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Original Article

A meta-analysis for association of three angiotensin-converting enzyme gene polymorphisms (SNPs rs4291A>T, rs4343A>G, and rs1800764T>C) with sporadic Alzheimer's disease susceptibility

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Abstract: The Angiotensin-converting enzyme (ACE) has been involved in the sporadic Alzheimer's disease (SAD) risk and investigated in numerous epidemiologic studies. ACE gene (SNPs rs4291A>T, rs4343A>G, and rs1800764T>C) polymorphisms may influence the risk for SAD. However, results thus far have been inconclusive. The purpose of the present study is to firstly investigate whether these three polymorphisms facilitate the susceptibility to SAD by a meta-analysis. The PubMed and EMBASE databases were searched for genetic association studies on three polymorphisms and SAD risk. 48 comparisons were identified and a meta-analysis was performed to evaluate the association between three polymorphisms and SAD risk by calculating combined odds ratios (ORs) and 95% confidence intervals (CIs). The combined results showed no significant association was found for rs4343A>G, rs4291A>T, and rs1800764C>T polymorphisms. However, the sensitivity analysis did not support the results for rs4921A>T (AA versus AT+TT: OR = 1.09, 95% CI: 1.01-1.18, *P* = 0.03 and AA versus AT: OR = 1.09, 95% CI: 1.00-1.18, *P* = 0.05). In summary, the meta-analysis results suggest rs4291 AA genotype within ACE gene is a risk factor for SAD and interactions might effect on ACE rs4291A>T genetic risk of SAD. However, the rs4343A>G or rs1800764T>C of ACE gene is not associated with SAD risk.

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

Introduction

Alzheimer's disease (AD) contributing to about two thirds of all dementias [13] is mainly characterized pathologically by the accumulation of extracellular amyloid-β (Aβ) deposits and the formation of neurofibrillary tangles in brain. Although the contribution of major factors to the pathogenesis of the sporadic AD (SAD) are incompletely understood, growing attention has been focused on the association between angiotensin-converting enzyme (ACE) and SAD. The epidemiological studies pointed to a link between risk factors of atherosclerotic vascular disease and AD [3, 29]. ACE prompts formation of angiotensin II in the rennin angiotensin system, and plays an important role in blood pressure and sodium homeostasis [25]. Neurons which are important elements for memory and cognition in the hippocampus and amygdala are excited by angiotensin II [28, 32]. The reducing availability of ACE could lead to an increase of the concentrations of the amyloid peptide Aβ in vitro studies [9, 12, 22], suggesting the role of ACE is as a possible Aβ degrading enzyme. Previous studies also reported that reduced concentrations of plasma ACE were associated with an increased risk of AD [9, 30]. ACE plays a role in AD pathology by acting on amyloid β-protein metabolism.

An insertion/deletion (I/D) polymorphism in intron 16 of the ACE gene on chromosome 17q23 has been identified, and results in the genotypes II, ID, and DD [1]. Previously published meta-analyses reported significant asso-

ciation between ACE I/D polymorphism and risk of SAD [16, 20]. The identification 3 SNPs of ACE gene (SNPs rs4291A>T, rs4343A>G, and rs1800764T>C) are in linkage disequilibrium with the ACE I/D polymorphism (0.462, 0.910, and 0.519 for rs4291A>T, rs4343G>A, and rs1800764C>T respectively, according to the r^2 metric) [11]. Two SNPs (SNPs rs4291A>T located -240 bp from the initiation codon, and rs4343A>G encoding a silent mutation in exon16) influence Aβ42 levels in the Cerebro Spinal Fluid [15]. It is interesting to note that rs1800764T>C has also been associated with elevated CSF Aβ42/Aβ40 ratio [14]. Three common polymorphisms associated with SAD risk have been investigated. However, results from individual studies are not consistent. In consideration of the extensive role of ACE in SAD process, the first meta-analysis was conducted to determine whether these three polymorphisms in the ACE gene were associated with risk of SAD.

Materials and methods

Identification of eligible studies

All the case-control studies were identified by a search of the MEDLINE and EMBASE using the following words and terms: ("angiotensin-converting enzyme" or "ACE") and ("genetic" or "polymorphism" or "mutation" or "genes") and ("Alzheimer's disease" or "AD"). References of the retrieved publications were also reviewed to ascertain additional studies. Only research articles were focused on studies conducted on human subjects and without language restriction. The genotype distribution of the control population of the studies had to be in Hardy-Weinberg equilibrium (HWE) (P>0.1).

Inclusion criteria

The identified studies met the following criteria: (a) the evaluation of 3 SNPs of ACE gene polymorphisms (SNPs rs4291A>T, rs4343A>G, or rs1800764T>C) and SAD risk, (b) the SAD was diagnosed clinically [18], (c) the case-control studies, (d) the available data for examining an odds ratio (OR) with 95% confidence interval (CI), (e) conforming Hardy-Weinberg equilibrium in the control group. The criterion for exclusion of study was (1) cases with a family history of AD, (2) case reports, editorials, and review articles, (3) duplicate.

Data extraction

Two investigators (Xiumin Wang, Xiaoguang Cao) extracted data independently and reached a consensus following discussion for ambiguity. The following characteristics of eligible studies were extracted: the first author, year of publication, nation and ethnicity of study population, numbers of genotype in cases and controls.

Statistical analysis

The strength of the association between the rs4291A>T polymorphisms and SAD risk was evaluated by OR with 95% CI. Five different ORs were calculated in our analysis: dominant model (AA+AT versus TT), recessive model (AA versus (AT+TT), homozygote comparison (AA versus TT), and heterozygote comparison (AA versus AT, AT versus TT). The same method was applied to the other two polymorphisms (rs4343A>G and rs1800764T>C).

Heterogeneity was evaluated with Cochran's Q statistic (P>0.10 was considered representative of homogeneity). When the homogeneity was present, a pooled OR was calculated using the fixed-effect model (the Mantel-Haenszel method) [17], whereas the random effects model (DerSimonian and Laird's method) was used [6]. Heterogeneity across studies was also detected using an I^2 test. I^2 values of <25% were considered low, I^2 values of 25% to 75% were considered moderate, and I^2 values of >75% were considered high [10]. The visual Begg's funnel plot was drawn to estimate the potential publication bias, and the Egger's linear regression test was utilized to quantitatively assess the publication bias. All genotype distribution of the control population for eligible studies was tested for deviation from Hardy-Weinberg equilibrium (HWE) using Chi-square test (P≤0.1 was considered to be significant). If the genotype distribution in control was not in accordance with Hardy-Weinberg Equilibrium, this study would be excluded in sensitivity analysis.

All statistical test for this meta-analysis were performed with STATA version 12.0 (Stata Corporation, College Station, TX, USA) and the Review Manager, version 5.2 (The Cochrane Collaboration, Oxford, England).

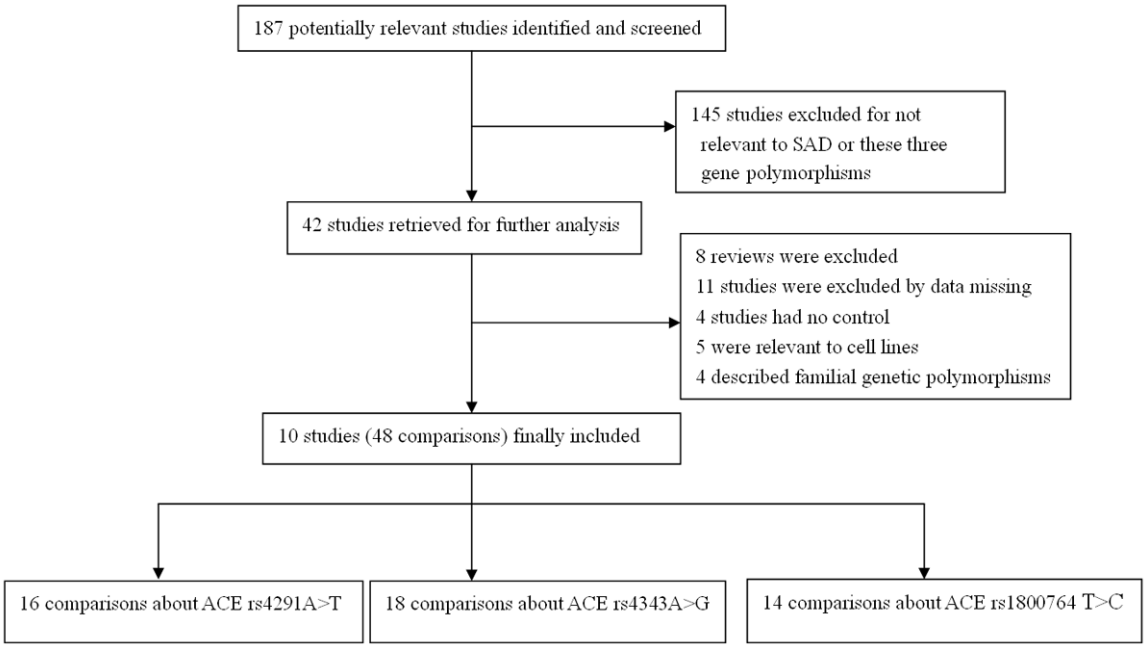

Figure 1. Flow chart of literature search and study selection.

Results

Study selection

A total of 187 articles were retrieved after a comprehensive search of the PubMed and EMBASE databases, and of which 145 of these studies were excluded as not relevant to SAD risk or the ACE (rs4291A>T, rs4343A>G, and rs1800764T>C) genetic polymorphisms. Based on their full articles, a further 32 studies were excluded including 8 reviews, 11 studies with missing data, 4 study with no controls, 5 studies relevant to cell lines, and 4 described familial genetic polymorphisms, Finally, 10 studies met the predetermined inclusion criteria [2, 4, 5, 7, 8, 15, 19, 21, 23, 26], the results of one study were conducted on different district populations, and were considered as different comparisons in the meta-analysis [2]. The PRISMA checklist was showed in **Figure 1**.

Consequently, 48 comparisons of the association of the ACE (rs4291A>T, rs4343A>G, and rs1800764T>C) genetic polymorphisms with the risk of SAD were included in the meta-analysis. 16 comparisons were about ACE rs4291A>T, 18 were about ACE rs4343A>G, and 14 were about ACE rs1800764T>C (**Table 1**).

Characteristics of included studies

Based on the search criteria, 10 studies (48 comparisons) were selected. The main study characteristics were summarized in **Table 1**. There are 16 case-control comparisons with 6,206 SAD cases and 13,095 controls concerning ACE (rs4291A>T) polymorphism [2, 4, 5, 7, 8, 15, 19], 18 case-control comparisons with 6,782 SAD cases and 13,685 controls concerning ACE (rs4343A>G) polymorphism [2, 4, 5, 7, 8, 15, 19, 21, 23, 26] and 5,033 cases and 5,781 controls concerning ACE (rs1800764T>C) polymorphism (14 comparisons) [2, 7, 15, 19, 21, 23]. There were one study of Asian descendents [21] and 9 studies of European descendents [2, 4, 5, 7, 8, 15, 19, 23, 26]. 6 studies used frequency-matched controls to the cases by the age, sex or ethnicity [4, 5, 7, 21, 23, 26]. Diagnoses of definite or probable AD were established according to NINCDS-ADRDA or CERAD in all analyzed articles. Genomic DNA was extracted from peripheral tissues according to standard procedure for 6 studies [2, 4, 5, 7, 8, 21]. Genotypes were determined after polymerase chain reaction (PCR) and TaqMan SNP Genotyping Assays for 4 studies [2, 4, 5, 21]. The distribution of the three ACE genetic polymorphisms was in Hardy-Weinberg equilibrium except 9 comparisons

Table 1. Characteristics of inclusive studies evaluating ACE gene polymorphisms and SAD risk

Author	Year	Country (Ethnicity)	Genotyping Method	SNP	Diagnosis Criteria	Matching Characteristics	HWE		Specimen
							X^2	P	
Helbecque	2009	France (Caucasian)	NP	rs4343	NINCDS-ADRDA	NP	1.83	0.18	Blood
				rs4291			10.45	0.001	
Ghebranious	2011	USA (Caucasian)	NP	rs4343	NINCDS-ADRDA	Gender	0.65	0.42	Blood
				rs4291			0.79	0.37	
				rs1800764			0.87	0.35	
Bruandet	2008	France (Caucasian)	TaqMan	rs4343	NINCDS-ADRDA	Gender, Age	0.21	0.65	Blood
				rs4291			1.26	0.26	
Belbin 1	2010	UK (Caucasian)	TaqMan	rs4343	NINCDS-ADRDA	NP	0.97	0.33	Blood
				rs4291			14.53	0.0001	
				rs1800764			4.05	0.05	
Belbin 2	2010	Germany (Caucasian)	TaqMan	rs4343	NINCDS-ADRDA	NP	0.70	0.40	Blood
				rs4291			0.30	0.58	
				rs1800764			0.86	0.35	
Belbin 3	2010	UK (Caucasian)	TaqMan	rs4343	NINCDS-ADRDA	NP	2.84	0.09	Blood
				rs4291			0.0003	0.99	
				rs1800764			0.45	0.50	
Belbin 4	2010	UK (Caucasian)	TaqMan	rs4343	NINCDS-ADRDA	NP	0.38	0.54	Blood
				rs4291			0.07	0.79	
				rs1800764			0.09	0.76	
Belbin 5	2010	UK (Caucasian)	TaqMan	rs4343	NINCDS-ADRDA	NP	0.55	0.46	Blood
				rs4291			0.68	0.41	
				rs1800764			3.00	0.08	
Belbin 6	2010	UK (Caucasian)	TaqMan	rs4343	NINCDS-ADRDA	NP	0.03	0.88	Blood
				rs4291			0.003	0.96	
				rs1800764			0.45	0.50	
Belbin 7	2010	Sweden (Caucasian)	TaqMan	rs4343	NINCDS-ADRDA	NP	3.81	0.05	Blood
				rs4291			7.17	0.01	
				rs1800764			6.05	0.01	
Belbin 8	2010	USA (Caucasian)	TaqMan	rs4343	NINCDS-ADRDA	NP	2.18	0.14	Blood
				rs4291			0.25	0.62	
				rs1800764			0.28	0.60	

Belbin 9	2010	USA (Caucasian)	TaqMan	rs4343	NINCDS-ADRDA	NP	0.005	0.94	Blood
				rs4291			0.52	0.47	
				rs1800764			0.003	0.96	
Belbin 10	2010	USA (Caucasian)	TaqMan	rs4343	NINCDS-ADRDA	NP	0.12	0.73	Blood
				rs4291			0.23	0.63	
				rs1800764			2.10	0.15	
Miners	2009	Sweden (Caucasian)	NP	rs4343	CERAD	NP	0.31	0.58	NP
				rs4291			0.02	0.89	
				rs1800764			0.75	0.39	
Prince	2001	Sweden (Caucasian)	PCR primers	rs4343	NINCDS-ADRDA	Gender	1.80	0.18	NP
Ning	2010	China (Asian)	TaqMan	rs4343	NINCDS-ADRDA	Gender	1.06	0.30	Blood
				rs1800764			0.66	0.42	
Kehoe	2003	Sweden (Caucasian)	Specific hybridization	rs4343	NINCDS-ADRDA	NP	1.42	0.23	NP
				rs4291			2.04	0.15	
				rs1800764			0.0008	0.98	
Cousin	2011	France (Caucasian)	TaqMan	rs4291	NINCDS-ADRDA	Age	1.44	0.23	Blood
Sarajärvi	2010	Finland (Caucasian)	Sequenom iPLEX	rs4343	NINCDS-ADRDA	Age	11.50	0.0007	NP

NP: Not Provided, HWE: Hardy-Weinberg equilibrium; NINCDS-ADRDA: the criteria of the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS) 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.

Table 2. The results of meta-analysis in overalls

Gene polymorphism	Genotype	No. of comparisons	Test of association			Model	Test of heterogeneity		
			OR	95% CI	*P*		Q	*P*	I^2 (%)
rs4291A>T	AA+AT vs. TT	16	1.08	0.94, 1.25	0.28	R	26.50	0.03	43%
	AA vs. TT	16	1.12	0.96, 1.30	0.14	R	25.50	0.04	41%
	AA vs. AT+TT	16	1.06	0.96, 1.18	0.27	R	27.45	0.03	45%
	AA vs. AT	16	1.05	0.94, 1.17	0.41	R	26.87	0.03	44%
	AT vs. TT	16	1.07	0.92, 1.24	0.41	R	26.37	0.03	43%
rs4343A>G	AA+AG vs. GG	18	1.03	0.95, 1.11	0.46	F	11.87	0.81	0%
	AA vs. GG	18	1.06	0.96, 1.17	0.24	F	14.80	0.61	0%
	AA vs. AG+GG	18	1.06	0.95, 1.18	0.29	R	28.39	0.04	40%
	AA vs. AG	18	1.05	0.93, 1.18	0.44	R	30.75	0.02	45%
	AG vs. GG	18	1.01	0.93, 1.10	0.73	F	14.61	0.62	0%
rs1800764T>C	TT+TC vs. CC	14	1.11	0.97, 1.26	0.13	R	20.27	0.09	36%
	TT vs. TC	14	1.00	0.92, 1.10	0.96	F	16.04	0.25	19%
	TT vs. TC+CC	14	1.03	0.94, 1.12	0.53	F	14.80	0.32	12%
	TT vs. CC	14	1.09	0.98, 1.22	0.12	F	16.46	0.23	21%
	TC vs. CC	14	1.12	0.97, 1.29	0.14	R	21.60	0.06	40%

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

(rs4291A>T: [8, 2 (1, 7)], rs4343A>G: [2 (3, 7)], rs1800764C>T: [2 (1, 5, 7), 26]) (**Table 1**), and these comparisons would be excluded for the sensitivity analysis.

Meta-analysis results

Association between the ACE rs4291A>T polymorphism and SAD risk: All 16 comparisons investigating the ACE rs4291A>T polymorphism reported on Caucasian populations [2, 4, 5, 7, 8, 15, 19]. There was between-study heterogeneity in all models, the results of our meta-analysis based on the random effects model (AA+AT versus TT: OR = 1.08, 95% CI = 0.94, 1.25, P = 0.28; AA versus AT: OR = 1.05, 95% CI = 0.94, 1.17, P = 0.41; AA versus AT+TT: OR = 1.06, 95% CI = 0.96, 1.18, P = 0.27; AA versus TT: OR = 1.12, 95% CI = 0.96, 1.30, P = 0.14; AT versus TT: OR = 1.07, 95% CI = 0.92, 1.24, P = 0.41) showed that rs4291A>T polymorphism was not related with SAD risk (**Table 2**).

Association between the ACE rs4343A>G polymorphism and SAD risk: For this SNP, 18 comparisons were conducted [2, 4, 5, 7, 8, 15, 19, 21, 23, 26]. For fixed-effect model, no association between the ACE (rs4343) polymorphism and SAD risk was observed in the overall populations in dominant model (AA+AG versus GG: OR = 1.03, 95% CI: 0.95-1.11, P = 0.46), homozygote comparison (AA versus GG: OR = 1.06, 95% CI: 0.96-1.17, P = 0.24), and heterozygote comparison (AG versus GG: OR = 1.01, 95% CI: 0.93-1.10, P = 0.73). For random effects model, no association was also found in recessive model (AA versus (AG+GG): OR = 1.06, 95% CI: 0.95-1.18, P = 0.29), and heterozygote comparison (AA versus AG: OR = 1.05, 95% CI: 0.93-1.18, P = 0.44) (**Table 2**). And the association was similar with overalls' between the ACE (rs4343A>G) polymorphism and SAD risk in Caucasians (AA+AG versus GG: OR = 1.03, 95% CI: 0.95-1.11, P = 0.46, AA versus GG: OR = 1.05, 95% CI: 0.95-1.16, P = 0.34, AG versus GG: OR = 1.02, 95% CI: 0.94-1.11, P = 0.61, AA versus (AG+GG): OR = 1.03, 95% CI: 0.95-1.12, P = 0.42, AA versus AG: OR = 1.03, 95% CI: 0.94-1.12, P = 0.54). The results of meta-analysis showed that rs4343A>G polymorphism is not related with SAD risk in overalls or Caucasians.

Association between the ACE rs1800764T>C polymorphism and SAD risk: 14 comparisons evaluated this polymorphism in Caucasians [2, 7, 15, 19, 23], one study on this polymorphism was performed in Asians [21]. For the fixed-effect model, we found no association between the ACE (rs1800764T>C) polymorphism and SAD risk in the overall populations in recessive

Table 3. The results of meta-analysis in overalls for sensitivity analysis

Gene polymorphism	Genotype	No. of comparisons	Test of association			Model	Test of heterogeneity		
			OR	95% CI	*P*		Q	*P*	I^2 (%)
rs4291A>T	AA+AT vs. TT	13	1.04	0.89, 1.22	0.63	R	21.47	0.04	44%
	AA vs. TT	13	1.10	0.93, 1.29	0.27	R	19.64	0.07	39%
	AA vs. AT+TT	13	1.09	1.01, 1.18	0.03	F	8.18	0.77	0%
	AA vs. AT	13	1.09	1.00, 1.18	0.05	F	3.99	0.98	0%
	AT vs. TT	13	1.10	0.98, 1.24	0.11	F	17.57	0.13	32%
rs4343A>G	AA+AG vs. GG	15	1.02	0.94, 1.11	0.70	F	8.38	0.87	0%
	AA vs. GG	15	1.06	0.96, 1.17	0.26	F	14.60	0.41	4%
	AA vs. AG+GG	15	1.09	0.96, 1.22	0.18	R	25.92	0.03	46%
	AA vs. AG	15	1.09	0.96, 1.23	0.19	R	25.23	0.03	45%
	AG vs. GG	15	0.99	0.91, 1.09	0.88	F	8.20	0.88	0%
rs1800764T>C	TT+TC vs. CC	11	1.05	0.92, 1.19	0.46	F	13.02	0.22	23%
	TT vs. TC	11	1.04	0.95, 1.15	0.38	F	7.95	0.63	0%
	TT vs. TC+CC	11	1.05	0.95, 1.16	0.32	F	11.26	0.34	11%
	TT vs. CC	11	1.07	0.92, 1.26	0.38	F	15.11	0.13	34%
	TC vs. CC	11	1.03	0.92, 1.15	0.57	F	9.81	0.46	0%

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

model (TT versus TC+CC: OR = 1.03, 95% CI: 0.94-1.12, P = 0.53), homozygote comparison (TT versus CC: OR = 1.09, 95% CI: 0.98-1.22, P = 0.12), heterozygote comparison (TT versus TC: OR = 1.00, 95% CI: 0.92-1.10, P = 0.96), dominant model (TT+TC versus CC): OR = 1.11, 95% CI: 0.97-1.26, P = 0.13, and heterozygote comparison (TC versus CC: OR = 1.12, 95% CI: 0.97-1.29, P = 0.14) (**Table 2**). We did not find an association between the ACE (rs1800164-T>C) polymorphism and SAD risk in Caucasians (TT+TC versus CC: OR = 1.12, 95% CI: 0.97-1.28, P = 0.13, TT versus TC+CC: OR = 1.03, 95% CI: 0.94-1.12, P = 0.53, TT versus CC: OR = 1.11, 95% CI: 0.99-1.24, P = 0.08, TC versus CC: OR = 1.09, 95% CI: 0.98-1.21, P = 0.12, TT versus TC: OR = 1.02, 95% CI: 0.93-1.11, P = 0.75). So the ACE rs1800764T>C polymorphism is not associated with SAD risk in overalls or Caucasians.

Sensitivity analysis and publication bias

Sensitivity analysis was conducted to determine whether modification of the inclusion criteria of the meta-analysis affected the final results. The included studies were limited to those conforming to HWE, however, the results were not consistent with overalls' for rs4291A>T (AA versus AT+TT: OR = 1.09, 95% CI: 1.01-1.18, *P* = 0.03 and AA versus AT: OR = 1.09, 95% CI: 1.00-1.18, *P* = 0.05) (**Table 3**). So an elevated risk was observed for AA versus AT and AA versus AT+TT after excluding the studies that deviated from HWE, and the increased risk of SAD might appear in populations among homozygous (AA) carriers.

Begg's funnel plot and Egger's regression test were performed to assess potential publication bias. For the ACE rs4291A>T AA versus AT and AA versus AT+TT in sensitivity analysis, visual inspection of the funnel plot (**Figures 2**, **3**) appeared to be approximately symmetrical distribution of OR estimations, suggesting no publication bias. In addition, the results of Egger's regression test also provided no evidence for publication bias (t = -0.84, *P* = 0.42 for AA versus AT+TT and t = -0.23, *P* = 0.82 for AA versus AT). So these results supported the robustness of our findings for sensitivity analysis.

Discussion

A key pathological feature of Alzheimer's disease is the abnormal extracellular accumulation of the amyloid-β (Aβ) peptide, and altered Aβ degradation could be a major contributor to the development of AD [24, 27]. Cumulative evidence strongly support the role of ACE widely expressed in the brain as an Aβ degrading enzyme [12, 31], In fact, rs4291A>T or rs4343A>G was reported to influence Ab42

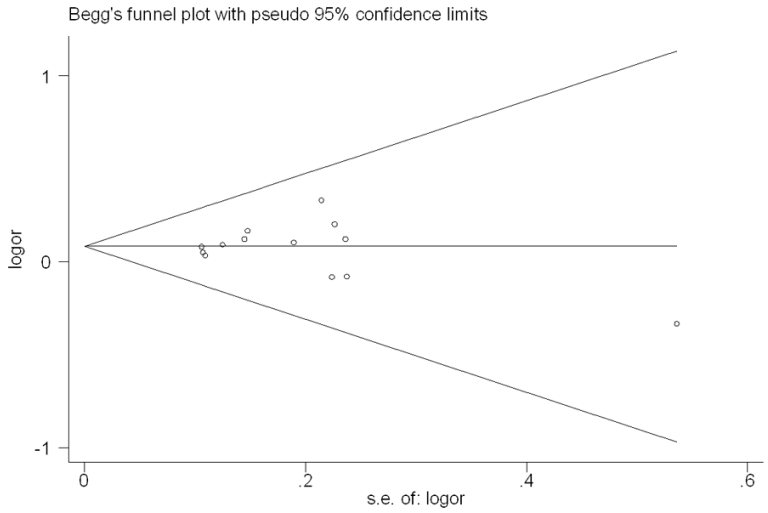

Figure 2. The plot for ACE rs4291A>T polymorphism (AA versus AT) and SAD risk for sensitivity analysis.

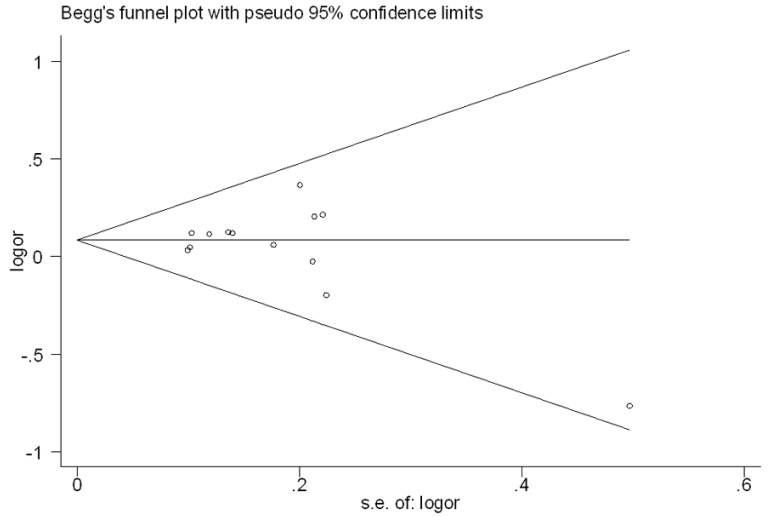

Figure 3. The plot for ACE rs4291A>T polymorphism (AA versus AT+TT) and SAD risk for sensitivity analysis.

level in cerebrospinal fluid (CSF) [15]. ACE rs1800764T>C has also been associated with elevated CSF Aβ42/Aβ40 ratio [14]. Several studies have been carried out to identify whether SNPs rs4291A>T, rs4343A>G, or rs1800764T>C polymorphism was associated with SAD risk, however, up to now the genome-wide association studies published reported no consensus for these SNPs as risk factors for SAD.

No gene (ACE rs4343A>G) effect could be evaluated on the risk of AD in the two France studies, Bruandet et al. reported the genotype distributions of the ACE SNPs rs4343A>G did not differ between AD cases and healthy controls [4], Cousin also failed to find the association [5]. In Finland [22] and Sweden [19], the similar results were found. However, in other France study, Helbecque et al. showed a statistically significant effect on the risk of AD based on 376 late-onset AD patients and 444 control subjects [8]. One study from Shanghai of China exhibited ACE rs4343-A>G might play a role in AD susceptibility [21]. In our meta-analysis, no association between ACE rs4343A>G genetic polymorphism and SAD risk. The results were consistent with that of most comparisons [4, 5, 19, 22]. Age onset is a well known risk factor for the development of SAD, Helbecque et al. exhibited the oldest patients bearing the ACE rs4343G was at reduced risk of SAD, and also showed a statistical difference between risk of AD and the haplotype ATI (rs4343/rs4291/rs1799752) in subjects aged 73 years and above [8]. In Asia, rs4343 A/G allele was related with SAD risk was firstly found in China [21]. So age onset, haplotypic effect or ethnicity might be an important factor on the ACE (rs4343A>G) genetic risk of SAD.

Bruandet et al. stated no genetic effect (SNP rs4291A>T) could be observed on the risk of AD [4], and no association was also found in Sweden [19]. Our meta-analysis on rs4291A>T confirmed this observation. However, the possible association was found by Ghebranious et al. [7] or Kehoe et al. [15], sensitivity analysis was performed in our meta-analysis, and the results showed that overalls' results were not statistically robust. An elevated risk was obser-

ved for AA versus AT and AA versus AT+TT after excluding the studies that deviated from HWE. Ghebranious et al. reported the association of the ACE SNPs with LOAD was varied by APOE4 allele status or different environments [7], the data also provided the evidence that gene/gene interaction is occurring in ACE genetic risk factor to SAD [8], so the interaction might be an important factor for ACE rs4291A>T genetic risk of SAD and effect on ACE rs4291A>T genetic risk of SAD.

Our meta-analysis results showed that the rs1800764T>C polymorphism was not associated with a risk of SAD in overalls. No association was detected in USA [7] and Sweden [19]. In only an Asian study, Ning et al. confirmed that rs1800764 T-allele was associated with risk of AD [21]. So a statistically significant decreased SAD risk might be found in Asian population but not in Caucasians. Actually, it might be common that the same polymorphism play different roles in SAD susceptibility among different ethnic populations, and the difference in ethnic backgrounds or the environment they lived in may influence the association between rs1800764T>C polymorphism and SAD risk.

Some limitations of our meta-analysis of observational studies should be taken into consideration. First, in our inclusive articles, the samples (blood and brain) were selected and different genotyping methods were used with different sensitivity and specificity, which might result in selection bias and clinic heterogeneity. However, we did not carry out subgroup analysis based on above factors due to lack of sufficient sample size. Second, the gene-gene or gene-environment interaction might affect the risk of SAD [7, 21]. However, the small sample size may have yielded false-positive or false-negative results due to lack of statistical power. Therefore, it is critical that larger-scale studies and well-designed research would be done to analyze the interactions. However, in all models of meta-analysis, the results of heterogeneity detected were I^2 values of <50%, and suggested the heterogeneity of inclusive comparisons were moderate and acceptable. At last, the sensitivity analysis exhibited the overalls' results of rs4291A>T (AA versus AT+TT and AA versus AT) was not robust. In contrast, the rs4291A>T might be a factor on genetic risk of SAD based on the sensitivity analysis, and gene-gene and gene-environment interactions could affect on rs4291A>T genetic risk of SAD, and studies with large sample size should be done to confirm these effects.

In conclusion, the first meta-analysis results suggest that the ACE (rs4291A>T) polymorphism might be associated with the susceptibility to SAD, the rs4291 AA genotype is a risk factor for SAD and gene-gene or gene-environment interaction plays a role in rs4291A>T genetic risk of SAD, whatever rs4343A>G or rs1800764T>C is be not a genetic factor of SAD risk. Further studies with large sample size, especially with the consideration of gene-gene and gene-environment interactions, will be needed to confirm our findings.

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Disclosure of conflict of interest

None.

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