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ORIGINAL ARTICLE

A gene–environment interplay between omega-3 supplementation and *APOE* ε4 provides insights for Alzheimer's disease precise prevention amongst high-genetic-risk population

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Abstract

Background and purpose: The present study aimed to explore whether and how omega-3 (ω-3) supplementation could interact with genetic factors to modulate cognitive functions, amyloid pathologies, and Alzheimer's disease (AD) risk.

Methods: A total of 1,670 non-demented participants (mean age 73 years, 47% females, 41% *APOE* ε4 carriers) were followed up for 10 years. Hierarchical regressions, linear mixed-effects models, and Cox proportional hazards models were used to examine the interaction effects of ω-3 supplementation with *APOE* ε4 and polygenic hazard scores, after adjusting for age, gender, education, cognitive diagnosis, insomnia, depression, anxiety, and cardiovascular risk score.

Results: Individuals who progress to AD during the follow-up tend to take a shorter duration of ω-3 at baseline than those stable, for whom the difference remained significant only amongst *APOE* ε4 carriers ($p < 0.01$). The interaction term (*APOE* ε4 × ω-3) accounted for a significant amount of variance in cognition and cerebral amyloid burden. Long-term ω-3 use protected cognition (especially memory function) and lowered amyloid burden and AD risk only amongst *APOE* ε4 carriers. Mediation analysis suggested that amyloid pathologies, brain reserve capacities, and brain metabolism mediated the relationships of ω-3 use with memory and global cognition for *APOE* ε4 (+) carriers. Similar interaction and mediation effects were also indicated amongst high-risk subjects defined by polygenic hazard scores.

Conclusions: Long-term ω-3 intake may have a role in AD prevention in genetically at-risk populations.

KEYWORDS
APOE ε4, environment, interaction, late-onset Alzheimer's disease, omega-3

Lin Li and Wei Xu contributed to the present work equally.

[†]The data used herein were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). The investigators contributed to the design and implementation of ADNI and/or provided data but did not participate in the analysis 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) is driven by both genetic [1,2] and environmental elements [3]. The apolipoprotein E (*APOE*) gene is the strongest common genetic determinant, followed by a large and still expanding genetic profile [1,2]. *APOE* ε4 carriers had an estimated 3 to 12 times increased risk of LOAD [4]. It was estimated that an average of roughly 26% were *APOE* ε4 carriers in global community-dwellers [5], further highlighting the importance of developing tailored prevention strategies for this specific population, especially considering that no modifying therapies are available for AD. Nonetheless, it is still unclear whether the hereditary predisposition could be modified by environmental factors. Thus, understanding the gene–environment interactions is of significant importance [6]. Dietary interventions are easily adopted primary prevention recommendations for the public to lower disease risk. Recently, omega-3 (ω-3), a long-chain polyunsaturated fatty acid (PUFA) [7] was proposed to interact with *APOE* ε4 to influence AD risk [8,9], although the evidence was contradictory in cognitively healthy subjects. Some observational studies [10–13] and clinical trials [14,15] reported that the cognitive benefits were achieved only amongst *APOE* ε4 carriers, whilst others suggested that the protection response was restricted to non-carriers [16–19]. Herein, the aim was to ask (i) whether ω-3 supplementation, especially for those long-term users, could modulate or counteract the deleterious effects of LOAD genetic factors on cognitive functions, cerebral amyloid burden and AD risk in pre-dementia stages, and (ii) whether ω-3 use was associated with cognition via the mediation of amyloid burden.

SUBJECTS AND METHODS

Participants

Data were derived from the Alzheimer's Disease Neuroimaging Initiative (ADNI) cohort (http://adni.loni.usc.edu/). Volunteers were continuously recruited from multiple centres across North America. The participants were older adults aged 55–90 years with normal cognition (NC), mild cognitive impairment (MCI) or mild AD dementia. The present study focused on subjects who were free of dementia at baseline, as determined via the National Institute of Neurological and Communicative Disorders and Stroke and Alzheimer's Disease and Related Disorders Association (NINDS-ADRDA) diagnostic criteria. Subjects were excluded if they (i) were clinically demented at entry, (ii) had no valid cognitive measures or amyloid positron emission tomography (PET) data and (iii) had data seen as extreme values (situated outside ±3 standard deviations). Finally, a total of 1670 subjects were included, amongst whom 1062 (64%) had cerebral amyloid PET data (Figure S1).

Exposure measurements

Omega-3 supplementation

Self-reported medication-taking information was recorded at the initial screening visit. ω-3 supplements were defined as fish oil, omega-3, PUFA, docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) or alpha linolenic acid. Participants who used ω-3 supplements for less than 1 year were treated as the non-exposed group. The duration of ω-3 use was defined as the time from initiation use to discontinuation. Subjects were roughly categorized into 'never user' (<1 year), 'medium user (MS)' (1–9 years) and 'long-term user' (≥10 years).

Genetic risk profile

The ADNI-1 samples were genotyped using the Illumina Human610-Quad BeadChip and ADNI GO/2 samples were genotyped by the Human OmniExpress BeadChip (Illumina Inc.). rs7412 and rs429358 were used to define the *APOE* ε2/ε3/ε4 isoforms [20]. A polygenic hazard score (PHS) for each subject was developed and validated based on a combination of *APOE* ε4 and 31 other LOAD genetic variants [21]. *APOE* ε4 status ('44/34/24' = 1) or PHSs were used to represent each subject's genetic predisposition.

Covariate measurements

The covariates include age, gender, education, cognitive diagnosis (MCI = 1, NC = 0), depression, anxiety, insomnia and cardiovascular conditions. A summary measure of cardiovascular risk score on a scale from 0 to 7 was constructed [22] by adding 1 point for the presence of each of the following conditions: hypertension, diabetes mellitus, hyperlipidaemia, smoking, obesity, stroke and coronary heart disease. Obesity (yes or no) was defined as body mass index ≥24 kg/m^2.

Cognitive assessments

General cognition was measured by the 85-point Alzheimer's Disease Assessment Scale 13-item cognitive subscale. The composite scores for memory, executive functioning and language were calculated using data from the ADNI neuropsychological battery via item response theory methods. The composite scores have been validated [23,24].

Positron emission tomography imaging

The PET data were collected as part of the linked study protocols. A PET scan was performed within 2 weeks before or after the baseline clinical assessments [25]. Standardized update value ratios were calculated with a standardized cortical anatomical automatic labelling volume-of-interest template placed on spatially normalized image volumes using a whole-cerebellum reference region [26]. The mean florbetapir AV45 uptake within each region was calculated by co-registering the florbetapir scan to the corresponding magnetic resonance imaging (MRI). Fluorodeoxyglucose (FDG) PET images were

registered to a PET template in Montreal Neurological Institute space [27] at an isotropic resolution of 3 mm using FLIRT.

Brain MRI

All participants received high-resolution structural brain MRI scans on 1.5-T scanners as specified by the ADNI protocol [28]. For volumetric analyses, 1 mm isotropic 3D T1 sequences without contrast injection were performed. Here, six brain regions were defined as regions of interest, including hippocampus, entorhinal, mid-temporal, parahippocampal region, posterior cingulate and precuneus area. These regions were known to be affected by AD and their atrophy in AD has been previously validated via MRI studies.

Diagnosis of AD dementia

Detailed information about neuropsychological testing and diagnostic criteria is available at the ADNI website (http://adni.loni.usc.edu/methods). Briefly, the AD patients had a Mini Mental State Examination (MMSE) score of 20–26 and a Clinical Dementia Rating Scale Sum of Boxes (CDR-SB) score of 0.5 or 1 and met the NINDS-ADRDA criteria for probable AD [29]. Cognitive diagnosis was recorded at each annual follow-up visit. Progressors were defined according to change in the latest follow-up diagnosis available in ADNI data, including NC to AD dementia and MCI to AD dementia; and individuals showing no changes during the full follow-up period were classified in the stable group. Time to AD dementia was defined as the time between the baseline visit and the date of AD dementia diagnosis.

Statistical analyses

Samples were categorized into *APOE* ε4 (+) and *APOE* ε4 (−) groups and the baseline between-group difference was compared by the Pearson chi-squared test or the independent-samples *t* test. First, the ω-3 total duration between progressors and stable subjects was compared and whether the difference varied with *APOE* ε4 status was tested. Secondly, cross-sectionally, hierarchical regression analyses were conducted to examine the interaction effects between *APOE* ε4 and ω-3 (status [yes vs. no] or duration [values and category variable (never, MS and long-term)]) on AD endophenotypes. The dependent variables (cognition or cerebral amyloid measures) were log-transformed to fit a Gaussian distribution. The overall significance of the interaction term was assessed by the *F*-ratio statistics comparing the full model and a nested model that did not include the interaction term, with R^2 representing the variance explained by the model. Simple slope analyses were performed to interpret the interaction effects, with either *APOE* ε4 status or ω-3 as the moderator variable. The statistical analyses were performed using SPSS version 18.0, R version 3.6.1 and Jamovi version 1.0.4.0. Longitudinal analyses were performed via the linear mixed-effects regression using the 'lme4' package in R. The linear mixed-effects models were employed because they could handle unbalanced and censored data as well as a continuous variable for time [30]. Fixed effects included main effects of long-term ω-3 supplementation (hereinafter referred to as 'ω-3'), *APOE* ε4 status, years of follow-up (time-varying variable, hereinafter referred to as 'visit'), as well as interaction terms of ω-3 × *APOE* ε4, visit × ω-3, visit × *APOE* ε4 and visit × ω-3 × *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. Regression diagnostics were conducted and outliers were excluded to indicate that all models met the necessary assumptions: model residuals were normally distributed and did not exhibit heteroscedasticity. Statistical comparison of model coefficients to determine the direction of group differences was performed using the Wald test via the 'aod' package.

Next, causal mediation analyses were conducted to examine whether amyloid pathology could modulate the relationship between ω-3 supplementation and cognition. The significance of the total effect (T), the direct effect (DE), the indirect effect (IE) and the proportion of mediation (IE/T) was estimated using 10,000 bootstrapped iterations. In each model, the total effect refers to the initial relationship of ω-3 supplementation on cognition, excluding the mediator. The DE represents the direct effect independent of the mediator. The IE represents the magnitude of the effect accounted for by the mediator, implying a possible causal relationship [31,32]. The mediating role of brain reserve or cerebral glucose metabolism in the relationship between ω-3 and cognitive functions was also explored. The 'lm', 'mediate' and 'car' packages were used to perform the above analyses.

Finally, the associations between *APOE* ε4 and incident probable AD stratified by ω-3 were studied by calculating cumulative incidence using the Kaplan–Meier method. Hazard ratio (HR) with 95% confidence interval (CI) was estimated using the time-dependent Cox proportional hazards model. The 'lm', 'survival', 'ggplot2', 'ggpubr', 'magrittr' and 'survminer' packages were used for these analyses.

On top of *APOE* ε4, the interaction of ω-3 supplementation with PHS was tested using the same method as described above. The distribution of PHS was divided into high (mean + 1SD), moderate (mean) and low (mean − 1SD) levels, representing high, moderate and low genetic risk. Moreover, sensitivity analyses were conducted by adding practice effects (number of prior exposures to the cognitive test [total visits completed − 1]) as covariates or by excluding those who were lost in the first 3 years during follow-up. Statistical tests were two-tailed, and an α-level of $p < 0.05$ was used to determine statistical significance; for interaction terms $p < 0.1$ was considered significant, and $0.1 < p < 0.15$ was considered a trend.

RESULTS

Stable *APOE* ε4 carriers have longer consumption of ω-3

Amongst 662 *APOE* ε4 carriers who were free of dementia at baseline, 208 (31.4%) diagnosed with AD since baseline were seen as progressors, leaving 454 without cognitive decline in the stable group. The average duration of ω-3 use was significantly longer for the stable *APOE* ε4 carriers (Figure 1a), whilst no difference was found amongst non-carriers (Figure 1b).

Long-term ω-3 use interacts with *APOE* ε4 to protect cognition and amyloid pathology

Cross-sectional relationship

A total of 1670 participants (46.8% females, 73 ± 7.0 years) were included (Table 1), amongst whom 64% (47.6% females, 72 ± 6.9 years) had amyloid PET at baseline (Table S1). The *APOE* ε4 carriers account for 41%. Compared with the non-carriers, the *APOE* ε4 carriers tended to be younger, less educated, with a higher proportion of MCI and a lower cardiovascular risk score (CVRS). In all, 391 (23.4%) subjects reported ω-3 use history, amongst whom 100 (23.8%) have used for at least 10 years (defined as 'long-term users') (Table 1). Details regarding the subgroups according to ω-3 duration is given in Table S2. A total of 1176 participants had data for interaction analyses with PHS (Table S3).

The interaction of *APOE* ε4 × ω-3 use accounted for a statistically significant amount of variance in memory ($R^2 = 0.001$, $p = 0.107$) and executive function ($R^2 = 0.001$, $p = 0.078$) (Table S4). The simple slope analyses indicated that long-term supplementation of ω-3 was associated with higher memory scores ($\beta = 0.014$, $p = 0.016$) only in the *APOE* ε4 (+) group, whereas with higher executive function ($\beta = 0.022$, $p = 0.004$) only in the *APOE* ε4 (−) group. No significant association was found with global cognition and language function (Table S5, Figure 2a–d). In addition, the associations of *APOE* ε4 with impairments in memory and global cognition were significant only in the 'never' and 'MS' groups ($p < 0.001$), whilst the associations became non-significant amongst 'long-term users'. Otherwise, the association of *APOE* ε4 with executive function was barely influenced by the ω-3 use (Table S5, Figure S2a–d). Hierarchical regression analyses showed that *APOE* ε4 could also interact with ω-3 supplementation to modulate amyloid pathology ($R^2 = 0.004$, $p = 0.016$). Long-term ω-3 use was associated with lower amyloid burden only in the *APOE* ε4 (+) group ($\beta = -0.005$, $p = 0.029$, Figure 2e). Interestingly, the effects of *APOE* ε4 on amyloid burden was 50% weaker for those long-term users ($\beta = 0.10$, $p = 0.028$) (Figure S2e).

On top of *APOE* ε4, significant interaction effects of PHS × long-term ω-3 supplementation on cognitive functions as well as amyloid burden were also found (Table S6). Long-term ω-3 use was associated with better memory and global cognition (Figure 2f–i) and lower amyloid burden (Figure 2j) only amongst high-risk samples. Similarly, the associations of PHS with cognition (memory and global cognition) or amyloid burden became non-significant in the long-term ω-3 user group, whilst the association of PHS with executive or language function was not influenced by ω-3 use (Table S7, Figure S2f–j).

Longitudinal relationships

All participants completed at least two evaluations and the maximum follow-up was 10 years (Figure S1). Finally, a total of 1452 participants (45% female, 73.4 ± 7.1 years) were included, amongst whom 46.3% had amyloid PET at baseline (Table S8).

The likelihood ratio test indicated that the three-way interaction of long-term ω-3 use (ω-3) × *APOE* ε4 × visit accounted for a significant amount of variance in cognitive performances, including general cognition ($\chi^2 = 6.41$, $p = 0.011$), memory ($\chi^2 = 6.18$, $p = 0.013$), executive function ($\chi^2 = 2.64$, $p = 0.100$) and language function ($\chi^2 = 5.89$, $p = 0.015$). Greater rates of decline were observed in the *APOE* ε4 (+) and never ω-3 use (*APOE* ε4 (+)/never) group, compared with other groups. Statistical comparison of the model coefficients indicated that *APOE* ε4 could predict greater cognitive decline in the never and MS groups, but not in the ω-3 long-term user group. Similarly, long-term ω-3 supplementation could predict slower cognitive decline only in the *APOE* ε4 (+) group but not in the *APOE* ε4 (−) group (Figure 3).

Also, the interaction of ω-3 × *APOE* ε4 × visit accounted for a statistically significant amount of variance in cerebral amyloid deposition ($\chi^2 = 11.39$, $p < 0.001$). The greatest rate of amyloid deposition was observed in the *APOE* ε4 (+)/never group (Figure 4a). *APOE* ε4 could predict greater amyloid deposition in never the ω-3 user and MS user groups, but not in the ω-3 long-term user group.

Long-term ω-3 use counteracts the influence of *APOE* ε4 status on incident AD risk

A total of 1320 participants were included (mean age 73.2 years, 44% females), amongst whom 258 subjects (19.5%) developed probable AD

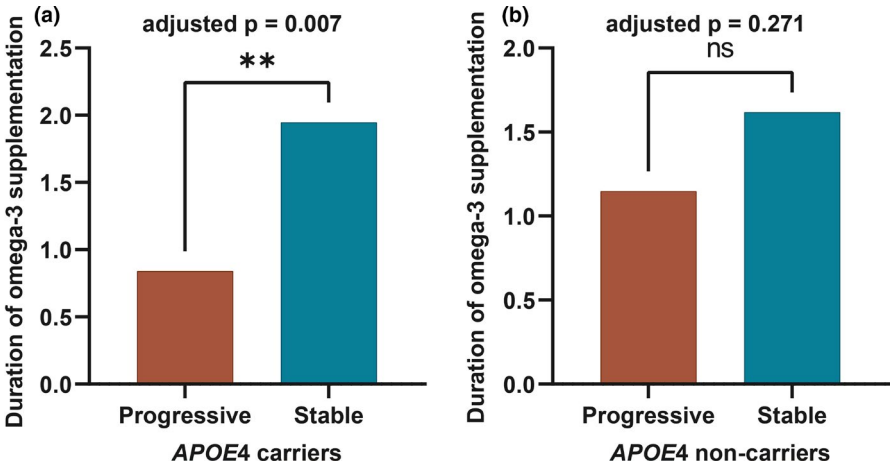

FIGURE 1 Difference of total ω-3 supplementation duration between the progressive and stable groups. The average ω-3 supplementation duration was significantly longer for the stable group compared to the progressive group (a). No significant difference was found in the non-carriers (b)

TABLE 1 Population characteristics at baseline

Variable	Total	*APOE* ε4 (+)	*APOE* ε4 (–)	*p* value
Number	1670	682	988	
Age, years, mean ± SD	73.03 ± 7.02	72.08 ± 6.82	73.68 ± 7.08	<0.001
Female, %	781 (46.8%)	318 (46.6%)	463 (46.9%)	0.92
Education, years	16.29 ± 2.59	16.13 ± 2.62	16.40 ± 2.57	0.04
MCI, %	923 (55.3%)	453 (66.4%)	470 (47.6%)	<0.001
ω-3 use, %	391 (23.4%)	165 (24.2%)	226 (22.9%)	0.53
ω-3 use duration	6.03 ± 4.58	6.63 ± 5.41	5.90 ± 4.47	0.16
MS-term use (1–9 years)	291 (17.4%)	115(16.9%)	176 (17.7%)	–
Long-term use (≥10 years)	100 (6.0%)	50 (7.3%)	50 (5.1%)	–
Cognitive functions				
ADAS-cog scores	13.80 ± 6.48	15.38 ± 6.81	12.71 ± 6.00	<0.001
MEM *z* scores	0.56 ± 0.74	0.38 ± 0.76	0.68 ± 0.70	<0.001
EF *z* scores	0.53 ± 0.91	0.37 ± 0.91	0.62 ± 0.91	<0.001
LAN *z* scores	0.49 ± 0.80	0.39 ± 0.82	0.55 ± 0.78	<0.001
Insomnia, %	125 (7.5%)	44 (6.5%)	81 (8.2%)	0.18
Depression, %	326 (19.5%)	144 (21.1%)	182 (18.4%)	0.17
Anxiety, %	107 (6.5%)	52 (7.6%)	55 (5.7%)	0.09
CVRS	1.33 ± 1.09	1.27 ± 1.07	1.38 ± 1.10	0.04
Hypertension, %	737 (44.1%)	300 (44.0%)	437 (44.2%)	0.98
Obesity, %	589 (35.3%)	201 (29.5%)	388 (39.3%)	<0.001
Diabetes, %	142 (8.5%)	54 (7.9%)	88 (8.9%)	0.48
Hyperlipidaemia, %	811 (48.6%)	364 (53.4%)	447 (45.2%)	0.001
Stroke, %	55 (3.3%)	21 (3.1%)	34 (3.4%)	0.68
CHD, %	138 (8.3%)	56 (8.2%)	82 (8.3%)	0.95
Current smoker, %	232 (13.9%)	96 (14.1%)	136 (13.8%)	0.86

Abbreviations: ADAS, Alzheimer's Disease Assessment Scale; *APOE*, apolipoprotein E gene; CHD, coronary heart disease; CVRS, cardiovascular risk score; EF, executive function; LAN, language; MCI, mild cognitive impairment; MEM, memory; MS, medium and short.

dementia over an average follow-up of 2.5 (SD = 1.6) years. Compared with the *APOE* ε4 (−) subjects, *APOE* ε4 carriers had a significantly increased risk of developing AD in the ω-3 never user group (HR 2.51, 95% CI 1.88–3.37, $p = 6.74 \times 10^{-10}$) and MS group (HR 2.56, 95% CI 1.37–4.78, $p = 0.003$). Nonetheless, the association of *APOE* ε4 with AD risk became non-significant in the ω-3 long-term user group ($p = 0.599$) (Table S9, Figure 4b). Similarly, it was found that each additional year of ω-3 supplementation was associated with 7% lower risk of developing AD (HR 0.93, 95% CI 0.87–1.00, $p = 0.037$) in the *APOE* ε4 (+) group but not in the *APOE* ε4 (−) group. These results were barely changed in the fully adjusted model or in the sensitivity analyses. Analyses of PHS-related risk obtained similar results (Table S10).

Amyloid pathologies, brain atrophy and metabolism mediated the association of ω-3 use with cognition

Causal mediation analyses indicated that amyloid pathology had significant mediation effects for the relationship of ω-3 use with memory (30.9%, $p = 0.026$). The effects can only be observed in the *APOE* ε4 (+) group but not in the non-carrier group. It was also found that volumes of entorhinal region could mediate the association of ω-3 use with memory (40.1%, $p = 8 \times 10^{-4}$) and global cognition (56.8%, $p = 0.011$) in the *APOE* ε4 (+) group; the FDG-PET mediated the association of ω-3 use with memory (29.6%, $p = 0.020$) in the *APOE* ε4 (+) group. No mediation effects were found for para-hippocampus, posterior cingulate or precuneus volume (Table S11, Figure 5a). Similar results were observed in analyses of PHS-related risk (Table S12, Figure 5b).

DISCUSSION

Herein, it is reported that (i) long-term ω-3 supplementation could mitigate the genetic predisposition to cognitive decline, amyloid deposition and AD risk in non-demented adults, and (ii) amyloid pathology, brain reserve and metabolism could mediate the relationship of ω-3 supplementation with cognition, especially memory function. These findings suggest that ω-3 supplementation could be used as a primary preventative approach to lowering AD risk in the long run.

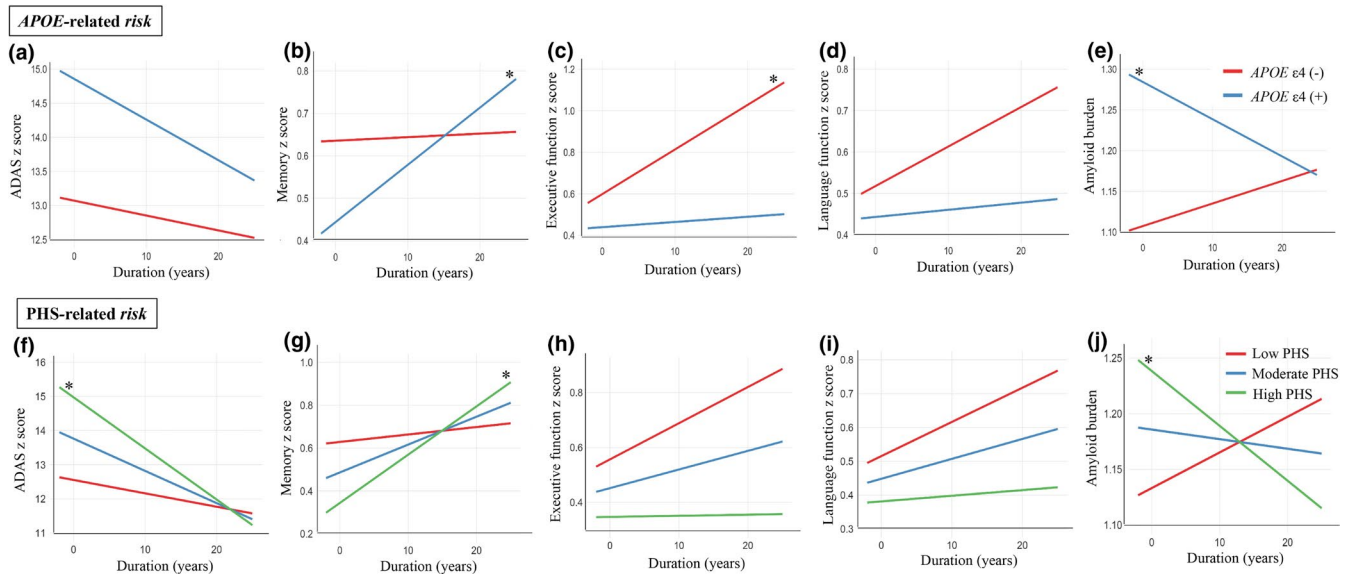

FIGURE 2 Simple slope analyses for the interaction effects of *APOE* ε4/PHS × ω-3 supplementation on cognitive function and amyloid burden. Stratified by the *APOE* ε4 status, long-term supplementation of ω-3 was associated with higher memory scores (b) only in the *APOE* ε4 (+) group, and with higher executive function scores (c) only in the *APOE* ε4 (−) group, but no significant association was found with global cognition and language function (a), (d). Similarly, longer ω-3 supplementation was associated with amyloid burden only in the *APOE* ε4 (+) group (e). Stratified by PHS status, longer ω-3 use was associated with better global cognition (f) and memory (g) as well as amyloid burden (j) only in the high PHS group. $^*p < 0.05$

Evidence from animal experiments [33,34], observational studies [10–13] and clinical trials [14,15] suggested that the cognitive or pathological benefits [11] associated with ω-3 supplementation were observed in *APOE* ε4 carriers. On the other hand, some reported that the cognitive outcomes linked to ω-3 supplements were observed in those at lower genetic risk [16–19]. The inconsistency might be due to heterogeneity in study design (sample size, dose or duration of ω-3, and assessed cognitive domains), baseline ω-3 consumption, and mixed population including individuals with AD dementia [19], in which stage ω-3 supplementation might not bring cognitive benefits [8,35]. Another possible explanation is that the underpinning pathways by which ω-3 PUFA plays protective roles are more vulnerable in a high-genetic-risk population. For example, it was recently found that *APOE* ε4 carriers were more susceptible to the impact of fatty acids on incident risk of cardiovascular disease and mortality [36]. In addition, DHA PET scan findings suggested greater brain DHA consumption in younger healthy *APOE* ε4 carriers, predisposing to ω-3 deficiency decades before the onset of cognitive decline [37]. Hence, a more likely explanation is that *APOE* ε4 carriers' cognition benefitted from the greater metabolic demand for DHA met by long-term ω-3 supplementation.

Our findings indicated that long-term ω-3 use was associated with improved memory, lower amyloid burden and AD risk only amongst high-genetic-risk subjects. The interaction might be explained by the following hypothesis: *APOE* ε4 is associated with reduced delivery of DHA and EPA to the brain before the onset of cognitive impairment [38]. The *APOE* ε4 carriers may thus have lower brain DHA/EPA uptake and levels and would benefit from ω-3 supplementation [39]. Consistent with our findings, it was recently found that aging-related declines in circulating plasmalogens, which act as reservoirs of ω-3 PUFA, was related to poorer cognition, greater amyloid pathology and an elevated AD risk [40].

The mediating findings provided primary clues indicating potential pathways for how ω-3 supplementation lowered AD risk. It is first demonstrated that the relationships of ω-3 use with cognition in *APOE* ε4 carriers were modulated by amyloid pathology, brain reserve and metabolism. However, the causal relationships warrant further investigations via in vivo or in vitro studies. Also, it remains to be determined whether other contributing factors exist. It has been postulated that the effects of ω-3 on cognitive functions might be influenced by amyloid status [41], total homocysteine [42] and cardiovascular health condition [43]. Uncovering the underlying mechanisms might help provide preventative or therapeutic targets for AD management.

There are several strengths in the present study. The studied population was restricted to those without dementia, which lowered the risk of bias due to population heterogeneity. The hypothesis was tested via both cross-sectional and longitudinal analyses in a large sample of subjects, amongst whom ~40% are *APOE* ε4 carriers. On top of *APOE* ε4, the interactions with PHS as well as the mediation effects were fully examined.

Limitations

The findings of the present study should be cautiously interpreted due to the following limitations. First, the total duration of supplementation but not the accurate dose was used for analyses, which might introduce a certain risk of measurement bias. According to previous publications, the ω-3 supplement dose varied slightly from

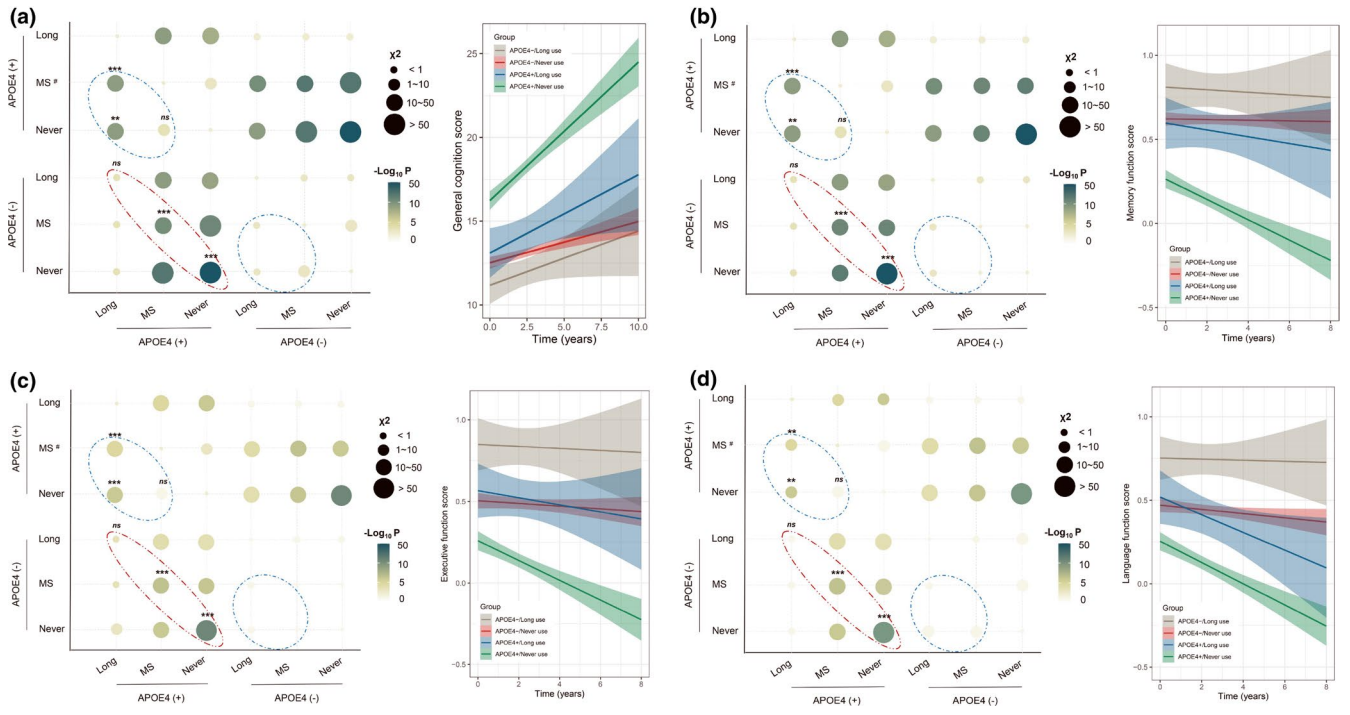

FIGURE 3 Longitudinal analyses for the interaction effects of long-term ω-3 supplementation and *APOE* ε4 status on cognitive decline. *APOE* ε4 could predict significantly greater cognitive decline in the never and MS groups, but not in the long-term group. Similarly, long-term ω-3 supplementation could predict significantly slower cognitive decline only in the *APOE* ε4 (+) group but not in the *APOE* ε4 (−) group. The conclusions were consistent across general cognition (a) and cognitive domains including memory (b), executive function (c) and language function (d)

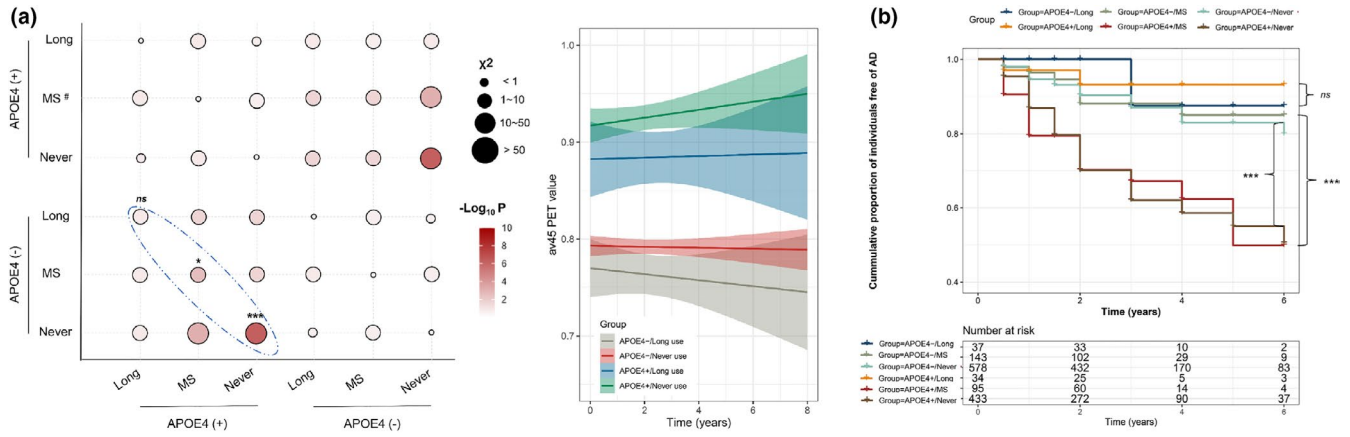

FIGURE 4 Longitudinal analyses for interaction effects of long-term ω-3 supplementation and *APOE* ε4 status on amyloid deposition and AD risk. The greatest rate of amyloid deposition was observed in the *APOE* ε4 (+)/never group. *APOE* ε4 could significantly predict greater amyloid deposition in the never and MS groups but not in the long-term group (a). Long-term ω-3 supplementation could predict lower AD risk only in the *APOE* ε4 (+) group. The association of *APOE* ε4 with AD risk became non-significant in the long-term group (b)

0.6 to 1.3 g/day for the general population [7,44,45]. Secondly, certain confounding factors might influence the identified associations, such as physical activity, although adjustments were made for insomnia, cardiovascular scores and psychological conditions in the final model. Thirdly, it was a post hoc retrospective analysis. The findings should be generalized with caution and further prospective multicentre studies are warranted to confirm the findings.

CONCLUSION

To sum up, taking ω-3 supplements regularly might be a promising approach to lowering AD risk in a population exposed to genetic risk. These findings also indicate that genetic risk factors of AD could be modified, and their adverse effects can be attenuated and even neutralized by long-term ω-3 supplementation.

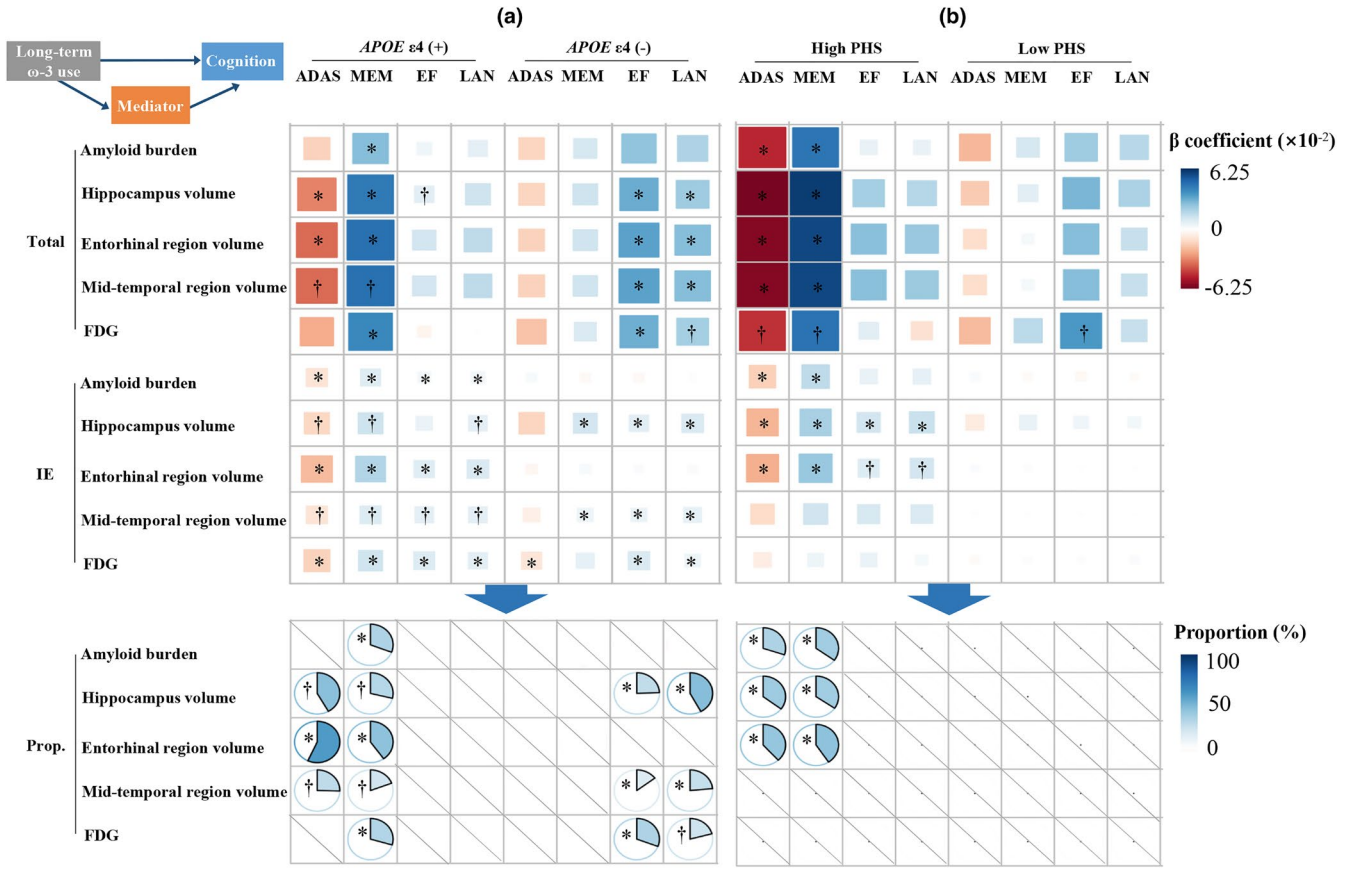

FIGURE 5 Mediation of amyloid pathologies, brain volume and metabolism for the relationship between ω-3 supplementation and cognitive function. The figure displays amyloid burden as the mediator, the estimate of the total effect (T), the indirect effect (IE) and the proportion of mediation (IE/T). (a) Amyloid pathology had significant mediation effects for the relationship of ω-3 use with memory only in the *APOE* ε4 (+) group. The volumes of entorhinal region and FDG-PET could mediate the association of ω-3 use with memory and global cognition in the *APOE* ε4 (+) group. No mediation effects were found for para-hippocampus, posterior cingulate or precuneus volume. (b) Amyloid pathology, hippocampus and entorhinal region volume also demonstrated the mediation effects for the relationship of global cognition (especially memory) with ω-3 use in the high-risk group defined by PHS. $^*p < 0.05$; †$p < 0.08$ (trend)

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CONFLICT OF INTEREST

None.

AUTHOR CONTRIBUTIONS

Lin Li: Formal analysis (equal); visualization (equal); writing—original draft (equal). Wei Xu: Conceptualization (equal); data curation

(equal); formal analysis (equal); methodology (equal). Chen-Chen Tan: Writing—review and editing (equal). Xi-Peng Cao: Writing—review and editing (equal). Bao-Zhen Wei: Writing—review and editing (equal). Cheng-Wen Dong: Writing—review and editing (equal). Lan Tan: Writing—review and editing (equal).

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The ADNI was approved by the institutional review boards of all participating centres, and written informed consent was obtained from all participants or authorized representatives according to the 1975 Declaration of Helsinki.

CONSENT FOR PUBLICATION

Not applicable.

DATA AVAILABILITY STATEMENT

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

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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