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Original Article

Polymorphisms of PICALM gene in Alzheimer's disease risk: a meta-analysis

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Abstract: Alzheimer's disease (AD) is the most common type of dementia. Recent genome-wide association studies have identified that PICALM is one of the numerous reproducible AD-associated risk genes, and variants in PICALMare shown to be involved in this disease. However, the results remain conflicting. In this meta-analysis, we searched the online electronic database to retrieve related articles concerning the role of PICALM polymorphisms in AD risk and to systematic reevaluate the exact association. Overall, we screened out ten case-control studies, including 6866 AD patients and 13205 controls. Our results found that A allele of rs3851179 (A vs. G: OR=0.84, 95% CI=0.75-0.94, P=0.002) and C allele of rs541458 (C vs. T: OR=0.85, 95% CI=0.74-0.97, P=0.02) were associated with AD incidence. This significant relationship was also found in GA+AA and AA genotypes of rs3851179, and CC+TC genotype of rs541458 variants (P<0.05). Subgroup analysis by ethnicity showed that rs3851179 variant was genetic factor for AD in Caucasians, while only A allele was associated in Asians; C allele and CC+TC genotype of rs541458 in Caucasians, while no association was found in Asians. For Chinese population, neither these variants was associated with AD risk. In conclusion, our results found that rs3851179 and rs541458 were associated with AD risk. However, more data about other ethnicities with large crowds were needed in the future studies.

Keywords: Alzheimer's disease, PICALM, polymorphism, meta-analysis

Introduction

Alzheimer's disease (AD) is a fatal brain disorder characterized by memory loss, abstract thinking, damage calculations, steady deterioration of cognition, and dementia [1, 2]. In United States, it is the leading cause of dementia in the elderly [3], and by 2050, an estimated 1.6 million deaths will occur among individuals aged 65 years or older with AD, comprising 43% of all older adult deaths [4]. The annual cost for this disease is increasing, and no effective therapeutic strategy is available in sight [5, 6]. Although the precise physiological changes that trigger the development of AD largely remain unknown, the main risk factors are thought to be the interaction between environmental aspect and known genetic mutations [7]. Thus, identification of new susceptibility genes may be helpful in exploring the underlying disease mechanisms and developing the targeting drugtherapy [8].

Genome-wide association studies have identified numerous genes which involve in the pathogenicity of AD risk. PICALM, located on human chromosome 11q14, contains 112 kb, and is expressed in multiple tissues and cells. It results in 23 alternative transcripts, and primary encodes phosphatidylinositol-binding clathrin assembly (PICALM) protein, which plays a key role in endocytosis [9], iron homeostasis and cell proliferation [10]. PICALM also binds to a nuclear exportinCRM-1 which used by the herpes simplex virus during its life cycle [11]. Recently, PICALM has been considered to be associated with AD risk. It influences AD risk primarily by modulating production, transportation, clearance of β-amyloid (Aβ) peptide, and other Aβ-independent pathways [12]. AD-associated single nucleotide polymorphisms (SNPs) in PICALM gene were discovered as well [13, 14]. Rs3851179, located upstream of PICALM, was the initially studied. This variant was associated with total PICALM expression [15]. The A allele of this variant was shown to be reduced the AD risk among Caucasians [16]. However, in Asians, this association was weak or not significant [17, 18]. Rs541458 in PICALM

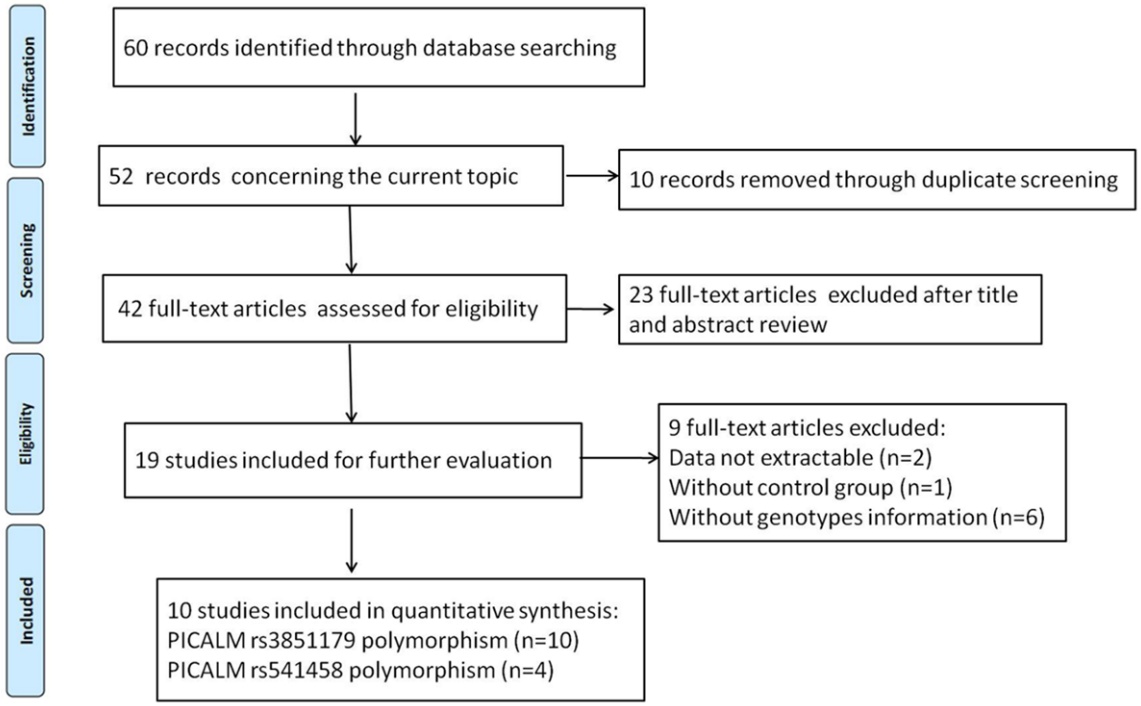

Figure 1. The process of relevant studies searching.

gene was shown to be associated with descending level of Aβ42 in cerebrospinal fluid [19]. While subsequent study suggested that PICALM variants did not affect AD risk via a mechanism resulting in a strong additive effect on cerebrospinal fluid levels of Aβ42 [20].

Although several studies evaluated PICALM polymorphisms in AD susceptibility, the functional effects of these SNPs remain to be determined.Gharesouran et al. identified rs3851179 polymorphism was significantly related with late-onset AD (LOAD) in Iranians [21]. While Liu et al. indicated that this polymorphism might not be an AD susceptibility locus in the Chinese population [22]. This may be due to the genetic heterogeneity in AD in different populations or the limited samples obtained. Therefore, we conducted this meta-analysis to summarize all the related published studies, and reevaluate this role of PICALM polymorphism in AD risk in population of total and subgroup by ethnicity.

Materials and methods

Publication search

Relevant studies were searched in online databases of CNKI (China National Knowledge Internet), Medline and PubMed. The following key terms: "Alzheimer's Disease or AD", "PICALM", "polymorphism or variant or mutation" and their combinations were used. References of related articles were searched manually to obtain more resources.Only published articles written in English or in Chinese were searched.

Inclusion criteria

Studies included should meet the following criteria: 1) case-control studies evaluating the role of PICALM variants in AD risk; 2) the patients should be met the DSM-IV diagnostic criteria (American Psychiatric Association 1994) [23]; 3) the controls should be ethnically and sex-matched controls without AD or other mental disorders; 4) the results were presented in odds ratio (OR) and 95% confidence interval (CI); and 5) the genotype information in cases and controls was available to extract.

Data extraction

Two of our authors assessed the quality of relevant articles independently. Any item was reached a final consensus. The following items were extracted from each article: the name of first author, country, ethnicity, sample size, genotype methods and distribution.

Table 1. Main characteristics of included studies in this meta-analysis

First author	Year	Country	Ethnicity	Mean Age		Sample size		Genotype methods	SNP
				Cases	Controls	Cases	Controls		
Harold D	2009	UK/Ireland	Caucasian	-	-	2227	4836	-	rs541458, rs3851179
		Germany	Caucasian	-	-	555	824	-	rs541458, rs3851179
		USA	Caucasian	-	-	1159	2188	-	rs541458, rs3851179
Li HL	2011	China	Asian	68.6±9.6	69.4±9.9	474	591	PCR-RFLP	rs3851179
Piaceri I	2011	Italy	Caucasian	71.0±6.1	74.5±6.2	349	359	PCR-RFLP	rs3851179
Yu JT	2011	China	Asian	77.0±6.6	76.7±5.9	266	343	MALDI-TOF MS assay	rs3851179
Chen LH	2012	China	Asian	-	-	462	350	Sequenom	rs541458, rs3851179
Ding D	2012	China	Asian	81.2±5.3	80.4±4.9	54	216	Taqman	rs541458, rs3851179
Ohara T	2012	Japan	Asian	83.2±6.5	60.2±11.5	825	2934	multiplex PCR-based Invader assay	rs3851179
Klimkowicz-mrowiec A	2013	Poland	Caucasian	73.9±5.2	73.8±6.9	253	240	PCR-RFLP	rs3851179
Belcavello L	2014	Brazil	Mixed	81.2±7.5	79.4±7.9	82	161	PCR-RFLP	rs 3851179
Gharesouran J	2014	Iran	Asian	76.1± 7.8	75.3±6.8	160	163	PCR-Sequencing	rs541458, rs3851179

Mixed, European origin and African origin; -, not available; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism; SNP, single nucleotide polymorphism.

Table 2. Distribution of alleles and genotypes for each polymorphism of included studies

First author	Cases					Controls				
rs3851179	GG	GA	AA	G	A	GG	GA	AA	G	A
Harold D-1	979	1004	244	2962	1492	1876	2240	720	5992	3680
Harold D-2	252	227	76	731	379	332	384	108	1048	600
Harold D-3	525	499	135	1549	769	880	1032	276	2792	1584
Li HL	161	258	55	580	368	196	321	74	713	469
Piaceri I	140	154	55	434	264	145	153	61	443	275
Yu JT	114	126	26	354	178	135	164	44	434	252
Chen LH	170	210	77	550	364	122	163	56	407	275
Ding D	23	19	9	65	37	84	100	28	268	156
Ohara T	310	394	121	1014	636	982	1434	518	3398	2470
Klimkowicz-mrowiec A	100	128	24	328	176	99	110	34	308	178
Belcavello L	7	42	33	56	108	3	81	77	87	235
Gharesouran J	24	65	71	113	207	2	29	132	33	293
rs541458	TT	TC	CC	T	C	TT	TC	CC	T	C
Harold D-1	1162	892	173	3216	1238	2260	2075	501	6595	3077
Harold D-2	263	231	61	757	353	366	375	83	1107	541
Harold D-3	592	475	92	1659	659	1040	940	208	3020	1356
Chen LH	122	215	112	459	439	94	154	92	342	338
Ding D	16	20	16	52	52	47	111	56	205	223
Gharesouran J	11	40	109	62	258	1	22	140	24	302

Table 3. Meta-analysis of PICALM polymorphisms in AD risk

SNP	Comparisons	Results of the Z-test		Between-study heterogeneity		
		OR (95% CI)	P	Ph	I^2	Model
rs3851179						
Total	A vs. G	0.84 (0.75, 0.94)	0.002	<0.00001	79%	R
	AA+GA vs. GG	0.83 (0.78, 0.89)	<0.00001	0.04	47%	F
	AA vs. GA+GG	0.77 (0.64, 0.94)	0.01	<0.00001	75%	R
Asian	A vs. G	0.76 (0.58, 0.99)	0.04	<0.00001	89%	R
	AA+GA vs. GG	0.84 (0.68, 1.05)	0.12	0.03	61%	R
	AA vs. GA+GG	0.72 (0.46, 1.12)	0.14	<0.00001	86%	R
Caucasian	A vs. G	0.86 (0.81, 0.90)	<0.00001	0.43	0%	F
	AA+GA vs. GG	0.83 (0.77, 0.89)	<0.00001	0.46	0%	F
	AA vs. GA+GG	0.80 (0.72, 0.89)	<0.0001	0.09	50%	F
rs541458						
Total	C vs. T	0.85 (0.74, 0.97)	0.02	0.003	73%	R
	CC+TC vs. TT	0.83 (0.77, 0.90)	<0.00001	0.15	39%	F
	CC vs. TC+TT	0.81 (0.64, 1.02)	0.07	0.01	67%	R
Asian	C vs. T	0.69 (0.38, 1.24)	0.21	0.0004	87%	R
	CC+TC vs. TT	0.63 (0.28, 1.41)	0.26	0.03	71%	R
	CC vs. TC+TT	0.73 (0.37, 1.43)	0.36	0.005	81%	R
Caucasian	C vs. T	0.86 (0.81, 0.91)	<0.00001	0.24	31%	F
	CC+TC vs. TT	0.83 (0.77, 0.90)	<0.00001	0.57	0%	F
	CC vs. TC+TT	0.84 (0.67, 1.04)	0.10	0.12	53%	R

OR, odds ratio; 95% CI, 95% confidence intervals; Ph, I^2, between-study heterogeneity; R, the random-effect model; F, the fixed-effect model.

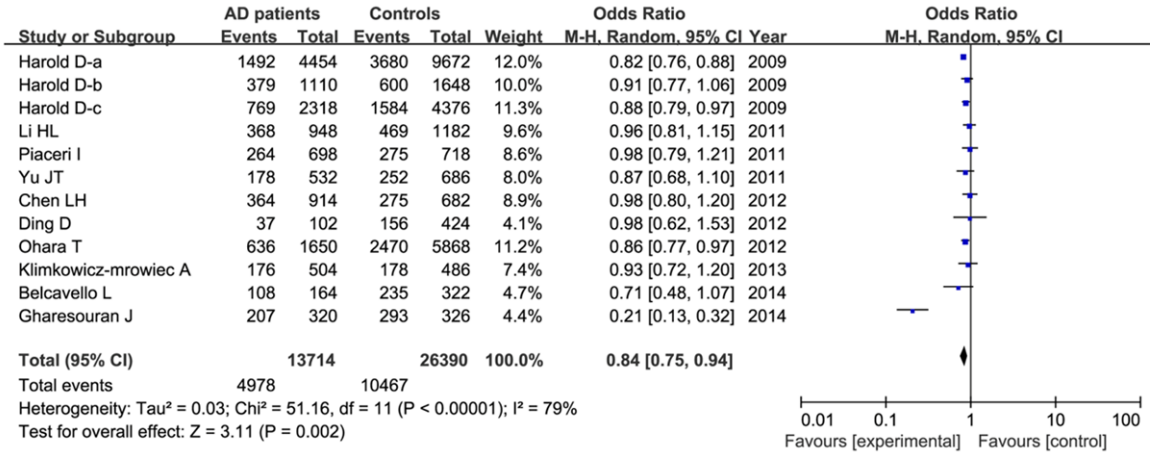

Figure 2. Forest plot of the association between PICALM rs3851179 polymorphism and AD risk in allelic model in total population in a random-effect model.

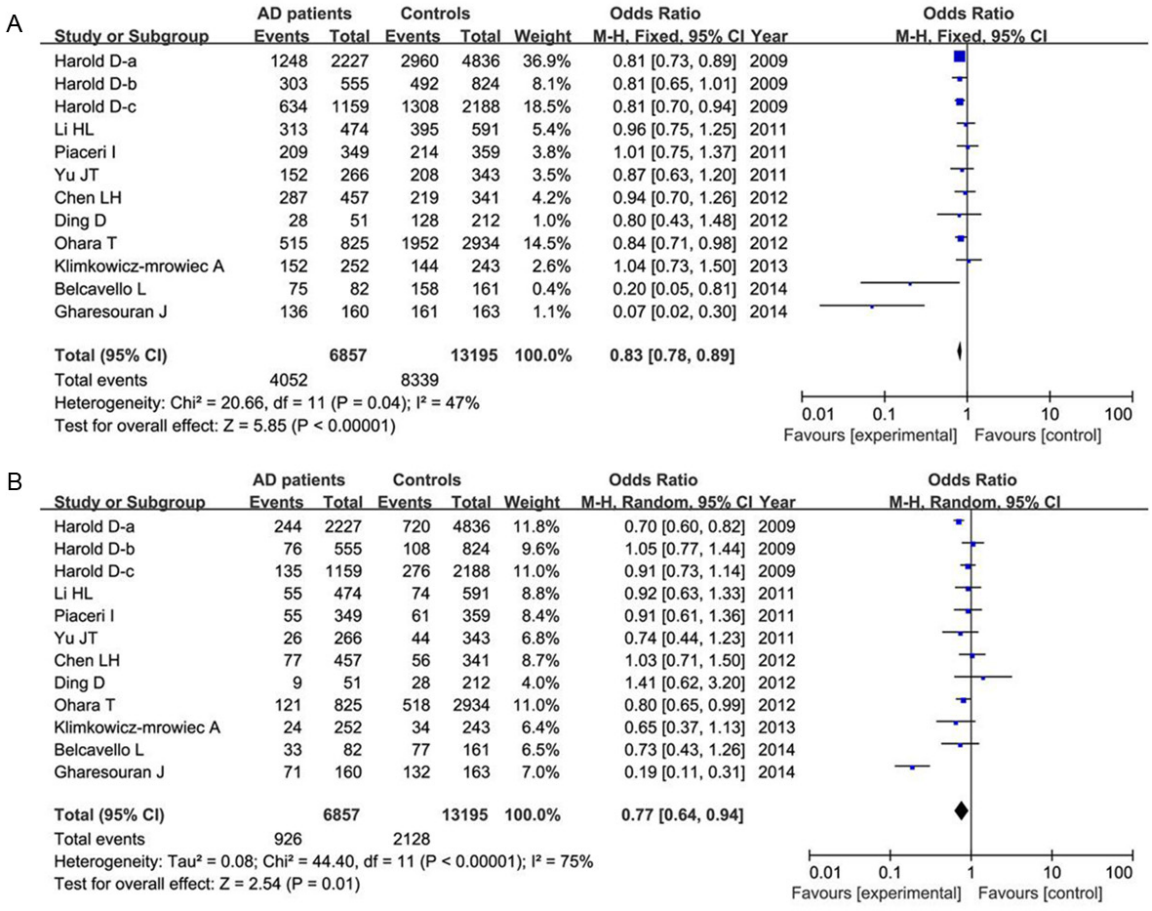

Figure 3. Meta-analysis of PICALM rs3851179 polymorphism in AD risk in dominant model (A) and recessive model (B).

Statistic analysis

The strength of association between PICALM gene polymorphisms and AD risk was measured by pooled ORs with its 95% CI. The statistical significance was determined by Z-test with a *P*-value less than 0.05 considered significant. For each variant, the allele model, dominant

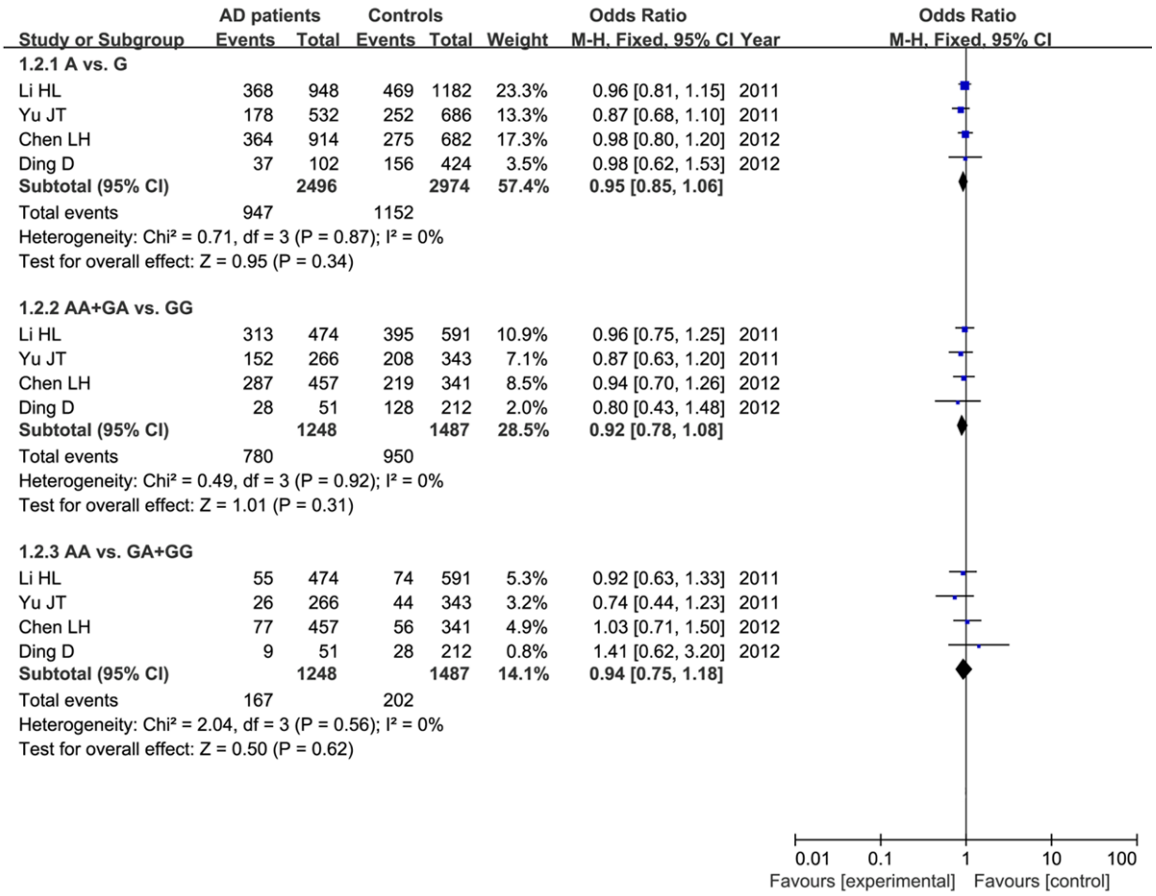

Figure 4. Meta-analysis of the association between rs3851179 polymorphism and AD risk in Chinese population in a fixed-effect model.

model and recessive model were calculated. Between-study heterogeneity was assessed by both the Q-test and the I^2 test. The fixed-effect model was employed when the effect were homologous (*P*-value ≥0.01 for the Q-test and I^2 ≤50% for the I^2 test), while the random-effect model was used in its opposite. All analyses were calculated using the RevMan5.2 program.

Results

Characteristics of included studies

After applying the inclusion criteria, total ten case-control studies (one in Chinese and nine in English) were screened out, including 6866 AD patients and 13205 controls. **Figure 1** presented the searching process. Of the ten studies, one contained three study population [16]. Six were Asian origin [17, 21, 24-27], three European origin [16, 28, 29], and one mixed origin (European origin and African origin) [30]. Two variants (rs541458 and rs3851179) in PICALM gene were involved. The sample size ranged from 270 to 7063. **Table 1** listed the main characteristics of included studies in this meta-analysis. **Table 2** exhibited the distribution information of alleles and genotypes for each polymorphism.

Association between PICALM rs3851179 variant and AD risk

All the ten studies concerned the rs3851179 polymorphism. **Table 3** showed the results of each genetic comparison model. Between-study heterogeneity was calculated, and the fixed-effect model or the random-effect model was used. Our results demonstrated that the A allele was significant associated and increased the risk of AD compared with the G allele (A vs. G: OR=0.84, 95% CI=0.75-0.94, P=0.002) in a random-effect model as shown

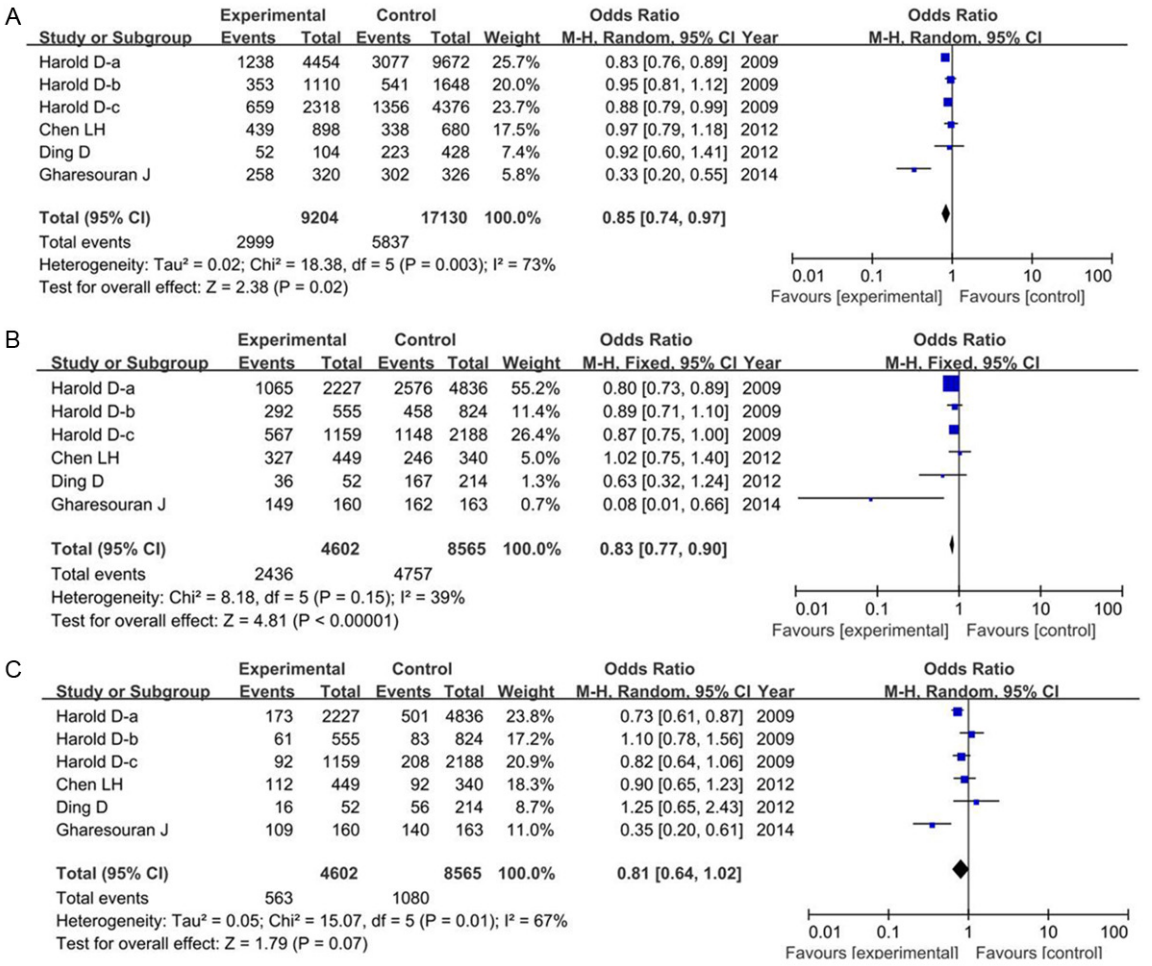

Figure 5. Forest plot of the association between PICALM rs541458 polymorphism and AD risk in the three genetic models (A. The allelic model; B. The dominant model; C. The recessive model).

in **Figure 2**. This relationship was also found in other two comparisons (AA+GA vs. GG: OR=0.83, 95% CI=0.78-0.89, P<0.00001; AA vs. GA+GG: OR=0.77, 95% CI=0.64-0.94, P=0.01) as shown in **Figure 3**.

Subgroup analysis by ethnicity showed that there was a positive association between rs3851179 polymorphism and AD risk in Caucasians (A vs. G: OR=0.86, 95% CI=0.81-0.90, P<0.00001; AA+GA vs. GG: OR=0.83, 95% CI=0.77-0.89, P<0.00001; AA vs. GA+GG: OR=0.80, 95% CI=0.72-0.89, P<0.0001). In Asians, only A allele was found to be associated with AD risk (A vs. G: OR=0.76, 95% CI=0.58-0.99, P=0.04). However, the significant association was not found in other genetic models (AA+GA vs. GG: OR=0.84, 95% CI=0.68-1.05, P=0.12; AA vs. GA+GG: OR=0.72, 95% CI=0.46-1.12, P=0.14).

We further evaluated the role of this variant in Chinese population. Four studies were included, containing 1248 cases and 1487 controls. Our results indicated that rs3851179 polymorphism was not associated with AD risk in Chinese population (A vs. G: OR=0.95, 95% CI=0.85-1.06, P=0.34; AA+GA vs. GG: OR=0.92, 95% CI=0.78-1.08, P=0.31; AA vs. GA+GG: OR=0.94, 95% CI=0.75-1.18, P=0.62) in a fixed-effect model as shown in **Figure 4**.

Association between PICALM rs541458 variant and AD risk

Four articles included 4602 patients and 8562 controls, involving six comparisons. Overall, our results suggested that the C allele and CC+TC genotype were associated with AD susceptibility (C vs. T: OR=0.85, 95% CI=0.74-0.97, P=0.02; CC+TC vs. TT: OR=0.83, 95% CI=0.77-0.90,

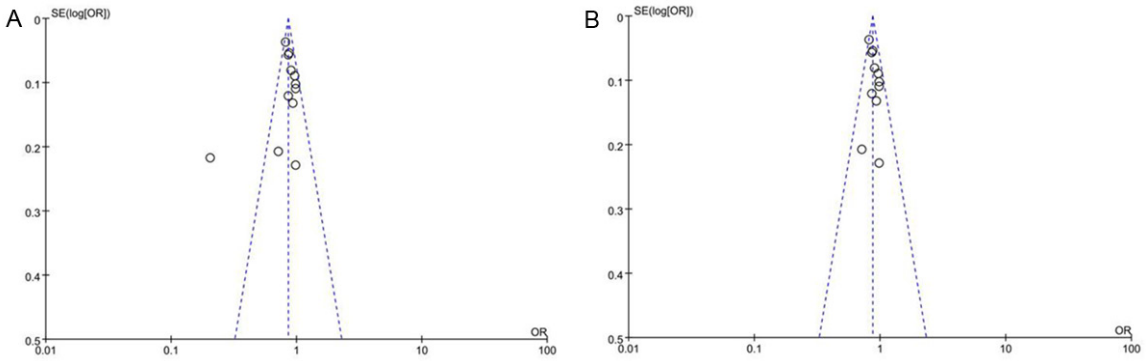

Figure 6. Funnel plot for publication bias analysis of rs3851179 polymorphism in the allelic model before (A) and after (B) deleting the study conducted by Gharesouran et al.

P<0.00001). While no association was found in CC genotype (CC vs. TC+TT: OR=0.81, 95% CI=0.64-1.02, P=0.07). **Figure 5** showed the results of PICALM rs541458 variant in AD risk. Subgroup analysis also found that only C allele and CC+TC genotype were associated with AD susceptibility (P<0.00001) in Caucasians. No association was found in Asians (P>0.05). Two studies concerned the Chinese population, and no significant role was found (P>0.05).

Sensitive analysis and publication bias

Each study was deleted at a time to determine whether individual study influenced the results. Our results showed that when the study of Gharesouran J et al. was omitted, the between-study heterogeneity disappeared, but the pooled OR was not significant changed. We carefully studied this article, but not found obvious difference with other included studies. The funnel plot also testified this result as shown in **Figure 6**.

Discussion

In this meta-analysis, we identified ten relevant articles. Our result showed that rs3851179 variant of PICALM gene was associated with AD risk in total population. This significance was also found in Caucasians by subgroup analysis. While only A allele was related with increased the occurrence of AD in Asians. Our result was not in accord with previous meta-analysis conducted by Liu et al. which screened out five articles (four in Asians and one in Caucasians), indicating that rs3851179 variant was associated with AD risk under three comparison models in Asians [31]. Forrs541458 variant, only C allele and CC+TC genotype were associated with incidence of AD in total population or Caucasians. No relationship was found in Asians. This is the first meta-analysis considering the role of rs541458 polymorphism in AD.

Recent studies investigated the mechanisms of PICALM in AD pathogenesis. PICALM was found associated with age at onset of AD in Down syndrome (P=0.011) [32]. Baig et al. firstly proved the presence of PICALM in endothelial cells of human brain tissue, suggesting an increase in PICALM expression in AD [33]. PICALM was found involving in the neurotransmitter release processes, thereby affecting memory functions [34]. It was implicated in intracellular amyloid precursor protein processing and plaque pathogenesis, and the overexpression of PICALM in vivo was detected to increases plaque deposition in AD transgenic mice [35]. PICALM might also regulate Aβ generation [36], andwork in autophagy-mediated Aβ clearance [37], which may influence accumulation of Aβ in brains with AD. Therefore, targeting PICALM might provide promising and novel avenues for AD therapy.

The increase of PICALM expression in the microvasculature may reduce AD risk. Genetic variants may affect PICALN expression. PICALM polymorphisms was estimated to account 5.3% of AD risk [38]. Several studies have evaluated the association between PICALM variants and AD risk. Carrasquillo et al. provided the first evidence that PICALM rs3851179 variant was associated with the risk of LOAD [39]. Subsequently, the G allele of this variant was shown to have an unexpected protective effect on incident mild cognitive impairment or LOAD [40]. Lambert et al. found that PICALM rs541458

polymorphism was genetic determinants of AD in European populations [41].Corneveaux et al. also demonstrated that rs541458 polymorphism was related with AD risk [42]. Other polymorphisms in PICALM gene may also affect AD incidence. Rs17817201 variant was shown to be associated withage at onset of AD susceptibility in Korean population [43]. PICALM rs17159904 variant was associated with AD risk in the Caribbean Hispanic cohort [44].

PICALM polymorphisms may interact with other genes. APOE is the well-known risk loci for AD, accounting for less than 20% of LOAD risk [45]. Previous meta-analysis showed that genotypes at PICALM confer risk predominantly in APOE ε4-positive subject. Thus, APOE and PICALM synergistically interact [46]. Morgen et al. suggested that a neural mechanism for APOE-PICALM interactions in patients with manifest AD and indicated that the PICALM genotype modulates both brain atrophy and cognitive performance in APOE ε4 carriers [47]. In our analysis, three articles considered the impact of rs3851179 genotype on the risk of LOAD stratified by the APOE-ε4 status. Our results found that there was no significant interaction between PICALM rs3851179 variant and APOE-ε4 carrier status (P=0.72).

Several limitations were presented. Firstly, the sample size was rather small compared with large-scale GWAS in Caucasian ancestry. Secondly, other genes which may interact with PICALM should be considered. For example, the PICALM-CLU interactions should be considered when investigating the impact of these two genetic variants on the brain [48]. Thirdly, other factors such as age, stage of disease also should be studied. Lastly, one included study conducted by Gharesouran et al. affected the between-study heterogeneity to a large extent even though the pooded ORs of each comparison models were not significantly changed when removing this article.

In conclusion, our result found that polymorphisms of rs3851179 and rs541458 in PICALM gene were associated with AD risk in total population or Caucasiansinvolving in the included studies. In Asians, this association was weak or disappeared. Therefore, subgroup analysis by ethnicity with large-scale should be included in the future researches.

Disclosure of conflict of interest

None.

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