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Original Article

Plasma neurofilament light mediates the effects of Apolipoprotein E on brain atrophy and cognitive decline in the comorbid Alzheimer's disease and cerebral small vessel disease

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ABSTRACT

Background: Alzheimer's disease (AD) and cerebral small vessel disease (CSVD) often coexist in older adults and contribute to cognitive impairment. The Apolipoprotein E (APOE) $\varepsilon 4$ allele and neuroaxonal injury, measured by plasma neurofilament light chain (NfL), are associated with an increased risk for both AD and CSVD. However, the relationship between APOE $\varepsilon 4$, plasma NfL, and their association with the comorbidity of AD and CSVD remains unclear.

Objective: To investigate the longitudinal relationship among APOE $\varepsilon 4$, elevated plasma NfL, brain atrophy, and cognitive decline in individuals with comorbid AD and CSVD.

Methods: We included 570 non-demented participants from the Alzheimer's Disease Neuroimaging Initiative (ADNI) study, categorizing them into four groups based on amyloid-β positivity and CSVD burden. Linear mixed-effects models examined the association among APOE $\varepsilon 4$, plasma NfL, brain volume measured by magnetic resonance imaging, and cognition over 2 years. Mediation analyses assessed the role of elevated plasma NfL in the relationship between APOE $\varepsilon 4$, brain atrophy, and cognitive decline.

Results: APOE $\varepsilon 4$ carriers showed elevated plasma NfL levels, brain atrophy, and cognitive decline. Plasma NfL mediated the effects of APOE $\varepsilon 4$ on brain atrophy and cognitive decline in participants with comorbid AD and CSVD.

Conclusion: Our findings suggest that neuroaxonal injury as a potential mechanism in the effects of APOE $\varepsilon 4$ on brain atrophy and cognitive decline, highlighting the clinical utility of plasma NfL as a potential biomarker for disease progression and response to therapeutic intervention in comorbid AD and CSVD.

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[2] Data used in the preparation of 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 analysis or writing of this report. A complete listing of ADNI investigators can be found at: http://adni.loni.usc.edu/wpcontent/uploads/how to apply/ADNI Acknowledgement List.pdf.

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1. Introduction

Dementia is a progressive neurodegenerative syndrome characterized by cognitive impairment which interfere with independence of daily living [1]. Alzheimer's disease (AD) and cerebral small vessel disease (CSVD) are two of the most prevalent causes of dementia in older adults [2]. AD is characterized by the accumulation of amyloid-β (Aβ) plaques, neurofibrillary tangles, and neuronal loss, which contribute to dementia symptoms [3]. In contrast, CSVD is characterized by structural brain changes observed on magnetic resonance imaging (MRI) such as white matter hyperintensities (WMH), lacunar infarcts, and cerebral microbleeds [4]. Approximately 50 % of dementia cases exhibit both AD and CSVD pathologies [5], and the coexistence of these pathologies significantly increases the risk of cognitive decline compared to either pathology alone [6,7]. Despite this, the exact mechanisms driving the effects of comorbid AD and CSVD on brain atrophy and cognitive impairment remain unclear.

Both AD and CSVD share key risk factors, such as the Apolipoprotein (APOE) ε4 allele, which contribute to the overlapping pathophysiological processes of these two conditions [8]. Neuroaxonal injury, a downstream consequence of both AD and CSVD, further exacerbates neurodegeneration and cognitive decline [9,10]. APOE ε4 is the most prominent genetic risk factor for sporadic AD [11,12], with carriers exhibiting greater Aβ plaque burden and more severe neurofibrillary tangles than non-carriers, increasing the likelihood of dementia progression [13]. APOE ε4 carriers also display volumetric decreases in specific medial temporal lobe structures, such as the hippocampus, amygdala, and entorhinal cortex [14]. Moreover, APOE ε4 is associated with an increased risk of CSVD [15], which negatively impacts white matter integrity by disrupting lipid transport and increasing WMH burden [16,17]. This relationship is supported by diffusion tensor imaging (DTI) studies, which show that cognitively healthy APOE ε4 carriers frequently exhibit increased radial and axial diffusivity, reflecting myelin degradation and axonal injury [18]. Therefore, APOE ε4 may represent a common risk factor in comorbid AD and CSVD, contributing to brain atrophy and cognitive impairment.

Neurofilament light chain (NfL) is a well-established biomarker for neuroaxonal injury, with elevated levels in both cerebrospinal fluid (CSF) and plasma reflecting axonal degeneration [19]. NfL levels show strong consistency with DTI metrics, particularly in reflecting the severity of axonal injury [20]. Plasma NfL has gained attention as a non-invasive biomarker due to its ease of detection and cost-effectiveness [21]. Studies show that plasma NfL levels are elevated in the early stages of AD [22] and strongly correlate with the Aβ burden, hippocampal atrophy, and cognitive decline [9]. Emerging studies show that elevated NfL levels are associated with increased WMH volumes and lacunar infarct burden [10], further supporting its role as a marker of neurodegeneration in CSVD. WMH indicate increased water content, demyelination, and axonal loss [23], while NfL serves as a nonspecific marker of axonal injury [24,25]. Increases in CSF NfL levels are observed with a higher number of WMH [26,27], and emerging studies show that plasma NfL levels correlate with WMH [10,28,29]. Thus, elevated plasma NfL levels may serve as a key biomarker for neuroaxonal injury, potentially representing a common mechanism underlying comorbid AD and CSVD, leading to brain atrophy and cognitive impairment.

APOE ε4 carriers, who are vulnerable to both AD and CSVD, may experience increased neuroaxonal injury due to lipid dysregulation and myelin breakdown, leading to axonal damage and elevated NfL release into CSF and plasma [18]. Significant differences in CSF NfL concentrations between APOE ε4 carriers and non-carriers have been observed in prodromal AD patients [30]. Furthermore, studies show that blood NfL concentrations are elevated in cognitively unimpaired (CU) APOE ε4 carriers compared to non-carriers [31]. While the roles of APOE ε4 and NfL in AD and CSVD are well-documented, the potential for NfL to reflect a shared neurodegenerative pathway in APOE ε4 carriers with comorbid AD and CSVD remains underexplored. Investigating plasma NfL as a marker of cumulative neuroaxonal damage may provide new insights into the pathogenesis of comorbid AD and CSVD.

This study aims to investigate the relationship among APOE ε4 status, neuroaxonal injury (measured by plasma NfL levels), brain atrophy, and cognitive function in individuals with comorbid AD and CSVD. We hypothesize that plasma NfL levels mediate the relationship between APOE ε4, brain atrophy and cognitive decline.

2. Methods

2.1. Data source

The data used in this study were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) study on March 27, 2024. The primary goal of ADNI is to assess whether serial MRI, positron emission tomography (PET), and various clinical, genetic, and neuropsychological markers can effectively measure the progression of mild cognitive impairment (MCI) and early AD. All participants received ethical approval from institutional review boards and provided written informed consent before any protocol-specific procedures were carried out in the ADNI study. The treatment of participants in this study was in accordance with the ethical standards established by the Declaration of Helsinki.

2.2. Participants

As previously described [32], ADNI enrolls participants aged 55–90 who are fluent in English or Spanish, have at least 6 years of education, a Geriatric Depression Scale score of less than 6, and a modified Hachinski Ischemic Scale score of less than 5 at enrollment. This study included 570 non-demented participants, comprising 224 CU individuals and 346 with MCI. Baseline data included clinical assessments, Aβ-PET, plasma NfL levels, APOE ε4 status, and WMH measurements, with longitudinal MRI scans and neuropsychological assessments conducted over a 2-year period. CU participants were required to have a Mini-Mental State Examination (MMSE) score greater than or equal to 24 and a Clinical Dementia Rating (CDR) score of 0, as well as the absence of memory concerns. MCI participants were required to have an MMSE score greater than or equal to 24, a CDR score of 0.5, objective memory loss, and normal daily activities. Detailed information regarding the ADNI inclusion and exclusion criteria can be accessed at http://adni.loni.usc.edu/.

According to the criteria established by the National Institute on Aging and the Alzheimer's Association, Aβ positivity (Aβ+) was considered an indicator of the AD continuum [33]. Aβ+ was defined based on a global standardized uptake value ratio (SUVR) cutoff greater than 1.11 for [18F]-florbetapir PET, as previously validated [34]. WMH, the most common radiological feature of CSVD, were used as a primary marker for the disease [35]. Following the guidelines proposed by Cedres et al., a high WMH burden was characterized by WMH volumes adjusted for total intracranial volume (TIV) exceeding 0.00321, while a low WMH burden was defined as volumes $\leq$ 0.00321 [36]. Lacunar infarcts, presumed to result from small vessel pathology, were the second most common ischemic brain lesions after WMH [37].

In this study, AD pathology was represented by Aβ positivity, while CSVD pathology was defined by the presence of either lacunar infarcts or a high WMH burden. Using baseline data and based on these criteria, participants were classified into the following four groups: Aβ-negative individuals with a low CSVD burden were categorized as Aβ−/CSVD− (controls); Aβ-negative individuals with a high CSVD burden were categorized as Aβ−/CSVD+; Aβ-positive individuals with a low CSVD burden were categorized as Aβ+/CSVD−; and Aβ-positive individuals with a high CSVD burden were categorized as Aβ+/CSVD+, indicating the comorbidity of AD and CSVD.

2.3. Plasma NFL measurement

Plasma NfL levels were measured using the single molecule array (Simoa) technique at the Clinical Neurochemistry Laboratory, University of Gothenburg, Mölndal Campus, Mölndal, Sweden. Further details can be found at: http://adni.loni.usc.edu. Plasma NfL data were obtained from the ADNI file ("ADNI_BLENNOWPLASMANFLLONG.csv").

2.4. APOE genotypes

APOE genotypes were determined using DNA extracted by Cogenics from a 3-mL aliquot of EDTA blood, as previously described [38]. Individuals with at least one $\varepsilon 4$ allele were classified as APOE $\varepsilon 4$ carriers, and those without were classified as APOE $\varepsilon 4$ non-carriers. APOE genotypes information were extracted from the ADNI file ("APOERES.csv").

2.5. Structural MRI data

All participants underwent whole-brain MRI scanning using standardized acquisition protocols designed and implemented by ADNI (http://adni.loni.usc.edu/methods/mri-tool/mri-analysis/). Structural MRI data were acquired using 3.0 T scanners with T1-weighted scans using a sagittal volumetric magnetization-prepared rapid acquisition gradient echo sequence at baseline, and at 1 year and 2 years. In total,107 regions of interest (ROIs) were automatically segmented according to the Jacob atlas defined by FreeSurfer [39], which included cortical volume, surface area, thickness average and thickness standard deviation of bilateral entorhinal, rostral anterior cingulate, caudal anterior cingulate; subcortical volume of bilateral amygdala, hippocampus, caudate, choroid plexus, inferior lateral ventricle; cortical volume and thickness average of bilateral isthmus cingulate, left superior and middle temporal, etc. The structural MRI neuroimaging data were downloaded from the ADNI file ("UCSFFSX51_11_08_19.csv").

2.6. WMH measurements

WMH volumes were measured using segmentation of high-resolution 3D T1 and fluid-attenuated inversion recovery sequences. An automated atlas was employed to remove non-brain structures from the 3D T1 images. The corresponding FLAIR image was then transformed and aligned to the 3D T1 image, as previously described [40,41]. Additional details on acquisition and segmentation can be found online (http://www.adni.loni.usc.edu). WMH volumes were extracted from the ADNI file ("ADNI_UCD_WMH_05_02_22.csv").

2.7. Quantification of lacunar infarcts

Lacunar infarcts were identified by a physician from ADNI specifically trained in MRI interpretation. Infarct size, location, and other imaging characteristics were recorded and confirmed by cross-checking against CSF density on T1 sequences and ensuring distinct separation from vessels in certain areas of the brain, as previously described [42]. For this analysis, only lesions equal to or greater than 3 mm in size were considered cerebral infarcts. Participants were then classified based on the presence or absence of infarcts. Data on lacunar infarcts were obtained from the ADNI file ("MRI_INFARCTS_01_29_21.csv").

2.8. PET image acquisition and processing

All detailed image acquisition procedures can be found on the ADNI website (http://adni.loni.usc.edu/methods/documents/). Aβ-PET images were acquired in four frames of 5 min each, 50–70 min post-injection. Aβ-PET values were calculated as the mean cortical grey matter SUVRs (frontal, lateral parietal, lateral temporal, anterior cingulate, and posterior cingulate) divided by the whole cerebellum [34]. Data on mean [18F]-florbetapir PET were obtained from the ADNI file ("UCBERKELEYAV45_04_26_22.csv").

2.9. Neuropsychological assessment

Global cognition was assessed using the AD assessment scale-cognitive subscale consisting of 11 (ADAS-Cog11) and 13 items (ADAS-Cog13). Composite scores for episodic memory (ADNI-MEM), executive function (ADNI-EF), language (ADNI-LAN), and visuospatial functioning (ADNI-VS) were retrieved from the ADNI-LONI database. Global cognitive outcomes examined included ADAS-Cog11, ADAS-Cog13 for which higher scores correspond to worse performance. Domain-specific cognitive outcomes included ADNI-MEM, ADNI-EF, ADNI-LAN, and ADNI-VS for which higher scores correspond to better cognition. Neuropsychological assessment data at baseline, 1 year, and 2 years were obtained from the ADNI files ("ADAS.csv," "UWNPSYCHSUM.csv").

2.10. Clinical assessments and data collection

History of hypertension was evaluated during screening as part of the Modified Hachinski Ischemic Scale. The presence of diabetes mellitus and hyperlipidemia was defined by self-reported diagnosis during medical history evaluations. Body mass index (BMI) was calculated using height and weight data. Information on the history of cardiovascular diseases and smoking status was obtained from ADNI based on self-reported information. All covariate data were compiled from the clinical evaluation files ("ADNIMERGE.csv," "MEDHIST," "RECCMEDS.csv," "MODHACH.csv," and "VITALS.csv").

2.11. Statistical analysis

TIV-adjusted WMH volumes, Aβ-PET, and plasma NfL levels were log2-transformed to achieve a normal distribution and correct for skewed distributions. For normally distributed data, one-way ANOVA with Bonferroni correction was used to account for multiple comparisons, while categorical variables were compared using the chi-square test. For non-normally distributed continuous variables, the Kruskal-Wallis test with Bonferroni correction was applied. Differences in baseline cognitive measures among four groups categorized by AD pathology and CSVD burden (Aβ−/CSVD−, Aβ−/CSVD+, Aβ+/CSVD−, and Aβ+/CSVD+) were assessed.

Multiple linear regression models were used to examine the association between APOE $\varepsilon 4$ status, baseline plasma NfL levels, structural MRI brain ROIs, and cognitive measures. Linear mixed models with random effects were used to explore the longitudinal relationship between APOE $\varepsilon 4$ status and plasma NfL levels with structural MRI ROIs, and cognitive measures. All models were adjusted for age, sex, and years of education.

Cognitive change rates were calculated by extracting subject-specific slopes from linear mixed models. Cognitive measures were treated as the dependent variable, and time from baseline was included as the independent variable, with random slopes and intercepts. This approach was similarly used to evaluate the rate of change in MRI brain ROIs.

To investigate whether the association between APOE $\varepsilon 4$ status and the change rates of MRI brain ROIs or cognitive measures was mediated by plasma NfL levels in the Aβ+/CSVD+ sample, a mediation analysis was conducted following the method proposed by Baron and Kenny [43]. The first equation regressed the mediator (plasma NfL levels) on the independent variable (APOE $\varepsilon 4$ status). The second equation regressed the dependent variable (change rates of MRI brain ROIs or cognitive measures) on the independent variable. The third equation regressed the dependent variable on both the independent variable and the mediator. A mediation effect was established if the following criteria were met: First, APOE $\varepsilon 4$ status was significantly associated with plasma NfL levels; Second, APOE $\varepsilon 4$ status was significantly associated with change rates of MRI brain ROIs and cognitive measures; Third, plasma NfL levels were significantly associated with change rates of MRI brain ROIs and cognitive measures; and Finally, the association between APOE $\varepsilon 4$ status and change rates of MRI brain ROIs and cognitive measures was reduced when plasma NfL levels (the mediator) were included

Table 1
Baseline characteristics of participants stratified by Aβ and CSVD status.

Indexes	Aβ-/CSVD- (N = 215)	Aβ-/CSVD+ (N = 94)	Aβ+/CSVD- (N = 127)	Aβ+/CSVD+ (N = 134)	*P*
Demographic					
Age(year)	69.16 (6.45)	74.25 (6.58)*	70.63 (6.44)†	75.27 (5.74)*,‡	**<0.001**
Female (%)	109 (51 %)	41 (44 %)	76 (60 %)	58 (43 %)‡	**0.031**
Education(year)	16.68 (2.42)	16.43 (2.49)	16.50 (2.65)	15.79 (2.83)*	**0.038**
Diagnosis					**<0.001**
CU/MCI	116/99 (54 %/ 46 %)	40/54 (43 %/ 57 %)	37/90 (29 %/71 %)*	31/103 (23 %/ 77 %)*,†	
Risk factors					
APOE ε4(%)	47 (22 %)	24 (26 %)	83 (65 %)*,†	72 (54 %)*,†	**<0.001**
BMI (kg/m^2)	28.22 (5.01)	27.35 (5.02)	27.74 (6.56)	27.20 (4.24)	0.069
Smoking (%)	74 (34 %)	36 (38 %)	64 (50 %)*	48 (36 %)	**0.024**
Hypertension (%)	86 (40 %)	55 (59 %)*	48 (38 %)†	83 (62 %)*,‡	**<0.001**
Diabetes mellitus (%)	15 (7.0 %)	6 (6.4 %)	6 (4.7 %)	12 (9.0 %)	0.6
Hyperlipidemia (%)	59 (27 %)	36 (38 %)	41 (32 %)	51 (38 %)	0.12
Biomarkers					
Aβ PET SUVR	0.02 (0.07)	0.03 (0.09)	0.45 (0.19) *,†	0.45 (0.17)*,†	**<0.001**
Lacunar infarcts (%)§	0 (0 %)	17 (20 %)*	0 (0 %)†	24 (18 %)*,‡	**<0.001**
WMH volume (mL)	−10.01 (1.13)	−7.63 (1.06) *	−9.82 (1.18)†	−7.27 (1.02) *,‡	**<0.001**
Plasma NfL (pg/mL;)	4.82 (0.63)	5.12 (0.70) *	5.03 (0.53)*	5.33 (0.58) *,‡	**<0.001**
Cognition					
ADNI-LAN	0.77 (0.74)	0.61 (0.71)	0.58 (0.74)	0.28 (0.79) *,†,‡	**<0.001**
ADNI-EF	0.92 (0.82)	0.47 (0.68)*	0.65 (0.87) *	0.27 (0.84) *,‡	**<0.001**
ADNI-MEM	1.10 (0.77)	0.79 (0.68)*	0.65 (0.88) *	0.33 (0.76) *,†,‡	**<0.001**
ADNI-VS	0.21 (0.68)	0.13 (0.62)	0.65 (0.89)	0.01 (0.72)	0.064
ADAS-Cog11	6.27 (3.40)	7.06 (3.44)*	0.65 (0.90)	9.83 (4.83)*,†,‡	**<0.001**
ADAS-Cog13	9.80 (5.04)	11.27 (5.28)	0.65 (0.91)	15.88 (7.17) *,†,‡	**<0.001**

Note: Data are presented as mean (SD), n (%), or median (interquartile range). WMH volume was adjusted for total intracranial volume (TIV) and log2-transformed. Plasma NfL and Aβ PET SUVR were log2-transformed. Bold values represent statistically significant results ($P < 0.05$). Aβ, amyloid-β; CSVD, cerebral small vessel disease; SD, standard deviation; CU, cognitively unimpaired; MCI, mild cognitive impairment; APOE, apolipoprotein E; BMI, body mass index; WMH, white matter hyperintensities; NfL, neurofilament light; PET, positron emission tomography; SUVR, standardized uptake value ratio; ADNI-MEM, memory function; ADNI-EF, executive function; ADNI-LAN, language function; ADNI-VS, visuospatial function; ADAS-Cog11 or 13, Alzheimer's Disease Assessment Scale 11- or 13-item subscale.

* Significant represented the difference between Aβ-CSVD- and other groups;.

† Significant represented the difference between Aβ-CSVD+ and Aβ+CSVD- or Aβ+/CSVD+;.

‡ Significant represented the difference between Aβ+CSVD- and Aβ+/CSVD+.

§ Missing data of 36 participants.

in the regression model. The magnitude of the indirect effect was estimated, and significance was determined through 1000 bootstrapped iterations using the "mediate" and "bruceR" packages. Each model path was controlled for age, years of education, and gender. The proportion mediated was calculated by dividing the indirect effect by the total effect.

Statistical significance was defined as a two-tailed *P*-value of less than 0.05. All statistical analyses were performed using R software (version 4.3.1, R Foundation for Statistical Computing).

3. Results

3.1. Demographic and clinical characteristics

This study included 570 participants, comprising 215 Aβ−/CSVD−, 94 Aβ−/CSVD+, 127 Aβ+/CSVD−, and 134 Aβ+/CSVD+ cases (for detailed participant selection procedures, refer to Figure S1). For the demographic characteristics, the Aβ+/CSVD+ group was significantly older (mean ± SD, 75.27 ± 5.74 years) compared to both the Aβ−/CSVD− group (69.16 ± 6.45 years) and the Aβ+/CSVD− group (70.63 ± 6.44 years). The Aβ+/CSVD+ group also had significantly fewer years of education and a higher prevalence of MCI compared to the Aβ−/CSVD− group. Additionally, the Aβ+/CSVD+ group had a lower proportion of female participants compared to the Aβ+/CSVD− group (see Table 1).

Compared to the Aβ−/CSVD− group, the Aβ+/CSVD+ group had a greater proportion of lacunar infarcts and APOE ε4 carriers, as well as higher Aβ PET SUVR levels, larger WMH volumes, and higher plasma NfL levels. When compared to the Aβ−/CSVD+ group, the Aβ+/CSVD+ group exhibited a greater proportion of APOE ε4 carriers and higher Aβ PET SUVR levels. Relative to the Aβ+/CSVD− group, the Aβ+/CSVD+ group had a greater proportion of lacunar infarcts, higher plasma NfL levels, and larger WMH volumes (see Table 1).

In terms of cognitive performance, the Aβ+/CSVD+ group, rather than the Aβ−/CSVD− group, demonstrated significant declines across all cognitive domains except visuospatial function (measured by ADNI-VS). Compared to the Aβ+/CSVD− group, the Aβ+/CSVD+ group had lower ADNI-LAN, ADNI-EF, and ADNI-MEM scores, as well as higher ADAS-Cog11 and ADAS-Cog13 scores, indicating poorer performance in language, executive function, memory, and global cognition. Similarly, compared to the Aβ−/CSVD+ group, the Aβ+/CSVD+ group showed lower ADNI-LAN and ADNI-MEM scores, and higher ADAS-Cog11 and ADAS-Cog13 scores, indicating poorer performance in language, memory, and global cognition (see Table 1).

3.2. Associations of APOE ε4 status with plasma NFL levels

APOE ε4 carriers showed significantly higher plasma NfL levels ($\beta = 0.152$, $P = 0.002$; see Table S1) compared to non-carriers in the entire cohort of non-demented individuals, after adjusting for age, gender, and years of education. When the total sample was stratified into four subgroups, a significant association between APOE ε4 carrier status and plasma NfL levels was observed only in the Aβ+/CSVD+ group ($\beta = 0.299$, $P = 0.002$; Model 1). The association remained significant after further adjustments for Aβ PET SUVR levels and clinical diagnosis (CU vs. MCI) ($\beta = 0.235$, $P = 0.026$; Model 2; see Table S1).

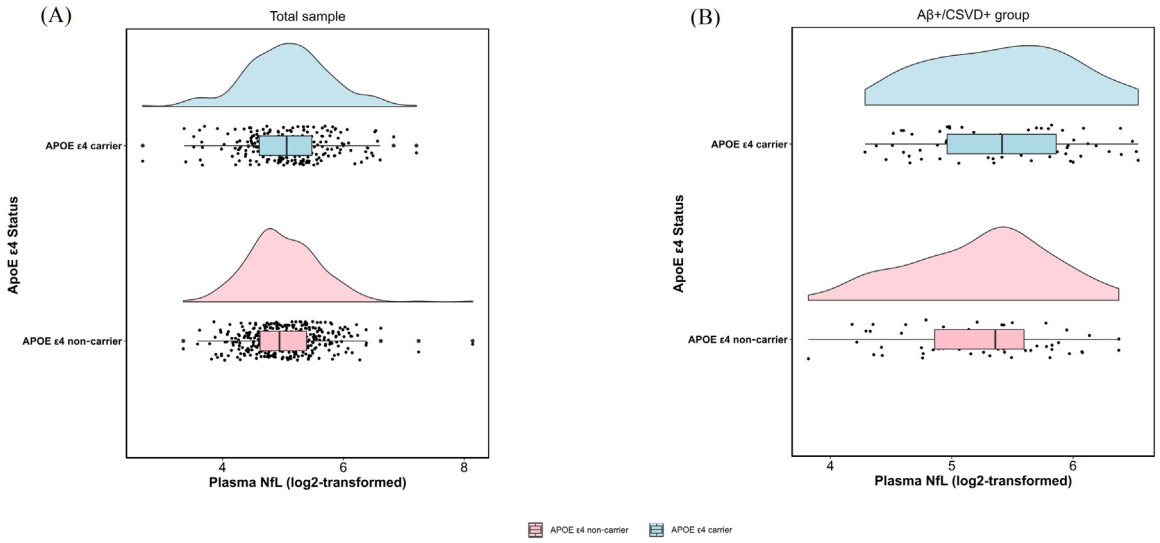

Fig. 1. APOE $\varepsilon 4$ status distributions. Raincloud plots illustrating the distribution of plasma NfL levels stratified by APOE $\varepsilon 4$ carrier status versus non-carriers, presented for the total sample (A) and the Aβ+/CSVD+ subgroup (B). APOE, apolipoprotein E; NfL, neurofilament light chain; Aβ, amyloid-β; CSVD, cerebral small vessel disease.

Table 2
Effects of ApoE ε 4 on baseline structural MRI brain ROIs and cognitive outcome in the Aβ+/CSVD+ participants.

Outcome	APOE $\varepsilon 4$ non-carrier (N = 62)	APOE $\varepsilon 4$ carrier (N = 72)	*P*	Adjusted *P*
Thickness average of right supramarginal	2.41 (0.14)	2.36 (0.15)	**0.043**	**0.021**
Subcortical volume of left amygdala	1338.95 (267.19)	1300.42 (223.81)	0.37	**0.046**
Thickness average of left entorhinal	3.34 (0.41)	3.21 (0.42)	0.078	**0.019**
Subcortical volume of left hippocampus	3483.26 (536.72)	3242.42 (479.21)	**0.007**	**<0.001**
Cortical volume of left middle temporal	9565.94 (1502.00)	9188.67 (1392.14)	0.13	**0.005**
Surface area of left middle temporal	2887.31 (386.45)	2818.69 (392.91)	0.31	**0.022**
Thickness average of right entorhinal	3.51 (0.42)	3.32 (0.49)	**0.019**	**0.001**
Surface area of right frontal pole	257.73 (37.21)	279.72 (51.30)	**0.006**	**0.01**
Thickness average of right frontal pole	2.58 (0.21)	2.48 (0.24)	**0.012**	**0.019**
Subcortical volume of right hippocampus	3591.95 (575.16)	3371.69 (502.66)	**0.019**	**<0.001**
ADNI-LAN	0.44 (0.75)	0.14 (0.81)	**0.031**	**0.005**
ADNI-EF	0.46 (0.85)	0.11 (0.79)	**0.014**	**0.003**
ADNI-MEM	0.61 (0.72)	0.08 (0.70)	**<0.001**	**<0.001**
ADNI-VS	0.06 (0.75)	−0.04 (0.69)	0.42	0.388
ADAS-Cog11	8.74 (4.82)	10.76 (4.67)	**0.015**	**0.014**
ADAS-Cog13	13.65 (6.91)	17.81 (6.86)	**<0.001**	**0.001**

Note: Data are presented as mean (SD). adjusted *P*-values are corrected for age, gender, and education. Bold values represent statistically significant results ($P < 0.05$). Aβ, amyloid-β; CSVD, cerebral small vessel disease; SD, standard deviation; APOE, apolipoprotein E; MRI, magnetic resonance imaging; ROIs, regions of interest; ADNI-MEM, memory function; ADNI-EF, executive function; ADNI-LAN, language function; ADNI-VS, visuospatial function; ADAS-Cog11 or 13, Alzheimer's Disease Assessment Scale 11- or 13-item subscale.

Fig. 1 shows the distribution of plasma NfL levels stratified by APOE $\varepsilon 4$ status.

3.3. Associations of APOE $\varepsilon 4$ status with baseline structural MRI and cognitive measures

At baseline, significant differences were observed in the 10 ROIs of Aβ+/CSVD+ individuals when stratified by APOE $\varepsilon 4$ carrier status, after adjusting for age, gender, and years of education. APOE $\varepsilon 4$ carriers exhibited significant cortical thinning in the right supramarginal gyrus, right frontal pole, and bilateral entorhinal cortices. Significant reductions in subcortical volumes were also observed in the bilateral hippocampus and left amygdala (all adjusted $P < 0.05$; see Table 2).

At baseline, APOE $\varepsilon 4$ carriers also demonstrated significantly poorer cognitive performance in language (ADNI-LAN), executive function (ADNI-EF), memory (ADNI-MEM), and global cognition (ADAS-Cog11, ADAS-Cog13) compared to non-carriers (all adjusted $P < 0.05$; see Table 2).

3.4. Associations of APOE $\varepsilon 4$ status with longitudinal structural MRI and cognitive measures

Over time, APOE $\varepsilon 4$ carriers exhibited significantly faster cortical thinning in the right supramarginal gyrus ($P = 0.029$), right frontal pole ($P = 0.01$), and bilateral entorhinal cortices ($P < 0.001$). Additionally, APOE $\varepsilon 4$ carriers showed accelerated reductions in the subcortical volume of the left amygdala ($P = 0.001$) and bilateral hippocampus (left: $P = 0.005$; right: $P = 0.003$), as well as a significant decrease in cortical volume in the left middle temporal region ($P = 0.001$) (see Table S2).

Cognitive analysis revealed that APOE $\varepsilon 4$ carriers exhibited a more rapid decline in memory (ADNI-MEM, $P = 0.045$) and global cognition (ADAS-Cog11, $P = 0.007$; ADAS-Cog13, $P = 0.005$) (see Table S3).

3.5. Associations of plasma NFL levels with longitudinal structural MRI and cognitive measures

Over time, higher plasma NfL levels were associated with faster cortical thinning in the right parahippocampal gyrus ($P = 0.044$) and bi-

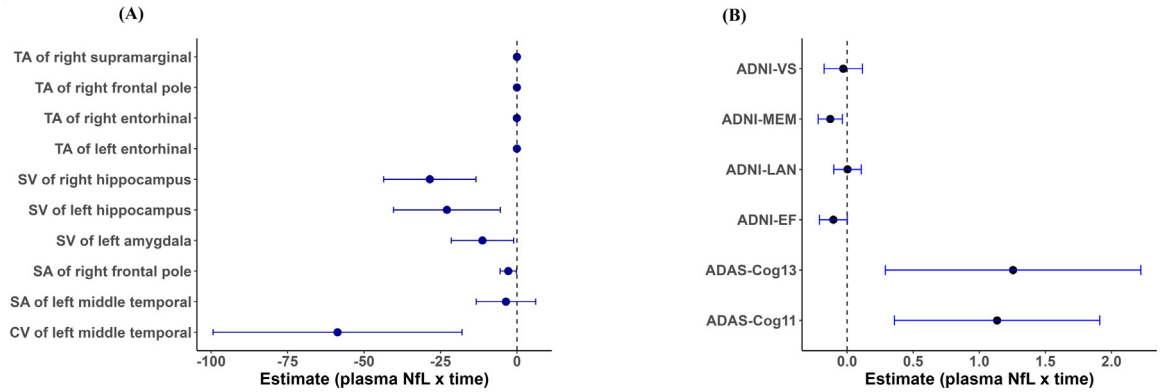

Fig. 2. Associations between plasma NfL levels and longitudinal structural MRI ROIs and cognitive outcome in the Aβ+/CSVD+ participants. Values represent standardized coefficients with bootstrapped 95 % confidence intervals for the interaction of baseline plasma NfL levels (log2-transformed) and time on brain volumetric(A) and cognitive (B) outcomes in the Aβ+/CSVD+ participants. Full model estimates and *P*-values are presented in Tables S4 and S5 in supporting information. NfL, neurofilament light; SV, Subcortical volume; TA, Thickness average; SA, Surface area; ADNI-MEM, memory function; ADNI-EF, executive function; ADNI-LAN, language function; ADNI-VS, visuospatial function; ADAS-Cog11 or 13, Alzheimer's Disease Assessment Scale 11- or 13-item subscale; Aβ, amyloid-β; CSVD, cerebral small vessel disease; MRI, magnetic resonance imaging; ROIs, regions of interest.

lateral entorhinal cortices (left: $P = 0.008$; right: $P = 0.003$). Elevated plasma NfL levels were also associated with faster reductions in the surface area of the right frontal pole ($P = 0.038$) and the cortical volume of the left middle temporal gyrus ($P = 0.005$). Additionally, elevated plasma NfL levels were associated with accelerated decreases in the subcortical volume of the left amygdala ($P = 0.031$) and bilateral hippocampus (left: $P = 0.01$; right: $P < 0.001$) (see Fig. 2 and Table S4).

Cognitive analyses further revealed that individuals with higher plasma NfL levels experienced more rapid declines in memory (ADNI-MEM, $P = 0.007$) and global cognition (ADAS-Cog11, $P = 0.005$; ADAS-Cog13, $P = 0.012$) (see Fig. 2 and Table S5).

3.6. Mediation effect of plasma NFL on the association between APOE ε4 and longitudinal changes in structural MRI

In the total sample ($n = 570$), plasma NfL partially mediated the association between APOE ε4 status and the rate of cortical thinning in the right entorhinal cortex (coefficient = −0.002; 95 % CI: [−0.004, −0.001]; mediation effect: 14.3 %) as well as the reduction in subcortical volume of the right hippocampus (coefficient = −2.098; 95 % CI: [−3.829, −0.741]; mediation effect: 16.3 %; see Table S6).

Notably, in Aβ+/CSVD+ individuals, plasma NfL significantly mediated the association between APOE ε4 status and the rate of cortical thinning in the right entorhinal cortex (coefficient = −0.006; 95 % CI: [−0.012, −0.002]; mediation effect: 27.3 %; see Table S7 and Fig. 3A) and the reduction in subcortical volume of the right hippocampus (coefficient = −3.389; 95 % CI: [−6.895, −0.421]; mediation effect: 15.5 %; see Table S7 and Fig. 3B). In contrast, among Aβ+/CSVD− individuals, plasma NfL did not significantly mediate the association between APOE ε4 status and the rate of cortical thinning in the right entorhinal cortex (coefficient = −0.002; 95 % CI: [−0.006, 0.001]) or the reduction in subcortical volume of the right hippocampus (coefficient = −1.697; 95 % CI: [−6.002, 0.849]; see Table S8)

3.7. Mediation effect of plasma NFL on the association between APOE ε4 and longitudinal changes in cognitive measures

In the total sample ($n = 570$), plasma NfL was found to partially mediate the relationship between APOE ε4 status and rapid memory deterioration (coefficient = −0.013; 95 % CI: [−0.024, −0.005]; mediation effect: 15.5 %) as well as global cognitive decline (coefficient = 0.100; 95 % CI: [0.035, 0.181]; mediation effect: 13.6 %; see Table S6).

Specifically, in Aβ+/CSVD+ individuals, plasma NfL significantly mediated the association between APOE ε4 status and the rate of memory deterioration (coefficient = −0.023; 95 % CI: [−0.043, −0.005]; mediation effect: 22.8 %; see Table S7, Fig. 3C) as well as global cognitive decline (coefficient = 0.164; 95 % CI: [0.026, 0.327]; mediation effect: 14.5 %; see Table S7, Fig. 3D). In contrast, no significant mediation by plasma NfL was found in the association between APOE ε4 status and either memory deterioration (coefficient = −0.011; 95 % CI: [−0.034, 0.007]) or global cognitive decline (coefficient = 0.098; 95 % CI: [−0.059, 0.293]) among Aβ+/CSVD− individuals (see Table S8).

3.8. Sensitivity analysis

To ensure the robustness of our findings, we performed a sensitivity analysis to reassess the mediation effect of plasma NfL on the relationship between APOE ε4 status and longitudinal changes in structural MRI brain ROIs and cognitive performance in Aβ+/CSVD+ individuals. This analysis controlled for various potential confounders, including age, gender, years of education, diabetes, hypertension, hyperlipidemia, smoking status, BMI, and diagnostic status (CU vs. MCI). Results were consistent with the primary analysis, confirming that plasma NfL significantly mediated the association between APOE ε4 status, cortical thinning in the right entorhinal cortex, subcortical volume loss in the right hippocampus, and cognitive decline, particularly in memory and global cognition (see Table S9). Adjusting for these covariates did not impact the magnitude or significance of the mediation effects, further supporting the robustness of the observed relationships.

4. Discussion

In this study, we examined the effects of APOE ε4 and plasma NfL on brain atrophy and cognitive decline in non-demented participants with comorbid AD and CSVD. Our key findings are as follows: First, in the Aβ+/CSVD+ group, which represents individuals with both AD and CSVD pathologies, we found a significantly higher prevalence of APOE ε4 carriers, elevated plasma NfL levels, and greater cognitive decline compared to the Aβ−/CSVD− group. Second, in the Aβ+/CSVD+ group, APOE ε4 carriers with elevated plasma NfL levels exhibited accelerated atrophy in medial temporal lobe structures, such as the hippocampus, entorhinal cortex, and amygdala, as well as in the supramarginal gyrus and frontal pole. We also observed greater decline in memory and global cognition among APOE ε4 carriers with elevated plasma NfL levels only in the Aβ+/CSVD+ group. Finally, plasma NfL significantly mediated the relationship between APOE ε4 and brain atrophy in the Aβ+/CSVD+ group, but not in the Aβ+/CSVD− group. Specifically, plasma NfL mediated the association between APOE ε4 and the rate of cortical thinning

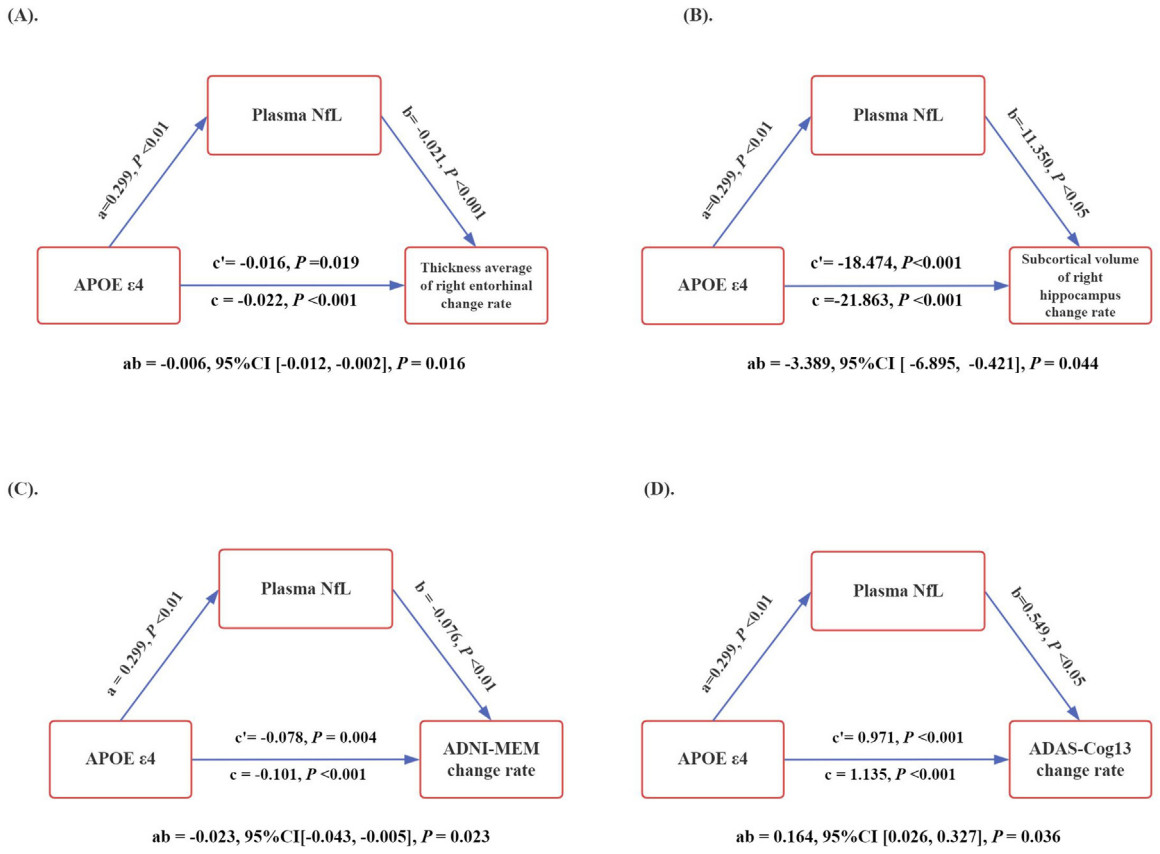

Fig. 3. Mediation effect of plasma NfL on the association between APOE ε4 and longitudinal structural MRI ROIs and cognitive measures in the Aβ+/CSVD+ participants. Plasma NfL levels were log2-transformed. The indirect effect (ab), direct effect (c'), and total effect (c) are reported. *P*-values were adjusted for age, gender, years of education. APOE, apolipoprotein E; NfL, neurofilament light chain; ADNI-MEM, memory function; ADAS-Cog11 or 13, Alzheimer's Disease Assessment Scale 11- or 13-item subscale; Aβ, amyloid-β; CSVD, cerebral small vessel disease; MRI, magnetic resonance imaging; ROIs, regions of interest.

in the right entorhinal cortex, as well as reductions in subcortical volumes of the right hippocampus. Additionally, plasma NfL mediated the relationship between APOE ε4 status and the rate of memory and global cognitive decline. Our results suggest that neuroaxonal injury could play a role in the effects of APOE ε4 status on brain atrophy and cognitive decline among persons with comorbid AD and CSVD. Hence, plasma NfL could serve as a valuable biomarker for identifying individuals at heightened risk for accelerated neurodegeneration and cognitive decline in comorbid AD and CSVD, offering novel pathways for targeted therapeutic interventions.

It is generally known that cognitive decline and brain aging are influenced by both neurodegenerative and vascular mechanisms. In this study, we used imaging biomarkers to detect both AD and CSVD pathologies. Amyloid PET was used to identify AD pathology, while conventional MRI markers, such as WMH and lacunar infarcts, were used to model CSVD-related changes. Our findings revealed that the Aβ+/CSVD+ group experienced more severe cognitive decline compared to the Aβ−/CSVD− group, consistent with previous research showing that the combination of Aβ pathology and vascular burden accelerates cognitive decline [44].

Previous research has identified a strong association between the APOE ε4 allele and plasma NfL levels in relation to Aβ pathology [45,46] and WMH progression [17,47]. However, studies on this association in comorbid AD and CSVD remain sparse. In the current study, we found a significantly higher prevalence of APOE ε4 carriers in the Aβ+/CSVD+ and Aβ+/CSVD− groups compared to the Aβ−/CSVD− group, with the highest plasma NfL levels observed in the Aβ+/CSVD+ group. These findings provide insights into the potential synergistic role of the APOE ε4 allele and plasma NfL in the comorbidity of AD and CSVD. Several biological mechanisms may explain these associations. First, APOE ε4 disrupts cholesterol transport and impairs myelin repair [48], increasing the susceptibility of axons to damage, particularly in white matter regions compromised by CSVD. Second, APOE ε4 is linked to cerebrovascular dysfunction [7], including blood-brain barrier (BBB) disruption and hypoperfusion, exacerbating white matter injury and accelerating WMH progression. Third, elevated neuroinflammation in APOE ε4 carriers further damage neurons and myelin [49], compounding axonal injury. Collectively, Aβ-related neurotoxicity and vascular injury in APOE ε4 contribute to accelerated brain atrophy and cognitive decline, with plasma NfL serving as a critical biomarker for these processes. Medial temporal lobe atrophy is a well-established hallmark of early AD [50], whereas CSVD−related cognitive impairment is often associated with subcortical ischemic lesions and frontal lobe atrophy [51]. Elevated plasma NfL levels are thought to capture both vascular injury, including the progression of white matter lesions, and neurodegenerative changes, such as medial temporal atrophy [47]. Our study found that Aβ+/CSVD+ individuals, particularly APOE ε4 carriers with high plasma NfL levels, exhibited accelerated atrophy in these regions. The presence of both Aβ and vascular mechanism likely accelerates the cognitive decline in Aβ+/CSVD+ individuals, hence forting previous research on the compounded impact of comorbid AD and CSVD on neurodegenerative processes [47].

Our findings are consistent with previous research indicating that APOE ε4 carriers exhibit elevated plasma NfL levels [52], particularly in the Aβ+/CSVD+ subgroup. While findings from Mielke et al. did not find a significant effect of APOE ε4 on plasma or CSF NfL levels in CU individuals [53], this discrepancy may arise from the possibility that NfL levels do not markedly increase until neurodegenerative processes

become more pronounced [54]. In cohorts with a higher proportion of APOE ε4 non-carriers, the absence of significant neurodegenerative changes might lead to stable NfL levels despite the presence of the APOE ε4 allele. In contrast, our study includes a more balanced representation of APOE ε4 carriers, likely reflecting a broader variation in NfL levels due to the compounded effects of AD and CSVD. This suggests that the interplay between these factors may heighten the sensitivity of plasma NfL as a biomarker in populations with comorbid pathologies.

While APOE ε4 and plasma NfL have been independently studied in relation to cognitive decline, few studies have explored the interactions of APOE ε4 and NfL on brain volumes and cognitive performance. Our study contributes to the existing literature by demonstrating that plasma NfL mediates the effects of APOE ε4 on brain atrophy, particularly in the right entorhinal cortex and hippocampus, in individuals with comorbid AD and CSVD. In contrast, no significant mediation effect was observed in Aβ+/CSVD− individuals, suggesting that CSVD exacerbates the impact of NfL on brain atrophy and cognitive decline through other mechanisms. These mechanisms may involve neuroinflammation, chronic hypoperfusion, and BBB dysfunction, and will require further clarification [55–57].

The right-sided atrophy observed in our study may reflect asymmetrical brain involvement in AD and vascular pathology. Previous research has documented asymmetry in hippocampal atrophy in AD, often varying by clinical stage or APOE ε4 status [58]. However, these studies have not fully accounted for the influence of coexisting vascular pathology, which may also affect atrophy patterns. The laterality of atrophy observed in this study suggests that vascular pathology may play a role in brain asymmetry, warranting further exploration in future studies. The link between right-sided atrophy and cognitive decline in our study also highlights the importance of considering laterality in cognitive assessments, as some tests may be less sensitive to non-dominant hemisphere functions.

To our knowledge, this is the first study to describe the mediating effects of plasma NfL on the association between APOE ε4 and both brain atrophy and cognitive decline in individuals with comorbid AD and CSVD. Specifically, we found that elevated plasma NfL levels serve as a significant mediator in the association with accelerated atrophy in critical regions, such as the right entorhinal cortex and hippocampus. This highlights the potential of plasma NfL as a valuable biomarker for identifying individuals at increased risk for cognitive decline and neurodegeneration, providing insights into the complex interplay between genetic, vascular, and neurodegenerative factors.

Several limitations should be acknowledged in this study. First, the data were retrospectively obtained from the ADNI database, which may have introduced selection bias. The study participants were predominantly from higher socioeconomic and educational backgrounds, potentially limiting the generalizability of the findings to more diverse populations. Second, the sample size was relatively small, particularly in subgroups defined by disease status or Aβ positivity. Additionally, the reliance on PET-derived Aβ burden as the primary biomarker of amyloid pathology likely further limited the sample size due to the high costs and limited availability of PET scans. Third, although WMH was included as the primary MRI-based marker of CSVD pathology, there is the potential for overlap between contributions from CSVD and cerebral amyloid angiopathy (CAA). However, previous analyses of the ADNI dataset suggest that WMH predominantly reflects non-CAA-related vascular changes [59]. Moreover, this study focuses on CU and MCI, in which the prevalence and severity of CAA are relatively low [60]. Future research should incorporate advanced biomarkers, such as high-resolution imaging techniques, and recruit larger, more diverse longitudinal cohorts to further delineate the respective contributions of CSVD and CAA to WMH burden, as well as to establish the relationship between AD and CSVD comorbidity.

In conclusion, our findings suggest that neuroaxonal injury may play a pivotal role in the relationship between APOE ε4 and the progression of brain atrophy and cognitive decline in individuals with comorbid AD and CSVD. These findings underscore the potential of plasma NfL as a valuable biomarker for monitoring disease progression and evaluating the efficacy of therapeutic interventions, particularly in individuals with a higher burden of Aβ and CSVD pathology.

Declarations

Ethics approval and consent to participate

Ethical approval was obtained from the institutional review boards of all participating ADNI sites, and the study was conducted in accordance with the Declaration of Helsinki.

Consent for publication

Consent for publication is not applicable to this article as no personal data were contained.

Data availability

Data used in this study are available from the ADNI database (adni.loni.usc.edu) upon registration and compliance with the data usage agreement.

Declaration of generative AI and AI-assisted technologies in the writing process

No.

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Declaration of competing interest

All authors have no conflicts of interest.

CRediT authorship contribution statement

Chunhua Zhang: Writing – original draft, Investigation, Data curation. **Bingyu Li:** Writing – review & editing, Data curation. **Kok Pin Ng:** Writing – review & editing, Data curation. **Yaojun Tai:** Supervision, Methodology. **Yuanming Tai:** Supervision, Conceptualization. **Xicheng Song:** Writing – review & editing, Conceptualization. **Min Kong:** Writing – review & editing. **Maowen Ba:** Methodology, Funding acquisition, Conceptualization.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.tjpad.2024.100054.

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