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PICALM Variation Moderates the Relationships of *APOE* ε4 with Alzheimer's Disease Cerebrospinal Biomarkers and Memory Function Among Non-Demented Population

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Abstract.
Background: *APOE* ε4 and *PICALM* are established genes associated with risk of late-onset Alzheimer's disease (AD). Previous study indicated interaction of *PICALM* with *APOE* ε4 in AD patients.
Objective: To explore whether *PICALM* variation could moderate the influences of *APOE* ε4 on AD pathology biomarkers and cognition in pre-dementia stage.
Methods: A total of 1,034 non-demented participants (mean age 74 years, 56% females, 40% *APOE* ε4 carriers) were genotyped for *PICALM* rs3851179 and *APOE* ε4 at baseline and were followed for influences on changes of cognition and cerebrospinal fluid (CSF) AD markers in six years. The interaction effects were examined via regression models adjusting for age, gender, education, and cognitive diagnosis.
Results: The interaction term of rs3851179 × *APOE ε4* accounted for a significant amount of variance in baseline general cognition ($p = 0.039$) and memory function ($p = 0.002$). The relationships of *APOE* ε4 with trajectory of CSF $A\beta_{42}$ ($p = 0.007$), CSF P-tau181 ($p = 0.003$), CSF T-tau ($p = 0.001$), and memory function ($p = 0.017$) were also moderated by rs3851179 variation.
Conclusions: *APOE* ε4 carriers experienced slower clinical and pathological progression when they had more protective A alleles of *PICALM* rs3851179. These findings firstly revealed the gene-gene interactive effects of *PICALM* with *APOE* ε4 in pre-dementia stage.

Keywords: Alzheimer's disease, amyloid, *APOE* ε4, cognition, interaction, memory, *PICALM*, tau protein

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[1]The data used in preparation for this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (http://adni.loni.usc.edu). As such, the investigators within the ADNI contributed to the design and implementation of ADNI and/or provided data but did not participate in the analyses or writing of this report. A complete listing of ADNI investigators can be found at: http://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf.

INTRODUCTION

Late-onset Alzheimer's disease (LOAD), the most common type of dementia, is characterized by cerebral abnormal accumulation of amyloid and tau proteins followed by cognitive deterioration [1]. It has been proven by the genome-wide association studies that both the ε4 allele of apolipoprotein E (*APOE* ε4) and phosphatidylinositol binding clathrin assembly protein (*PICALM)* single nucleotide polymorphisms are strongly associated with the risk of LOAD [2, 3]. Moreover, *APOE* ε4 was associated with faster rates of AD pathology accumulation [4–6] and cognitive decline among non-demented population [7–11]. *In vitro* evidence showed that *APOE* ε4 could disturb the formation of endosome and endosomal-lysosomal degradation pathway [12–14]. *PICALM* is mainly involved in clathrin-mediated endocytosis, intracellular trafficking, and signaling [3]. We previously reported that its top signal rs38511179 (allele A) was associated with a 9% to 29% reduced AD risk in numbers of ethnicities [15] and associated with better memory scores among non-demented population [16]. Rs3851179 is a transcription factor binding site located in *PICALM* [15], and its A allele was associated with up-regulated *PICALM* expression [17].

Though *APOE* ε4 and *PICALM* are two established risk genes of AD, it is rarely investigated whether they could interact for each other to influence AD occurrence, though there are potential overlapping pathways, such as production and clearance of amyloid proteins, cholesterol metabolism [18], and autophagic-endolysosmal network [12, 19]. Experimental studies indicated that increased cellular dose of *PICALM* uncouples the presence of *APOE* ε4 from its detrimental effects on endocytosis [20]. Understanding their gene-gene interaction role can help clarify the genetic underpinning of AD etiology and also contribute to precise prediction and classification of genetic high-risk population. Recently, population-based studies indicated that interaction of *PICALM* rs3851179 G allele with *APOE* ε4 can promote cognitive impairments among AD population [2, 21, 22]. However, no study has ever explored their interactive roles in pre-dementia stage. Thus, the present study aimed to explore whether *PICALM* rs3851179 could moderate the relationship of *APOE* ε4 with cerebrospinal fluid (CSF) AD biomarkers and cognitive functions among non-demented population.

METHODS

Participants

The data used in the present study was acquired from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (http://adniloni.usc.edu), a multi-site longitudinal study launched in 2003. ADNI recruited volunteers from multiple centers in North America. Participants were older adults aged from 55 to 90 years with normal cognition, mild cognitive impairment, or mild AD dementia. The CSF AD biomarkers and cognitive functions were assessed simultaneously for each participant at baseline and repeatedly each year during follow-up. The present study only focuses on Caucasian with normal cognition or mild cognitive impairment at enrollment. All subjects or their proxies had provided written consent obtained from all participants or authorized representatives after extensive description of the ADNI according to 1975 Declaration of Helsinki.

Genotyping of APOE and PICALM

In ADNI-1, GenomeStudio v2009.1 (Illumina) was used to process the array data. The ADNI-GO/2 samples were genotyped by the Human OmniExpress BeadChip (Illumina Inc., San Diego, CA). All samples and genotypes underwent stringent QC with following criteria before association analyses: call rates for individuals > 95%, call rates for SNPs > 95%, Hardy-Weinberg equilibrium test $p > 0.001$, and minor allele frequencies > 0.2. The ADNI-1 and ADNI-GO/2 datasets consisted of 620,901 and 710,618 genotyped variants respectively, both of which included rs3851179 (*PICALM*) and the two loci (rs7412 and rs429358) used to define the *APOE* 2/3/4 isoforms [15].

CSF AD biomarker measurements

CSF procedural protocols have been previously described [23]. CSF was collected by lumbar puncture in 10 ml polypropylene tubes before being sent to the lab within 2 h. The samples were centrifuged at 2000 g for 10 min. The thaw/freezing cycle was limited so as not to surpass two times. CSF $A\beta_{42}$, T-tau, and P-tau_{181} (pg/ml) were measured using the INNOBIA AlzBio3 immunoassay (Fujirebio, Belgium). The within-batch precision values were 5.1–7.8%, 4.4–9.8%, and 5.1–8.8% for $A\beta_{1-42}$, T-tau, and P-tau_{181} respectively. $A\beta_{42}$ levels below 976.6 pg/ml

was categorized as A (+). CSF P-tau$_{181}$ levels above 21.8 pg/ml and CSF T-tau levels above 245 pg/ml were categorized as T (+) and N (+), respectively.

Cognitive assessments

General cognitive functions were measured by the 85-point Alzheimer's Disease Assessment Scale 13-item cognitive subscale (ADAS-cog13, ranging from 0 to 85). ADAS-cog13 scores were transformed into z-scores to aid in figure presentation. The composite scores for memory (ADNI_MEM) and executive function (ADNI_EF) were calculated using data from the ADNI neuropsychological battery via item response theory methods. Item parameters (loadings and thresholds) from the baseline model were used to compute scores at each follow-up visit. All composite scores have been validated previously [24, 25]. A lower ADAS-cog13 score indicates a better cognition, while a higher ADNI_MEM and ADNI_EF scores indicate a better memory and executive function.

Covariate measurement

The covariates include baseline age (continuous variable), gender (female = 1, male = 0), education (continuous variable), cognitive status (mild cognitive impairment = 1, normal cognition = 0), and baseline self-reported medical history, including insomnia, stroke, depression, cancer, anxiety, smoking, and body mass index (BMI). BMI is measurement of one's weight in relation to their weight (weight (kg) / height^2 (m^2)).

Statistical analyses

APOE ε4 status was dichotomized ("44/34/24" = 1 or ε4 (+), "33/22/23" = 0 or ε4 (–)) and *PICALM* rs3851179 was categorized into three groups (AA = 2, AG = 1, GG = 0) to better reflect the dose-response relationship. Participants were accordingly sub grouped into six groups (*APOE* ε4 (+) / AA, *APOE* ε4 (+) / AG, *APOE* ε4 (+) / GG, *APOE* ε4 (–) / AA, *APOE* ε4(–) / AG, and *APOE* ε4(–) / GG).

Before the regression models were run, the normality of residuals of dependent variables in all regression models were examined by Kolmogorov-Smirnov test ($p > 0.05$) via "nortest" package of R software. Next, the dependent variables were normalized via "car" package in case of skewed distribution. Finally, the model performances were re-tested using the "performance" package to ensure that the residuals meet the normality. As for the cross-sectional analyses, multiple linear regression models were conducted to examine the interactive effects of rs3851179 × *APOE* ε4 on cognitive scores and CSF AD biomarker. Simple slope analyses were performed to interpret the direction of interaction effects. Linear mixed-effects regression was conducted via the "lme4" package to test the longitudinal interaction effects. The linear mixed effects models were employed because they could handle unbalanced and censored data as well as a continuous variable for time [26]. Fixed effects included main effects of *PICALM* rs3851179, *APOE* ε4 status, years of follow-up (time-varying variable, "time"), as well as interaction terms of rs3851179 × *APOE* ε4, time × rs3851179, time × *APOE* ε4, and time × rs3851179 × *APOE* ε4. The overall significance of the three-way interaction term was assessed by the likelihood ratio test comparing the full model and a nested model that did not include the three-way interaction term. $p < 0.05$ was considered significant for interaction terms.

All models were calculated adjusting for covariates including age, gender, education, and cognitive status (baseline diagnose, mild cognitive impairment = 1, normal cognition = 0). Subgroup analyses were performed by cognitive status (MCI versus NC) and age strata (midlife versus late-life) at baseline, because former studies indicated that *APOE*-related cognitive decline varied with age [9, 27]. Sensitivity analyses were conducted by adding more variables (history of insomnia, stroke, depression, cancer, anxiety, smoking, and BMI). To preclude the potential influences of practice effect in the cognition cohort, we also included the follow-up times (proxy of the times of cognitive measurements) in the model. The significance of the findings did not change after adding these covariates. R software 4.2.1, Jamovi version 2.3.16.0, and Prism Graphpad 9 were applied for statistical analyses and figure preparation. The 'nlme', 'ggplot2', 'car', 'nortest', 'performance', 'spearman' packages were used in R software.

RESULTS

Population characteristics

A total of 699 non-demented participants (mean age = 73 years, 55% females, 39% *APOE* ε4 carriers) were included for CSF AD biomarkers analysis (Table 1), in which 467 participants has at least one follow-up visit (6 years, average follow-up

Table 1
Baseline characteristics of participants for CSF biomarker cohort by *APOE* ε4 and *PICALM* rs3851179

N = 699	Total	*APOE* ε4 carrier, N = 274			*p*	*APOE* ε4 non-carrier, N = 425			*p*
		AA	AG	GG		AA	AG	GG	
Number of samples	699	29 (10.6%)	102 (37.2%)	143 (52.2%)		50 (11.8%)	190 (44.7%)	185 (43.5%)	
Age, y, mean (SD)	73.2 ± 7.2	72.6 ± 5.3	72.1 ± 8.2	71.9 ± 6.7	0.52	73.6 ± 7.6	74.2 ± 6.9	73.9 ± 7.1	0.94
Female, %	395 (56.5%)	15 (51.7%)	63 (61.8%)	81 (56.6%)	0.56	26 (52.0%)	110 (57.9%)	105 (56.8%)	0.76
Education, y, mean (SD)	16.1 ± 2.7	15.6 ± 2.5	16.1 ± 2.8	16.2 ± 2.7	0.0003	16.2 ± 2.5	16.1 ± 2.5	16.0 ± 3.0	0.87
MCI, %	471 (67.4%)	22 (75.9%)	77 (75.5%)	118 (82.5%)	0.37	30 (60.0%)	116 (61.1%)	108 (58.4%)	0.87
Depression, %	131 (18.7%)	5 (17.2%)	38 (17.6%)	28 (19.6%)	0.004	10 (20.0%)	91 (47.9%)	32 (17.3%)	< 0.0001
Anxiety, %	44 (6.3%)	1 (3.4%)	14 (9.8%)	9 (6.3%)	0.07	2 (4.0%)	28 (14.7%)	8 (4.3%)	0.0008
BMI, kg/m^2, mean (SD)	27.14 ± 4.8	27.1 ± 5.5	27.1 ± 5.0	26.4 ± 4.3	0.0005	27.6 ± 4.3	27.4 ± 5.0	27.3 ± 4.9	0.94
Insomnia, %	55 (7.9%)	7 (24.1%)	13 (12.7%)	35 (24.5%)	0.06	9 (18.0%)	23 (12.1%)	37 (20.0%)	0.11
Stroke, %	22 (3.1%)	1 (3.4%)	3 (3.4%)	0 (0.0%)	–	1 (2.0%)	6 (3.2%)	11 (5.9%)	0.29
HTN, %	322 (46.1%)	13 (44.8%)	49 (48.0%)	61 (42.7%)	0.71	24 (48%)	81 (42.6%)	94 (50.1%)	0.28
DM2, %	45 (6.4%)	1 (3.4%)	8 (7.8%)	6 (4.2%)	0.41	2 (4%)	19 (10.0%)	9 (4.9%)	0.10
Cancer, %	120 (17.2%)	5 (17.2%)	38 (19.6%)	19 (13.3%)	< 0.0001	6 (12.0%)	82 (43.2%)	32 (17.3%)	< 0.0001
Smoking, %	128 (18.3%)	6 (20.7%)	18 (17.6%)	21 (14.7%)	0.67	14 (28.0%)	37 (19.5%)	32 (17.3%)	0.24
CSF Aβ_{42}, pg/ml, median [Q1-Q3]	950.1 [649.4–1524.5]	744.4 [535.0–992.0]	709.1 [563.1–1047.1]	696.5 [544.0–921.4]	0.03	1,275.0 [736.2–1603.2]	1,180.0 [822.7–1722.5]	1,243.0 [790.5–1788.0]	0.53
CSF P-tau$_{181}$, pg/ml, median [Q1-Q3]	22.48 [16.7–31.9]	30.1 [20.2–37.0]	25.6 [19.9–38.4]	27.8 [27.1–39.8]	0.001	19.1 [14.9–26.6]	19.8 [15.2–26.5]	19.9 [15.8–28.6]	0.66
CSF T-tau, pg/ml, median [Q1-Q3]	247.2 [188.2–328.2]	188.1 [312.6–374.6]	267.6 [216.5–380.5]	285.1 [228.9–392.2]	< 0.0001	236.1 [165.4–284.7]	220.7 [174.5–287.7]	228.8 [177.8–302.8]	0.58
A (+), %	364 (52.1%)	21 (72.4%)	76 (74.5%)	111 (77.6%)	0.77	17 (34.0%)	68 (35.8%)	71 (38.4%)	0.76
T/N (+), %	382 (54.6%)	20 (68.9%)	69 (67.6%)	105 (73.4%)	0.60	22 (44.0%)	81 (42.6%)	85 (45.9%)	0.81

MCI, mild cognitive impairment; HTN, hypertension; DM2, type 2 diabetes mellitus; BMI, body mass index; CSF Aβ_{42}, amyloid-β-protein in cerebrospinal fluid, pg/ml; CSF T-tau, total tau protein in cerebrospinal fluid, pg/ml; CSF P-tau$_{181}$, phosphorylated tau protein in cerebrospinal fluid, pg/ml; A(+), Aβ_{42} levels below 976.6 pg/ml; TN(+), CSF P-tau$_{181}$ levels above 21.8 pg/ml or CSF T-tau levels above 245 pg/ml.

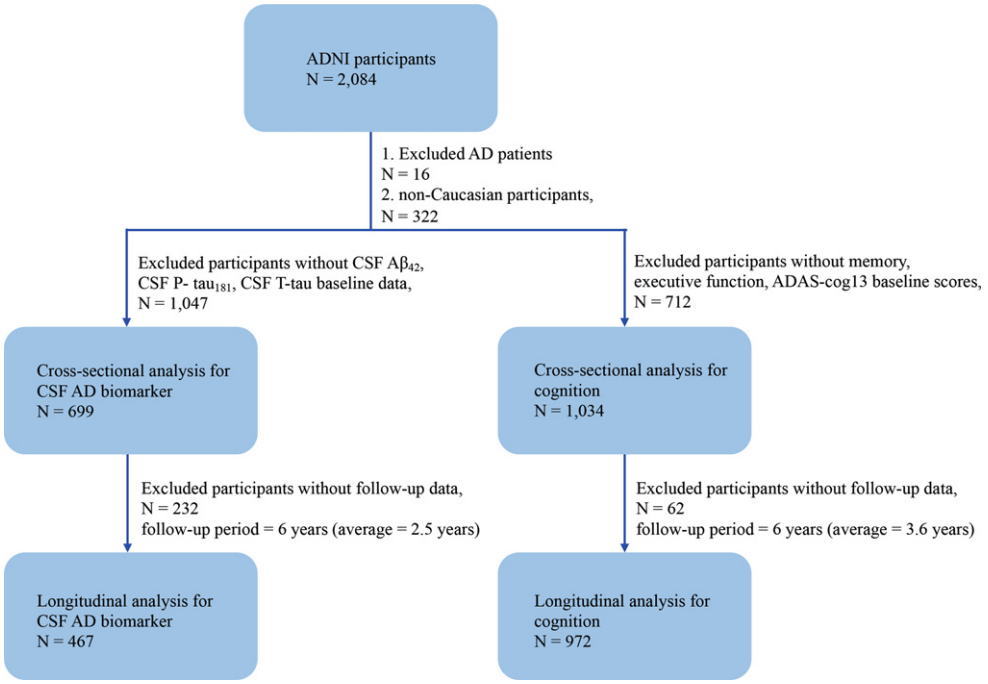

Fig. 1. Study population flowchart. A total of 2,084 participants were screened. After excluding non-Caucasian ($N = 16$) and demented participants ($N = 322$), participants were divided into two groups for CSF AD biomarker ($N = 699$) and cognitive cohort ($N = 1,034$) respectively.

period = 2.5 years) (Fig. 1). A total of 1,034 non-demented participants (mean age = 74 years, 56% females, 40% *APOE* ε4 carriers) were included for cognition analysis (Table 2), in which 972 participants has at least one follow-up visit (6 years, average follow-up period = 3.6 years) (Fig. 1).

rs3851179 allele A moderates the relationships of APOE ε4 with CSF AD biomarkers

The interaction of rs3851179 × *APOE* ε4 was not significantly associated with levels of CSF $A\beta_{42}$ (Fig. 2A), CSF P-tau_{181} (Fig. 2B), and CSF T-tau (Fig. 2C) at baseline. Longitudinal analysis via the likelihood ratio test indicated that the three-way interaction of time × rs3851179 × *APOE* ε4 accounted for a significant amount variance in CSF $A\beta_{42}$ ($\chi^2 = 7.816$, $p = 0.007$, Fig. 3A), CSF T-tau ($\chi^2 = 8.56$, $p = 0.003$, Fig. 3B), and CSF $Ptau_{181}$ ($\chi^2 = 10.61$, $p = 0.001$, Fig. 3C). Rs3851179 A allele was associated with slower decrease of CSF $A\beta_{42}$ and slower elevation of CSF T-tau and CSF $Ptau_{181}$ among *APOE* ε4 carriers. The above-mentioned interaction effects remained significant in stratified analyses by cognitive status (Supplementary Table 2) and age strata (Supplementary Table 3).

rs3851179 allele A moderates the relationships of APOE ε4 with cognition

For cross-sectional analyses, the interaction of rs3851179 × *APOE* ε4 accounted for a statistically significant amount of variance in ADAS-cog13 scores ($\beta = -0.29$, $p = 0.04$, Fig. 2D) and memory ($\beta = 0.17$, $p = 0.002$, Fig. 2E), but not executive function (Fig. 2F). The A allele of rs3851179 was associated with lower ADAS-cog13 scores and higher memory scores only among *APOE* ε4 carriers. The likelihood ratio test indicated that the three-way interaction of time × rs3851179 × *APOE* ε4 accounted for a statistically significant amount variance on ADAS-cog13 scores ($\chi^2 = 4.47$, $p = 0.03$, Fig. 3D) and memory ($\chi 2 = 5.70$, $p = 0.02$, Fig. 3E). The presence of rs3851179 A allele was associated with slower memory decline among *APOE* ε4 carriers. No interactive effects were for found for trajectory of executive function (Fig. 3F).

Subgroup analyses showed that the interaction effects on memory and ADAS-cog13 scores were significant in both NC and MCI groups (Supplementary Table 2). Interaction effects of rs3851179 × *APOE* ε4 on baseline ADAS-cog13 scores and memory remained significant in late-life group, but not in

Table 2
Baseline characteristics of participants for cognition cohort by *APOE* ε4 and *PICALM* rs3851179

N = 1,034	Total	*APOE* ε4 carrier, *N* = 427			*p*	non - *APOE* ε4 carrier, *N* = 607			*p*
		AA	AG	GG		AA	AG	GG	
Number of samples	1,034	45 (10.5%)	173 (40.6%)	209 (48.9%)		79 (13.0%)	274 (45.2%)	254 (41.8%)	
Age, y, mean (SD)	73.8 ± 7.0	73.7 ± 5.6	73.7 ± 7.5	72.6 ± 6.6	0.42	74.3 ± 7.0	74.7 ± 7.0	74.3 ± 7.0	0.81
Female, %	449 (43.4%)	23 (51.1%)	100 (57.8%)	120 (57.4%)	0.69	43 (54.5%)	158 (57.7%)	145 (57.1%)	0.88
Education, y, mean (SD)	16.0 ± 2.8	15.5 ± 2.5	15.5 ± 2.8	16.1 ± 2.8	0.44	16.1 ± 2.7	16.0 ± 2.7	16.1 ± 3.0	0.95
MCI, %	679 (65.7%)	22 (48.9%)	129 (74.6%)	165 (78.9%)	< 0.01	47 (59.5%)	159 (58.0%)	145 (57.1%)	0.93
Depression, %	200 (19.3%)	7 (15.5%)	37 (21.4%)	44 (21.0%)	0.86	13 (16.5%)	54 (19.7%)	45 (17.7%)	0.74
Anxiety, %	57 (5.5%)	1 (2.2%)	12 (6.9%)	14 (6.7%)	0.49	3 (3.8%)	16 (6.4%)	11 (4.3%)	0.64
BMI, kg/m^2, mean (SD)	26.90 ± 4.7	26.48 ± 5.4	26.63 ± 4.7	26.32 ± 4.3	0.80	27.11 ± 4.2	27.20 ± 4.9	27.26 ± 4.6	0.97
Insomnia, %	69 (6.7%)	2 (4.4%)	7 (4.0%)	35 (16.7%)	< 0.01	9 (11.4%)	23 (8.4%)	37 (14.6%)	0.08
Stroke, %	36 (3.5%)	2 (4.4%)	9 (5.2%)	0 (0.0%)	< 0.01	3 (3.8%)	9 (3.3%)	13 (5.1%)	0.56
Cancer, %	171 (16.5%)	8 (17.8%)	35 (20.2%)	27 (12.9%)	0.15	9 (11.4%)	47 (17.1%)	45 (17.7%)	0.40
HTN, %	479 (46.3%)	23 (51.1%)	81 (46.8%)	94 (44.9%)	0.75	37 (46.8%)	116 (42.3%)	128 (50.3%)	0.18
DM2, %	73 (7.1%)	1 (2.2%)	12 (6.9%)	10 (4.8%)	0.37	3 (3.8%)	24 (8.8%)	23 (9.1%)	0.30
ADAS-cog13 z score, mean (SD)	0 ± 0.99	–0.002 ± 1.01	0.27 ± 0.99	0.35 ± 0.98	0.09	–0.16 ± 0.97	–0.19 ± 0.93	–0.22 ± 0.97	0.86
ADNI_MEM z score, mean (SD)	0.44 ± 0.74	0.45 ± 0.74	0.24 ± 0.74	0.18 ± 0.74	0.08	0.52 ± 0.72	0.64 ± 0.69	0.43 ± 0.71	0.27
ADNI_EF z score, mean (SD)	0.37 ± 0.88	0.04 ± 0.82	0.26 ± 0.89	0.23 ± 0.80	0.29	0.52 ± 0.89	0.46 ± 0.88	0.47 ± 0.91	0.88

MCI, mild cognitive impairment; HTN, hypertension; DM2, Type2 Diabetes Mellitus; BMI, body mass index; ADAS, Alzheimer's Disease Assessment Scale; MEM, memory; EF, executive function.

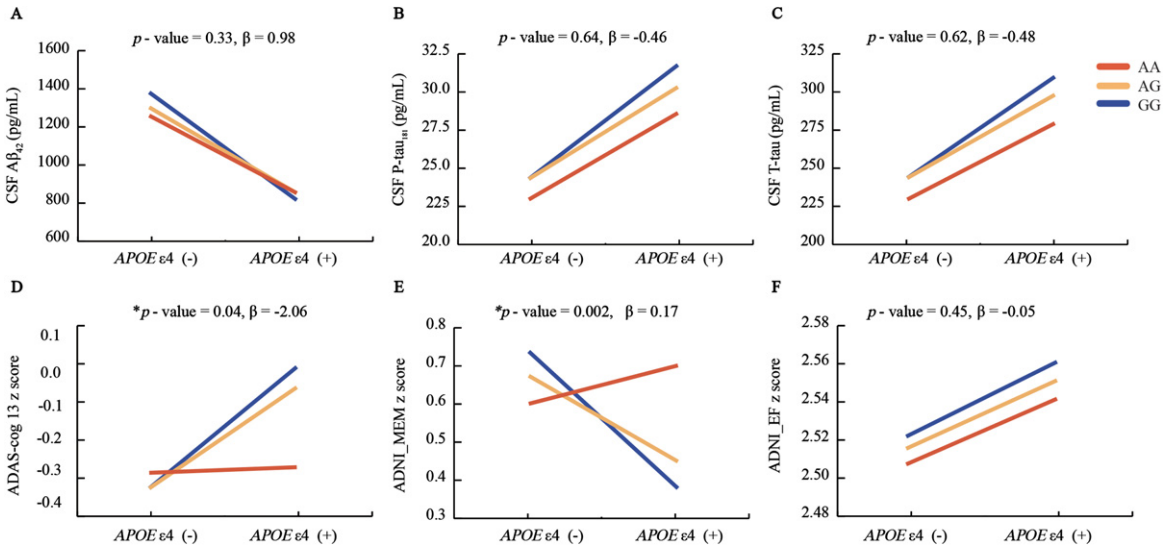

Fig. 2. Roles of *PICALM* rs3851179 in moderating the relationship of *APOE* ε4 with CSF AD biomarker and cognition at baseline. The interaction of rs3851179 × *APOE* ε4 was not associated with CSF levels of $A\beta_{42}$ (A), CSF P-tau_{181} (B), and CSF T-tau (C). The interaction of rs3851179 × *APOE* ε4 accounted for a statistically significant amount of variance in ADAS-cog13 scores (D), and memory (E), but not executive function (F). ADAS, Alzheimer's Disease Assessment Scale; MEM, memory; EF, executive function; $*p < 0.05$; Estimate (β), the amount of change in the dependent variable corresponding to each one-unit change in the independent variable.

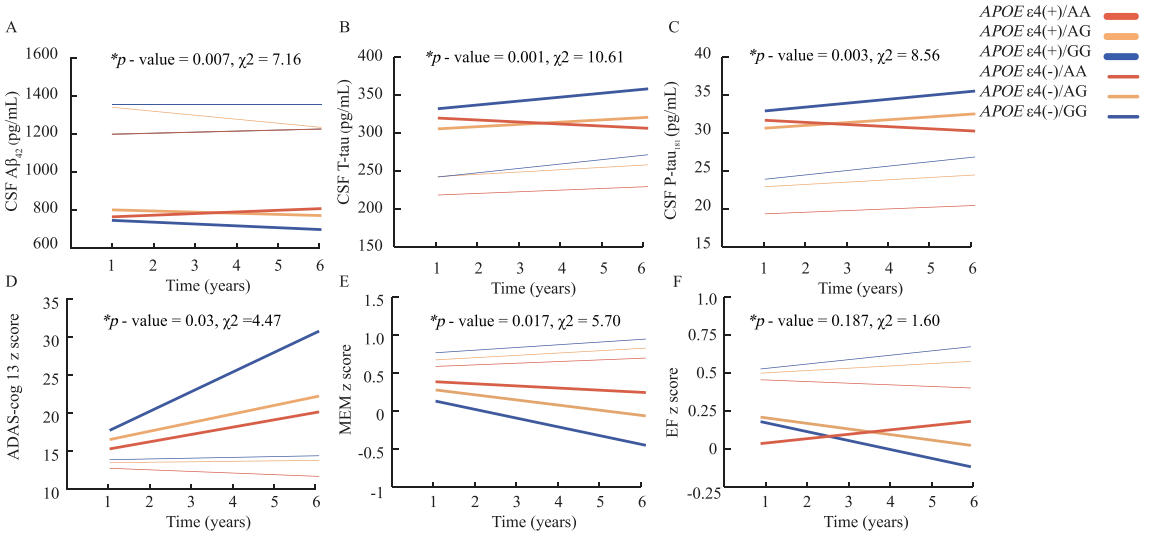

Fig. 3. Roles of *PICALM* rs3851179 in moderating the relationship of *APOE* ε4 with trajectory of CSF AD biomarker and cognitive functions. The three-way interaction of time × rs3851179 × *APOE* ε4 accounted for a significant amount variance in longitudinal changes of CSF $A\beta_{42}$ (A), CSF T-tau (B), CSF $Ptau_{181}$ (C), general cognition (D), and memory function (E). No interactive effect was found for longitudinal changes of executive function (F). ADAS, Alzheimer's Disease Assessment Scale; MEM, memory; EF, executive function; $*p < 0.05$. A "square" symbol was used to represent "*APOE* ε4(+)/AA" group, while a "triangle" symbol was used to highlight the "*APOE* ε4(+)/GG" group.

mid-life group (Supplementary Table 3). The interaction effects on trajectory of ADAS-cog13 scores was significant in late-life group, but not mid-life group (Supplementary Table 3).

DISCUSSION

In the present study, we reported that rs3851179 allele A of *PICALM* could alleviate the impacts of

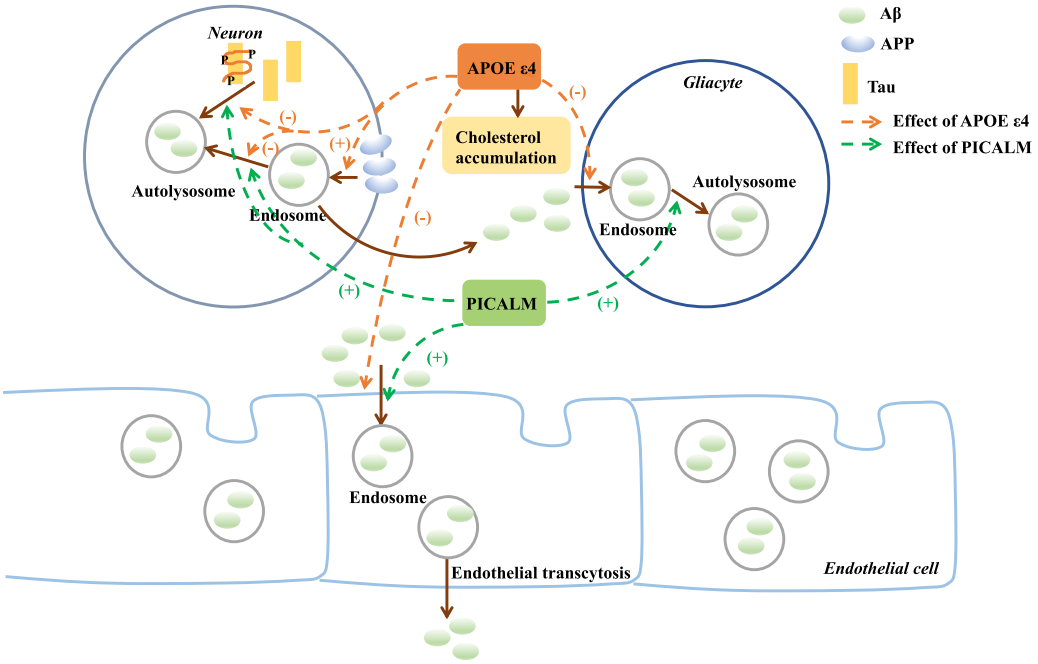

Fig. 4. Potential mechanisms by which PICALM interact with *APOE* ε4 to influence AD pathologies. The red line represents the detrimental effects of *APOE* ε4. "(+)" means facilitation while "(–)" means inhibition. *APOE* ε4 can lead to more production and less elimination of Aβ via endosomal-lysosomal degradation pathway. *PICALM* rs3851179 allele A was associated with elevated expression of PICALM proteins, which could play an active role in eliminating Aβ by promoting endocytosis, autophagy of Aβ in neurons and gliacytes, and facilitating extracellular Aβ protein to cross blood-brain barrier. In addition, *PICALM* can also eliminate abnormal tau proteins by facilitating autophagy in neurons.

APOE ε4 on CSF levels of AD biomarkers and cognitive functions among non-demented population, strengthening the potential roles of gene-gene interaction in AD occurrence.

The identified interaction of *PICALM* × *APOE* ε4 on CSF Aβ levels could be explained by their interaction in several common pathways including autophagy, endocytosis, and endothelial transcytosis, which are involved in the production and elimination of Aβ (Fig. 4). Normally, full-length amyloid precursor protein on the cell surface is endocytosed into endosomes where it can be cleaved to produce Aβ and subsequently released into extracellular matrix and CSF or otherwise eliminated through autophagy [28, 29]. Specifically, *APOE* ε4 was associated with increased production and hampered elimination of amyloid proteins. First, *APOE* ε4 can promote cholesterol accumulation in neurons and extracellular environment [14, 30], which could lead to enhanced volume of endosome and more production of Aβ in neurons [20]. Second, *APOE* ε4 is associated with dysfunctional degradation process, resulting in less elimination of Aβ in neurons [14]. Third, *APOE* ε4 could damage endocytosis in gliacytes and endothelial transcytosis, causing less removal of extracellular Aβ across the brain-blood barrier and more accumulation of Aβ in brain matrix [14, 31, 32]. As a potential defender against AD, *PICALM* acts in eliminating Aβ via clathrin-mediated endocytosis (CME) pathway in neurons and gliacytes [33, 34]. Also, *PICALM* is associated with removal of extracellular Aβ across the brain-blood barrier into CSF [33, 35–38], compensating the dysfunction of endothelial transcytosis caused by *APOE* ε4.

Degradation of excessive abnormal tau protein can be also disturbed by *APOE* ε4 [19], which process might be rescued by *PICALM*. Abnormal tau proteins are generally eliminated through ubiquitin-proteasome system, chaperon-mediated autophagy, and endosomal micro autophagy in neuron cell [39]. Autophagy can keep pace with the degradation of tau aggregates under normal condition. However, impairments of autophagy caused by *APOE* ε4 allele may lead to overwhelmed accumulation of abnormal tau proteins in neurons, and then propagate between neurons, and spread from neurons to microglia, astrocytes, and oligodendrocytes [39]. *PICALM* can to

some extent eliminate excessive tau proteins by facilitating autophagy through CME, compensating the deficiency in autophagy associated with *APOE* ε4 (Fig. 4).

We also found that rs3851179 A allele could alleviate the impacts of *APOE* ε4 on cognition, especially for memory function. This can be explained by the role of *PICALM* in fighting against neurotransmission dysfunction caused by *APOE* ε4. Specifically, normal endocytosis and exocytosis of synaptic vesicle cycle underpinned chemical neurotransmission between related neurons [40]. *APOE* ε4 leads to endosomal-lysosomal degradation dysfunction [41], which can affect the normal functioning of synaptic vesicle cycle. Also, *APOE* ε4 is associated with apoptosis induction and synaptic loss [42], which can directly lead to cognitive impairment. *PICALM* can facilitate neurotransmission by maintaining normal endocytosis [43] and alleviate the negative effects of *APOE* ε4 on neurons and cognitive functions..

To the best of our knowledge, we for the first time reported how interaction of *PICALM* with *APOE* ε4 influence AD occurrence in pre-dementia stages based on population-based study, The interaction of *PICALM* with *APOE* ε4 might help predict prognosis of pathological and clinical outcomes in early stages of AD continuum and also help recognition and stratification of high-risk population for apoe4 carriers in clinical settings. In addition, future drug development experiments could target picalm-related pathways for early therapy of AD associated with *APOE* ε4.

There are several limitations in the present study. First, limited sample size and attrition bias due to loss to follow-up in longitudinal analysis could impact the stability of the conclusions. Larger and better designed cohort are expected to validate these findings. Second, these are preliminary findings based on observational design which cannot equal to causal relationship. Third, PET and blood biomarkers are not analyzed due to limited sample size, which could jeopardize the statistical power especially for uncovering interaction effects. Fourth, the generalizability of our findings might be constrained because the participants were Caucasian volunteers. The interactive effects should be explored in independent population of other race or ethnicity.

Conclusions

The present study found that the rs3851179 A allele of *PICALM* could alleviate the negative influences of *APOE* ε4 on AD core biomarkers and cognition among non-demented population. These findings can help understand the gene-gene interactive roles in AD occurrence.

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CONFLICT OF INTEREST

Wei Xu is an Editorial Board Member of this journal but was not involved in the peer-review process nor had access to any information regarding its peer-review.

All other authors have no conflict of interest to report.

DATA AVAILABILITY

All data are available upon reasonable request or can be obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu).

SUPPLEMENTARY MATERIAL

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