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Review Article

Angiotensin-converting enzyme (insertion/deletion) gene polymorphism does not contribute to sporadic Alzheimer's disease risk in caucasian individuals: a meta-analysis

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Abstract: Despite extensive research on the role of ACE in the development of sporadic Alzheimer's disease (SAD) in Caucasian individuals, studies about the influence of ACE Insertion/Deletion (I/D) polymorphism on the risk of SAD were inconsistent. To explore whether the ACE I/D polymorphism confers susceptibility to SAD in Caucasian individuals, a meta-analysis comprising 12,108 SAD cases and 6180 controls from 34 case-control comparisons was performed by searching electronic databases. The results showed no significant differences in five models involving genotypes II, ID, and DD: II versus (ID + DD) (odds ratio (OR) = 1.02, 95% confidence interval (CI) = 0.94-1.12, *P* = 0.59), II versus DD (OR = 1.07, 95% CI = 0.97-1.19, *P* = 0.16), II versus ID (OR = 1.00, 95% CI = 0.91-1.09, *P* = 0.93), ID versus DD (OR = 1.10, 95% CI = 0.98-1.23, *P* = 0.11), and (II + ID) versus DD (OR = 1.09, 95% CI = 0.98-1.22, *P* = 0.12) on the basis of all studies. When a subgroup analysis was performed based on the age at onset or the epsilon 4 allele of apolipoprotein E (APOEε4) carrier status, significant correlations were demonstrated, but they were not consistent with the overall results. The pooled results suggested that ACE I/D polymorphism might not be a risk factor for SAD in Caucasians, and genetic interactions with the age at onset or APOEε4 carrier status might affect these correlations. Large-sample studies are needed to confirm these findings in Caucasians.

Keywords: Alzheimer's disease, angiotensin-converting enzyme, gene, polymorphism, caucasians, meta-analysis

Introduction

Alzheimer's disease (AD), a progressive neurodegenerative disorder, accounts for impairment in cognitive function. The essential pathological features of AD are characterized by a selective neuronal loss associated with neurofibrillary tangles and deposition of amyloid in the medial temporal lobe structures and cortical areas of the brain [1, 2]. Although the contribution of major factors to the pathogenesis of the sporadic AD (SAD) is incompletely understood, increasing attention has been focused on the association between angiotensin-converting enzyme (ACE) and SAD.

ACE prompts the formation of angiotensin II in the renin-angiotensin system and plays an important role in blood pressure and sodium homeostasis [3]. Neurons, which are important elements for memory and cognition in the hippocampus and amygdala, are excited by angiotensin II [4, 5]. The levels of amyloid b-protein (Ab) have been found to be lowered by ACE via promoting its degradation [6, 7], and the activity or serum levels of ACE increased in the brain of patients with SAD [8, 9]. These studies suggested the contribution of ACE to the pathogenesis of AD.

An insertion/deletion (I/D) polymorphism in intron 16 of the ACE gene on chromosome 17q23 has been identified and constitutes the genotypes II, ID, and DD [10]. ACE I/D polymorphism was found to be associated with the lev-

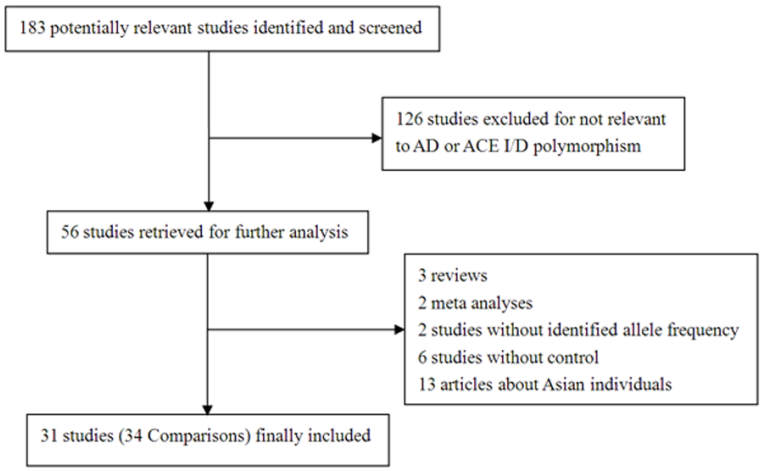

Figure 1. Flow chart of literature search and study selection.

els and activity of ACE [11]. A potential correlation between ACE I/D polymorphism and SAD risk was reported [12] in Caucasians. However, this association is still controversial.

Previously published meta-analyses reported a significant correlation between ACE I/D polymorphism and risk of SAD [13, 14]. However, it remains unclear whether ethnicity could affect this correlation. Since then, several studies on this correlation using a large sample size have been reported. The present study investigated the possible association of ACE I/D polymorphism with the risk of SAD through a meta-analysis in Caucasian individuals. Subgroup analyses were performed on the basis of the age at onset and the epsilon 4 allele of apolipoprotein E (APOEε4) carrier status.

Materials and methods

Search strategy

Studies were identified by searching the standard databases: MEDLINE, EMBASE, and HuGEnet, without language restriction, and the search focused on studies conducted on human subjects only. The following Medical Subject Headings and text words were used: Alzheimer's disease, Alzheimer disease, AD in combination with ACE, angiotensin-converting enzyme, polymorphism, genotype, gene, or mutation. Two investigators (Juanjuan Cao and Liping Ding) independently reviewed abstracts or full text of all citations to identify eligible studies. The identified articles had to meet the following criteria: (1) SAD was diagnosed clinically; (2) it was a case-control study; (3) the frequency of people and individual ACE I/D genotype (II, ID, DD) in cases and controls was reported; and (4) both cases and controls were Caucasian individuals. The exclusion criteria were as follows: (1) a family history of dementia in cases and (2) case reports, editorials, and review articles.

Data extraction

All studies were checked by two investigators (Juanjuan Cao and Liping Ding) independently according to the prespecified selection criteria. The relevant data of eligible studies were extracted and entered separate databases. The discrepancy was resolved by discussion. The following information was extracted from eligible studies: first author, year of publication, ethnicity, clinical characteristics, number of genotype (II, ID, DD) of cases and controls, and genotyping methods.

Statistical analysis

For dichotomous outcomes, the odds ratios (ORs) and their 95% confidence intervals (CIs) were calculated using the Review Manager software, version 5.2. Five different ORs were calculated in the present study: dominant model (II + ID vs DD), recessive model [II vs (ID + DD)], homozygote comparison (II vs DD), and heterozygote comparison (ID vs DD; II vs ID). The statistical significance was determined by the Z test. A P value ≤ 0.05 was considered statistically significant. Subgroup analyses were conducted on the basis of patients with the APOEε4 carrier status and age at onset (the criterion for the age at onset ≥ 65 years was defined as late-onset AD).

The genotype distribution of the control population in eligible studies was tested for deviation from the Hardy-Weinberg equilibrium (HWE) using the chi-square test (with $P \leq 0.1$ considered as significant). If the genotype distribution was not in accordance with HWE, the study was excluded. The test for heterogeneity between studies was performed with Cochran's Q statistic ($P > 0.10$ was considered the representative

Table 1. Characteristics of inclusive studies evaluating ACE I/D polymorphism and SAD risk

Author	Year	Country (Ethnicity)	Genotyping Method	Diagnosis Criteria	Matching Characteristics	Specimen	HWE	
							χ^2	P
Alvarez et al. [51]	1999	Spain (European)	PCR-RFLP	NINCDS-ADRDA	-	-	1.2633	0.26102
Bowirrat et al. [48]	2005	Israel (European)	PCR-RFLP	NINCDS-ADRDA	-	-	2.3939	0.12181
Buss et al. [56]	2002	Germany et al. (European)	PCR-RFLP	NINCDS-ADRDA	Age and Gender	Blood	0.2491	0.61773
Camelo et al. [25]	2004	Colombia (European)	PCR-RFLP	NINCDS/ADRDA	Age and Gender	Blood	0.0393	0.84285
Carbonell et al. [35]	2003	UK (European)		NINCDS-ADRDA	-	Blood	0.0149	0.90278
Chapman et al. [49]	1998	Israel (European)	PCR-RFLP	NINCDS-ADRDA DSM-III-R	Age	Blood	0.0985	0.75368
Crawford et al. [28]	2000	USA (American)	PCR-RFLP	CERAD		Blood	5.5274	0.01872
Farrer et al. [54] a	2000	Russia (European)	PCR-RFLP	NINCDS-ADRDA	Age	Blood	1.4962	0.22126
Farrer et al. [54] b	2000	Canada (American)	PCR-RFLP	NINCDS-ADRDA	Age	-	6.1123	0.01342
Helbecque et al. [32]	2009	France et al. (European)		DSM-III-R NINCDS-ADRDA	Age and Gender	Blood	0.0013	0.9707
Isbir et al. [41]	2001	Turkey (European)	PCR-RFLP	NINCDS-ADRDA	Age and Gender	Blood	0.8522	0.35592
Kehoe et al. [11] a	1999	UK (European)	-	-	-	-	0.0518	0.81989
Kehoe et al. [11] b	1999	UK (European)	-	-	-	-	1.3195	0.25068
Kehoe et al. [11] c	1999	UK (European)	-	-	-	-	0.4877	0.48493
Kehoe et al. [60]	2003	Sweden (European)	-	NINCDS-ADRDA CERAD	-	-	0.3027	0.58222
Keikhaee et al. [42]	2006	Iran (European)	PCR-RFLP	NINCDS-ADRDA	Age and Gender	Blood	0.06	0.80649
Kŏlsch et al. [31]	2005	Germany (European)	PCR-RFLP	DSM IV	Age and Gender	Blood	1.8477	0.17405
Lendon et al. [46]	2002	UK (European)	PCR-RFLP	NINCDS-ADRDA DSM-III-R	-	Blood, Brain	0.8202	0.36513
Mattila et al. [55]	2000	Finland (European)	PCR-RFLP	NINCDS-ADRDA CERAD	Age and Gender	Blood, Brain	0.0233	0.87859
Miners et al. [7]	2009	Sweden (European)	Fluorescent peptide assay	CERAD	-	-	0.4828	0.48715
Monastero et al. [30]	2002	Italy (European)		NINCDS-ADRDA	Age and Gender		0.5853	0.44426
Myllykangas et al. [47]	2000	Finland (European)	PCR-RFLP	NINCDS-ADRDA DSM-III-R	Age and Gender		2.1167	0.1457
Nacmias et al. [27]	2007	Italy (European)	PCR-RFLP	DSM-IV	Age and Gender	Blood	0.3657	0.54535
Narain et al. [44]	2000	UK (European)	PCR-RFLP	CERAD	Age and Gender	Blood	12.452	0.00042
Nirmal et al. [36]	2011	India (Asian)	-	DSM-IV	-	Blood	0.0062	0.93709
Palumbo et al. [33]	1999	Italy (European)	-	NINCDS-ADRDA	Age and Gender	Blood	2.5671	0.10911
Panza et al. [53]	2002	Italy (European)	-	-	-	-	2.4875	0.11475
Perry et al. [62]	2001	USA (American)	-	-	-	-	10.855	0.00099
Prince et al. [63]	2001	Sweden (European)	-	-	-	-	1.7971	0.18006
Richard et al. [64]	2001	French (European)	PCR-RFLP	NINCDS-ADRDA DSM-III-R	Age and Gender	-	2.2219	0.13606
Scacchi et al. [52]	1998	Italy (European)	-	NINCDS-ADRDA DSM-III-R	Gender	Blood	0.09	0.76415
Seripa et al. [26] a	2003	USA (European)	PCR-RFLP	NINCDS-ADRDA	Age and Gender	Brain	2.7409	0.09781
Seripa et al. [26] b	2003	Italy (European)	PCR-RFLP	NINCDS-ADRDA	Age and Gender	Blood	0.1402	0.70812
Shcherbatykhet al. [34]	2001	USA (American)	-	NINCDS-ADRDA	Age and Gender	Blood	1.9297	0.16479
Sleegers et al. [19]	2005	Netherlands (European)	PCR-RFLP	DSM-IIIR	-	-	0.0086	0.92606
Trebunova et al. [50]	2008	Slovakia (European)	PCR-RFLP	NINCDS-ADRDA	Age and Gender	Blood	0.7991	0.37136
Vardy et al. [61]	2009	UK (European)	Fluorescent peptide assay	NINCDS-ADRDA	Age and Gender	Blood	0.0006	0.98016
Schǎchter	1994	France (European)	PCR-RFLP	-	-	Blood	2.1603	0.14162
Zuliani et al. [45]	2001	Italy (European)	PCR-RFLP	NINCDS-ADRDA	Age and Gender	Blood	0.9994	0.31745

Neurological and Communicative Disorders and Stroke (NINCDS) and the Diagnostic and Statistical Manual of Mental Disorders, third edition, revised (DSM-III-R) and the Alzheimer's Disease and Related Disorders Association (ADRDA).
CERAD: The Consortium to Establish a Registry for Alzheimer's disease. Part I. Clinical and neuropsychological assessment of Alzheimer's disease. PCR-RFLP: polymerase chain reaction (PCR)-restriction fragment length polymorphism.

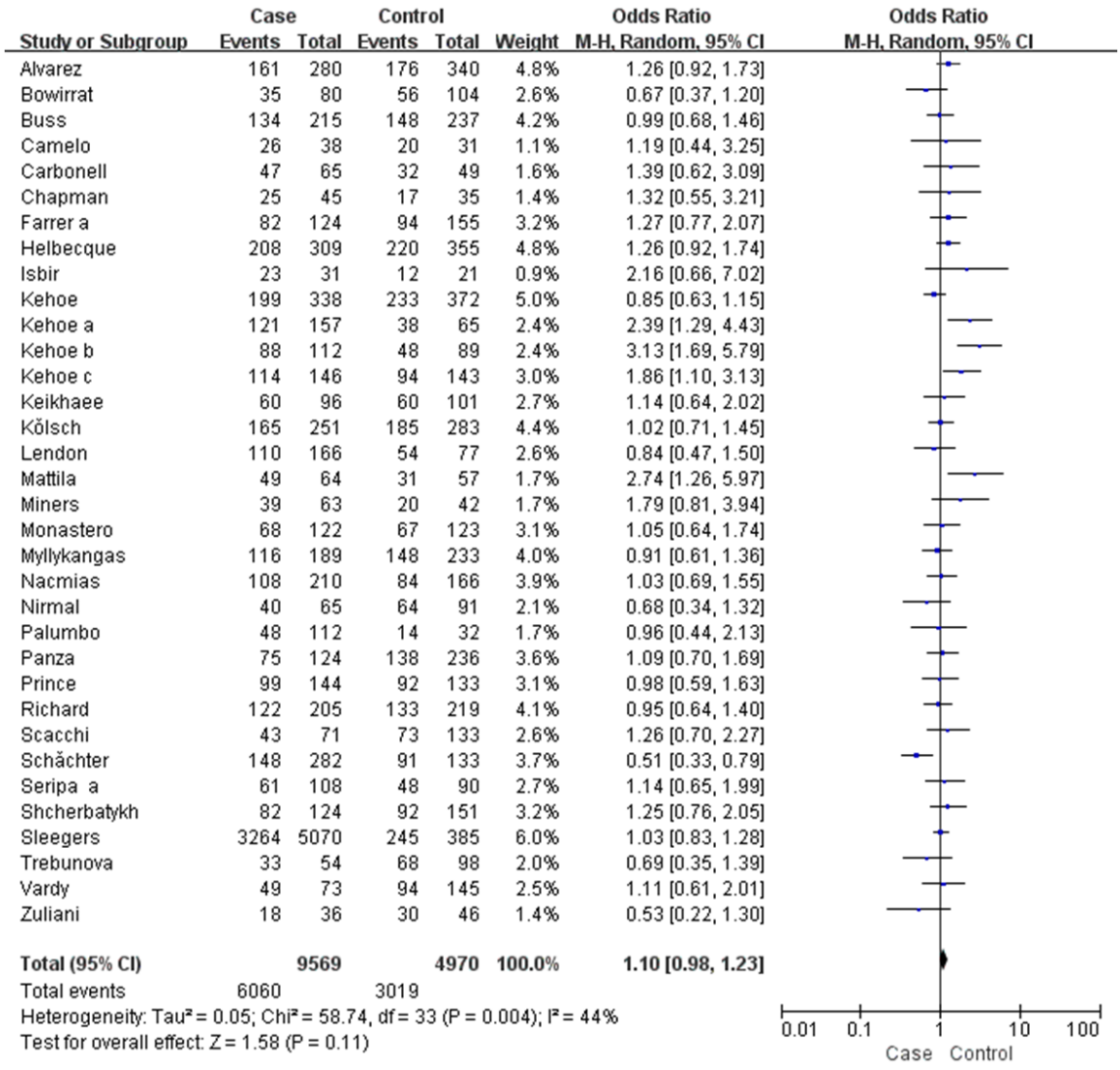

Figure 2. Forest plot for ACE I/D polymorphism (ID versus DD) and SAD risk.

of homogeneity). A pooled OR was calculated using the fixed-effects model (Mantel-Haenszel method) in the case of homogeneity; otherwise, the random-effects model (Der Simonian-Laird) was adopted.

The stability of the results was detected by performing a sensitivity analysis. The higher heterogeneity studies involved in the meta-analysis were deleted to reflect the influence of the related data on the pooled ORs. The Begg's funnel plot was used to explore publication bias, and the Egger's linear regression test was used to quantitatively assess the publication bias ($P \leq 0.10$ was considered statistically significant) (version 12.0, Stata Corp).

Results

Identification of eligible studies

The 183 potentially relevant studies were retrieved using the search criteria, and 126 of these articles were excluded as irrelevant to SAD risk and ACE I/D polymorphism. Abstracts from 57 articles were reviewed, and 5 studies were excluded (3 reviews [15-17] and 2 meta-analyses [13, 14]); 2 studies with unidentified allele frequency [18, 19] and 6 studies with no controls [20-25] were excluded. Also, 13 articles on Asian individuals were excluded [26-38]. No additional relevant studies were found from the references reviewed. Thus, 31 articles

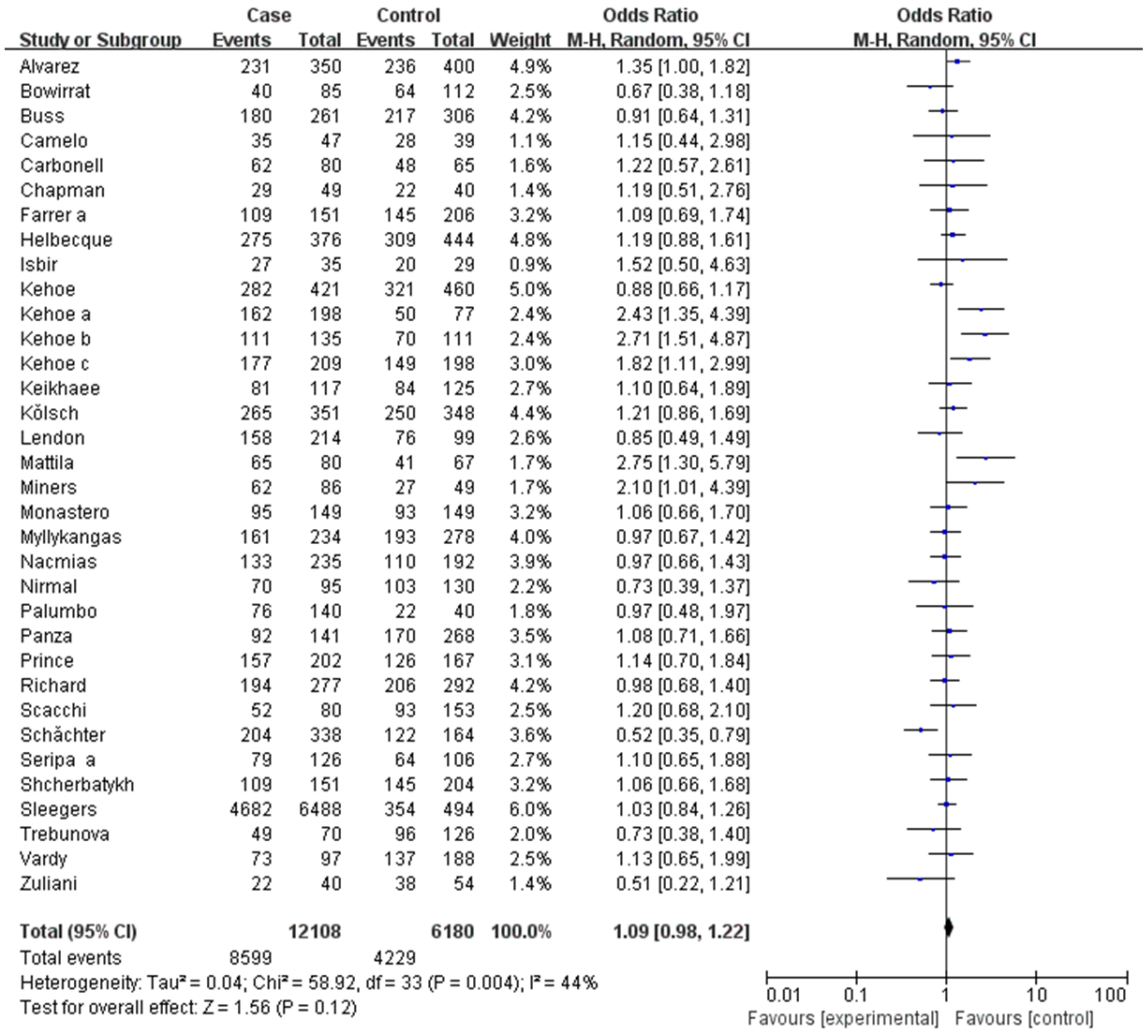

Figure 3. Forest plot for ACE I/D polymorphism (II+ID versus DD) and SAD risk.

[8, 12, 20, 39-65, 31] met the inclusion criteria (**Figure 1**). Different comparisons were made based on population distribution in the three articles [12, 40, 57]. The genotype distribution for the control group in four comparisons did not follow HWE [42, 49, 57, 62], and these comparisons were exclusive in the present meta-analysis. The characteristics of included studies are presented in **Table 1**.

A total of 34 comparisons consisting of 12,108 patients with SAD and 6180 controls were included in this meta-analysis. For most studies, the polymerase chain reaction (PCR)-restriction fragment length polymorphism was performed. Also, the diagnosis of definite or probable SAD was established according to the criteria of the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA), the age- or sex-matched controls to the cases were found, and genomic DNA was isolated from peripheral tissues according to the standard procedure (**Table 1**).

Meta-analysis database

The combined results showed no significant difference in II versus (ID + DD) (OR = 1.02, 95% CI = 0.94-1.12, P = 0.59), II versus DD (OR = 1.07, 95% CI = 0.97-1.19, P = 0.16), and II versus ID (OR = 1.00, 95% CI = 0.91-1.09, P = 0.93) under the fixed-effects model, and ID versus DD (OR = 1.10, 95% CI = 0.98-1.23, P = 0.11) and II + ID versus DD (OR = 1.09, 95% CI

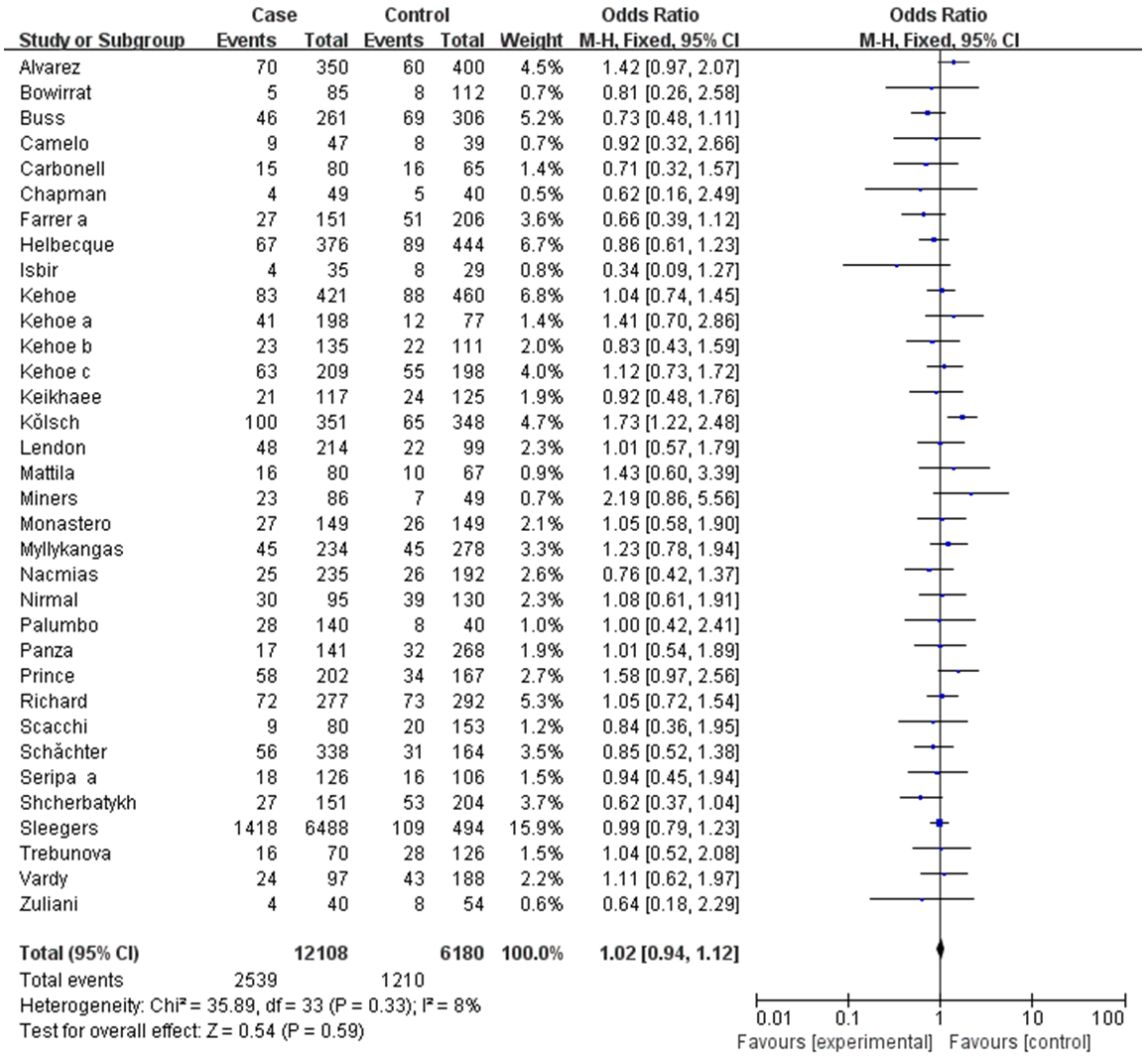

Figure 4. Forest plot for ACE I/D polymorphism (II versus ID + DD) and SAD risk.

= 0.98-1.22, *P* = 0.12) (**Figures 2**-**6**) under the random-effects model. All results for genetic models and the test for heterogeneity are summarized in **Table 2**.

Subgroup analysis

A statistically significant difference was observed in late-onset AD (II vs DD: OR = 0.65, 95% CI = 0.48-0.86, *P* = 0.003 < 0.05; II vs ID: OR = 0.49, 95% CI = 0.38-0.65, *P* < 0.05; ID vs DD: OR = 1.29, 95% CI = 1.03-1.61, *P* = 0.03 < 0.05; or II versus ID + DD: OR = 0.55, 95% CI = 0.42-0.70, *P* < 0.05) (**Table 2**), but no statistical significance in early-onset AD was found. When stratified by the APOEε4 carrier status, a higher risk for SAD was also observed for the APOEε4 carrier status (ID vs DD: OR = 1.54, 95% CI = 1.10-2.15, *P* = 0.01 < 0.05; II + ID vs DD: OR = 1.58, 95% CI = 1.15-2.16, *P* = 0.005 < 0.05); however, the results were not pronounced for the non-APOEε4 carrier status (**Table 2**).

Sensitivity analysis

The sensitivity analysis indicated that three independent comparisons [12, 58] were the main origin of heterogeneity. The heterogeneity was removed after the exclusion of these three comparisons [test for heterogeneity for ID vs DD (I^2 = 12%, *P* = 0.28) and (II + ID) vs DD (I^2 = 14%, *P* = 0.25)], and the corresponding pooled ORs were not materially altered [II vs (ID + DD) (OR = 1.02, 95% CI = 0.94-1.12, *P* = 0.59), II vs DD (OR = 1.07, 95% CI = 0.97-1.19, *P* = 0.16), II vs ID (OR = 1.00, 95% CI = 0.91-1.09, *P* = 0.93),

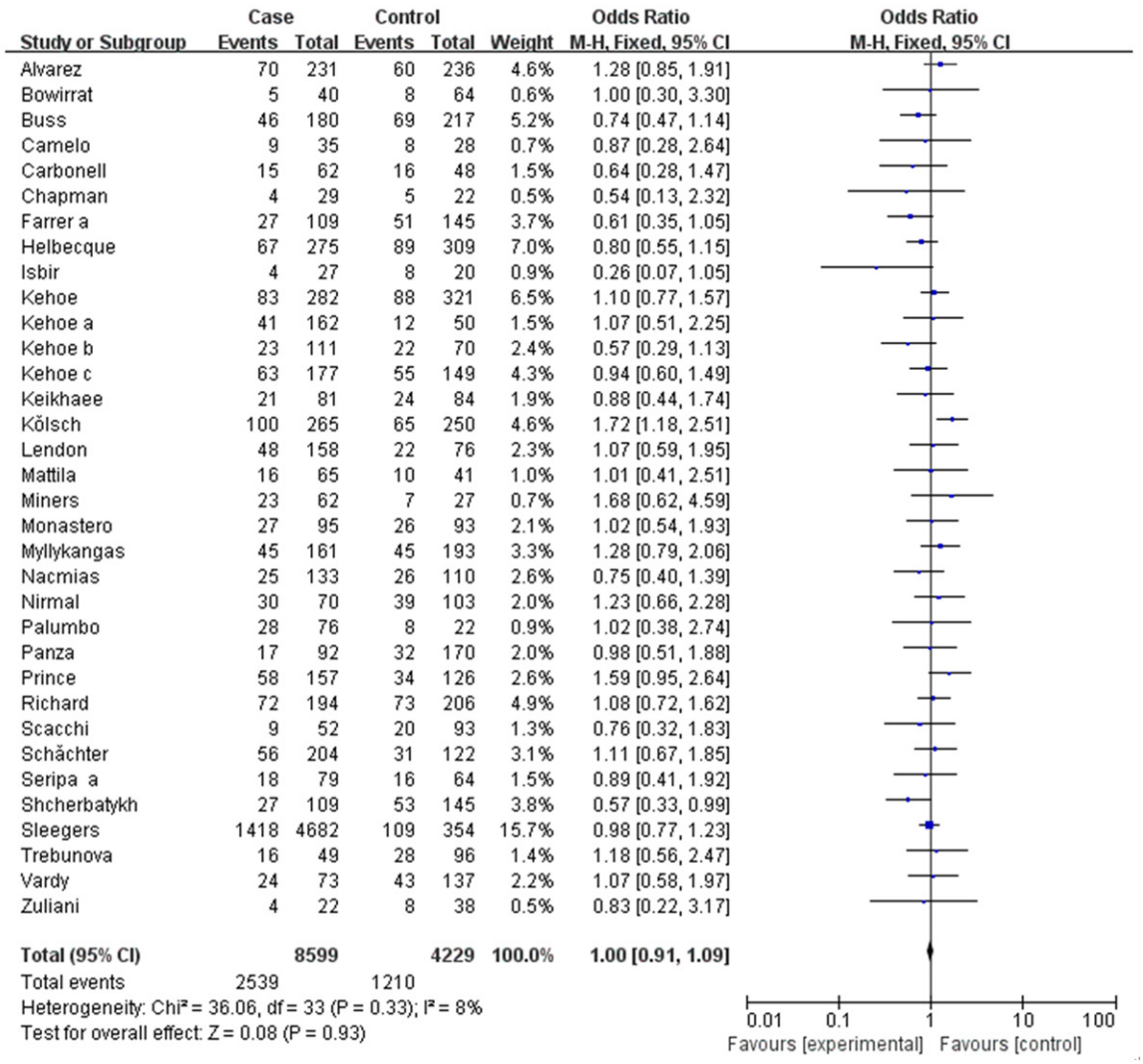

Figure 5. Forest plot for ACE I/D polymorphism (II versus ID) and SAD risk.

ID vs DD (OR = 1.10, 95% CI = 0.98-1.23, *P* = 0.11), and II + ID vs DD (OR = 1.09, 95% CI = 0.98-1.22, *P* = 0.12) under the fixed-effects model]. No effect of any study on the pooled results in the aforementioned five models was found. The results of sensitivity analysis indicated the stability of the results.

Publication bias

The shape of the funnel plots in genetic models seemed symmetrical, indicating no evidence for obvious publication bias (**Figures 7-11**). Further, the Egger's test showed no significant publication bias in the genetic model [II vs (ID + DD), *P* = 0.249; II vs DD, *P* = 0.873; II vs ID, *P* = 0.141; ID vs DD, *P* = 0.22, and (II + ID) vs DD, *P* = 0.326)]. Therefore, the potential publication bias did not materially alter the combined risk estimate.

Discussion

Epidemiological and pathogenetic evidences strongly suggested a correlation between genetic factors and the risk for SAD. On the basis of this hypothesis, the contribution of various candidate genes to the risk for SAD was investigated, and one of the candidate genes that have been analyzed as a risk factor for SAD was found to be the ACE gene. ACE modulates the progression of AD via degradation of Ab or components of renin-angiotensin system in the brain [6, 7].

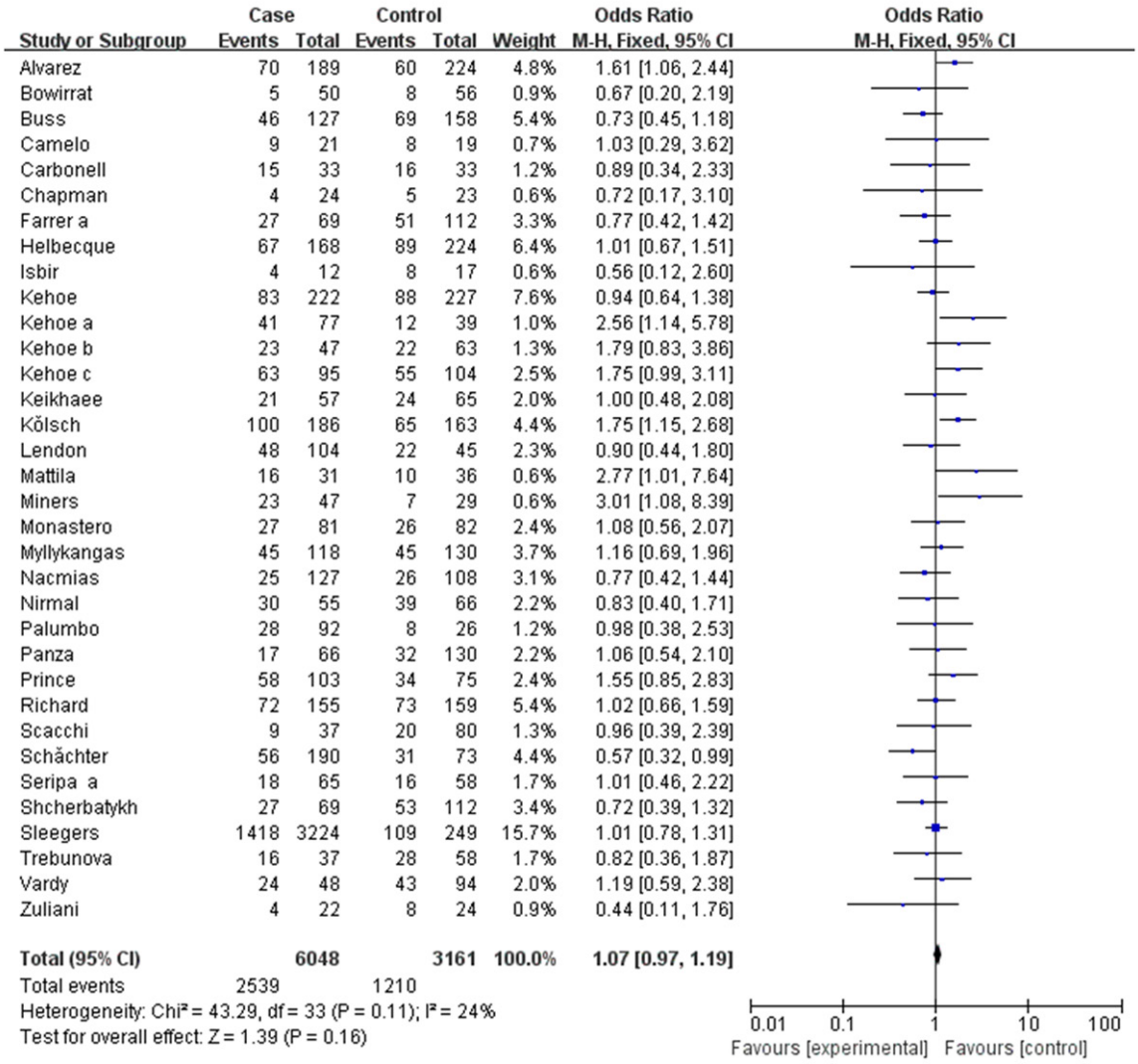

Figure 6. Forest plot for ACE I/D polymorphism (II versus DD) and SAD risk.

A collaborative study demonstrated a possible correlation between ACE gene I allele and the risk for SAD [12]. Since then a considerable number of studies were performed to replicate these results. In Colombian patients, ACE I/D polymorphism does not appear to confer an added risk for SAD [39]. However, ambiguous results have been presented. The ACE I/D genotypes are associated with an increased risk for SAD in patients with German origin [44]. A possible protective role for the II genotype of ACE was observed by Shcherbatykh *et al.* [47]. The failure to reproduce replicated studies might be due to the small sample size used. In inclusive 34 comparisons, the pooled results confirmed that ACE I/D polymorphism had no effect on the risk for SAD. However, the results of *I*-square and Cochran's Q statistic showed heterogeneity in the two models (**Table 2**). The most plausible explanation for the heterogeneity may be the presence of an interaction between age at onset, APOEε4 carrier status, and ACE I/D polymorphism for the risk of SAD.

The association with genetic risk factors might be dependent on the heterogeneity of age at onset. Helbecque *et al.* showed that the ACE D allele was at a reduced risk for SAD for the oldest patients with SAD [45]. However, no correlation of ACE with the age at onset was found for patients with SAD in Italy [40]. On the basis of age at onset, statistically significant difference was found in late-onset AD. The present study further supported the previous reports of an

Table 2. Meta-analysis of the association between ACE I/D polymorphism and SAD risk

Comparison	Population	No. of comparisons	Test of association				Mode	Test of heterogeneity	
			OR	95% CI	*P* value	Z		*P* value	I^2 (%)
II vs. DD	Overall	34	1.07	0.97, 1.19	0.16	1.39	F	0.11	24
	Early-onset	6	0.95	0.72, 1.27	0.75	0.31	F	0.30	17
	Late-onset	6	0.65	0.48, 0.86	0.003	2.96	F	0.90	0
	APOEε4	7	1.31	0.86, 2.00	0.21	1.26	F	0.18	33
	Non-APOEε4	7	1.17	0.87, 1.56	0.30	1.04	F	0.22	28
II vs. ID	Overall	34	1.00	0.91, 1.09	0.93	0.08	F	0.33	8
	Early-onset	6	0.83	0.64, 1.08	0.16	1.39	F	0.28	20
	Late-onset	6	0.49	0.38, 0.65	< 0.05	5.15	F	0.54	0
	APOEε4	7	0.82	0.55, 1.21	0.32	1.00	F	0.91	0
	Non-APOEε4	7	0.81	0.62, 1.06	0.13	1.53	F	0.89	0
ID vs. DD	Overall	34	1.10	0.98, 1.23	0.11	1.58	R	0.004	44
	Early-onset	6	1.16	0.92, 1.47	0.22	1.23	F	0.99	0
	Late-onset	6	1.29	1.03, 1.61	0.03	2.19	F	0.85	0
	APOEε4	7	1.54	1.10, 2.15	0.01	2.54	F	0.35	0
	Non- APOEε4	7	1.36	0.97, 1.90	0.07	1.80	R	0.06	51
II + ID vs. DD	Overall	34	1.09	0.98, 1.22	0.12	1.56	R	0.004	44
	Early-onset	6	1.09	0.88, 1.36	0.42	0.81	F	0.88	0
	Late-onset	6	1.07	0.86, 1.32	0.54	0.61	F	0.94	0
	APOEε4	7	1.58	1.15, 2.16	0.005	2.84	F	0.19	31
	Non-APOEε4	7	1.28	0.92, 1.78	0.15	1.44	R	0.06	51
II vs. ID + DD	Overall	34	1.02	0.94, 1.12	0.59	0.54	F	0.33	8
	Early-onset	6	0.87	0.68, 1.11	0.27	1.11	F	0.19	32
	Late-onset	6	0.55	0.42, 0.70	< 0.05	4.70	F	0.62	0
	APOEε4	7	0.98	0.68, 1.42	0.92	0.10	F	0.62	0
	Non-APOEε4	7	0.92	0.72, 1.18	0.52	0.64	F	0.77	0

R: random-effects model; F: fixed-effect model.

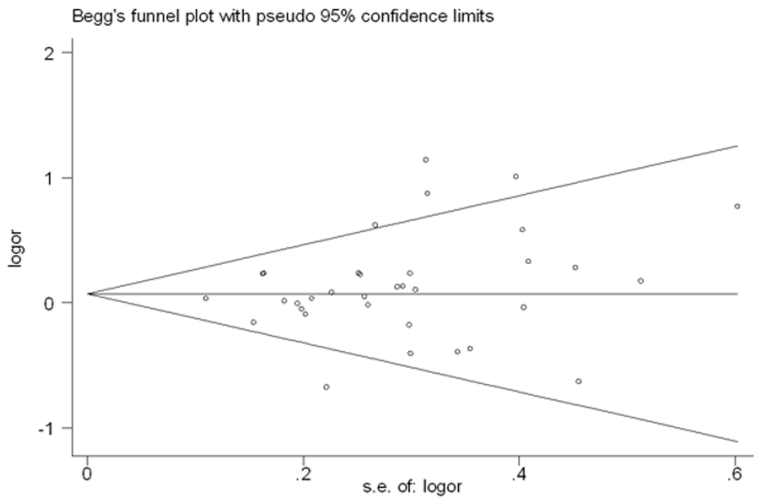

Figure 7. Begg's funnel plot for ID versus DD.

interaction between age at onset and ACE I/D polymorphism for the risk for SAD [26, 29, 45].

A gene-gene interaction analysis should be explored. When cases and controls were stratified according to the APOEε4 carrier status, no significant difference was revealed [42]. Otherwise, an increase in the risk of developing SAD was found in subjects carrying both the ACE DD genotype and the APOEε4 allele (OR = 10.32, 95% CI = 2.67-39.81) [53]. A statistically significant difference in the APOEε4 carrier status was observed between ACE I/D polymorphism and the risk for SAD in the present meta-analysis. Hence, a synergistic interaction existed between ACE I/D polymorphism and APOEε4 carrier status for the risk of SAD, and ACE I/D polymorphism might

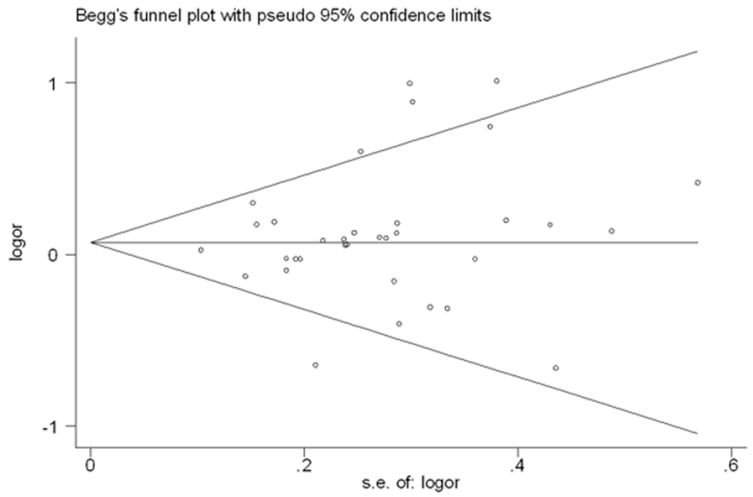

Figure 8. Begg's funnel plot for (II+ID) versus DD.

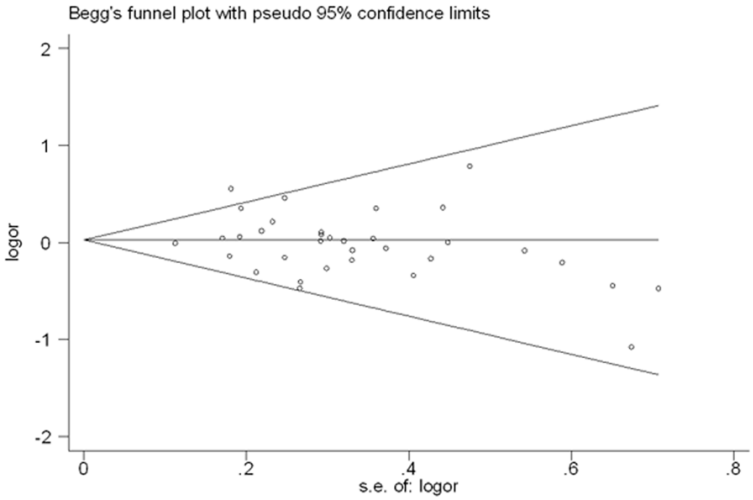

Figure 9. Begg's funnel plot for II versus (ID+DD).

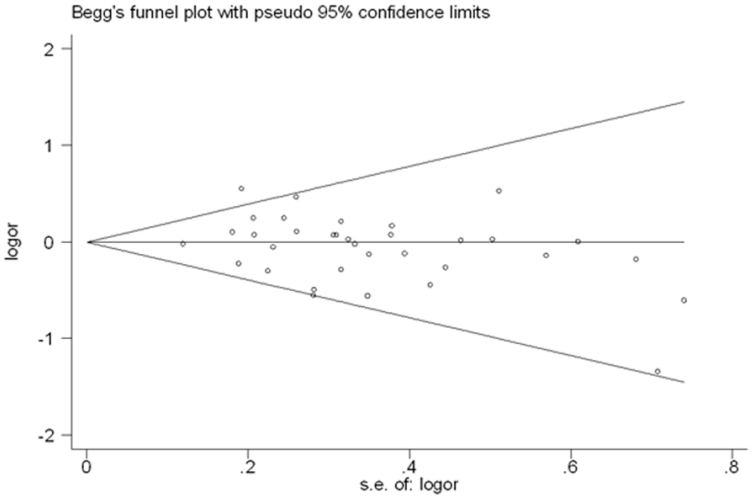

Figure 10. Begg's funnel plot for II versus ID.

be a genetic risk for patients with SAD having the APOEε4 carrier status.

The present meta-analysis of observational studies had some limitations. First, the effects of clinical heterogeneity (age, gender, and so on) on the conclusions were explored. However, the small sample size might have yielded false-positive results due to the lack of statistical power. Therefore, it is critical that larger-scale, well-designed studies should be performed to identify these effects. On the contrary, the samples (blood and brain) were selected, and different genotyping methods were used with different sensitivity and specificity, which might have also resulted in selection bias and clinical heterogeneity. If the heterogeneity was removed by sensitivity analysis, the overall results were not materially altered, suggesting the stability of the results. Third, an important issue that is often raised in a methodological meta-analysis is publication bias. Publication bias was not detected by the Begg's funnel plot and Egger's test in this meta-analysis. However, publication bias might not be ruled out completely. It is possible that relevant unpublished articles with null results might not have been included, leading to publication bias.

Despite the aforementioned limitations, this meta-analysis demonstrated that the ACE I/D polymorphism might not be associated with the risk for SAD. It was suggested that the interaction between ACE I/D polymorphism and age at onset or APOEε4 carrier status might account for the risk for SAD. On the basis of SAD with multifactorial etiology, the re-

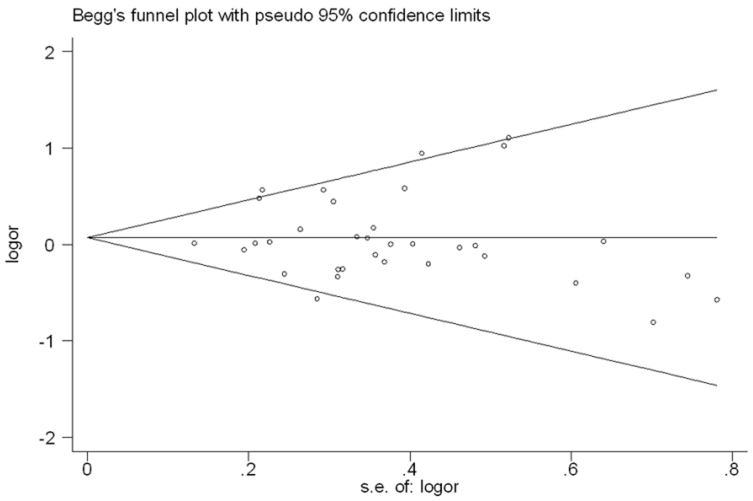

Figure 11. Begg's funnel plot for II versus DD.

sults of the present meta-analysis should be properly replicated in future prospective cohort studies, taking into consideration the interactions.

Disclosure of conflict of interest

None.

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