

Cerebrospinal fluid Visinin-like protein-1 was associated with the relationship of body mass index with Alzheimer's disease pathology and cognition in non-demented elderly

Yayu Wang¹ , Siqi Yu¹ , Man Zhang¹ , Huaiyuan Zhu^{2,3,4} , Shujian Chen¹ , Yajun Zhou¹ , Xia Zhou¹, Zhongwu Sun¹ , Xianfeng Yu¹ , Xiaoqun Zhu¹  and for the Alzheimer's Disease Neuroimaging Initiative[§]

Abstract

Background: The relationship and mechanisms between body mass index (BMI) and cognition are complex and inconclusive. Additionally, the role of neuronal calcium dysfunction, reflected by cerebrospinal fluid (CSF) Visinin-like protein 1 (VILIP-1), in the mechanisms linked with BMI and Alzheimer's disease (AD) has not been investigated.

Objective: To investigate the relationship between CSF VILIP-1, BMI, and AD pathologies in non-demented elderly at early stages of AD.

Methods: Baseline CSF AD core biomarkers (amyloid- β_{42} [$A\beta_{42}$], phosphorylated tau [P-tau], and total tau [T-tau]) were measured for 1201 non-demented participants, selected from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database, among whom 128 had measurements of CSF VILIP-1. Multivariate linear regression, causal mediation analyses, and linear mixed effects models were conducted to detect these associations.

Results: The average age of participants was 72.6. CSF VILIP-1 was decreased in A+/TN- (A-positive/T- and N- negative) group and elevated in A-/TN+ (A-negative/T- or N-positive) and A+/TN+ groups, as compared with A-/TN- group. In total participants, BMI was negatively related to CSF P-tau, T-tau, P-tau/ $A\beta_{42}$ and T-tau/ $A\beta_{42}$. Noticeable associations were also presented between CSF VILIP-1 and AD core biomarkers, but not with $A\beta_{42}$ after stratification by A/T/N scheme. Furthermore, the influences of BMI on CSF tau pathology were mediated by CSF VILIP-1. Higher baseline CSF VILIP-1 correspond to faster longitudinal cognitive decline.

Conclusions: Our findings indicated that CSF VILIP-1 changed dynamically and might be a key mediator in the associations between BMI and tau pathology, providing new insights into understanding the mechanisms underlying BMI-related cognitive deficits in non-demented elderly.

Keywords

Alzheimer's disease, body mass index, cerebrospinal fluid biomarkers, cognition, Visinin-like protein 1

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¹Department of Neurology, The First Affiliated Hospital of Anhui Medical University, Hefei, China

²Department of Clinical Pharmacy, The First Affiliated Hospital of Xinxiang Medical University, Weihui, China

³Clinical Pharmacy, Henan Province Key Subjects of Medicine, the First Affiliated Hospital of Xinxiang Medical University, Weihui, China

⁴Xinxiang Key Laboratory for Individualized Drug Use Research for Immune Diseases, Weihui, China

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Corresponding authors:

Xiaoqun Zhu, Department of Neurology, The First Affiliated Hospital of Anhui Medical University, NO. 210 Jixi Rode, Shushan District, Hefei 230031, China.

Email: zxq_ayfy@163.com

Xianfeng Yu, Department of Neurology, The First Affiliated Hospital of Anhui Medical University, NO. 210 Jixi Rode, Shushan District, Hefei 230031, China.

Email: Dr.YXF@outlook.com



Introduction

Alzheimer's disease (AD) is characterized by the extracellular accumulation of amyloid- β (A β) plaques and intracellular tau neurofibrillary tangles. Neuronal calcium dysfunction has been pinpointed as a notable feature of AD pathologies.¹ Visinin-like protein 1 (VILIP-1), a neuronal Ca²⁺-sensor protein,^{2,3} modulates calcium homeostasis and multiple Ca²⁺-dependent cell signaling pathways.⁴ It is predominantly expressed in neuronal pericaria, dendrites and some axons,⁵ and involved in the regulation of neuronal growth, differentiation,⁶ as well as synaptic plasticity.⁷ Previous articles provide evidential supports that elevated concentrations of CSF VILIP-1 are closely related to multiple neurological disorders, such as cerebral ischemic infarction,⁴ dementia,^{3,4,7-9} and brain atrophy.¹⁰ Taken together, these findings indicated CSF VILIP-1-related neuronal calcium dysfunction and injury may play a vital role in the progression of AD.

Body mass index (BMI) is regarded as a modifiable risk factor for AD, but its association with cognitive function and its role in the pathogenesis of AD remain controversial and unclear. Multiple epidemiological evidence suggested that higher BMI in midlife was associated with an increased risk of cognitive decline.^{11,12} Whereas in older adults, the relationship between BMI and cognitive function was more complex and the prevailing perspectives propose that overweight or obesity might serve as a potential protective factor against AD.¹³⁻¹⁵ Besides, the pathological mechanisms between BMI and AD remain poorly understood. Currently, the widely recognized mechanisms linked with BMI in the progression of AD include metabolism and nutritional supports,¹⁶ inflammation and immune regulation,¹⁷ neuroendocrine changes,^{18,19} etc. However, to our knowledge, the role of neuronal calcium dysfunction and injury, as reflected by CSF VILIP-1, in the mechanisms linked with BMI and AD pathology and cognition has not been investigated.

Hence, we aimed to (1) ascertain the relationships of CSF VILIP-1 with BMI and AD core biomarkers (A β ₄₂, phosphorylated tau [P-tau], and total tau [T-tau]) and cognitive function; (2) determine whether CSF VILIP-1 serves as a mediating factor in the correlation between BMI and tau pathology; and (3) explore the predictive value of CSF VILIP-1 in cognitive decline and AD risk.

Methods

Study participants

The study participants were screened from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database.²⁰ The ADNI was launched in 2003 as a public-private partnership, led by Principal Investigator Michael W. Weiner, MD. The original goal of ADNI was to test whether serial magnetic resonance imaging, positron emission tomography (PET), other biological markers, and clinical and

neuropsychological assessment can be combined to measure the progression of mild cognitive impairment (MCI) and early AD. The current goals include validating biomarkers for clinical trials, improving the generalizability of ADNI data by increasing diversity in the participant cohort, and to provide data concerning the diagnosis and progression of Alzheimer's disease to the scientific community. For up-to-date information, see <https://adni.loni.usc.edu/>. The detailed inclusion and exclusion criteria in ADNI are available at <https://adni.loni.usc.edu/>. ADNI has received approval from the institutional review board of all participating centers, and written informed consent was obtained from all participants or their authorized relatives.

Participants lacking data of CSF biomarker and those with data 3 standard deviations (SDs) greater or smaller than the mean value were excluded. To study the early stages of AD, we also excluded demented participants. Ultimately, a sum of 1201 participants with baseline CSF AD core biomarkers and longitudinal scores of cognitive performance were selected from the ADNI database. Among these, 76 individuals with MCI (Mini-Mental State Examination [MMSE] > 24, Clinical Dementia Rating Sum of Boxes [CDR] = 0.5) and 52 cognitively normal individuals (CNs, MMSE > 24, CDR = 0) had available CSF VILIP-1 data to mediation analyses (refer to Supplemental Figure 1 for a detailed explanation of the sample selection process).

CSF measurements of VILIP-1 and AD core biomarkers

CSF procedural protocols in ADNI have been described in previous published literature.²¹ A sandwich ELISA was approached utilizing the Erenna[®] immunoassay system for the detection of CSF VILIP-1.²² CSF A β ₄₂, P-tau, and T-tau were detected using Roche automated immunoassay platform (Cobase601) and immunoassay reagents, as described previously.^{23,24} The measurements of all CSF biomarkers were duplicated, and the mean values were calculated. The interbatch coefficients of variation were 15%. The original data mentioned above and detailed assay protocols are accessible in the ADNI database.

Cognitive assessments

Cognitive function was evaluated through a range of scales. According to the neuropsychological batteries, specific cognitive tests for memory (MEM), executive function (EXF) and language (LAN) were developed and used to reflect cognitive domains.²⁵⁻²⁷ In detail, EXF measures encompass Category Fluency, WAIS-R Digit Symbol, Trails A and B, Digit Span Backwards, and clock drawing. MEM measures encompass relevant items of the Rey Auditory Verbal Learning Test, ADAS, Logical Memory, and

MMSE. LAN measures encompass Boston Naming Test, the Animal Fluency test, and the naming portion of AD Assessment Scale-Cog. AD Assessment Scale 13-item cognitive subscale (ADAS13)²⁸ and MMSE²⁹ were employed to reflect the global cognitive function. Obviously, higher ADAS 13 scores and lower MEM, EF, LAN, and MMSE scores indicate worse cognitive function.

The A/T/N classification methods

Based on the A/T/N scheme³⁰ proposed by National Institute on Aging and Alzheimer's Association criteria in 2018,³¹ "A+" was defined as CSF A β ₄₂ concentrations <977 pg/ml, while "T+" was defined as CSF P-tau concentrations >22 pg/ml, and "N+" was defined as CSF T-tau concentrations >245 pg/ml in this study.³² It has been demonstrated that the establishment of these cutoff values for CSF biomarkers exhibits strong agreement with the PET classification in ADNI.²³ We combined T and N groups together for statistical convenience due to their high correlation, thus generating the TN group: "TN+" denotes either T+ or N+, and "TN−" signifies both T− and N−.^{32,33}

Statistical analyses

First, Chi-square tests were utilized for categorical variables, while Mann-Whitney U tests were applied for continuous variables to examine inter-group variations in baseline characteristics. Skewed data of CSF biomarkers were transformed to achieve or approximate a normal distribution ($p < 0.05$ by the Kolmogorov-Smirnov test) using the "car" package in R software.

Analysis of covariance (ANCOVA) was approached to evaluate the difference in CSF VILIP-1 levels across the four biological groups, with subsequent Bonferroni correction conducted. Multiple linear regressions (MLR) were conducted to investigate interrelationships of BMI with CSF biomarkers and cognitive function, with adjustments for covariates (including sex, age, education, and APOE ϵ 4 carrier status) in whole cohort. Moreover, Exploratory analyses were conducted within subgroups based on sex, APOE ϵ 4 carrier status (non-carriers versus carriers), cognitive status (CN versus MCI), and four A/TN groups to examine the aforementioned interrelationships.

Following the methodology proposed by Baron and Kenny,³⁴ mediation analyses were performed to ascertain whether CSF VILIP-1 (the mediator) could mediate the correlation between BMI and CSF AD biomarkers and cognitive function. Significance was assessed through 10,000 bootstrap resamples, and the direct and indirect effects, as well as the proportion mediated were estimated accordingly. The same covariates included in the previous MLR models were also adjusted within each path of the mediation models. Sensitivity analyses were also conducted by constructing various MLR models with adjusting more

potential confounding factors, including depression, anxiety, hypertension, DM2, smoking and stroke.

Linear mixed effects (LME) models were conducted to identify whether the baseline level of CSF VILIP-1 could predict longitudinal changes in cognitive function. These LME models were created using cognitive scores as dependent variables. Time since baseline (years), VILIP-1, and their interaction were included as fixed effects. Age, sex, education, APOE ϵ 4 status, and their interactions with time were included as covariates. Finally, influence of BMI and CSF VILIP-1 on the risk of incident AD were detected with the method of Kaplan-Meier.

A two-sided p-value below 0.05 was deemed statistically significant. Data analyses and graphical representation were conducted utilizing R version 4.4.1 and GraphPad Prism 9.00 software.

Results

Baseline participants characteristics

The detailed demographic features, cognitive scores and CSF biomarker levels stratified by cognitive status were showed in Table 1 and Supplemental Table 1. A cohort with a total of 1201 non-demented participants (51.9% males, 72.6 ± 7.0 years, Supplemental Table 1) were selected, among whom 128 participants (64.8% males, 75.2 ± 6.1 years, Table 1) with CSF VILIP-1 data available were incorporated into the mediation analysis.

CSF VILIP-1 levels in different biological stages of AD

Participants were categorized into four biological groups based on the A/T/N framework. In order to evaluate the correlation between CSF VILIP-1 and AD pathologies, we first explored the variations in CSF VILIP-1 concentrations and found significant disparities among the four groups ($p = 7.7 \times 10^{-15}$, Figure 1). Specifically, A+/TN− ($n = 20$) had the significantly lower levels of CSF VILIP-1 compared to the A−/TN− group ($n = 27$) ($p < 0.001$), whereas the A−/TN+ ($n = 23$) revealed a significant increase ($p < 0.0001$), followed by A+/TN+ group ($n = 58$) ($p < 0.001$). However, the results did not unveil notable difference between the A−/TN+ and the A+/TN+ group ($p > 0.05$).

Associations between BMI and CSF AD core biomarkers and cognitive function

In all participants, MLR models showed that continuous BMI variables were negatively correlated with the concentration of CSF P-tau ($\beta = -0.29$, $p = 3.76 \times 10^{-5}$), T-tau ($\beta = -2.51$, $p = 8.53 \times 10^{-5}$), P-tau/A β ₄₂ ($\beta = -6.51 \times 10^{-4}$, $p = 3.39 \times 10^{-6}$), and T-tau/A β ₄₂ ($\beta = -5.60 \times 10^{-3}$, $p = 2.71 \times 10^{-6}$), but the positive association with CSF A β ₄₂ was at the threshold of significance ($\beta = 6.31$,

Table 1. Characteristics of participants with available CSF VILIP-I data.

	Total	CN	MCI	p
N	128	52	76	—
Age, y	75.21 ± 6.14	76.21 ± 5.03	74.52 ± 6.72	0.158
Sex, n _{Male} /n _{Female}	83/44	29/33	55/21	0.087
Education, y	16.06 ± 2.88	15.71 ± 3.20	16.31 ± 2.61	0.316
Positive APOE ε4 Carrier Status, n (%)	50 (39.4%)	11 (21.2%)	40 (52.6%)	<0.001
BMI (kg/m ²)	26.58 ± 4.34	27.59 ± 4.82	25.88 ± 3.79	0.029
MMSE Score	27.87 ± 1.79	27.77 ± 1.99	27.93 ± 1.64	0.628
ADAS Score	14.53 ± 7.05	9.06 ± 3.76	18.33 ± 6.26	<0.001
MEM	0.27 ± 0.61	0.80 ± 0.40	0.10 ± 0.43	<0.001
EXF	0.45 ± 0.47	0.66 ± 0.39	0.31 ± 0.47	<0.001
LAN	0.46 ± 0.44	0.69 ± 0.46	0.31 ± 0.35	<0.001
CSF biomarkers (pg/ml)				
Aβ ₄₂	1006.44 ± 603.08	1293.31 ± 669.76	807.55 ± 456.34	<0.001
P-tau	27.44 ± 12.38	22.97 ± 7.99	30.53 ± 13.85	0.001
T-tau	282.61 ± 106.57	248.23 ± 74.12	306.44 ± 118.47	0.005
VILIP-I	163.63 ± 56.73	161.83 ± 56.16	163.17 ± 57.12	0.045

Continuous variables were presented as mean ± SD, and categorical variable was showed as number and proportion. Significance at the level of p < 0.05 were showed in bold.

BMI: weight (in kg)/ height² (in m²); MMSE: Mini-Mental State Examination; ADAS: Alzheimer's Disease Assessment Scale; CSF: cerebrospinal fluid; MEM: memory function; EXF: executive function; LAN: language; Aβ: amyloid-β; P-tau: phosphorylated tau; T-tau: total tau protein; VILIP-I: Visinin-like protein-I; SD: standard deviation.

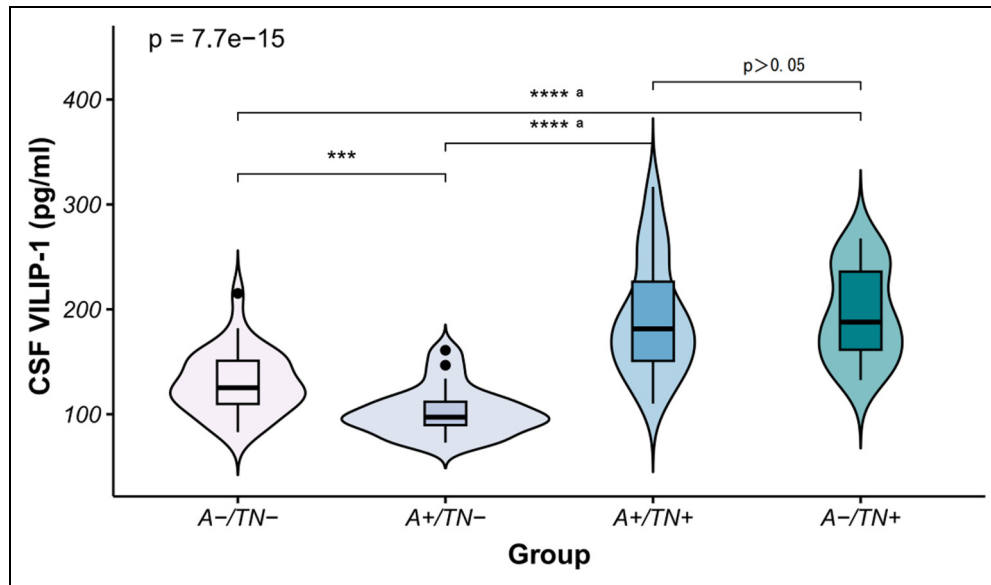


Figure 1. Dynamic changes of CSF VILIP-I in the preclinical phase of AD stratified by A/T/N criteria.

p-values were calculated using one-way ANCOVAs, controlling for age, sex, education, and APOE ε4 status. "a" indicated a significant p value after Bonferroni corrected post hoc pairwise comparisons.

A: Aβ pathology biomarker status; T: tau pathology biomarker status; N: neurodegeneration biomarker status.

p < 0.001; *p < 0.0001.

p = 0.059) after controlling for aforementioned covariates (Figure 2 and Supplemental Table 2A). Subgroup analyses (stratified by cognitive diagnosis, gender or APOE ε4 carrier status) indicated that positive relationships of continuous BMI variables with tau pathology remained significant (all p < 0.05, Figure 2) except in CN group. But the positive association with CSF Aβ₄₂ was

statistically significant only in MCI (β = 9.16, p = 0.03) and APOE ε4 carrier group (β = 17.52, p = 1.68 × 10⁻⁵). As for cognitive function, any association between continuous BMI variables and cognitive scores was observed across all participants. Further subgroup analyses showed that BMI had noticeable correlation with ADAS13 score in female, MCI and APOE ε4 carrier subgroups. Furthermore, higher

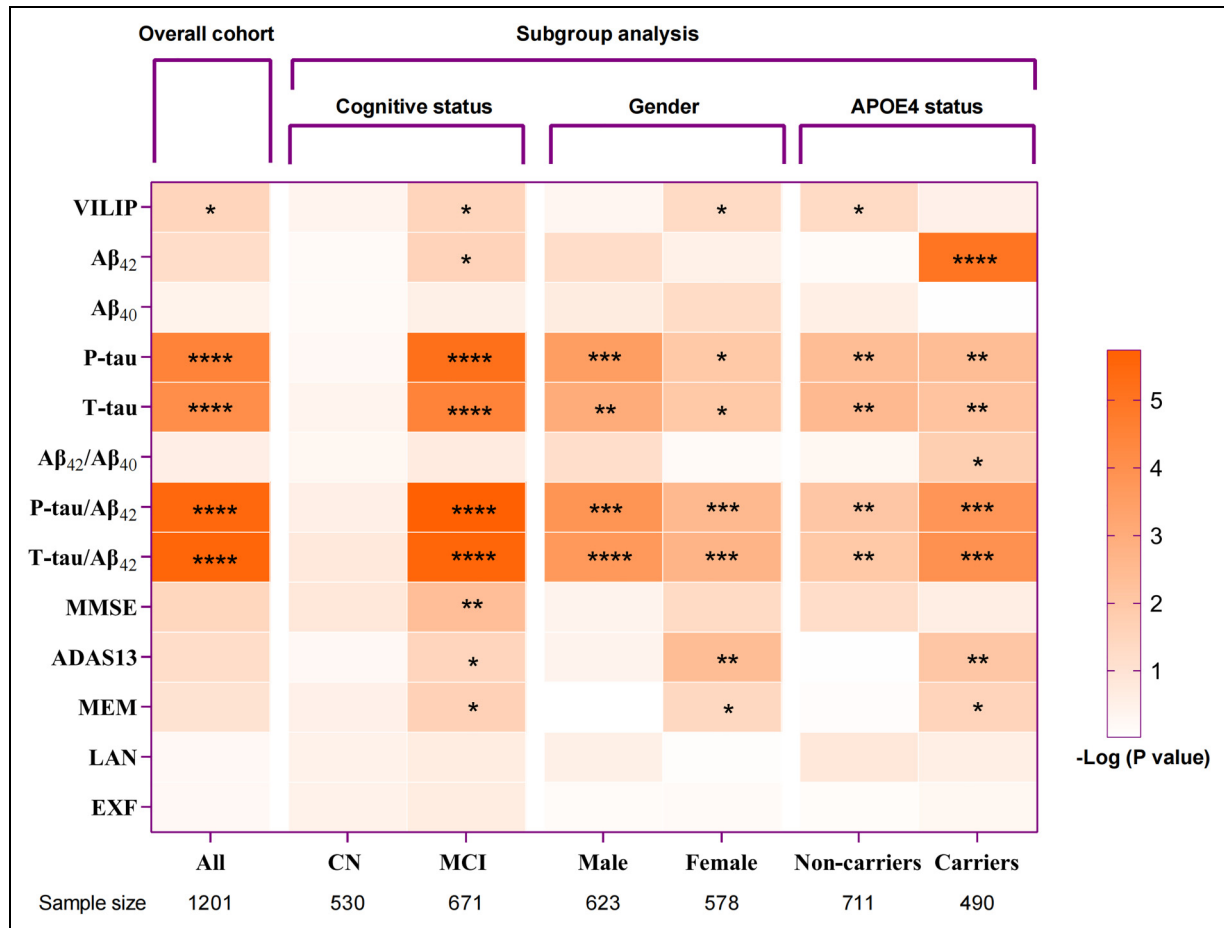


Figure 2. Association of BMI with CSF biomarkers and cognitive function.

BMI were significant associated with CSF P-tau and CSF Tau in total and subgroups but only was significant associated with CSF Aβ₄₂ in MCI group.

VILIP-I, Visinin-like protein-I; Aβ: amyloid-β; P-tau: phosphorylated tau; T-tau: total tau protein; APOE: apolipoprotein gene E; MMSE: Mini-Mental State Examination; ADAS: Alzheimer's Disease Assessment Scale; MEM: memory function; EXF: executive function; LAN: language.

*p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001.

BMI was positively related to MMSE only in MCI group (Figure 2 and Supplemental Table 2A). Within different biological groups, significant correlations with P-tau/Aβ₄₂ and T-tau/Aβ₄₂ were only observed in A+/TN+ group (Supplemental Table 2B). The abovementioned significant results were barely changed when BMI was considered as a categorical variable in the correlation analysis (Supplemental Table 3). We also found older adults with obesity exhibited a decreased risk of AD utilizing the Kaplan-Meier method (p = 0.044, Supplemental Figure 3A).

Associations between CSF VILIP-I and AD core biomarkers and cognitive function

As shown in Figure 3 and Supplemental Table 4, baseline level of CSF VILIP-I was associated with AD core

biomarkers (Aβ₄₂, β = 2.41, p < 8.63 × 10⁻³; P-tau, β = 0.16, p < 2 × 10⁻¹⁶; T-tau, β = 1.48, p < 2 × 10⁻¹⁶; P-tau/Aβ₄₂, β = 1.76 × 10⁻⁴, p < 1.68 × 10⁻⁴ and T-tau/Aβ₄₂, β = 1.50 × 10⁻³, p < 3.16 × 10⁻⁴), but not with cognitive function (MMSE, ADAS13, MEM, EXF, or LAN scores) in all participants. All results exhibited negligible alterations in the sensitivity analyses after controlling for the potential confounders, such as depression, anxiety, hypertension, DM2, smoking, and stroke (Supplemental Table 4). Subgroup analyses revealed that abovementioned significant associations were persistent both within the CN and MCI groups (Figure 3A–E). However, elevated CSF VILIP-I were linked exclusively to CSF tau pathology, but not with CSF Aβ₄₂ in all different biological groups (Figure 3F and G). Significant correlations with P-tau/Aβ₄₂ and T-tau/Aβ₄₂ were only

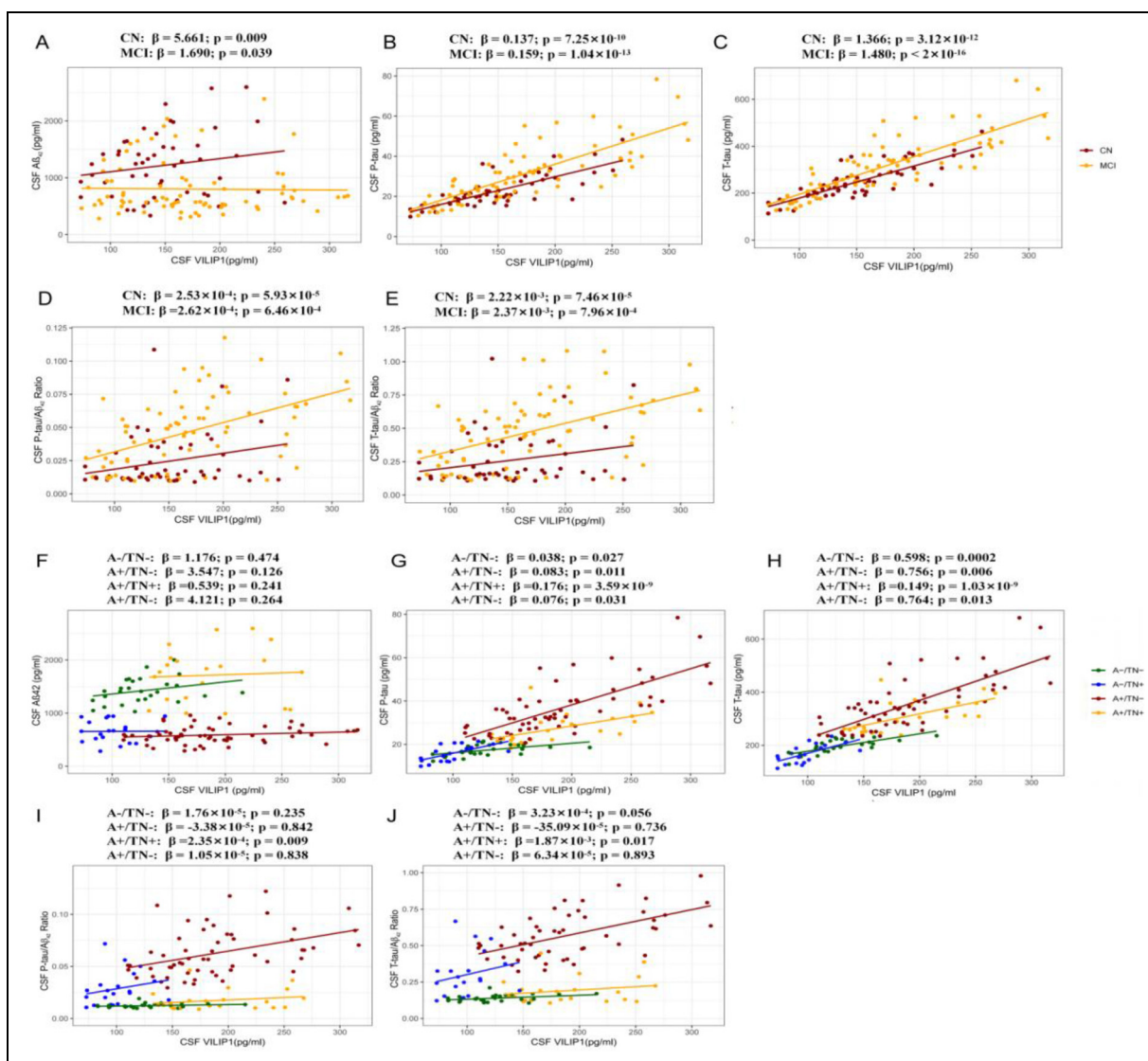


Figure 3. Relationship of CSF VILIP-I with CSF AD biomarkers.

The positive association of CSF VILIP-I and AD core biomarkers both in CN and MCI group were depicted (A–E). After stratification by the A/T/N scheme, the positive association were only significant with CSF P-tau (G) and T-tau (H). Multiple linear regression models, controlling for age, sex, education, and *APOE* $\epsilon 4$ status, were employed to determine the p-values.

CSF: cerebrospinal fluid; VILIP-I: Visinin-like protein-I; A β : amyloid- β ; P-tau: phosphorylated tau protein; T-tau: total tau protein.

observed in A+/TN+ group (Figure 3I and J). Our findings suggested that CSF VILIP-I-related disturbed calcium homeostasis might be accompanied by the pathogenesis of AD.

CSF VILIP-I mediated the association between BMI and tau pathology

Mediation analyses were performed on CSF P-tau and T-tau rather than A β_{42} or cognitive functions, due to their non-robust significance in relation to BMI. The mediation analyses showed that CSF VILIP-I partially mediated the

association between BMI and P-tau (the mediation proportion: 53.7%, $p = 0.018$), and completely mediated the association with T-tau (the mediation proportion: 64.2%, $p = 0.014$) in total participants (Figure 4A and B). Subgroup analyses revealed that, for MCI group, CSF VILIP-I mediated completely the effect of BMI on tau pathology (Supplemental Figure 2). Owing to the constraint of sample size, it was not feasible to perform meaningful analyses within the different biological subgroups. To better control for potential confounding factors, we conducted sensitivity analyses and found the mediating role of CSF VILIP-I in total participants and MCI group were persistent and robust (Supplemental Table 6A–C).

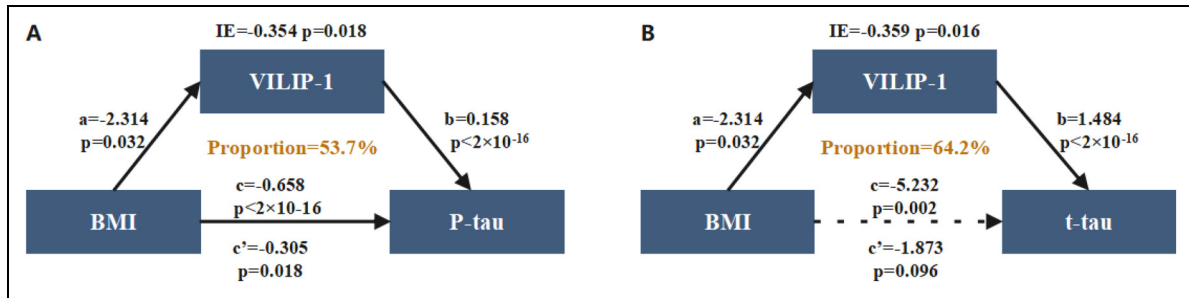


Figure 4. CSF VILIP-I mediated association between BMI and tau pathology.

CSF VILIP-I mediated the association between BMI and tau pathology in total participants (A, B). Mediation analyses with 10,000 bootstrapped iterations were conducted to examine mediation effects. Each path of the model was adjusted for age, sex, education, and APOE $\epsilon 4$ status.

VILIP-I: Visinin-like protein-I; P-tau: phosphorylated tau protein; t-tau: total tau protein; IE: indirect effect.

Association between baseline CSF VILIP-I levels and longitudinal cognitive changes and risk of AD

Given the above findings, we postulated that CSF VILIP-1 might have a predictive role in cognitive changes or AD risk. In order to confirm our assumption, LME models were performed. Baseline levels of CSF VILIP-1 showed an inverse relationship with MMSE, MEM, EXF, and LAN scores ($p = 4 \times 10^{-4}$, 0.036, 0.004 and 6×10^{-4} , respectively, Table 2), and positively associated with ADAS13 scores ($p = 0.009$) during the follow-up. However, Kaplan–Meier test showed baseline CSF VILIP-1 failed to predict the risk of incident AD as revealed by a 10-year follow-up of 128 non-demented participants (Supplemental Figure 3B).

Discussion

In our study, we probed the dynamic alterations of CSF VILIP-1 and found the concentration exhibited an initial decline, reaching a nadir in the A+/TN- group, followed by a gradual ascent across various biological stages of AD. Mediation analysis indicated that CSF VILIP-1 played a mediating part in the pertinence of BMI and tau pathology (CSF P-tau and T-tau) especially amongst MCI group. Longitudinal data revealed that individuals with lower CSF VILIP-1 had less cognitive decline. Overall, our results suggested that CSF VILIP-1, which could be implicated in the interplay between BMI and AD pathology, was predominantly active in the early stages of AD and potentially affected subsequent cognitive function.

In line with prior research, the present study consolidated that a higher BMI served as a protective factor in cognitive function among elderly non-demented individuals. Specifically, subgroup analyses demonstrated that significant correlations were observed with CSF AD core biomarkers in MCI,³⁵ whereas not in CN group.¹⁵ These results indicated that MCI with normal or low BMI might be more prone to have amyloid and tau-induced cognitive impairment. The above significant correlations were barely changed

Table 2. Association of CSF VILIP-I with longitudinal cognitive changes.

	VILIP-I $\times$ time		
	β	t	p
MMSE	-3.572×10^{-3}	-3.554	4×10^{-4}
ADAS13	5.823×10^{-3}	2.623	0.009
MEM	-2.714×10^{-4}	-2.106	0.036
EXF	-4.856×10^{-4}	-2.878	0.004
LAN	-4.778×10^{-4}	-3.462	6×10^{-4}

p-values were evaluated via mixed-effect models controlling for age, sex, education, and APOE $\epsilon 4$ status. The results revealed the protective influence of lower baseline levels of CSF VILIP-1 on the deterioration of cognitive functions, as reflected by MMSE, ADAS13, MEM, EXF, and LAN. Significance at the level of $p < 0.05$ were showed in bold. MMSE: Mini-Mental State Examination; ADAS13: Alzheimer's Disease Assessment Scale 13; MEM: memory function; EF: executive function; LAN: language.

after further grouping by sex or APOE $\epsilon 4$ carrier status. However, no correlation with CSF A β_{42} was observed in all different biological groups. A potential explanation for the lack of statistical difference is the limited sample size in each biomarker-specific subgroup.

CSF VILIP-1 reflects disrupted calcium homeostasis and neuronal injury and may potentially be involved in signal transduction and neurotransmission.^{2,3} Its expression was reduced in the intracellular compartments of AD neurons yet markedly increased in extracellular plaques and neurofibrillary tangles.³ Accumulating evidence from AD subjects showed there existed a growing trend of CSF VILIP-1.^{8,9} The loss of neurons expressing VILIP-1,² as well as the pathological decrease in VILIP-1 mRNA levels³⁶ were observed in animal models with AD pathology. Coincidentally, individuals with MCI displayed higher concentrations of CSF VILIP-1 than those with cognitively normal status in our study.³⁷ Our cross-sectional analysis also provided additional evidence that CSF VILIP-1 levels exhibit dynamic alterations,⁹ as opposed to a sustained upward or downward trend, inferring it

declined with the formation of amyloid plaques in the earliest stage and subsequently increased with the formation of tau pathology in the late stages of AD pathology. One potential explanation for the early reduction of CSF VILIP-1 level may be that neurons expressing VILIP-1 appear to be selectively vulnerable against disturbed calcium homeostasis induced by A β plaque,⁷ and VILIP-1 is involved in the formation of A β plaque aggregates.³ Another possible hypothesis is that microglia, in the initial phases, downregulate their own exaggerated response to noxious A β plaques, thereby providing neuroprotective effects.³⁸ However, in later phases, overloaded pathological burdens foster more severe neuroinflammatory responses, eventually inducing neuronal injuries and contributing to an increase in CSF VILIP-1. It was also plausible to deduce that CSF VILIP-1 might be involved in neuronal damage in other neurodegenerative diseases apart from AD,^{3,9} since it was found to be the most concentrated in A-/TN+ group.

Significant positive correlations between CSF VILIP-1 and tau pathology were detected both in the whole cohort and in different subgroups. All results were negligible alteration in sensitivity analyses after further adjustments. These results were favored by the previous findings that VILIP-1 enhanced the hyperphosphorylation of tau protein and the formation of neurofibrillary tangles.^{4,9,39} Additionally, we observed that CSF VILIP-1 was positively associated with A β ₄₂, which seemed counterintuitive. In our further analysis, this correlation was observed in both the CN group and MCI group (albeit weak), yet this correlation did not prove statistically significant within any of the biological groups. Further studies with larger sample size are warranted to validate our findings.

In light of CSF VILIP-1 reflecting neuronal impairment, we propose that its higher concentrations could be linked to the progression or severity of dementia. Indeed, our findings revealed no correlation between the CSF VILIP-1 and cognitive function at baseline, differing from the findings of Lee et al., which reported an association with MMSE score.⁸ Our cohort excluded patients with dementia and had a higher mean age, but Lee's study encompassed a significant portion of AD patients. Differences in the subjects and age profiles in these cohorts may explain this inconsistency. Interestingly, analyses of the longitudinal follow-up data revealed that baseline lower levels of CSF VILIP-1 were indicative of preserved longitudinal cognitive functions.²² Those findings were in line with a study conducted from the memory clinic-based Amsterdam Dementia Cohort.⁴⁰ The mechanism by which CSF VILIP-1 was involved in the cognitive decline might be through the regulation of signal transduction,² neuronal growth and differentiation,⁶ synaptic plasticity, dendritic spine density,⁷ and nAChR activity in the hippocampus.⁴¹

Our findings suggested that CSF VILIP-1 acted as a mediator in the relationship between BMI and tau pathology and provided further support for the underlying mechanism.

Previous studies demonstrated that body weight and fat were positively associated with leptin in obese individuals.¹⁸ Leptin regulates tau phosphorylation by activating downstream signaling pathways, such as the phosphoinositide 3-kinase⁴² and adenosine monophosphate-activated protein kinase⁴³ pathways, which are essential in regulating the opening of calcium ion channels and maintaining calcium homeostasis in neurons.⁴⁴ In mouse models, exogenous leptin confers protection to hippocampal and hypothalamic cell lines against neurotoxic effects by inhibiting calcium elevation.⁴⁵ In light of CSF VILIP-1 being a hallmark of neuronal injury and calcium overload,^{3,4} it is reasonable to postulate that calcium homeostasis may have a mediating role in the relationship between BMI and the progression of AD. Besides, our observations provide evidence that interventions targeting at CSF VILIP-1-related neuronal calcium dysfunction may benefit the prevention of tau pathology. Future studies combining different omics fields such as metabolomics, proteomics, and genomics may provide a more comprehensive understanding of the biomolecular mechanisms of BMI, VILIP-1, and other factors including adiponectin signaling,¹⁸ insulin resistance,¹⁹ and nutritional status¹⁶ in the progression of AD.

This study is the first to explore the correlations between CSF VILIP-1 and BMI, AD pathologies and cognition in a non-demented population at early stages of AD. However, several limitations should be addressed. Firstly, BMI is a non-specific index that cannot fully represent the distribution of fat and the basic components of body composition in older adults. Nonetheless, it remains a universally applied health evaluation metric due to its simplicity and accessibility. Future studies with other body composition measures like waist circumference, body roundness index or waist-to-hip ratio (not accessible in ADNI), are needed to deeply examine the role of body weight in AD.⁴⁶ Secondly, the potential for a reverse causal relationship between BMI and AD cannot be dismissed. Future researches involving other confounding factors such as inflammation,¹⁷ insulin resistance,¹⁹ and chronic diseases⁴⁷ are necessary to elucidate the causal link. Thirdly, it is important to note that mediating associations are only indicative but not synonymous with causation. Thus, more validations in cross-sectional and longitudinal cohorts with larger sample sizes should be conducted in forthcoming studies. Finally, CSF VILIP-1 only provides an indirect measurement to assessing cerebral disturbed calcium homeostasis and neuronal injuries, unless confirmed by autopsy. Therefore, additional *in vitro* and *in vivo* research might help to better elucidate the role of neuronal injuries and disturbed calcium homeostasis in the mechanism between BMI and primary AD pathologies.

In conclusion, the present study demonstrated that CSF VILIP-1 changed dynamically and might play a role in mediating the associations of BMI with tau pathology. This provides a novel theoretical basis for advocating

older adults maintaining a higher weight at early stages of AD, since targeting CSF VILIP-1 may reduce tau deposition. Our results indicated that CSF VILIP-1-related disruption of calcium homeostasis could as a promising target for future management of AD.

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ORCID iDs

Yayu Wang  <https://orcid.org/0009-0004-7736-7991>
 Siqi Yu  <https://orcid.org/0009-0003-4364-1465>
 Man Zhang  <https://orcid.org/0009-0004-7510-3415>
 Huaiyuan Zhu  <https://orcid.org/0009-0008-2440-7901>
 Shujian Chen  <https://orcid.org/0009-0004-1324-5426>
 Yajun Zhou  <https://orcid.org/0009-0009-8989-1914>
 Zhongwu Sun  <https://orcid.org/0000-0002-7230-9328>
 Xianfeng Yu  <https://orcid.org/0000-0001-7293-8776>
 Xiaoqun Zhu  <https://orcid.org/0000-0001-9042-0057>

Statements and declarations

Ethical considerations

The research received approval from the institutional review boards of all participating institutions.

Consent to participant

Written informed consent was obtained from all participants or their legal guardians in accordance with the Declaration of Helsinki (Research Consent).

Author contributions/CRedit

Ya-Yu Wang (Data curation; Formal analysis; Investigation; Writing – original draft; Writing – review & editing); Si-Qi Yu (Formal analysis); Man Zhang (Formal analysis); Huai-Yuan Zhu (Formal analysis); Shu-Jian Chen (Investigation); Ya-Jun Zhou (Investigation); Xia Zhou (Investigation); Zhong-Wu Sun (Investigation); Xian-Feng Yu (Conceptualization; Formal analysis; Investigation; Methodology; Visualization; Writing – original draft; Writing – review & editing); Xiao-Qun Zhu (Conceptualization; Formal analysis; Investigation; Methodology; Visualization; Writing – original draft; Writing – review & editing).

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Conflicting interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Data availability

The data analyzed in this study can be downloaded from the ADNI (<https://adni.loni.usc.edu/>).

Supplemental material

Supplemental material for this article is available online.

References

1. Baracaldo-Santamaría D, Avendaño-Lopez SS, Ariza-Salamanca DF, et al. Role of calcium modulation in the pathophysiology and treatment of Alzheimer's disease. *Int J Mol Sci* 2023; 24: 9067.
2. Braunewell K, Riederer P, Spilker C, et al. Abnormal localization of two neuronal calcium sensor proteins, visinin-like proteins (vilips)-1 and -3, in neocortical brain areas of Alzheimer disease patients. *Dement Geriatr Cogn Disord* 2001; 12: 110–116.
3. Groblewska M, Muszyński P, Wojtulewska-Supron A, et al. The role of visinin-like protein-1 in the pathophysiology of Alzheimer's disease. *J Alzheimers Dis* 2015; 47: 17–32.
4. Schnurra I, Bernstein HG, Riederer P, et al. The neuronal calcium sensor protein VILIP-1 is associated with amyloid plaques and extracellular tangles in Alzheimer's disease and promotes cell death and tau phosphorylation in vitro: a

- link between calcium sensors and Alzheimer's disease? *Neurobiol Dis* 2001; 8: 900–909.
5. Bernstein HG, Baumann B, Danos P, et al. Regional and cellular distribution of neural visinin-like protein immunoreactivities (VILIP-1 and VILIP-3) in human brain. *J Neurocytol* 1999; 28: 655–662.
 6. Braunewell KH and Gundelfinger ED. Low level expression of calcium-sensor protein VILIP induces cAMP-dependent differentiation in rat C6 glioma cells. *Neurosci Lett* 1997; 234: 139–142.
 7. Braunewell KH. The visinin-like proteins VILIP-1 and VILIP-3 in Alzheimer's disease-old wine in new bottles. *Front Mol Neurosci* 2012; 5: 20.
 8. Lee JM, Blennow K, Andreasen N, et al. The brain injury biomarker VLP-1 is increased in the cerebrospinal fluid of Alzheimer disease patients. *Clin Chem* 2008; 54: 1617–1623.
 9. Halbgebauer S, Steinacker P, Riedel D, et al. Visinin-like protein 1 levels in blood and CSF as emerging markers for Alzheimer's and other neurodegenerative diseases. *Alzheimers Res Ther* 2022; 14: 175.
 10. Tarawneh R, Head D, Allison S, et al. Cerebrospinal fluid markers of neurodegeneration and rates of brain atrophy in early Alzheimer disease. *JAMA Neurol* 2015; 72: 656–665.
 11. Whitmer RA, Gunderson EP, Barrett-Connor E, et al. Obesity in middle age and future risk of dementia: a 27 year longitudinal population based study. *BMJ* 2005; 330: 1360.
 12. Xu WL, Atti AR, Gatz M, et al. Midlife overweight and obesity increase late-life dementia risk: a population-based twin study. *Neurology* 2011; 76: 1568–1574.
 13. Alosco ML, Duskin J, Besser LM, et al. Modeling the relationships among late-life body mass index, cerebrovascular disease, and Alzheimer's disease neuropathology in an autopsy sample of 1421 subjects from the National Alzheimer's Coordinating Center data set. *J Alzheimers Dis* 2017; 57: 953–968.
 14. Bell SP, Liu D, Samuels LR, et al. Late-life body mass index, rapid weight loss, apolipoprotein E ϵ 4 and the risk of cognitive decline and incident dementia. *J Nutr Health Aging* 2017; 21: 1259–1267.
 15. Sun Z, Wang ZT, Sun FR, et al. Late-life obesity is a protective factor for prodromal Alzheimer's disease: a longitudinal study. *Aging (Albany NY)* 2020; 12: 2005–2017.
 16. Danat IM, Clifford A, Partridge M, et al. Impacts of overweight and obesity in older age on the risk of dementia: a systematic literature review and a meta-analysis. *J Alzheimers Dis* 2019; 70: S87–s99.
 17. Guillemot-Legris O and Muccioli GG. Obesity-induced neuroinflammation: beyond the hypothalamus. *Trends Neurosci* 2017; 40: 237–253.
 18. Aisike G, Kuerbanjiang M, Muheyati D, et al. Correlation analysis of obesity phenotypes with leptin and adiponectin. *Sci Rep* 2023; 13: 17718.
 19. Barber TM, Kyrou I, Randeve HS, et al. Mechanisms of insulin resistance at the crossroad of obesity with associated metabolic abnormalities and cognitive dysfunction. *Int J Mol Sci* 2021; 22: 546.
 20. Weiner MW, Aisen PS, Jack CR Jr., et al. The Alzheimer's disease neuroimaging initiative: progress report and future plans. *Alzheimers Dement* 2010; 6: 202–211.e207.
 21. Shaw LM, Vanderstichele H, Knapik-Czajka M, et al. Cerebrospinal fluid biomarker signature in Alzheimer's disease neuroimaging initiative subjects. *Ann Neurol* 2009; 65: 403–413.
 22. Tarawneh R, D'Angelo G, Macy E, et al. Visinin-like protein-1: diagnostic and prognostic biomarker in Alzheimer disease. *Ann Neurol* 2011; 70: 274–285.
 23. Hansson O, Seibyl J, Stomrud E, et al. CSF Biomarkers of Alzheimer's disease concord with amyloid- β PET and predict clinical progression: a study of fully automated immunoassays in BioFINDER and ADNI cohorts. *Alzheimers Dement* 2018; 14: 1470–1481.
 24. Clark CM, Xie S, Chittams J, et al. Cerebrospinal fluid tau and beta-amyloid: how well do these biomarkers reflect autopsy-confirmed dementia diagnoses? *Arch Neurol* 2003; 60: 1696–1702.
 25. Crane PK, Carle A, Gibbons LE, et al. Development and assessment of a composite score for memory in the Alzheimer's Disease Neuroimaging Initiative (ADNI). *Brain Imaging Behav* 2012; 6: 502–516.
 26. Gibbons LE, Carle AC, Mackin RS, et al. A composite score for executive functioning, validated in Alzheimer's Disease Neuroimaging Initiative (ADNI) participants with baseline mild cognitive impairment. *Brain Imaging Behav* 2012; 6: 517–527.
 27. Choi SE, Mukherjee S, Gibbons LE, et al. Development and validation of language and visuospatial composite scores in ADNI. *Alzheimers Dement (N Y)* 2020; 6: e12072.
 28. Mohs RC, Knopman D, Petersen RC, et al. Development of cognitive instruments for use in clinical trials of antidementia drugs: additions to the Alzheimer's disease assessment scale that broaden its scope. The Alzheimer's Disease Cooperative Study. *Alzheimer Dis Assoc Disord* 1997; 11: S13–S21.
 29. Folstein MF, Folstein SE and McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res* 1975; 12: 189–198.
 30. Jack CR Jr., Bennett DA, Blennow K, et al. A/T/N: an unbiased descriptive classification scheme for Alzheimer disease biomarkers. *Neurology* 2016; 87: 539–547.
 31. Jack CR Jr., Bennett DA, Blennow K, et al. NIA-AA Research framework: toward a biological definition of Alzheimer's disease. *Alzheimers Dement* 2018; 14: 535–562.
 32. Suárez-Calvet M, Morenas-Rodríguez E, Kleinberger G, et al. Early increase of CSF sTREM2 in Alzheimer's disease is associated with tau related-neurodegeneration but not with amyloid- β pathology. *Mol Neurodegener* 2019; 14: 1.
 33. Xu W, Tan CC, Cao XP, et al. Neuroinflammation modulates the association of PGRN with cerebral amyloid- β burden. *Neurobiol Aging* 2021; 103: 52–59.
 34. Baron RM and Kenny DA. The moderator-mediator variable distinction in social psychological research: conceptual, strategic, and statistical considerations. *J Pers Soc Psychol* 1986; 51: 1173–1182.

35. Mathys J, Gholamrezaee M, Henry H, et al. Decreasing body mass index is associated with cerebrospinal fluid markers of Alzheimer's pathology in MCI and mild dementia. *Exp Gerontol* 2017; 100: 45–53.
36. Loring JF, Wen X, Lee JM, et al. A gene expression profile of Alzheimer's disease. *DNA Cell Biol* 2001; 20: 683–695.
37. Dulewicz M, Kulczyńska-Przybik A and Mroczko B. Neurogranin and VILIP-1 as molecular indicators of neurodegeneration in Alzheimer's disease: a systematic review and meta-analysis. *Int J Mol Sci* 2020; 21: 8335.
38. Lan G, Chen X, Yang J, et al. Microglial reactivity correlates with presynaptic loss independent of β -amyloid and tau. *Ann Neurol* 2024; 95: 917–928.
39. Muszyński P, Kulczyńska-Przybik A, Borawska R, et al. The relationship between markers of inflammation and degeneration in the central nervous system and the blood-brain barrier impairment in Alzheimer's disease. *J Alzheimers Dis* 2017; 59: 903–912.
40. Kester MI, Teunissen CE, Sutphen C, et al. Cerebrospinal fluid VILIP-1 and YKL-40, candidate biomarkers to diagnose, predict and monitor Alzheimer's disease in a memory clinic cohort. *Alzheimers Res Ther* 2015; 7: 59.
41. Lin L, Jeanclos EM, Treuil M, et al. The calcium sensor protein visinin-like protein-1 modulates the surface expression and agonist sensitivity of the $\alpha 4\beta 2$ nicotinic acetylcholine receptor. *J Biol Chem* 2002; 277: 41872–41878.
42. Hamilton K, Morrow K, Markantoni E, et al. Leptin prevents aberrant targeting of tau to hippocampal synapses via PI 3 kinase driven inhibition of GSK3 β . *J Neurochem* 2023; 167: 520–537.
43. Greco SJ, Sarkar S, Johnston JM, et al. Leptin regulates tau phosphorylation and amyloid through AMPK in neuronal cells. *Biochem Biophys Res Commun* 2009; 380: 98–104.
44. McGuire MJ and Ishii M Leptin dysfunction and Alzheimer's disease: evidence from cellular, animal, and human studies. *Cell Mol Neurobiol* 2016; 36: 203–217.
45. Gomes S, Martins I, Fonseca AC, et al. Protective effect of leptin and ghrelin against toxicity induced by amyloid- β oligomers in a hypothalamic cell line. *J Neuroendocrinol* 2014; 26: 176–185.
46. Cova I, Pomati S, Maggiore L, et al. Nutritional status and body composition by bioelectrical impedance vector analysis: a cross sectional study in mild cognitive impairment and Alzheimer's disease. *PLoS One* 2017; 12: e0171331.
47. Morys F, Dadar M and Dagher A. Association between midlife obesity and its metabolic consequences, cerebrovascular disease, and cognitive decline. *J Clin Endocrinol Metab* 2021; 106: e4260–e4274.