

## Original Article

# The association of three BACE1 gene polymorphisms (exon5 C/G, intron 5 T/G and 3'UTR T/A) with sporadic Alzheimer's disease susceptibility: a meta-analysis

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**Abstract:** Despite biological support for a role of Beta-site APP-cleaving enzyme 1 (BACE1) in sporadic Alzheimer's disease (SAD), studies about the BACE1 genetic polymorphisms in SAD are inconsistent. To explore whether the BACE1 polymorphisms confers susceptibility to SAD, the current meta-analysis was conducted to evaluate the gene-disease association in relevant studies. The serious databases were researched to identify studies. The association between BACE1 (exon5 C/G, intron 5 T/G or 3'UTR T/A) polymorphism and SAD risk was evaluated by odds ratios (ORs) together with their 95% confidence intervals (CIs). The combined results showed no significant difference in all models on the basis of all studies for BACE1 (exon5 C/G, intron 5 T/G or 3'UTR T/A) polymorphisms. When subgroup analysis was performed based on ethnicity and the epsilon 4 allele of apolipoprotein E (APOE $\epsilon$ 4) carriers status, significant associations were demonstrated (CC versus CG+GG: OR=1.37, 95% CI=1.04-1.82,  $P=0.03<0.05$  and CC versus CG: OR=1.49, 95% CI=1.11-2.01,  $P=0.01<0.05$ ) for APOE $\epsilon$ 4 carriers status. The pooled results suggest the BACE1 (exon5 C/G, intron 5 T/G or 3'UTR T/A) polymorphism could be not a risk factor for SAD. However, individuals with CC genotype have higher risk of SAD with APOE $\epsilon$ 4 carrier status, and gene-gene interaction might affect on the association. Further studies with large sample size, especially in subgroup analysis, should be done to confirm these findings.

**Keywords:** BACE1, gene, polymorphism, alzheimer's disease, meta-analysis

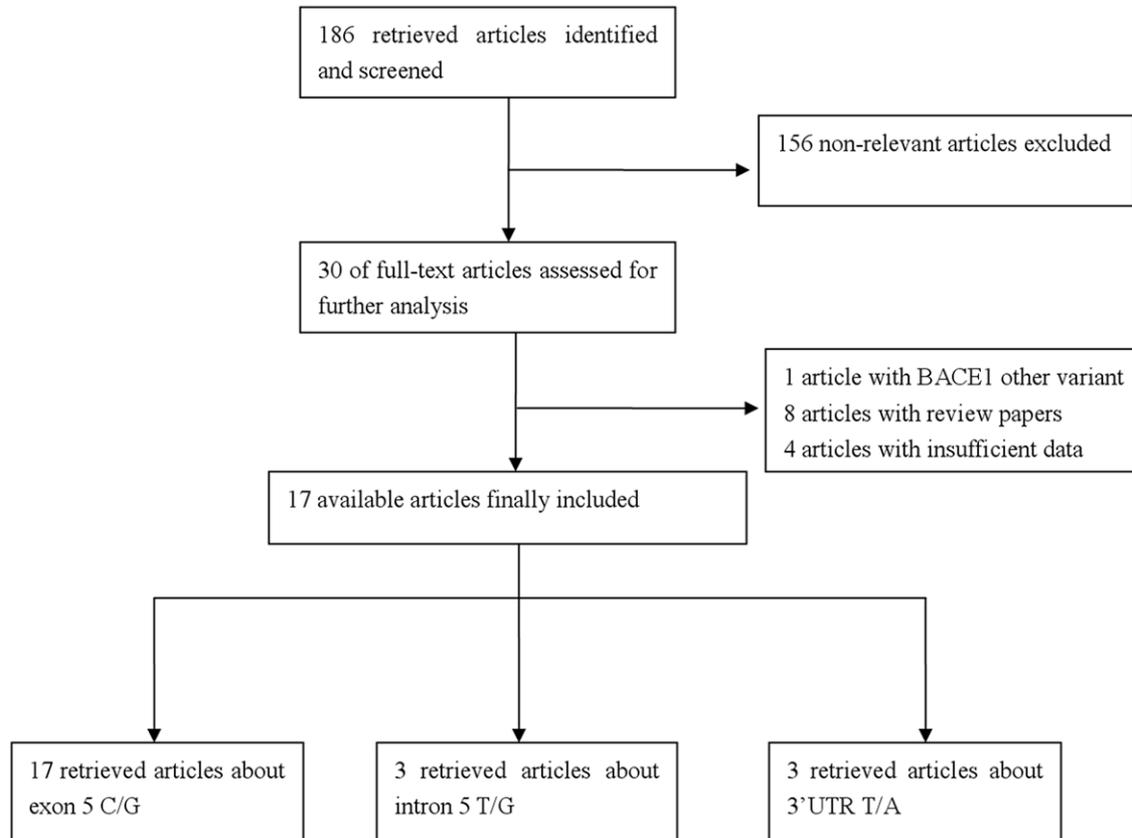
## Introduction

Alzheimer's disease (AD), a progressive neurodegenerative disorder, accounts for impairment in cognitive function. The essential pathological features of AD are characterized by extracellular amyloid beta (A $\beta$ )-containing senile plaques and intraneuronal fibrillary tangles [1, 2]. A $\beta$ -peptide is generated via sequential proteolytic cleavage of the  $\beta$ -amyloid precursor protein (APP) by  $\beta$ - and  $\gamma$ -secretase [3]. Beta-site APP-cleaving enzyme 1 (BACE1) as an important  $\beta$ -secretase is predominantly expressed in neuronal cells and cleaves APP at Asp1 and Glu11 of A $\beta$  [4, 7, 8, 26-28, 31]. Furthermore, previous articles exhibited the higher in BACE1 protein levels and enzymatic

activity in AD was showed in the brains of patients with AD versus age-matched controls [5, 6, 29, 30], and its protein and activity levels increase with both aging and in brain regions affected by amyloid deposition [29, 30], and in transgenic mice, BACE1 is the major  $\beta$ -secretase for A $\beta$  peptide generation by neurons [7, 8]. So BACE1 plays an important role in developing Alzheimer's disease. And its gene polymorphisms have been also taken into account in increased risk of AD by modulating the A $\beta$  production.

BACE1 located on chromosome 11q23.2-3, closed to the region with increased LOD score for AD [9], has been identified, and variations in BACE1 gene might be associated with the risk

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**Figure 1.** Flow chart of the process about eligible articles selection in our meta-analysis.

for AD has been speculated. The polymorphisms of BACE1 (exon5 C/G, intron 5 T/G and 3'UTR T/A) have been reported to be associated with the risk for AD [10-12, 14], but others generated conflicting result [13, 15-23], and several studies described a association of the exon5 (C/G) with AD in the epsilon 4 allele of apolipoprotein E (APOE $\epsilon$ 4) carriers [11, 12, 15, 17, 19, 24]. So the large-scale studies should be pooled to refute gene-disease associations. Previously published meta-analysis reported a significant association between exon 5 C/G polymorphism and risk of SAD in Asians [15]. However, it remains unclear whether ethnicity (Asians or Caucasians) and other gene could affect the association. Since then, additional many studies with a large sample size about this association have been reported. We investigated the possible association of BACE1 polymorphisms (exon5 C/G, intron 5 G/T and 3'UTR T/A) with SAD risk by an update meta-analysis and subgroup analyses on basis of ethnicity and the APOE $\epsilon$ 4 carriers status to derive a more precise estimation of the relationships.

### Materials and methods

#### Search strategy

Studies were identified by searching the serious databases: MEDLINE, EMBASE and HuGENet without language restriction, and the searched studies were conducted on human subjects. The following Medical Subject Heading (MESH) terms and text words were used: Alzheimer's disease, Alzheimer disease, AD in combination with Beta-site APP-cleaving enzyme 1, BACE1, polymorphism, genotype, gene, or mutation. Two investigators (Kang Ling and Xunping Du) independently reviewed abstracts or full text of all citations to identify eligible studies. The identified articles had to meet the following information: (1) the SAD was diagnosed clinically; (2) the case-control design study; (3) frequency of people and individual BACE1 genotype (exon5 C/G, intron 5 G/T and 3'UTR T/A) in cases and controls were reported. The exclusion criterion was (1) a family history of dementia in cases; (2) case reports, editorials, and review articles.

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## Data extraction

All studies were checked by two investigators (Kang Ling and Xunping Du) independently according to the prespecified selection criteria, the relevant data of eligible studies were extracted or calculated, and entered separate databases. Discrepancy was resolved following discussions. The following characteristics of eligible studies were extracted: first author, year of publication, ethnicity, clinical characteristics, numbers of genotype (exon5 C/G, intron 5 T/G or 3'UTR T/A) of cases and controls, and genotyping methods.

## Statistical analysis

For dichotomous outcomes, the odds ratios and their 95% confidence intervals were calculated using STATA, version 12.0. For exon5 C/G, five different ORs were calculated in our analysis: dominant model (CC+CG versus GG), recessive model (CC versus (CG+GG)), homozygote comparison (CC versus GG), and heterozygote comparison (CG versus GG; CC versus CG). The statistical significance was determined by the Z-test ( $P \leq 0.05$  was considered statistically significant). Subgroup analyses were conducted on the basis of patients with APOE $\epsilon$ 4 carrier status and ethnicity. The same methods were applied to two other polymorphisms (intron 5 T/G and 3'UTR T/A).

All genotype distribution of the control population of eligible studies was tested for deviation from Hardy-Weinberg equilibrium (HWE) using Chi-square test ( $P \leq 0.05$  was considered to be significant). If the genotype distribution was not in accordance with Hardy-Weinberg Equilibrium, this study would be excluded for sensitivity analysis. The test for heterogeneity between studies was performed with Cochran's Q statistic ( $P > 0.10$  was considered representative of homogeneity). A pooled OR was calculated using the fixed-effect model (the Mantel-Haenszel method) when there was homogeneity [32]. Otherwise, the random effects model (Der Simonian-Laird) was adopted [33].

The stability of conclusion was detected by performing sensitivity analysis. The higher heterogeneity studies involved in the meta-analysis were deleted to reflect the influence of the related data to the pooled ORs. The visual Begg's funnel plot was utilized to explore publication bias, and the Egger's linear regression test was taken to quantitatively assess the

publication bias ( $P \leq 0.05$  was considered statistically significant) (version 12.0, STATA Corp., College Station, TX, USA).

## Results

### Identification of eligible studies

The 186 potentially relevant studies were retrieved through the search criteria, and 156 of these articles were excluded as irrelevant to SAD risk and BACE1 polymorphisms. The full-text from 30 articles was reviewed and 13 studies were excluded (eight reviews, four studies with not identified allele frequency and one article with BACE1 other variant). Thus, 17 papers were found to match our inclusion criteria (**Figure 1**). Different comparisons were distinguished based on population distribution for one article [11]. The genotype distribution for control group in three studies did not follow HWE for exon 5 C/G [19, 23, 25], and these studies were exclusive in our sensitivity analysis. Characteristics of studies were presented in **Table 1**.

A total of 17 articles were included in our meta-analysis [10-25, 35]. For most studies, the polymerase chain reaction (PCR)-restriction fragment length polymorphism was performed, the diagnosis of definite or probable SAD was established according to NINCDS-ADRDA [34], the age or sex-matched controls to the cases were found, and genomic DNA was isolated from peripheral tissues according to standard procedure. There were consisted of 11 European samples [10, 14, 16, 17, 19-23, 25, 35] and 7 Asian populations [11-13, 15, 18, 24] (**Table 1**).

### Meta-analysis database

For exon 5 C/G, the combined results showed no significant difference in CC+CG versus GG (OR=0.99, 95% CI: 0.78-1.26,  $P=0.96$ ), CC versus (CG+GG) (OR=0.99, 95% CI: 0.82-1.19,  $P=0.89$ ), CC versus GG (OR=0.91, 95% CI: 0.74-1.12,  $P=0.38$ ), CG versus GG (OR=1.03, 95% CI: 0.74-1.43,  $P=0.86$ ) and CC versus CG (OR=1.01, 95% CI: 0.81-1.25,  $P=0.95$ ) under the random-effects model. No effect on genetic risk of SAD was exhibited for exon 5 C/G. All results for genetic models and the test of heterogeneity were summarized in **Table 2**.

All of the three European studies were evaluated for intron 5 T/G and 3'UTR T/A respectively.

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**Table 1.** Characteristics of inclusive studies evaluating BACE1 genetic polymorphisms and SAD risk

Gene/Author	Year	Specimen	Ethnicity	Diagnosis Criteria	Cases					Control					HWE	
					F	Age	CC	CG	GG	F	Age	CC	CG	GG	$\chi^2$	P
exon5 C/G																
Jo et al. [15]	2008	Blood	Asian	NINCDS-ADRDA	184	71.6±8.7	109	111	28	137	68.3±6.3	97	104	23	0.4013	0.53
Murphy et al. [16]	2001	--	Caucasian	CERAD	153	81.2±7.8	42	83	87	202	82.1±3.8	54	145	130	1.5597	0.21
Nowotny et al. [17]	2001	--	Caucasian	--	123	--	44	119	99	163	74.5±9.0	39	136	99	0.4996	0.48
Nicolaou et al. [35]	2001	--	Caucasian	NINCDS-ADRDA	--	76.1±7.8	39	86	48	--	69.6±12.5	33	69	53	1.3827	0.24
Liu et al. [18]	2003	--	Asian	NINCDS-ADRDA	54	77.3±7.8	36	50	13	69	77.3±7.8	57	63	8	3.0121	0.08
Gold et al. [19]	2003	blood	Caucasian	--	51	79.9±9.3	20	42	30	78	74.7±7.4	21	85	44	3.8699	0.05
Kirschling et al. [10]	2003	blood	Caucasian	NINCDS-ADRDA	132	72.9±8.1	17	97	70	131	71.7±9.2	41	111	95	0.7774	0.38
Shi (Guangzhou) et al. [11]	2004	blood	Asian	NINCDS-ADRDA	153	76.7±8.8	129	109	19	128	80.0±7.6	95	123	24	3.0503	0.08
Shi (Chengdu) et al. [11]	2004	blood	Asian	NINCDS-ADRDA	72	81.5±7.8	62	42	8	62	78.2±8.1	47	53	13	0.1116	0.74
Kan et al. [12]	2005	blood	Asian	NINCDS-ADRDA	48	79.2±6.3	39	51	15	39	68.1±2.8	68	51	12	0.2927	0.59
Cai et al. [24]	2005	blood	Asian	--	72	74.51	18	55	43	153	57.8	52	147	72	2.2349	0.14
Randall et al. [25]	2009	blood	Caucasian	NINCDS-ADRDA	128	--	0	326	74	62	--	65	35	100	81.649	0
Todd et al. [20]	2008	blood	Caucasian	NINCDS-ADRDA	293	77.9±7.3	82	194	185	268	75.3±9.0	68	187	161	1.1981	0.27
Wang et al. [13]	2010	--	Asian	NINCDS-ADRDA	260	71.3±7.2	146	208	72	216	72.5±8.1	120	174	49	1.2346	0.27
Cousin et al. [22]	2011	blood	Caucasian	NINCDS-ADRDA	--	64.9±9.9	154	180	69	--	66.2±10.8	189	198	67	1.627	0.2
Clarimón et al. [14]	2003	blood	Caucasian	NINCDS-ADRDA	101	76.6±5.3	20	57	59	45	74.9±5.3	11	51	25	3.6057	0.06
Cruts et al. [23]	2001	--	Caucasian	--	--	--	12	40	45	--	--	17	40	60	5.1332	0.03
Laws et al. [21]	2011	blood	Caucasian	--	--	69.0±9.1	154	214	70	--	66.8±12.0	102	143	45	0.1936	0.66
3'UTR T/A																
Gold et al. [19]	2003	blood	Caucasian	--	--	--	64	16	0	--	--	127	32	1	0.4505	0.5
Todd et al. [20]	2008	blood	Caucasian	NINCDS-ADRDA	--	--	377	98	6	--	--	330	77	11	5.7951	0.01
Clarimón et al. [14]	2003	blood	Caucasian	NINCDS-ADRDA	--	--	109	24	3	--	--	66	20	1	0.1439	0.7
intron5 T/G																
Murphy et al. [16]	2001	--	Caucasian	CERAD	--	--	106	87	19	--	--	158	87	23	4.5281	0.03
Kirschling et al. [10]	2003	blood	Caucasian	NINCDS-ADRDA	--	--	45	71	12	--	--	81	103	21	2.0101	0.16
Clarimón et al. [14]	2003	blood	Caucasian	NINCDS-ADRDA	--	--	48	69	19	--	--	28	52	7	6.3123	0.01

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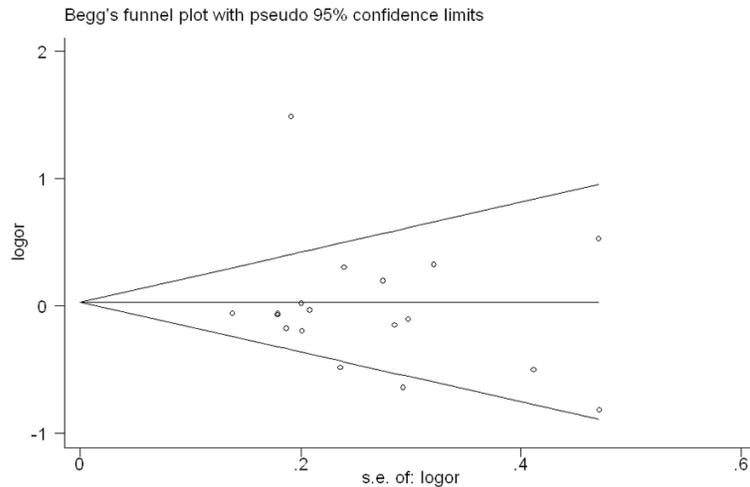
**Table 2.** Meta-analysis of the association between BACE1 genetic polymorphisms and SAD risk

Comparison	Population	No. of comparisons	Test of association			Mode	Test of heterogeneity		
			OR	95% CI	P value		$\chi^2$	P value	$I^2$ (%)
Exon 5 C/G									
CC+CG VS GG	Overall	18	0.99	0.78, 1.26	0.96	R	80.49	0.00	78.9
	European	11	1.09	0.80, 1.48	0.61	R	66.14	0.00	84.9
	Asian	7	0.82	0.66, 1.02	0.07	F	8.85	0.18	32.2
	APOE $\epsilon$ 4	7	0.77	0.42, 1.42	0.41	R	15.95	0.01	62.4
	Non-APOE $\epsilon$ 4	7	1.08	0.83, 1.42	0.60	R	10.98	0.09	45.4
CC VS CG+GG	Overall	18	0.99	0.82, 1.19	0.89	R	50.05	0.00	66.0
	European	11	0.97	0.73, 1.29	0.84	R	34.94	0.00	71.4
	Asian	7	1.00	0.75, 1.32	0.97	R	17.52	0.008	65.7
	APOE $\epsilon$ 4	7	1.37	1.04, 1.82	0.03	F	8.74	0.19	31.3
	Non-APOE $\epsilon$ 4	7	1.16	0.88, 1.54	0.30	R	16.12	0.01	62.8
CCVSGG	Overall	18	0.91	0.74, 1.12	0.38	R	32.70	0.01	48.0
	European	11	0.95	0.74, 1.21	0.67	R	18.93	0.04	47.2
	Asian	7	0.85	0.57, 1.27	0.43	R	13.81	0.03	56.6
	APOE $\epsilon$ 4	7	0.90	0.44, 1.83	0.77	R	14.38	0.03	58.3
	Non-APOE $\epsilon$ 4	7	1.17	0.79, 1.74	0.44	R	15.57	0.02	61.5
CC VS CG	Overall	18	1.01	0.81, 1.25	0.95	R	57.56	0.00	70.5
	European	11	0.96	0.67, 1.36	0.81	R	48.45	0.00	79.4
	Asian	7	1.05	0.82, 1.34	0.71	R	12.35	0.06	51.4
	APOE $\epsilon$ 4	7	1.49	1.11, 2.01	0.01	F	4.60	0.60	0.0
	Non-APOE $\epsilon$ 4	7	1.17	0.92, 1.49	0.19	R	10.47	0.10	42.7
CG VS GG	Overall	18	1.03	0.74, 1.43	0.86	R	135.5	0.00	87.5
	European	11	1.17	0.74, 1.85	0.51	R	124.9	0.00	92.0
	Asian	7	0.80	0.64, 1.01	0.06	F	4.01	0.68	0.0
	APOE $\epsilon$ 4	7	0.69	0.40, 1.21	0.20	R	12.07	0.06	50.3
	Non-APOE $\epsilon$ 4	7	1.03	0.86, 1.25	0.75	F	5.10	0.53	0.0
Intron 5 T/G									
TT VS TG+GG	Overall	3	0.81	0.63, 1.05	0.11	F	2.12	0.35	5.7
TT+TG VS GG	Overall	3	0.87	0.57, 1.33	0.53	F	1.53	0.47	0.0
TT VS GG	Overall	3	0.81	0.52, 1.27	0.37	F	0.45	0.80	0.0
TT VS TG	Overall	3	0.82	0.63, 1.06	0.13	F	3.33	0.19	39.9
TG VS GG	Overall	3	0.97	0.63, 1.50	0.90	F	2.77	0.25	27.7
3'UTR T/A									
TT+TA VS AA	Overall	3	1.64	0.70, 3.84	0.25	F	1.26	0.53	0.0
TT VS TA+AA	Overall	3	1.02	0.79, 1.33	0.86	F	0.60	0.74	0.0
TT VS TA	Overall	3	0.98	0.75, 1.29	0.89	F	1.27	0.53	0.0
TA VS AA	Overall	3	1.66	0.70, 3.97	0.25	F	1.84	0.40	0.0
TT VS AA	Overall	3	1.64	0.70, 3.85	0.26	F	1.11	0.58	0.0

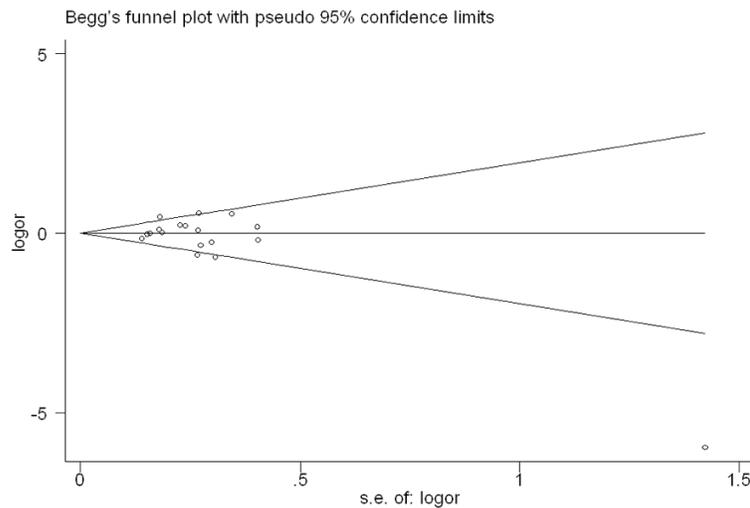
We used fix-effect to pool the results and found no statistic difference for intron 5 T/G ((TT+TG versus GG (OR=0.87, 95% CI: 0.57-1.33,  $P=0.53$ ), TT versus (TG+GG) (OR=0.81, 95% CI: 0.63-1.05,  $P=0.11$ ), TT versus GG (OR=0.81, 95% CI: 0.52-1.27,  $P=0.37$ ), TG versus GG (OR=0.97, 95% CI: 0.63-1.50,  $P=0.90$ ) and TT versus TG (OR=0.82, 95% CI: 0.63-1.06,

$P=0.13$ )) and 3'UTR T/A ((TT+TA versus AA (OR=1.64, 95% CI: 0.70-3.84,  $P=0.25$ ), TT versus (TA+AA) (OR=1.02, 95% CI: 0.79-1.33,  $P=0.86$ ), TT versus AA (OR=1.64, 95% CI: 0.70-3.85,  $P=0.26$ ), TA versus AA (OR=1.66, 95% CI: 0.70-3.97,  $P=0.25$ ) and TT versus TA (OR=0.98, 95% CI: 0.75-1.29,  $P=0.89$ ) based on the homogeneity of including studies (**Table 2**).

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**Figure 2.** Funnel plots for publication bias of BACE1 exon 5 C/G polymorphism and SAD risk in the overall (dominant model: CC+CG versus GG).



**Figure 3.** Funnel plots for publication bias of BACE1 exon 5 C/G polymorphism and SAD risk in the overall (recessive model: CC versus CG+GG).

### Subgroup analysis

For exon 5 C/G, no statistical significance was found in Caucasian or Asian populations (data were showed in **Table 2**). However, in the subgroup analysis by APOE $\epsilon$ 4 carriers status, higher SAD risk was also observed for APOE $\epsilon$ 4 carriers status (CC versus CG+GG: OR=1.37, 95% CI=1.04-1.82,  $P=0.03$  and CC versus CG: OR=1.49, 95% CI=1.11-2.01,  $P=0.01$ ), and people within CC genotype have higher risk of SAD. However, the results were not pronounced among non-APOE $\epsilon$ 4 carriers status (**Table 2**). So the APOE $\epsilon$ 4 carrier status might play an

important role in exon 5 C/G genetic risk of SAD.

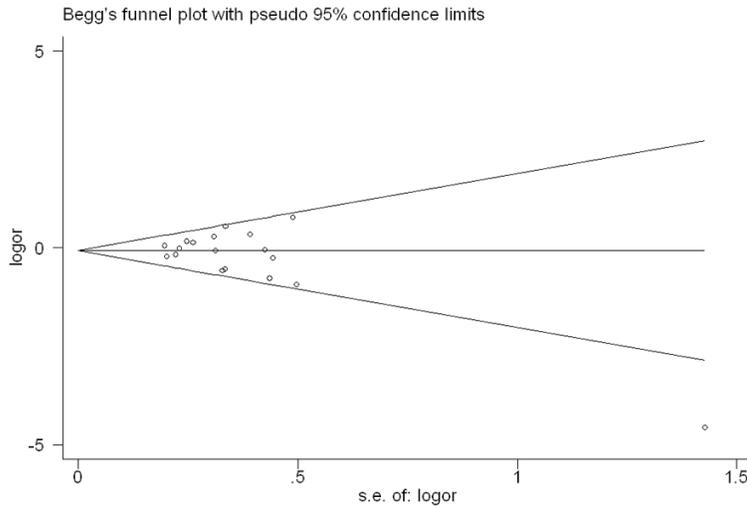
### Sensitivity analysis

Sensitivity analysis indicated that four independent articles [10, 11, 19, 25] were the main origin of the heterogeneity. The heterogeneity decreased after exclusion of four studies based on Galbraith plots analysis (the results for the test of heterogeneity were (CC+CG versus GG ( $I^2=6.8\%$ ,  $P=0.38$ ), CC versus (CG+GG) ( $I^2=37.5\%$ ,  $P=0.08$ ), CC versus GG ( $I^2=26.4\%$ ,  $P=0.17$ ), CG versus GG ( $I^2=0.0\%$ ,  $P=0.73$ ) and CC versus CG ( $I^2=21.4\%$ ,  $P=0.22$ ), and the corresponding pooled ORs were not materially altered CC+CG versus GG (OR=0.93, 95% CI: 0.82-1.04,  $P=0.20$ ), CC versus (CG+GG) (OR=1.03, 95% CI: 0.92-1.14,  $P=0.66$ ), CC versus GG (OR=0.97, 95% CI: 0.83-1.11,  $P=0.55$ ), CG versus GG (OR=0.91, 95% CI: 0.80-1.03,  $P=0.80$ ) and CC versus CG (OR=1.05, 95% CI: 0.94-1.18,  $P=0.36$ ) under the fix-effect model. Although the genotype distributions in three of the included studies did not follow HWE [19, 23, 25], the corresponding pooled ORs were not materially altered without these studies (CC+CG versus GG (OR=0.91, 95% CI: 0.82-1.45,  $P=0.09$ ), CC versus (CG+GG) (OR=0.97, 95% CI: 0.87-1.14,  $P=0.73$ ), CC versus GG (OR=0.90, 95% CI: 0.79-1.04,  $P=0.15$ ), CG versus GG (OR=0.91, 95% CI: 0.81-1.02,  $P=0.10$ ) and CC versus CG (OR=1.00, 95% CI: 0.86-1.18,  $P=0.97$ ). Sensitivity analysis suggested that the pooled results were robust.

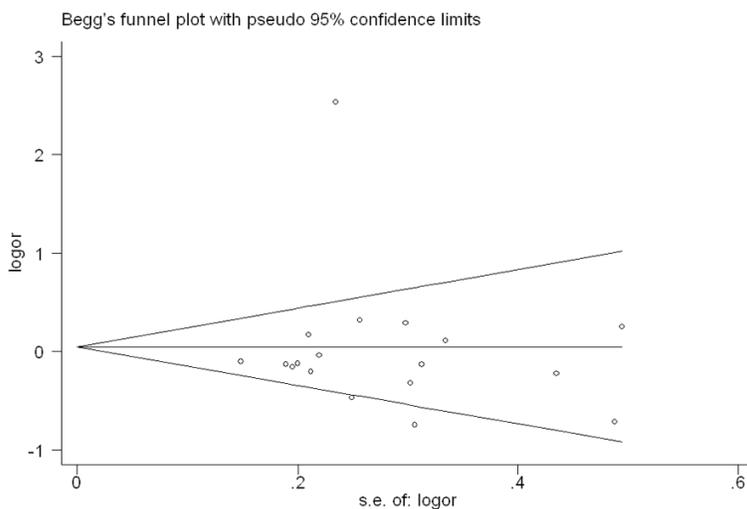
### Publication bias

The shape of the funnel plots in genetic models seemed symmetrical, indicating that there were no evidences for obvious publication bias (**Figures 2-6**). Further, Egger's test was used to

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**Figure 4.** Funnel plots for publication bias of BACE1 exon 5 C/G polymorphism and SAD risk in the overalls (homozygote comparison: CC versus GG).



**Figure 5.** Funnel plots for publication bias of BACE1 exon 5 C/G polymorphism and SAD risk in the overalls (heterozygote comparison: CG versus GG).

assess publication bias and provided the similar result that there were no significant publication bias in genetic models (CC+CG versus GG:  $t=-0.59$ ,  $P=0.562$ , CC versus CG+GG:  $t=-1.64$ ,  $P=0.12$ , CC versus GG:  $t=-1.67$ ,  $P=0.113$ , CG versus GG:  $t=-0.12$ ,  $P=0.904$ , and CC versus CG:  $t=-1.76$ ,  $P=0.098$ ). The potential publication bias therefore did not materially alter the combined risk estimates.

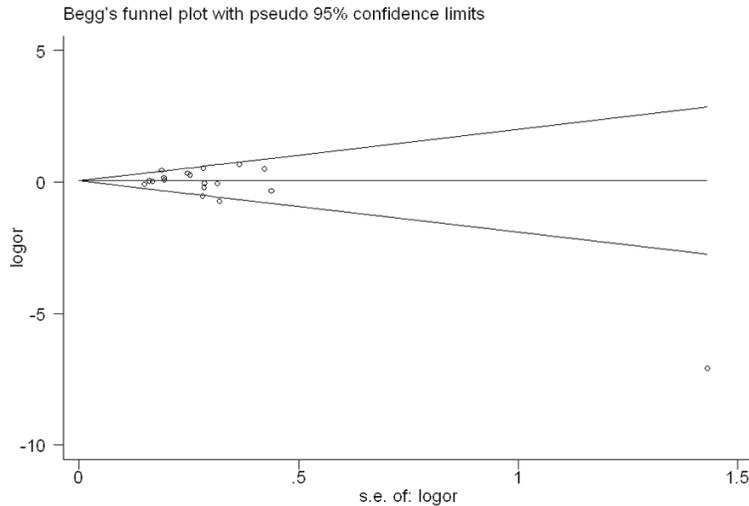
### Discussion

Epidemiological and pathogenetic evidences strongly suggest an association between genetic factors and SAD risk. Based on this

hypothesis, the contribution of various candidate genes to SAD risk has been investigated, and one of the candidate genes that has been analyzed as an SAD risk factor is the BACE1 gene. In Caucasians, Nowotny et al. firstly reported no association between the BACE1 exon 5 genotypes and AD risk [17]. Since then a considerable number of papers were used to replicate these results. The data revealed no association between the BACE1 polymorphism and SAD risk from two UK studies [16, 20]. Similar results were found in Switzerland [19], Australia [21], France [22] and Netherlands [23]. However, Ambiguous results have been presented. In Germany, Kirschling et al. exhibited the G-allele of the exon 5 C/G polymorphism was associated with an increased SAD risk, and BACE polymorphism played an important role in the development of AD by influencing Ab42 levels [10]. An association between BACE1 exon 5 GG genotype and AD ( $P=0.014$ ) was observed in Spain [14]. In Asians, Shi et al. found an associated with AD risk (Guangzhou cohort,  $OR=1.56$ ,  $95\% CI=1.09-2.23$ ; Chengdu cohort,  $OR=1.74$ ,  $95\% CI=1.03-2.95$ ) for BACE1 exon5

C/G [11]. Similar results were established in one other Chinese study [12]. However, in Taiwan of China, no significant association of this polymorphism with the occurrence of AD could be found [18]. In the Korean population, the distribution of BACE1 C/G genotypes was also not significantly different between 248 AD cases and 224 healthy controls [15]. The C or G allele was not associated with Alzheimer's disease ( $P=0.069$ ) in two other Chinese studies [13, 24]. The failure to reproduce replicated studies may be due to the small sample size used. In inclusive articles, our pooled results confirm that BACE1 exon5 C/G genetic poly-

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**Figure 6.** Funnel plots for publication bias of BACE1 exon 5 C/G polymorphism and SAD risk in the overalls (heterozygote comparison: CC versus CG).

morphism has no effect on SAD risk, and the results are consistent with that of most studies. The conclusion of allele C versus G effect also shows no significant difference (OR=0.96, 95% CI: 0.87-1.06,  $P>0.05$ ) by meta-analysis of 17 studies on BACE1 exon 5 C/G polymorphism in AlzGene database (<http://www.alzgene.org/meta.asp?geneID=53>). And no significant difference in the genotypes distribution in cases and controls was found after exclusion of studies deviating from HWE. However, evidence of heterogeneity was found. Between-study heterogeneity decreased after sensitivity analysis and the corresponding pooled ORs were not materially altered, and no publication bias was found in all the inherited models. So the results of our meta-analysis were robust.

The different ethnic backgrounds as confounding factor in genetic studies should be taken into account. In Asia, persons who were C allele carriers had increased risk of SAD in three articles [11], however, an association of the G-allele with LOAD was found [13]. In one Asian meta-analysis, there was not a difference between AD patients and controls ( $P=0.0555$  for genotypes) [15]. The results of our meta-analysis identified that of this meta-analysis, and no significant difference between SAD risk and BACE1 exon 5 C/G polymorphism for Caucasian population was also found. Small samples might be important factor for contradictory conclusions for including studies. The implication of these stratified conclusions should be further explored.

It has been speculated that variations in BACE1 might be associated with the risk for SAD in combination with the APOE $\epsilon$ 4 genotypes. Gene-gene interaction analysis should be explored. A synergistic interaction between the G-allele and APOE $\epsilon$ 4 carriers status on the risk of LOAD (OR=1.91, 95% CI 1.23-2.95,  $P=0.003$ ) was explored, and suggested that BACE1 gene polymorphism exon 5 C/G might act as an APOE $\epsilon$ 4 allele-dependent risk factor for developing LOAD [12]. In the Korea [15], USA [17], Switzerland [19] and Germany [10], the exon 5 C/G polymorphism

was a significant risk factor for AD in APOE $\epsilon$ 4 carriers status. Clarimón et al. did not detect this association in subjects carrying APOE $\epsilon$ 4 in Spain [14]. There was statistical difference between BACE1 exon 5 C/G polymorphism and SAD risk for our meta-analysis in APOE $\epsilon$ 4 carriers status and no association in non-APOE $\epsilon$ 4 carriers status, persons within the CC phenotype have more effect on risk of SAD among cases with at least one APOE $\epsilon$ 4 allele. So there is a synergistic interaction between APOE $\epsilon$ 4 carriers status and BACE1 exon 5 C/G polymorphism for risk of SAD, and BACE1 exon 5 C/G polymorphism could be genetic risk of SAD patients with APOE $\epsilon$ 4 carriers status.

For 3'UTR T/A or intron 5 T/G, Kirschling et al. found no association for the intron 5 T/G with SAD ( $P=0.425$ ) [10], there was no significant influence on genetic susceptibility to SAD in Northern Irish population for 3'UTR T/A [20]. In Switzerland [19], Spain [14], UK [16], no association was also established. The results of our meta-analysis are consistent with including studies. However, because the low occurrence of the genotypes of the two genetic polymorphisms will lead to poor statistical power, these results would be needed to be further confirmed with larger sample sizes in future studies.

Some limitations of our meta-analysis of observational studies should be attended. First, a relatively small number of studies were includ-

ed, and there was no sufficient power to estimate the association between intron 5 T/G or 3'UTR T/A polymorphism and SAD risk. On the other hand, the samples (blood or brain) were selected and different genotyping methods were used with different sensitivity and specificity, which could also result in selection bias and clinic heterogeneity. Otherwise, the heterogeneity was removed by sensitivity analysis and the overall results were not materially altered, so it suggested the stability of our 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, and so it could not play an important role in results of our meta-analysis.

### Conclusion

Despite the above-mentioned limitations, this meta-analysis demonstrated that in APOE $\epsilon$ 4 carrier status, the BACE1 exon5 C/G polymorphism could be associated with SAD risk and individuals with CC genotype could have increased risk of SAD. It might be suggested that interaction between BACE1 exon 5 C/G polymorphism and APOE $\epsilon$ 4 carrier status might account for SAD risk. However, 3'UTR T/A or intron 5 T/G might not affect risk of SAD. Based on SAD with multifactorial etiology, the results of our meta-analysis should be properly replicated in future prospective cohort study, including consideration into interactions.

### Disclosure of conflict of interest

None.

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