicting conclusions.[24–36] In this study, we performed meta-analyses and corresponding stratified analyses using currently available data to clarify the effects of eNOS T786C, G894T, and 27-bp-VNTR polymorphisms on the risk of IAs.

MATERIALS AND METHODS

Publication Search

We searched the articles using the following terms: ''intracranial aneurysm,'' ''cerebral aneurysm,'' ''brain aneurysm,'' ''SAH,'' ''subarachnoid hemorrhage'' in combination with ''polymorphism,'' ''variant'' in combination with ''eNOS,'' and ''endothelial nitric oxide synthase'' in PubMed, Web of Science, Ovid & Embase, and Chinese Wanfang databases from the established date to July 15, 2014. The reference lists of relevant articles were also retrieved to find additional articles. There was no limitation on language or publication year.

Selection Criteria

Studies selected for further meta-analysis must meet the following criteria: case–control studies, reports about associations between eNOS T786C, G894T, or 27-bp-VNTR polymorphism and risk of IA, and inclusion of genotype frequencies of case and control subjects to perform the related statistical analysis. Duplicated reports, reviews, meta-analysis articles, and meeting abstracts without adequate information were excluded. If multiple studies involved same subjects, only the complete one was used in the analysis. Results of article selection were compared and discrepancies were resolved by consensus.

Data Extraction

The following data was extracted carefully and independently by 2 authors from each eligible study: first author's last name, publication year, country, size of the study population (case/control), race, source of the control subjects, genotyping method, endpoint of IAs, percentage of female gender, mean age, and related genotype numbers of cases and controls. Any disagreements were resolved by discussion between the 2 authors.

Statistical Analysis

The Hardy–Weinberg equilibrium (HWE) for control subjects of each study was evaluated by Pearson goodness-of-fit χ^2 test. To evaluate the relationship between eNOS polymorphisms (T786C, G894T, 27-bp-VNTR) and risk of IAs, crude odds ratios (ORs) and their 95% confidence intervals (CIs) were applied. Three genetic models, for example, codominant, dominant, and recessive models, were used to determine the pooled OR. For T786C polymorphism, the codominant model was represented by heterozygous comparison of TC vs TT and homozygous comparison of CC vs TT, the dominant model was CC + TC vs TT and the recessive model was CC vs TC + TT. For G894T, the codominant model was heterozygous comparison of TG vs GG and homozygous comparison of TT vs GG, the dominant model was TT + TG vs GG and the recessive model was TT vs TG + GG. For 27-bp-VNTR polymorphism, the codominant model was represented by heterozygous comparison of ab vs bb and homozygous comparison of aa vs bb, the dominant model was aa + ab vs bb and the recessive model was aa vs ab + bb. Since the frequency of mutation homozygous genotype was equal to zero, studies by Song et al[29] for T786C polymorphism and Kim et al[32] for G894T polymorphism were excluded in recessive model and homozygous comparison of codominant model.[37]

Heterogeneity between studies was evaluated by Q test and I^2 statistic. Heterogeneity was considered significant when $P < 0.05$ in Q test.[38] Low heterogeneity is considered when $I^2 < 25\%$, moderate heterogeneity when $I^2 = 25\%$ to 50%, and high heterogeneity $I^2 > 50\%$.[39]

The fixed-effects model was subsequently used to calculate the pooled ORs when $P > 0.05$. Otherwise, the random-effects model was applied. Subgroup analyses were performed by endpoint of IAs (RIA and mixed), racial descent (Asian and Caucasian), and control source (hospital-based [HCC] and population-based case–control [PCC] study). Sensitivity analysis was conducted by sequential removing each individual study and possible publication bias was calculated by the Begg[40] and the Egger tests.[41] The STATA software version 12.0 (STATA Corporation, College Station, TX) was used to carry out all statistical analysis.

RESULTS

Study Selection and Subject Characteristics

The flow diagram of study selection procedure was shown in Figure 1. A total of 88 articles were originally identified, including 22 from PubMed, 26 from Web of Science, 36 from Ovid & Embase, and 4 from Chinese Wanfang database. No additional finding through screening article reference was retrieved. After evaluating articles according to selection

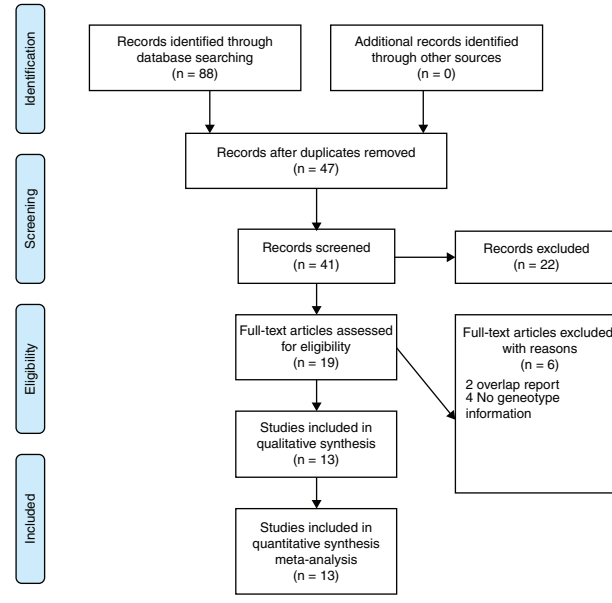

FIGURE 1. Flowchart showing the different phases of the meta-analysis.

TABLE 1. Study Characteristics of Individual Studies Included in the Meta-Analysis

Author	Year	Country in Which Conducted	Racial Descent	Control Source	Gender*	Age†	Method	Cases Pathologic
Khurana	2003	USA	Caucasian	PCC	67.0/49.0	54.0/69.0	Microarray	RA
Khurana	2004	USA	Caucasian	PCC	69.0/49.0	54.0/69.0	Microarray, microfluidic chip	RA
Akagawa	2005	Japan	Asian	HCC	65.5/40.2	59.3/63.7	Sequencing	Mixed
Akagawa	2005	Korea	Asian	HCC	71.7/42.4	55.2/61.7	Sequencing	RA
Krex	2006	German	Caucasian	PCC	NA	NA	Sequencing, PCR-RFLP	Mixed
Krischek	2006	Japan	Asian	HCC	47.7/56.8	58.0/54.7	Sequencing	Mixed
Song	2006	Korea	Asian	HCC	58.6/54.0	53.7/61.8	Sequencing	RA
Koshy	2008	India	Caucasian	HCC	42.6/50.9	51.5/46.7	Sequencing, PCR-RFLP	RA
Ozum	2008	Turkey	Caucasian	PCC	67.9/66.6	54.2/50.0	PCR-RFLP	RA
Xu	2009	China	Asian	PCC	53.4/43.3	46.3/43.9	PCR-RFLP, Sequencing	RA
Kim	2011	Korea	Asian	PCC	62.4/62.0	52.9/55.2	PCR-RFLP	Mixed
Bi	2010	China	Asian	HCC	55.0/54.6	46.7/42.8	Sequencing	Mixed
Liu	2013	China	Asian	PCC	NA	NA	PCR-RFLP	Mixed
Staalsø	2014	Denmark	Caucasian	HCC	70.0/50.8	56.0/55.0	Taqman, PCR-RFLP	RA

HCC = hospital-based case–control study, PCC = population-based case–control study, NA = not applicable, PCR-RFLP = polymerase Chain reaction-restriction fragment length polymorphism, RA = ruptured aneurysm.

* Indicates % of males in cases/% of males in controls.

† Indicates mean age of cases/mean age of controls.

criteria, 13 articles including 11 published journal articles and 2 unpublished academic dissertations were eligible for this meta-analysis. Two studies by Khurana et al[24,25] shared some common subjects. The study[24] only concerning T786C polymorphism contains more cases than the other about T786C, G894T, and 27-bp-VNTR polymorphisms. Therefore, the former study was included in the meta-analysis of T786C polymorphism and the later was employed in G894T and 27-bp-VNTR polymorphisms. One article by Akagawa et al[26] reported 2 studies in Japan and Korea and both of the studies met the selection criteria; therefore, we included them as single study.

Characteristics of the Studies

Table 1 summarizes the characteristics of included studies. The ruptured IA (RIA) in all studies was verified during surgery and the unruptured IA (UIA) was diagnosed by angiography or

TABLE 2. Genotype Information for Main Meta-Analysis

Polymorphism				Case			Control			
T786C	Author	Year	Cases/Controls	TT	TC	CC	TT	TC	CC	HWE
	Khurara *	2003	52/90	12	35	5	28	46	16	0.699
	Akagawa *	2005	220/214	176	41	3	179	34	1	0.648
	Akagawa†	2005	191/191	144	46	1	149	41	1	0.304
	Krex	2006	135/184	48	60	27	71	86	27	0.908
	Krischek	2006	405/176	326	72	7	145	23	8	<0.01
	Song	2006	132/113	106	26	0	100	13	0	0.516
	Koshy	2008	122/224	68	51	3	136	81	7	0.219
	Kim	2011	149/121	122	24	3	99	22	0	0.271
	Liu	2013	82/82	59	19	4	69	12	1	0.569
	Staalsø	2014	331/498	145	147	39	197	233	68	0.946
G894T				GG	GT	TT	GG	GT	TT	
	Khurara	2004	51/90	20	25	6	20	55	15	0.032
	Krex	2006	142/190	64	67	11	96	76	18	0.602
	Krischek	2006	405/176	349	50	6	145	23	8	<0.01
	Koshy	2008	122/224	85	35	2	159	61	4	0.501
	Ozum	2008	53/60	26	4	23	34	12	14	<0.01
	Xu	2009	58/67	27	17	14	45	15	7	<0.01
	Kim	2011	149/121	125	24	0	98	23	0	0.248
	Liu	2013	82/82	49	25	8	36	38	8	0.656
	Staalsø	2014	331/498	151	153	27	233	216	49	0.918
27-bp-VNTR				bb	ab	aa	bb	ab	aa	
	Khurara	2004	51/90	25	25	1	70	16	4	0.028
	Krex	2006	142/189	98	41	3	126	55	8	0.525
	Krischek	2006	405/176	325	70	10	143	30	3	0.341
	Koshy	2008	122/224	77	40	5	143	77	4	0.077
	Bi	2010	80/107	59	18	3	91	16	1	0.753
	Kim	2011	149/121	122	24	3	96	25	0	0.205
	Staalsø	2014	332/498	254	71	7	344	145	9	0.156

HWE = *P* value of goodness-of-fit test for Hardy–Weinberg equilibrium, VNTR = variable number of tandem repeat.

* Study performed in Japan.

† Studies performed in Korea.

TABLE 3. Pooled ORs and 95% CIs of Meta-Analysis

			Codominant						Dominant			Recessive		
			TC vs TT			CC vs TT			CC + TC vs TT			CC vs TC + TT		
Polymorphism T786C	Study[#]	Case/Control	OR (95% CI)	P_h	I^2 (%)	OR (95% CI)	P_h	I^2 (%)	OR (95% CI)	P_h	I^2 (%)	OR (95% CI)	P_h	I^2 (%)
Total	10	1819/1893	1.137 (0.972–1.331)	0.384	6.2	0.957 (0.705–1.301)	0.263	20.2	1.120 (0.963–1.303)	0.378	6.9	0.948 (0.712–1.264)	0.193	28.3
Racial descent														
Asian	6	1179/897	1.294 (1.025–1.634)	0.622	0.0	1.092 (0.529–2.254)	0.114	46.3	1.277 (1.019–1.600)	0.593	0.0	1.043 (0.506–2.150)	0.113	46.5
Caucasian	4	640/996	1.019 (0.823–1.262)	0.267	24.0	0.930 (0.663–1.305)	0.433	0.0	1.005 (0.820–1.233)	0.304	17.5	0.932 (0.681–1.274)	0.278	22.1
Control source														
PCC	4	418/477	1.193 (0.867–1.641)	0.353	8.1	1.497 (0.885–2.531)	0.370	4.6	1.267 (0.932–1.721)	0.466	0.0	1.280 (0.799–2.050)	0.149	43.8
HCC	6	1401/1416	1.120 (0.935–1.342)	0.285	19.7	0.757 (0.516–1.110)	0.536	0.0	1.075 (0.904–1.279)	0.281	20.2	0.795 (0.552–1.145)	0.476	0.0
Type														
RAs	5	828/1116	1.097 (0.895–1.344)	0.159	39.3	0.784 (0.527–1.168)	0.995	0.0	1.058 (0.869–1.288)	o.171	37.6	0.784 (0.541–1.137)	0.830	0.0
Mixed*	5	991/777	1.201 (0.936–1.540)	0.610	0.0	1.300 (0.795–2.126)	0.086	50.9	1.215 (0.960–1.538)	0.651	0.0	1.281 (0.805–2.038)	0.079	52.2
G894T	Study[#]	Case/Control	GT vs GG			TT vs GG			TT + GT vs GG			TT vs GT + GG		
			OR (95% CI)	P_h	I^2 (%)	OR (95% CI)	P_h	I^2 (%)	OR (95% CI)	P_h	I^2 (%)	OR (95% CI)	P_h	I^2 (%)
Total	9	1393/1508	0.974 (0.819–1.159)	0.063	45.9	0.940 (0.567–1.559)	0.023	56.7	0.959 (0.738–1.245)	0.026	54.2	1.002 (0.628–1.601)	0.036	53.4
Racial descent														
Asian	4	694/446	0.846 (0.617–1.162)	0.098	52.4	0.921 (0.233–3.636)	0.006	80.2	0.906 (0.519–1.579)	0.018	70.3	0.965 (0.285–3.268)	0.015	76.2
Caucasian	5	699/1062	1.034 (0.841–1.272)	0.116	46.0	0.955 (0.590–1.545)	0.194	34.2	1.028 (0.787–1.342)	0.189	34.8	1.008 (0.622–1.632)	0.159	39.4
Control source														
PCC	6	535/610	0.803 (0.498–1.295)	0.020	62.7	1.177 (0.585–2.366)	0.033	61.9	0.957 (0.603–1.520)	0.007	68.2	1.311 (0.732–2.348)	0.088	50.6
HCC	3	858/898	1.052 (0.838–1.320)	0.823	0.0	0.940(0.567–1.559)	0.244	29.1	0.983 (0.792–1.219)	0.466	0.00	0.657 (0.365–1.182)	0.277	22.1
Type														
RAs	5	778/596	1.017 (0.813–1.273)	0.083	51.5	1.211 (0.601–2.440)	0.027	63.6	1.091 (0.736–1.619)	0.037	60.9	1.292 (0.693–2.410)	0.047	58.5
Mixed	4	615/939	0.913 (0.695–1.201)	0.103	51.5	0.635 (0.338–1.194)	0.282	21.0	0.828 (0.578–1.187)	0.139	45.3	0.663 (0.352–1.247)	0.263	15.1
27-bp-VNTR	Study[#]	Case/Control	ab vs bb			aa vs bb			aa + ab vs bb			aa vs ab + bb		
			OR (95% CI)	P_h	I^2 (%)	OR (95% CI)	P_h	I^2 (%)	OR (95% CI)	P_h	I^2 (%)	OR (95% CI)	P_h	I^2 (%)
Total	7	1281/1406	1.115 (0.760–1.634)	0.001	73.5	1.291 (0.761–2.191)	0.498	0.0	1.132 (0.794–1.612)	0.002	71.1	1.277 (0.757–2.156)	0.447	0.0
Racial descent														
Asian	3	634/405	1.055 (0.701–1.589)	0.245	29.0	2.332 (0.816–6.661)	0.562	0.0	1.144 (0.764–1.713)	0.230	32.0	2.299 (0.807–6.547)	0.577	0.0
Caucasian	4	647/1001	1.182 (0.640–2.183)	0.000	84.6	0.995 (0.524–1.886)	0.434	0.0	1.142 (0.657–1.983)	0.001	82.1	0.990 (0.526–1.863)	0.344	9.8
Control source														
PCC	3	342/400	1.415 (0.557–3.592)	0.001	85.3	0.829 (0.316–2.174)	0.333	9.0	1.353 (0.596–3.073)	0.003	82.3	0.756 (0.293–1.947)	0.292	18.7
HCC	4	939/1006	0.944 (0.664–1.343)	0.087	54.5	1.577 (0.828–3.002)	0.614	0.0	1.009 (0.695–1.466)	0.048	61.9	1.636 (0.859–3.118)	0.708	0.0
Type														
RAs	4	696/486	1.391 (0.671–2.882)	0.000	86.4	1.475 (0.739–2.945)	0.521	0.0	1.397 (0.711–2.742)	0.000	82.5	1.435 (0.725–2.841)	0.447	0.0
Mixed	3	585/920	0.933 (0.694–1.255)	0.735	0.0	1.081 (0.480–2.435)	0.255	26.8	0.951 (0.714–1.265)	0.803	0.0	1.091 (0.486–2.447)	0.249	28.0

CI = confidence interval, I^2 = the proportion of the total variation across studies due to heterogeneity, OR = odds ratio, Ph = *P* value for heterogeneity test, , RAs = ruptured aneurysms, RIA = ruptured IA, UIA = unruptured IA.

* Includes study cases in which the endpoint was mixed of UIA and RIA.

[#] The number of studies.

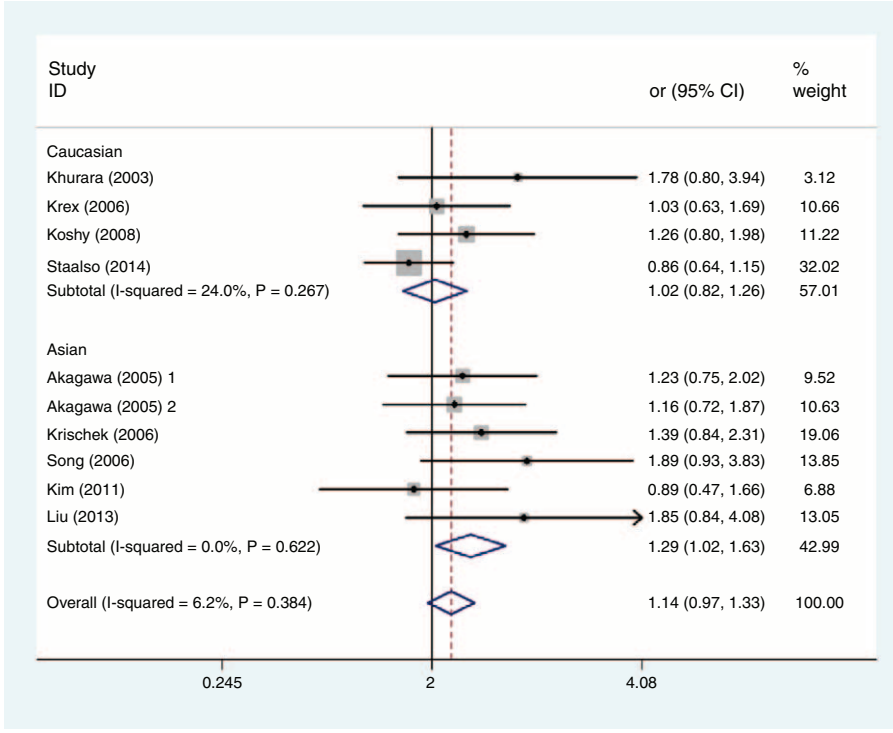

FIGURE 2. Forest plot of ORs with a fixed-effect model for association between the eNOS T786C polymorphism and subgroup IA risk under codominant model (TC vs TT). eNOS = endothelial nitric oxide synthase, OR = odds ratio.

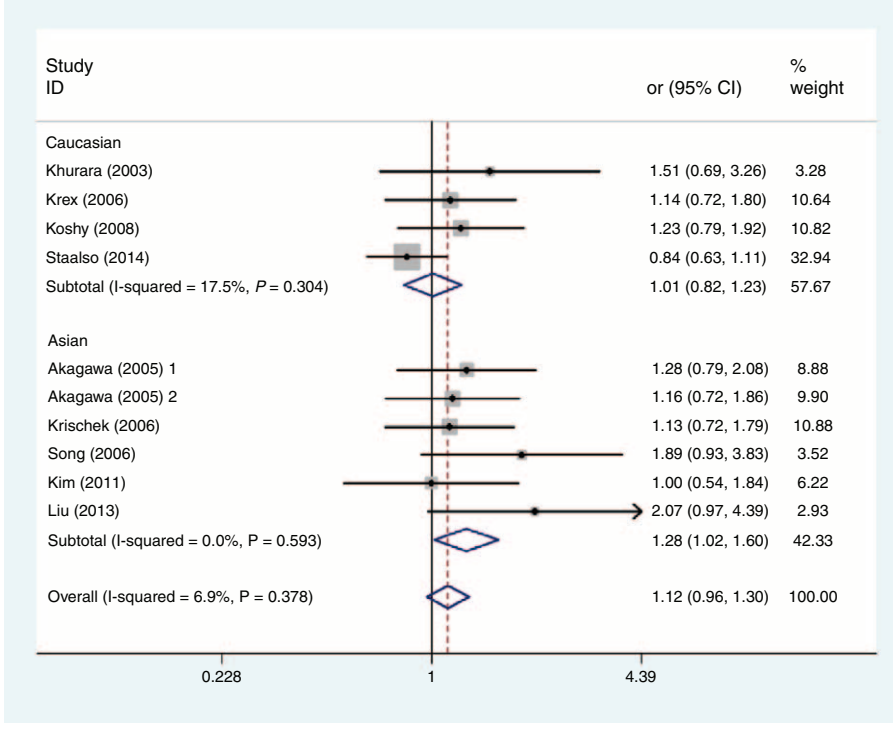

FIGURE 3. Forest plot of ORs with a fixed-effect model for association between the eNOS T786C polymorphism and subgroup IA risk under dominant model (TC + CC vs TT). eNOS = endothelial nitric oxide synthase, OR = odds ratio.

surgical findings. Completed genotype information was obtained and HWE tests in the controls were calculated (Table 2). Genotype distribution in most control groups was consistent with HWE with some exceptions.[25,28,31,35] There were 10 studies in 9 articles including 1819 cases and 1893 controls for the eNOS T786C polymorphism. Five studies were carried out in Asians and 5 in Caucasians. Four studies used population-based control subjects and 6 used hospital-based control subjects (Tables 1 and 2). Furthermore, 5 studies used RIA patients as case subjects and the other 5 used both RIA and UIA patients.

For eNOS G894T polymorphism IAs analysis, 9 studies involving 1393 cases and 1508 controls were identified for our meta-analysis. In the 9 studies, 4 were Asians and 5 Caucasians. Three of these studies were of PCC design, whereas the other 6 studies were of HCC design. Cases in the 5 studies came from RIA patients and 4 were from RIA and UIA patients (Tables 1 and 2).

eNOS 27-bp-VNTR polymorphism was conducted for 1281 cases and 1406 controls in 7 studies. Subjects of 3 studies originated from Asian populations, whereas the other 4 were from Caucasian populations. Controls were recruited randomly from hospitals in 4 studies or the general population in 3 studies. RIA patients were used as cases in 4 studies, and RIA and UIA patients were used in 3 studies (Tables 1 and 2).

Meta-Analysis

Table 3 shows the main results of this meta-analysis and the heterogeneity test of the eNOS T786C, G894T, and 27-bp-VNTR polymorphisms and IAs. For the 3 polymorphisms, the combined results based on all studies did not show any significant associations between the polymorphisms and IAs risk for all genetic models (Table 3). As stratified by ethnicity, our results showed that T786C polymorphism was associated with increased risk of IA in dominant model (CC + TC vs TT; OR = 1.277, 95% CI = 1.019–1.600) and heterozygous comparison of codominant model (TC vs TT; OR = 1.294, 95% CI = 1.025–1.634) among Asians (Figures 2 and 3), but the association did not emerge in the other genetic models of Asians or among Caucasians (Table 3). Furthermore, there was no evidence for the association between the other 2 polymorphisms and IA risk in stratified analysis based on the source of controls, ethnicity, or endpoint of IA (Table 3).

Test of Heterogeneity and Sensitivity Analyses

The heterogeneity test showed that there was no significant between-study heterogeneity in terms of the eNOS T786C polymorphism (Table 3). However, significant heterogeneity in G894T polymorphism (homozygous comparison of codominant model: $P_h = 0.023$, $I^2 = 56.7\%$; dominant model: $P_h = 0.026$, $I^2 = 54.2\%$; and recessive model: $P_h = 0.036$, $I^2 = 53.4\%$), and 27-bp-VNTR polymorphism (homozygous comparison of codominant: $P_h = 0.001$, $I^2 = 73.5\%$; dominant model: $P_h = 0.001$, $I^2 = 71.1\%$) (Table 3) was observed. To explore the potential sources of heterogeneity across studies, we assessed the pooled ORs under all comparisons via subgroup and sensitivity analyses. In the subgroup analysis by race, the heterogeneity of G894T was significant in the Asian studies. When stratified by source of control and endpoint of cases, heterogeneity of G894T in population-based studies and RIA cases group was significant in all models except heterozygous comparison of codominant models (Table 3). It is interesting that when we excluded the Asian population-based controls

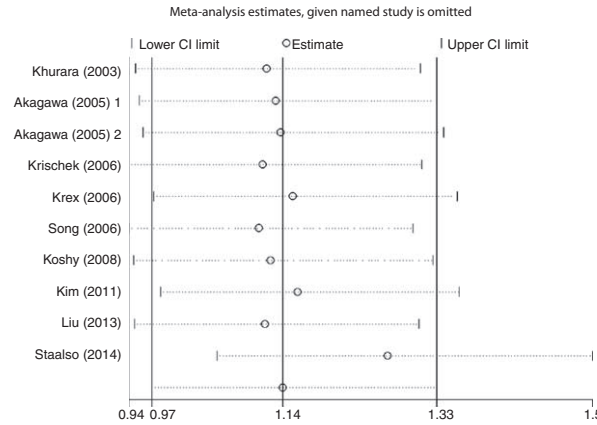

FIGURE 4. Sensitivity analyses of eNOS T786C polymorphism in TC vs TT model. eNOS = endothelial nitric oxide synthase.

study with RIA cases by Xu,[35] the heterogeneity was significantly decreased in all genetic models. So we can say that the study by Xu[35] contributed to substantial heterogeneity of G894T polymorphism. In the stratified study of 27-bp-VNTR polymorphism, heterogeneity was significant in Caucasian and population-based studies when subgrouped by racial and source of control. Similarly, when we excluded a study with Caucasian subjects and population-based controls by Khurana et al,[25] the heterogeneity of homozygous comparison of codominant and dominant models for 27-bp-VNTR polymorphism was obviously reduced. So the study by Khurana et al[25] may contribute to heterogeneity of 27-bp-VNTR polymorphism. Furthermore, sensitivity analysis was performed to evaluate the stability of the results by excluding 1 single study at a time. The overall association between T786C polymorphism and IA risk was significantly influenced by the study of Staalsø et al.[34] When excluding the study by Staalsø et al, an increased risk of significance was associated with T786C polymorphism in heterozygous comparison of codominant model (OR = 1.266, 95% CI = 1.050–1.526) and dominant model (OR = 1.255, 95% CI = 1.049–1.503) (Figures 4 and 5). The pooled ORs and corresponding 95% CIs were not significantly altered when any individual study was excluded for the other models of T786C and all models of G894T and 27-bp-VNTR polymorphisms (data not shown).

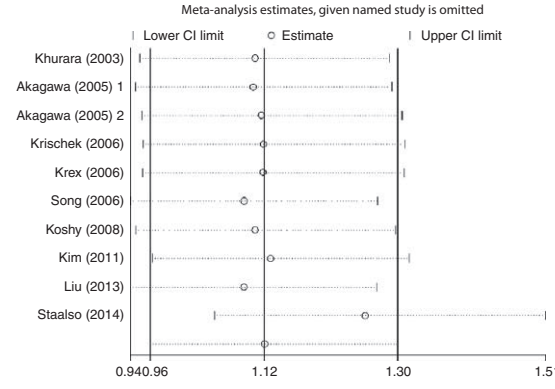

FIGURE 5. Sensitivity analyses of eNOS T786C polymorphism in TC + CC vs TT model. eNOS = endothelial nitric oxide synthase.

TABLE 4. *P* Values for Publication Bias Tests

Polymorphism	Codominant		Dominant	Recessive
T786C	TC vs TT	CC vs TT	CC + TC vs TT	CC vs TC + TT
Begg test	0.074	0.251	0.032	0.348
Egger test	0.005	0.268	0.001	0.508
G894T	GT vs GG	TT vs GG	GT + TT vs TT	TT vs GT + GG
Begg test	0.175	0.902	0.602	0.902
Egger test	0.177	0.880	0.682	0.914
27-bp VNTR	ab vs bb	aa vs bb	aa + ab vs bb	aa vs ab + bb
Begg test	0.230	0.368	0.230	0.368
Egger test	0.024	0.315	0.015	0.504

VNTR = variable number of tandem repeat.

Publication Bias

The Begg and the Egger tests were applied to evaluate the publication bias. The results of the Begg and the Egger tests suggest possible evidence of publication bias in the meta-analysis of T786C codominant model TC vs TT (Begg $P = 0.074$, Egger $P = 0.005$) and dominant model (Begg $P = 0.032$, Egger $P = 0.001$), also in 27-bp-VNTR codominant model ba vs bb (Begg $P = 0.230$, Egger $P = 0.024$) and dominant model (Begg $P = 0.230$, Egger $P = 0.015$). But no evidence of publication bias in all genetic models of G894T polymorphism was observed (Table 4). The Begg funnel plot was also constructed and the shape of the plot is consistent with the calculation results (Figures 6–8).

DISCUSSION

Because of the versatile NO that has a role in the regulation of vascular tone, hemodynamic changes, and remolding vessel wall, a lot of studies have been done to investigate the contribution made by eNOS polymorphisms to the formation and progression of various vascular diseases.[18] Khurana et al[24] first investigated the relationship between the T786C polymorphism and IA susceptibility and no significant association was found. After that, 9 studies from 8 articles had been done,[26–30,32–34] and the result of 5 studies was negative.[27,30,32–34] The other 4 Asian studies have analyzed genotype of cases and controls but did not conclude the association.[26,28,29] For G894T, 3 of 9 studies showed significant associations with IA risk,[31,33,35] and

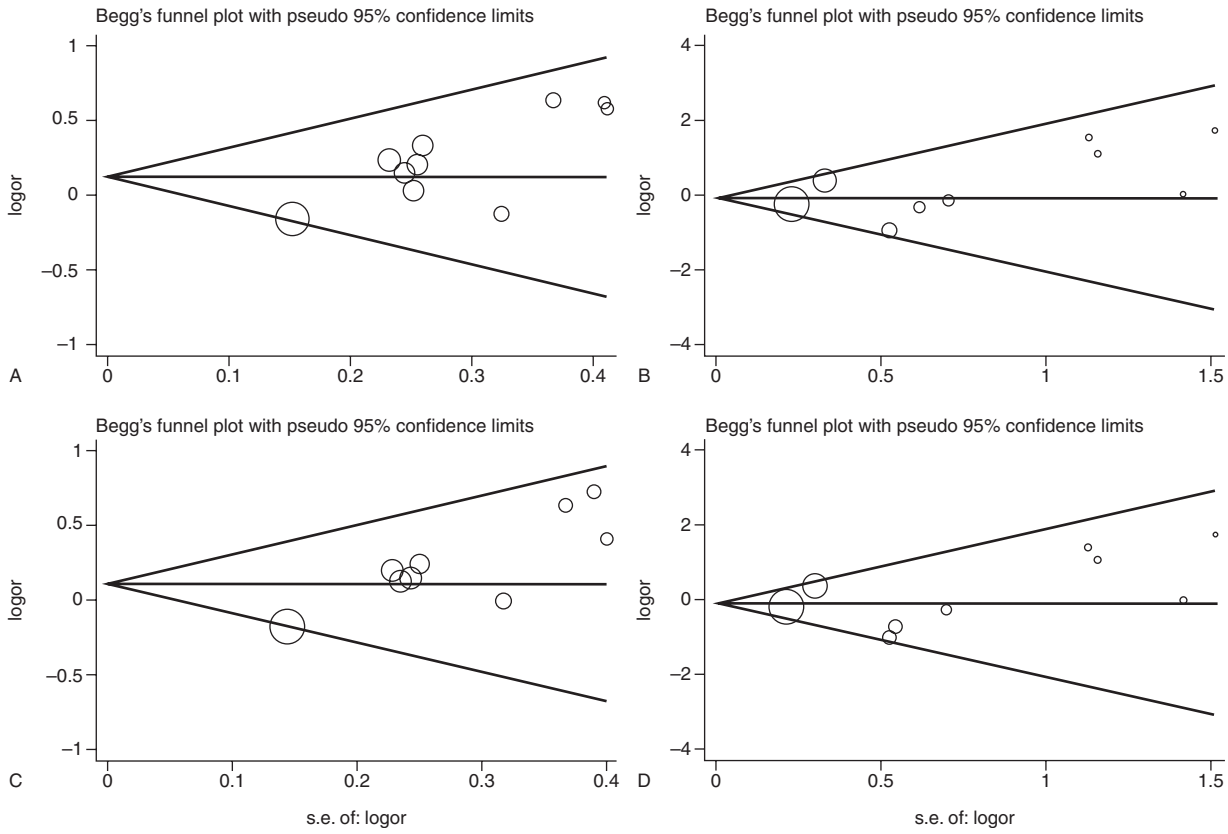

FIGURE 6. Begg funnel plot for publication bias test of T786C. (A) TC vs TT. (B) CC vs TT. (C) CC + TC vs TT. (D) CC vs TC + TT, SE = Standard Error.

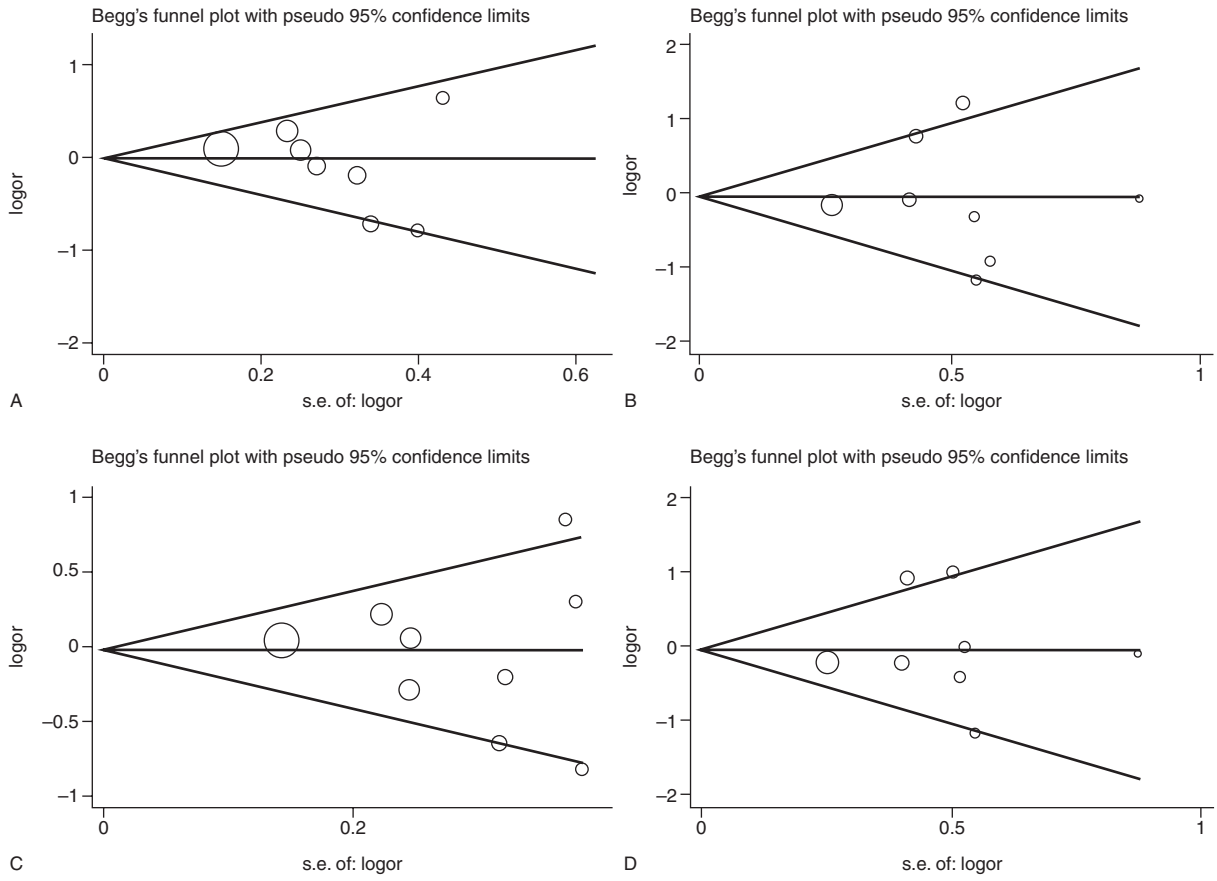

FIGURE 7. Begg funnel plot for publication bias test of G894T. (A) GT vs GG. (B) TT vs GG. (C) TT + GT vs GG. (D) TT vs GT + GG, SE = Standard Error.

the other 6 got inverse result.[25,27,28,30,32,34] Seven studies investigated 27-bp-VNTR and the risk of IA, and significant association was found in 3 of them,[25,34,36] but was not found in the remaining 4.[27,28,30,32]

A meta-analysis study focusing on the relationship between the 3 eNOS polymorphisms and risk of IA was performed in 2010.[42] In that meta-analysis, T786C polymorphism was significantly associated with IA risk, but neither G894T nor 27-bp-VNTR showed significant association. After that, several articles holding different viewpoints about that topic have been published. Here, we conducted a comprehensive meta-analysis to shed light on the role of eNOS polymorphisms in IA risk.

One thousand eight hundred nineteen cases and 1893 controls from 10 studies in 9 articles were eligible for the meta-analysis and no evidence for the association between eNOS T786C polymorphism and IA susceptibility was found in the overall result. However, in subgroup analysis by ethnicity, significant association was found in Asians in heterozygote comparison (OR = 1.294, 95% CI = 1.025–1.634) and the dominant model (OR = 1.277, 95% CI = 1.019–1.600), but not in Caucasians. Given that people in different region share little in environmental backgrounds and lifestyles, this discrepancy in the results between Asians and Caucasians suggested that eNOS T786C polymorphism may play a penetrance role in IA susceptibility in an ethnicity-specific manner. In fact, differences in the distribution of eNOS polymorphisms in Asians and Caucasians have been reported by Tanus-Santos et al.[44] In sensitivity analysis, when we deleted 1 Caucasian study by Staalsø et al,[34] significant association was observed in heterozygous comparison (OR = 1.266, 95% CI = 1.050–1.526) and dominant model (OR = 1.255, 95% CI = 1.049–1.503). The results of sensitivity analysis indicated that the main meta-analysis may be influenced powerfully by the study of Staalsø et al for its Caucasian subjects and biggest sample size (case = 331, control = 498), as well as the relatively small simple size of the whole study. Therefore, this finding make the result of overall analysis should be explained carefully and confirmed in future studies.

For eNOS G894T and 27-bp-VNTR polymorphisms, there were 9 studies containing 1393 cases vs 1508 controls and 7 studies about 1281 cases vs 1406 controls eligible. We found no significant association between both of the polymorphisms and IA risk, which were consistent with the majority but not all previous studies.[25,27,28,30–36] The studies by Özüm et al,[31] Xu,[35] and Liu et al[33] found association between increased risk of IA and G894T polymorphism. Studies by Khurana et al[25] and Kim et al[32] found association between increased risk of IA and 27-VNTR polymorphism. The inconsistency of these studies may be explained by differences in population background, source of controls, sample size, and also by chance. In the subgroup according to study design, race, and endpoint of IAs, similar trends with overall results were observed. In sensitivity analysis, our results were not meaningfully influenced by any individual study in all models of G894T and 27-bp-VNTR polymorphisms.

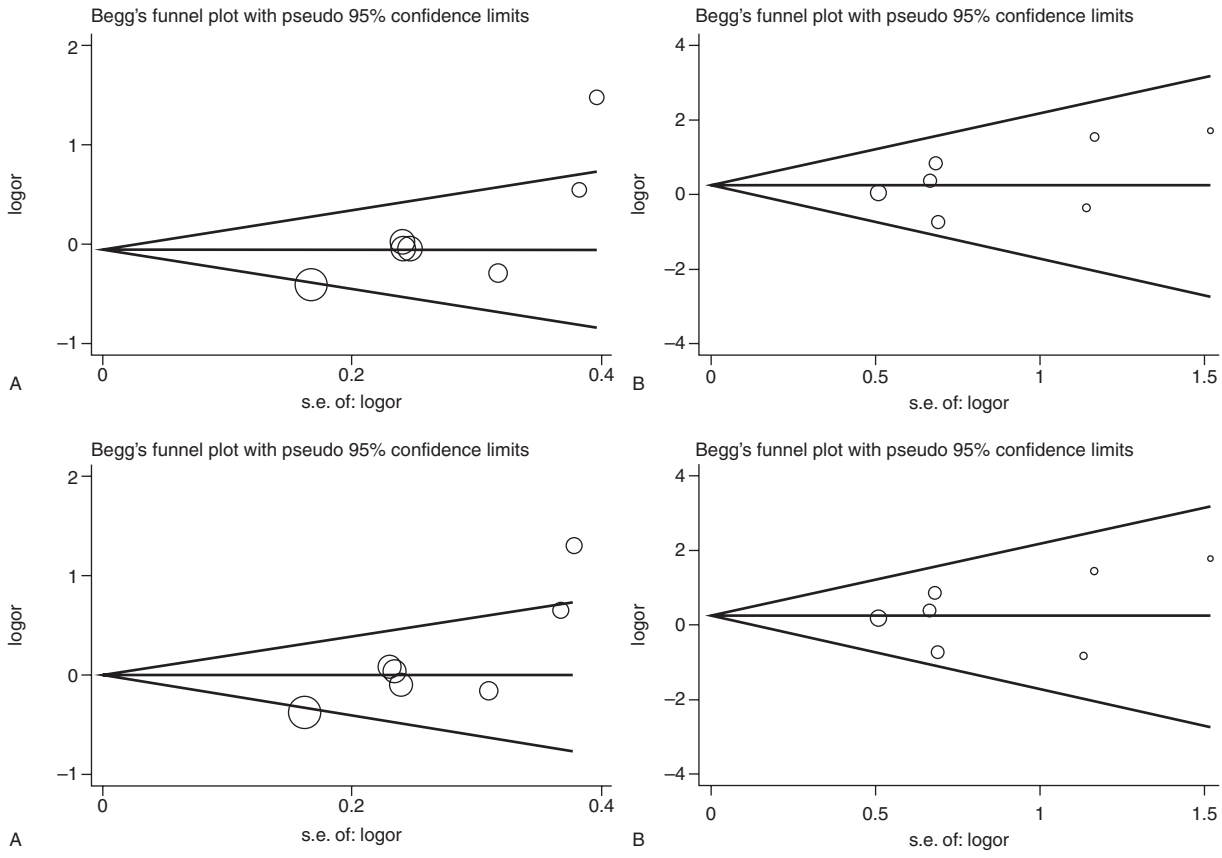

FIGURE 8. Begg funnel plot for publication bias test of 27-bp-VNTR. (A) ab vs bb. (B) aa vs bb. (C) aa + ab vs bb. (D) aa vs ab + bb. VNTR = variable number of tandem repeat, SE = Standard Error.

For the 3 polymorphisms of eNOS, 2 studies[28,43] tried to investigate their effect to the rupture risk of IAs. The study by Khurana et al[43] found evidence of association between all of the 3 polymorphisms and rupture risk of IAs but inverse result was found by Krischek et al.[28] In our present meta-analysis, RIA was applied in 7 studies and mixed RIA and UIA in 6 studies (Table 1), and no study involved the UIA. To explore the potential influence of different endpoint, subgroup analysis by different endpoint was performed, and significant association was found in neither of the group in different models of the 3 eNOS polymorphisms.

Heterogeneity was observed in G894T polymorphism (homozygous comparison of codominant model: $P_h = 0.023$, $I^2 = 56.7\%$; dominant model: $P_h = 0.026$, $I^2 = 54.2\%$; and recessive model: $P_h = 0.036$, $I^2 = 53.4\%$), and 27-bp-VNTR polymorphism (homozygous comparison of codominant: $P_h = 0.001$, $I^2 = 73.5\%$; dominant model: $P_h = 0.001$, $I^2 = 71.1\%$). The heterogeneity might arise from different characteristics of selected studies, such as study design, sample sizes, inclusion criteria, ethnicity, endpoint of IAs, and different genotyping methodologies. In the stratification and sensitivity analyses, we found that the study by Xu[35] did contribute to potential heterogeneity of G894T polymorphism and the study by Khurana et al[25] produced heterogeneity of 27-bp-VNTR polymorphism, whereas influence analysis suggested that the pooled ORs for the G894T and 27-bp-VNTR polymorphisms were not influenced by the 2 studies. In this view, the results of our meta-analysis were reliable.

Evidence of publication bias was observed in codominant model heterozygous comparison and dominant model for both T786C and 27-bp-VNTR polymorphisms in the Begg and the Egger tests. To some extent, the publication bias was inevitable for this study because of the limited databases searched, the little number of eligible studies, and only English and Chinese publications included. Therefore, some unpublished and published studies in other databases or in other languages that may meet the inclusion criteria were likely to be missed.

Some limitations should be admitted when explaining the results of our meta-analysis. First, the number of eligible studies and subjects of studies was not large enough for an integrated analysis, especially for subgroup analyses. Therefore, our results should be explained with caution. Second, some inclusion studies[25,28,31,34] whose genotype distribution in control group was not consistent with HWE may do contribute to the bias of the meta-analysis, although the results were not affected by these studies in sensitivity analysis. Finally, our results were based on single-factor estimates without adjustments for other risk factors. Further evaluation of IA risk should pay more attention to the potential interactions among gene–gene, gene–environment, and even different polymorphism loci of the same gene.

In conclusion, our meta-analysis indicates that eNOS T786C polymorphism is associated with elevated IA risk among Asians but not in Caucasians, whereas G894T and 27-bp-VNTR polymorphisms might have no influence on the susceptibility of IA. However, large well-designed studies are needed to be

performed using standardized genotyping methods, homogeneous cases, and well-matched controls. In addition, further studies investigating the effect of gene–gene and gene–environment interactions may eventually lead to our better, comprehensive understanding of the association between the eNOS polymorphisms and IA risk.

REFERENCES

1. Rinkel GJ, Djibuti M, Algra A, et al. Prevalence and risk of rupture of intracranial aneurysms: a systematic review. *Stroke.* 1998;29:251–256.
2. Pakarinen S. Incidence, aetiology, and prognosis of primary subarachnoid haemorrhage: a study based on 589 cases diagnosed in a defined urban population during a defined period. *Acta Neurol Scand.* 1967;43:1–28.
3. van Gijn J, Kerr RS, Rinkel GJ. Subarachnoid haemorrhage. *Lancet.* 2007;369:306–318.
4. Nieuwkamp DJ, Setz LE, Algra A, et al. Changes in case fatality of aneurysmal subarachnoid haemorrhage over time, according to age, sex, and region: a meta-analysis. *Lancet Neurol.* 2009;8:635–642.
5. Karamanakos PN, von Und Zu Fraunberg M, Bendel S, et al. Risk factors for three phases of 12-month mortality in 1657 patients from a defined population after acute aneurysmal subarachnoid hemorrhage. *World Neurosurg.* 2012;78:631–639.
6. Malmivaara K, Juvela S, Hernesniemi J, et al. Health-related quality of life and cost-effectiveness of treatment in subarachnoid haemorrhage. *Eur J Neurol.* 2012;19:1455–1461.
7. Wiebers DO, Whisnant JP, Huston J 3rd et al. Unruptured intracranial aneurysms: natural history, clinical outcome, and risks of surgical and endovascular treatment. *Lancet.* 2003;362:103–110.
8. Caranci F, Briganti F, Cirillo L, et al. Epidemiology and genetics of intracranial aneurysms. *Eur J Radiol.* 2013;82:1598–1605.
9. Turjman AS, Turjman F, Edelman ER. Role of fluid dynamics and inflammation in intracranial aneurysm formation. *Circulation.* 2014;129 (3):373–382.
10. Chalouhi N, Hoh BL, Hasan D. Review of cerebral aneurysm formation, growth, and rupture. *Stroke.* 2013;44:3613–3622.
11. Palmer RM, Ferrige AG, Moncada S. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature.* 1987;327:524–526.
12. Knowles RG, Palacios M, Palmer RM, et al. Formation of nitric oxide from L-arginine in the central nervous system: a transduction mechanism for stimulation of the soluble guanylate cyclase. *Proc Natl Acad Sci USA.* 1989;86:5159–5162.
13. Rudic RD, Shesely EG, Maeda N, et al. Direct evidence for the importance of endothelium-derived nitric oxide in vascular remodeling. *J Clin Invest.* 1998;101:731–736.
14. Napoli C, Paolisso G, Casamassimi A, et al. Effects of nitric oxide on cell proliferation: novel insights. *J Am Coll Cardiol.* 2013;62:89–95.
15. Wolf A, Zalpour C, Theilmeier G, et al. Dietary larginine supplementation normalizes platelet aggregation in hypercholesterolemic humans. *J Am Coll Cardiol.* 1997;29:479–485.
16. Kubes P, Suzuki M, Granger DN. Nitric oxide: an endogenousmodulator of leukocyte adhesion. *Proc Natl Acad Sci USA.* 1991;88:4651–4655.
17. Oemar BS, Tschudi MR, Godoy N, et al. Reduced endothelial nitric oxide synthase expression and production in human atherosclerosis. *Circulation.* 1998;97:2494–2498.
18. Wang XL, Wang J. Endothelial nitric oxide synthase gene sequence variations and vascular disease. *Mol Genet Metab.* 2000;70:241–251.
19. Faraci FM, Brian JE. Nitric oxide and the cerebral circulation. *Stroke.* 1994;25:692–703.
20. Marsden PA, Heng HH, Scherer SW, et al. Structure and chromosomal localization of the human constitutive endothelial nitric oxide synthase gene. *J Biol Chem.* 1993;268:17478–17488.
21. Nakayama M, Yasue H, Yoshimura M, et al. T-786C mutation in the 5-flanking region of the endothelial nitric oxidesynthase gene is associated with coronary vasospasm. *Circulation.* 1999;99:2864–2870.
22. Wang XL, Sim AS, Wang MX, et al. Genotype dependent and cigarette specific effects on endothelial nitric oxide synthase gene expression and enzyme activity. *FEBS Lett.* 2000;471:45–50.
23. Wang XL, Mahaney MC, Sim AS, et al. Genetic contribution of the endothelial constitutive nitric oxide synthase gene to plasma nitric oxide levels. *Arterioscler Thromb Vasc Biol.* 1997;17:3147–3153.
24. Khurana VG, Sohni YR, Mangrum WI, et al. Endothelial nitric oxide synthase T-786C single nucleotide polymorphism: a putative genetic marker differentiating small versus large ruptured intracranial aneurysms. *Stroke.* 2003;34:2555–2559.
25. Khurana VG, Sohni YR, Mangrum WI, et al. Endothelial nitric oxide synthase gene polymorphisms predict susceptibility to aneurysmal subarachnoid hemorrhage and cerebral vasospasm. *J Cereb Blood Flow Metab.* 2004;24:291–297.
26. Akagawa H, Kasuya H, Onda H. et al Influence of endothelial nitric oxide synthase T-786C single nucleotide polymorphism on aneurysm size. *J Neurosurg.* 2005;102:68–71.
27. Krex D, Fortun S, Kuhlisch E, et al. The role of endothelial nitric oxide synthase (eNOS) genetic variants in European patients with intra-cranial aneurysms. *J Cereb Blood Flow Metab.* 2006;26:1250–1255.
28. Krischek B, Kasuya H, Akagawa H, et al. Inoue I. Using endothelial nitric oxide synthase gene polymorphisms to identify intracranial aneurysms more prone to rupture in Japanese patients. *J Neurosurg.* 2006;105:717–722.
29. Song MK, Kim MK, Kim TS, et al. Endothelial nitric oxide gene T-786C polymorphism and subarachnoid hemorrhage in Korean population. *J Korean Med Sci.* 2006;21:922–926.
30. Koshy L, Easwer HV, Neetha NV, et al. Role of endothelial nitric oxide synthase gene polymorphisms in predicting aneurysmal subarachnoid hemorrhage in South Indian patients. *Dis Markers.* 2008;24:333–339.
31. Özüm Ü, Bolat N, Gül E, et al. Endothelial nitric oxide synthase gene [G894T.] polymorphism as a possible risk factor in aneurysmal subarachnoid haemorrhage. *Acta Neurochir (Wien).* 2008;15:57–61.
32. Kim TG, Kim NK, Baek MJ, et al. The relationships between endothelial nitric oxide synthase polymorphisms and the formation of intracranial aneurysms in the Korean population. *Neurosurg Focus.* 2011;30:E23.
33. Liu L, Huang XS, Cai YL, et al. Association of G-894T and T-786C polymorphisms of endothelial nitric oxide synthase gene with sporadic intracranial aneurysms. *Nan Fang Yi Ke Da Xue Xue Bao.* 2013;33:1733–1737.
34. Staalsø JM, Edsen T, Kotinis A, et al. Association of the NOS3 intron-4 VNTR polymorphism with aneurysmal subarachnoid hemorrhage: clinical article. *J Neurosurg.* 2014;121:587–592.
35. Xu L. The Relationship Between G894T Polymorphism of Endothelial Nitric Oxide Synthase Gene and Spontaneous Aneurismal Subarachnoid Hemorrhage [D]. Suzhou, Jiangsu, China: Soochow University; 2009:1–42.
36. Bi Z. Study of the Relationship Between the 27-bp Repeat Polymorphism in Intron 4 of the eNOS Gene, the Plasminogen Activator Inhibitor-1 (PAI-1) promotor 4G/5G Insersion/Deletion Polymorphism and Intracrianal Aneurysm [D]. Beijing, China: Capital University of Medical Science; 2006:1–103.

37. Friedrich JO, Adhikari NKJ, Beyene J. Inclusion of zero total event trials in meta-analyses maintains analytic consistency and incorporates all available data. *BMC Med Res Methodol.* 2007;7:5.

38. Zintzaras E, Ioannidis J. Heterogeneity testing in meta-analysis of genome searches. *Genet Epidemiol.* 2005;28:123–137.

39. Higgins JP, Thompson SG, Deeks JJ, et al. Measuring inconsistency in meta-analyses. *BMJ.* 2003;327:557–560.

40. Begg CB, Mazumdar M. Operating characteristics of a rank correlation test for publication bias. *Biometrics.* 1994;50:1088–1101.

41. Egger M, Davey Smith G, Schneider M, et al. Bias in metaanalysis detected by a simple, graphical test. *BMJ.* 1997;315:629–634.

42. McColgan P, Thant KZ, Sharma P. The genetics of sporadic ruptured and unruptured intracranial aneurysms: a genetic meta-analysis of 8 genes and 13 polymorphisms in approximately 20,000 individuals: clinical article. *J Neurosurg.* 2010;112:714–721.

43. Khurana VG, Meissner I, Sohni YR, et al. The presence of tandem endothelial nitric oxide synthase gene polymorphisms identifying brain aneurysms more prone to rupture. *J Neurosurg.* 2005;102:526–531.

44. Tanus-Santos JE, Desai M, Flockhart DA. Effects of ethnicity on the distribution of clinically relevant endothelial nitric oxide variants. *Pharmacogenetics.* 2001;11:719–725.