# The effects of CSF neurogranin and APOE ε4 on cognition and neuropathology in mild cognitive impairment and Alzheimer's disease

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**Research**

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# Abstract

**Background:** Cerebrospinal fluid (CSF) neurogranin (Ng) has emerged as a promising biomarker for cognitive decline in mild cognitive impairment (MCI) and Alzheimer's disease (AD). Apolipoprotein E ε4 (*APOE* ε4) allele is by far the most consistent genetic risk factor for AD. However, it is not known whether the pathophysiological roles of Ng in MCI or AD are related to *APOE* ε4.

**Methods:** We stratified 250 participants from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database into cognitively normal (CN) ε4 negative (CN ε4-, n = 48), CN ε4 positive (CN ε4+, n = 17), MCI ε4 negative (MCI ε4-, n = 50), MCI ε4 positive (MCI ε4+, n = 72), AD ε4 negative (AD ε4-, n = 17), and AD ε4 positive (AD ε4+, n = 46) according to whether the subjects carried ε4. Spearman correlation was used to test the relationships between biomarkers. Overall diagnostic accuracy (area under the curve (AUC)) was obtained from receiver operating curve (ROC) analyses. Cox proportional hazard models tested the effect of CSF Ng measures on the conversion from CN to MCI or AD and MCI to AD. Relationships between the CSF Ng levels and diagnostic groups were tested with linear regressions. Linear mixed-effects models and linear regression models were used to evaluate CSF Ng as predictors of AD features, including cognition as measured with the Mini-Mental State Examination (MMSE) and Alzheimer's disease assessment scale (ADAS)-cog 11, brain structure as measured by magnetic resonance imaging (MRI), and functional measures of cortical glucose metabolism as measured with 18F-Fluorodeoxyglucose-PET (FDG-PET).

**Results:** CSF Ng levels were significantly increased in *APOE* ε4 carriers compared to *APOE* ε4 non-carriers with MCI. In addition, CSF Ng identified MCI ε4 + versus CN ε4-, but not MCI ε4- versus CN ε4-. Similarly, CSF Ng negatively correlated with MMSE scores at baseline in the MCI ε4 + group. Above phenomena between the *APOE* ε4 carriers and *APOE* ε4 non-carriers with CN and AD did not observe.

**Conclusions:** Our findings support the use of CSF Ng as a biomarker of synaptic pathology for AD. We propose that the roles of CSF Ng in the pathophysiology of MCI may be dependent of *APOE* ε4.


# Background

Alzheimer's disease (AD) is the most prominent cause of dementia and a major health problem all over the world. The main pathological features of AD include extracellular depositions of β-amyloid (Aβ) peptides and intracellular neurofibrillary tangles consisting of hyperphosphorylated tau in the forebrain, while there is almost no neurodegeneration in the cerebellum [1]. The etiology and pathogenesis of AD are still unclear, but more than 15 genome-wide studies have shown that apolipoprotein E ε4 (*APOE* ε4) allele is associated with AD and is by far the most consistent genetic risk factor [2, 3]. Compared with non-carriers, *APOE* ε4 carriers tend to show an accelerated decline in cognitive ability. A large number of evidences show that *APOE* ε4 affects clinical AD to a large extent through Aβ metabolism and triggers a cascade of events, which eventually leads to the formation or propagation of neurofibrillary tangles and cognitive impairment [4–7].

Synaptic dysfunction and degeneration are central events in AD pathology [8]. Synaptic loss has been identified as an early event of AD progression, as well as the basic cause of progressive cognitive decline [8]. Furthermore, compared with Aβ deposits and neurofibrillary tangles, synapse loss is more related to the degree of dementia, especially in certain areas of the brain, such as the hippocampus [9, 10]. Therefore, synaptic proteins are promising tools for early AD diagnosis in addition to monitoring disease progression and evaluating drug effects on synaptic dysfunction and degeneration in clinical trials of disease-modifying therapies for AD. Neurogranin (Ng) is a 78-amino acid-long post synaptic protein,which is enriched in dendritic spines and plays an important role in long-term potentiation and memory consolidation [11–13], where it regulates calmodulin levels in response to the levels of intracellular calcium after neuronal excitation [14–16]. Several studies have shown that the levels of Ng are increased in cerebrospinal fluid (CSF) [17–21] but reduced in the brain of AD patients [22–24]. In the mild cognitive impairment (MCI) patients, high baseline CSF Ng levels predict cognitive decline at clinical follow-up [20]. In addition, high baseline CSF Ng levels in MCI correlate with longitudinal reductions in cortical glucose metabolism and hippocampal volume at clinical follow-up [20]. Furthermore, within the progressive MCI group, elevated CSF Ng levels are associated with accelerated deterioration in Alzheimer's disease assessment scale (ADAS)[20]. Another study has also demonstrated CSF Ng is associated with brain atrophy [25].

However, so far, the relationship between *APOE* ε4 and Ng is poorly understood, and it is not known whether the above-mentioned roles of Ng are related to *APOE* ε4. In this study, we show results on CSF Ng in the Alzheimer's Disease Neuroimaging Initiative (ADNI) cohort, which includes cognitively normal (CN) control, participants with MCI, and dementia due to AD. We verified the specific hypotheses that CSF Ng is increased in *APOE* ε4 positive subjects compared to *APOE* ε4 negative participants in each diagnostic group and CSF Ng reflects neurodegeneration dependently of *APOE* ε4.

# Methods

### Database description

Data used in preparation of this article were obtained from the ADNI database. The ADNI was launched in 2003 as a public-private partnership, led by Principal Investigator Michael W. Weiner, MD. The primary goal of ADNI has been to test whether serial magnetic resonance imaging (MRI), positron emission tomography (PET), biological markers, and clinical and neuropsychological assessments can be combined to measure the progression of MCI and early AD. Further information can be found at http://www.adni-info.org.

From the dataset, we selected all participants between 55–90 (inclusive) years of age who had completed lumbar puncture, Mini–Mental State Examination (MMSE), ADAS-cog 11, Clinical Dementia Rating scale (CDR), MRI, and 18F-Fluorodeoxyglucose-PET (FDG-PET). Selected individuals were classified as CN (n=65), MCI (n=122), and dementia due to AD (n=63) according to clinical and behavioral measures provided by ADNI. Individuals who have at least one ε4 allele were considered as ε4 carriers.

According to whether the subjects carried ε4, they were divided into CN ε4 negative (CN ε4-, n=48), CN ε4 positive (CN ε4+, n=17), MCI ε4 negative (MCI ε4-, n=50), MCI ε4 positive (MCI ε4+, n=72), AD ε4 negative (AD ε4-, n=17), and AD ε4 positive (AD ε4+, n=46).

### Classification criteria

The criteria for CN included an MMSE score ranging between 24–30, and a CDR score of 0 [26, 27]. The criteria for MCI included the presence of a subjective memory complaint, with an MMSE score between 24–30, a CDR of 0.5, preserved activities of daily living, and an absence of dementia [28]. In addition to the NINCDS/ADRDA criteria for probable AD, AD dementia subjects had MMSE scores between 20–26 and a CDR of 0.5 or 1.0 [29]. (Further information about the inclusion/exclusion criteria may be found at www.adni-info.org [accessed July 2020].)

### Standard protocol approvals and patient consents

The ADNI study was approved by the Institutional Review Boards of all the participating institutions. Informed written consent was obtained from all subjects at each center.

### CSF analyses

CSF Aβ42, total-tau (T-tau), and phosphorylated-tau at threonine 181 (P-tau) were measured by using the multiplex xMAP Luminex platform (Luminex Corp, Austin, TX) and Innogenetics INNO-BIA AlzBio3 (Innogenetics, Ghent, Belgium) immunoassay reagents as described previously [30]. CSF Ng was analyzed by electrochemiluminescence technology (Meso Scale Discovery, Gaithersburg, Maryland, USA) using Ng7, which is a monocloncal antibody specific for Ng, as coating antibody and polyclonal Ng anti-rabbit (ab 23570, Upstate) as detector antibody. Values are given as pg/ml. All of the CSF data used in this study were obtained from the ADNI files “UPENNBIOMK5-8.csv” and “BLENNOWCSFNG.csv”, (accessed July 2020). Further details of ADNI methods for CSF acquisition and measurements and quality control procedures can be found at www.adni-info.org.

### Cognitive assessment

To assess the global cognitive performance, we used MMSE and ADAS-Cog 11 scores. Because of the lack of many data during the follow-up period, we only collected MMSE and ADAS-cog 11 scores at baseline. The data used in this study was obtained from the ADNI files “MMSE.csv” and “ADAS_ADNI1.csv”, (accessed July 2020).

### Neuroimaging methods

To investigate neurodegeneration, we used the hippocampal and ventricular volumes. Those data were obtained from the ADNI files “FOXLABBSI_08_04_17.csv” and “UCSDVOL.csv”, (accessed July 2020). All the imaging data were selected at baseline because there were too many missing data during the follow-

up period. The neuroimaging methods used by ADNI have been described previously [31]. Further details for ADNI image acquisition and processing can be found at www.adni-info.org/methods.

### 18F-Fluorodeoxyglucose-PET

ADNI PET image data were acquired at baseline and processed as described online (adni.loni.ucla.edu/about-data-samples/image-data/ and adni.loni.ucla.edu/research/pet-post-processing/, respectively). For detailed instructions, see Landau et al. [7]. Briefly, we used the average PET counts of the lateral and medial frontal, anterior cingulate, posterior cingulate, lateral parietal, and lateral temporal regions [20].

### Statistical methods

Analysis of covariance (ANOVA) and chi-square analyses were performed to test for significant differences between groups on baseline demographics. Associations between the CSF Ng and diagnostic groups were tested with multiple-variable linear regression, adjusted for age and gender. To evaluate whether *APOE* ε4 influenced these associations, we included the interaction between diagnosis and *APOE* ε4 positivity as a predictor in the model.

Spearman correlation was used to test associations between Ng and other core biomarkers. Overall diagnostic accuracy (area under the receiver operator characteristics curve, AUC) was obtained for each biomarker by using Receiver operating curve (ROC) analyses. The differences between two AUCs derived from all pairs of two different variables were tested by using bootstrap methods.

The associations of Ng with the incidence of AD were assessed by calculating hazard ratios (HR) with 95% CIs using Cox proportional hazard regression analysis with adjustment of age, education, and gender. Ng was categorized into two groups by the median of each biomarker when conducting COX proportional hazard regression analysis.

For MMSE, ADAS-cog 11, hippocampal and ventricular volumes, and FDG-PET, intercepts (baseline values) were derived by using linear mixed effects models. The intercept was then used as outcomes in linear regression models with Ng as predictor (adjusted for age and gender; and for education for MMSE and ADAS-cog 11; and for intracranial volume for hippocampal and ventricular volumes) within diagnostic groups. All statistics were done using R (v. 3.4.2) and SPSS version 20. Statistical significance was defined as $p<0.05$ for all analyses.

## Results

### Characteristics of subjects

The demographics and biomarker characteristics of the study participants are listed in Table 1. There were no differences in age and education among the groups. Compared with the CN □4- and AD □4+ groups, there were significantly fewer female subjects in the MCI □4- group. Between CN □4- and CN □4+ and MCI □4- and MCI □4+, Aβ42 levels in *APOE* ε4 positive subjects decreased significantly. There was no

similar phenomenon between AD 4- and AD 4+. Between MCI 4- and MCI 4+, the levels of T-tau and P-tau in *APOE* ε4 positive participants increased significantly. However, there was no similar finding between CN 4- and CN 4+, and between AD 4- and AD 4+. MMSE scores were lower in MCI 4-, MCI 4+, AD 4-, and AD 4+ groups compared with CN 4- and CN 4+ subjects, and lower in AD 4- and AD 4+ groups compared with MCI 4- and MCI 4+. On the contrary, ADAS-Cog 11 scores were higher in MCI 4-, MCI 4+, AD 4-, and AD 4+ groups compared with CN 4- and CN 4+ participants, and higher in AD 4- and AD 4+ groups compared with MCI 4- and MCI 4+ patients.

### CSF Ng levels in *APOE* ε4 positive and negative participants in different diagnostic groups

CSF Ng levels were significantly higher in patients with MCI 4+, AD 4-, and AD 4+ (all $p < 0.001$) compared with CN 4-. Higher Ng levels were also found in both MCI 4+ ($p < 0.05$) and AD 4- ($p < 0.05$) compared with CN 4+. Between MCI 4- and MCI 4+, CSF Ng levels in *APOE* ε4 positive participants increased significantly ($p < 0.001$). However, there were no differences between CN 4- and CN 4+, and similarly between AD 4- and AD 4+ (Fig. 1).

### CSF Ng levels in relation to Aβ and tau

Ng and Aβ42 were negatively correlated in CN 4- participants ($r = -0.353$, $p = 0.014$). However, there were no significant associations between Ng and Aβ42 in CN 4+, MCI 4-, MCI 4+, AD 4-, and AD 4+ subjects ($r = -0.095$, $p = 0.717$; $r = -0.194$, $p = 0.177$; $r = -0.023$, $p = 0.845$; $r = 0.172$, $p = 0.509$; $r = 0.080$, $p = 0.596$, respectively) (Fig. 2A). Ng was strongly correlated with T-tau and P-tau in CN 4- ($r = 0.550$, $p < 0.001$ for T-tau; $r = 0.519$, $p < 0.001$ for P-tau), CN 4+ ($r = 0.858$, $p < 0.001$ for T-tau; $r = 0.841$, $p < 0.001$ for P-tau), MCI 4- ($r = 0.799$, $p < 0.001$ for T-tau; $r = 0.784$, $p < 0.001$ for P-tau), MCI 4+ ($r = 0.746$, $p < 0.001$ for T-tau; $r = 0.726$, $p < 0.001$ for P-tau), AD 4- ($r = 0.869$, $p < 0.001$ for T-tau; $r = 0.906$, $p < 0.001$ for P-tau), and AD 4+ subjects ($r = 0.747$, $p < 0.001$ for T-tau; $r = 0.726$, $p < 0.001$ for P-tau) (Fig. 2B and C).

### Diagnostic accuracy of CSF Ng and core CSF biomarkers

ROC analyses were performed to test CSF biomarkers in relation to clinical diagnoses for MCI 4-, MCI 4+, AD 4-, and AD 4+. All CSF biomarkers had significant diagnostic accuracy for MCI 4+ (Table 2 and Fig. 3B), AD 4- (Table 2 and Fig. 3C), and AD 4+ (Table 2 and Fig. 3D) but not MCI 4- (Table 2 and Fig. 3A) compared with CN 4-. Compared with T-tau and P-tau, Ng had almost the same range of diagnostic accuracy for MCI 4+, AD 4-, and AD 4+ (Table 2 and Fig. 3B-D). However, compared with T-tau and P-tau, the combination of Ng, T-tau or P-tau did not significantly improve the diagnostic accuracy for MCI 4-, MCI 4+, AD 4-, and AD 4+ (Table 2 and Fig. 3A-D).

### CSF Ng predict conversion from CN to MCI or AD and MCI to AD

Among the subjects, 18 CN individuals progressed to MCI or AD and 73 MCI participants progressed to AD during follow-up. We investigated whether CSF Ng predicted conversion from CN to MCI or AD and MCI to AD. Cox proportional hazard models were performed for Ng as a continuous variable. HRs were then calculated for Ng as a dichotomous variable using the median values of Ng as a cutoff (adjusting

for age, education, and gender). CSF Ng did not significantly predict conversion from CN to MCI or AD (Fig. 4A) and MCI to AD (Fig. 4B).

### CSF Ng and *APOE* ε4 in relation to cognition

High CSF Ng levels associated with low MMSE scores at baseline in the MCI 4+ group (β = −0.18, p = 0.036), but not in the MCI 4- and other groups (Fig. 5A). CSF Ng did not correlate with ADAS-cog 11 scores at baseline in every diagnostic group (Fig. 5A). Although there was trend for associations between CSF Ng and with ADAS-cog 11 in the AD 4- group, but this did not reach statistical significance (β = −0.22, p = 0.064) (Fig. 5B).

### CSF Ng and *APOE* ε4 in relation to brain structure

CSF Ng did not correlate with baseline FDG-PET or ventricular volumes in different diagnostic groups (Fig. 6A and B). However, high CSF Ng levels were associated with low hippocampal volumes in the MCI 4- group (β = −0.39, p = 0.007), the MCI 4+ group (β = −0.25, p = 0.036), and the AD 4+ group (β = −0.42, p = 0.003) (Fig. 6C), whereas in the AD 4- and other groups, no such associations were found.

## Discussion

The present study performed a relatively comprehensive assessment about the characteristic of CSF Ng of subjects with MCI or AD from the ADNI database. We have the following main findings: first, the levels of Ng in *APOE* ε4 positive participants increased significantly between MCI 4- and MCI 4+. However, there was no similar finding between CN 4- and CN 4 + and between AD 4- and AD 4+. Secondly, CSF Ng was strongly correlated with T-tau and P-tau but not Aβ42 in every diagnostic group. Our third main finding is that CSF Ng had almost the same range of diagnostic accuracy for MCI 4+, AD 4-, and AD 4 + compared with T-tau and P-tau. However, the combination of Ng, T-tau or P-tau did not significantly improve the diagnostic accuracy. Finally, high CSF Ng levels only associated with low MMSE scores at baseline in the MCI 4 + group. Moreover, CSF Ng correlated with hippocampal volumes at baseline in the MCI 4-, MCI 4+, and AD 4 + groups.

Previous studies have shown that, compared with healthy controls, CSF Ng was significantly increased in progressive MCI and AD, and progressive MCI participants had higher levels of CSF Ng than stable MCI subjects [20, 32]. To investigate whether the levels of CSF Ng can be used to identify MCI and AD subjects with an underlying *APOE* ε4 pathology, each group included in the present study was dichotomized into either *APOE* ε4-positive or *APOE* ε4-negative. The CSF Ng levels were significantly increased in the MCI 4+, AD 4-, and AD 4 + groups compared to CN 4- group. Interestingly, higher CSF Ng levels were also found in the MCI 4 + group than MCI 4- group, but there was no similar finding between CN 4- and CN 4 + and between AD 4- and AD 4+, validating that CSF Ng may be an early pathophysiological marker of AD-related synaptic degeneration [20], and suggesting the roles of CSF Ng in the pathophysiology of MCI may be related to *APOE* ε4.

Previous studies demonstrated that CSF Ng was particularly elevated in patients with MCI and AD with Aβ pathologic features [20, 33]. However, the relationship between CSF Ng and Aβ pathology remains controversial. Some studies have shown that CSF Ng positively or negatively correlated with Aβ42 or Aβ40 in AD patients [17, 19, 21, 34]. Moreover, other researchers have reported that the CSF Ng levels did not correlate to Aβ42 in AD samples [21, 35]. In the present study, except for CN ε4- group, no correlation between CSF Ng and Aβ42 was found in other diagnostic groups. Most likely, it indicates that the degeneration of synapses is weakly related to the axonal damage induced by Aβ. In line with previous studies [17, 19–21, 33–36], we have investigated that elevated levels of CSF Ng were positively associated with levels of T-tau and P-tau in every diagnostic group. Interestingly, T-tau and P-tau levels were significantly increased in *APOE* ε4 carriers compared to *APOE* ε4 non-carriers with MCI, but the levels of T-tau and P-tau between the *APOE* ε4 carriers and *APOE* ε4 non-carriers with CN and AD did not differ. These suggest that synaptic degeneration especially in MCI subjects may be related to the axonal damage induced by tau or *APOE* ε4.

We next sought to test whether CSF Ng could improve the differential diagnosis of MCI and AD dementia when compared to the standard AD biomarkers, CSF T-tau and P-tau. All biomarkers identified MCI ε4 + versus CN ε4-, AD ε4- versus CN ε4-, and AD ε4 + versus CN ε4-, but not MCI ε4- versus CN ε4-, and combinations did not improve the diagnostic accuracy compared with using individual biomarkers. In terms of diagnostic accuracy, the different performance of CSF Ng on MCI ε4- and MCI ε4 + may be due to the fact that less MCI ε4- subjects would progress to AD, while more MCI ε4 + subjects would progress to AD. In addition, unfortunately, our study indicated that CSF Ng did not significantly predict conversion from CN to MCI or AD and MCI to AD, suggesting that CSF Ng may be not sensitive in predicting progression in cognitively normal subjects or MCI patients.

Ng is a post synaptic protein involved in memory consolidation as well as a potential biomarker for cognitive decline and brain injury in AD [13, 37]. However, the correlation between CSF Ng and cognitive evaluation scores as measured by MMSE and ADAS-cog is still inconsistent. De Vos, et al. investigated that no correlations were found between CSF Ng and clinical parameters, such as MMSE scores (at baseline), nor the change in MMSE per year or disease duration in control, MCI, and AD groups [17]. Hellwig, et al also thought that the MMSE scores did not correlate to Ng levels in any of the groups [21]. Portelius, et al. found that CSF Ng did not relate to baseline MMSE and ADAS-cog scores, but high CSF Ng levels correlated significantly to a more rapid elevation in ADAS-cog scores in progressive MCI over time [20]. Mattsson, et al. demonstrated that Ng was associated with worsening MMSE and ADAS-cog scores during the clinical follow-up period in subjects with Aβ positive[34]. In the present study, we only collected MMSE and ADAS-cog 11 scores at baseline because there were so many missing data during the follow-up period. We found that CSF Ng negatively associated with MMSE scores at baseline in the MCI ε4 + group, whereas CSF Ng did not correlate with ADAS-cog 11 scores at baseline in every diagnostic group. This result again suggests that the pathophysiological effects of CSF Ng in MCI may be related to *APOE* ε4. Finally, we tested whether CSF Ng correlated with structural measures of hippocampal and ventricular volumes as measured by MRI and with functional measures of cortical glucose metabolism as measured with FDG-PET. CSF Ng did not correlate with baseline FDG-PET or

ventricular volumes in different diagnostic groups, but CSF Ng was associated with hippocampal volumes in the MCI 4-, MCI 4+, and AD 4 + groups. In this respect, the role of CSF Ng in MCI is not found to be related to *APOE* ε4.

This study has limitations. Firstly, our study did not include non-AD neurodegenerative diseases. In addition, the ADNI database was volunteered by highly educated individuals for research focused on AD research. This may give rise to bias in choice because the study population is a self-selected individual who may have concerns about their cognition. Finally, this study lacks follow-up data and cannot dynamically observe the relevant indicators.

# Conclusions

In conclusion, our findings support the use of CSF Ng as a biomarker of synaptic pathology for AD. CSF Ng levels were significantly increased in *APOE* ε4 carriers compared to *APOE* ε4 non-carriers with MCI. In addition, CSF Ng identified MCI 4 + versus CN 4-, but not MCI 4- versus CN 4-. Similarly, CSF Ng negatively correlated with MMSE scores at baseline in the MCI 4 + group. Above phenomena between the *APOE* ε4 carriers and *APOE* ε4 non-carriers with CN and AD did not observe. We propose that the roles of CSF Ng in the pathophysiology of MCI may be dependent of *APOE* ε4. Future studies will further explore the relationship between *APOE* ε4 and CSF Ng and related mechanisms, providing more evidence for the potential roles of Ng in clinical research, trials and practice of AD and other neurodegenerative diseases.

# Declarations


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## Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Authors' Contributions

YF: study concept, design, analysis and interpretation of data, composition of figures, and manuscript drafting. YG: study design, composition of figures, manuscript drafting, and critical review of manuscript for intellectual content. JL: analysis and interpretation of data. MB: analysis and interpretation of data. HZ: study concept, design, study supervision, and critical review of manuscript for intellectual content. All authors read and approved the final manuscript.

## Ethics approval and consent to participate

The ADNI study was approved by the Institutional Review Boards of all the participating institutions. Informed written consent was obtained from all participants at every center.

## Consent for publication

Not applicable.

## Competing interests

The authors report no competing interests.

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# Abbreviations

Aβ: amyloid-β; AD: Alzheimer's disease; ADAS-cog: Alzheimer's disease assessment scale-cog; ADNI: Alzheimer's disease Neuroimaging Initiative; ANOVA: Analysis of covariance; *APOE*: Apolipoprotein E; AUC: area under the curve; CDR: Clinical Dementia Rating scale; CN: cognitively normal; CSF: cerebrospinal fluid; FDG-PET: 18F-Fluorodeoxyglucose-PET; HR: hazard ratios; MCI: mild cognitive impairment; MMSE: Mini-mental State Examination; MRI: magnetic resonance imaging; NFT: neurofibrillary tangles; Ng: neurogranin; PET: positron emission tomography; ROC: receiver operating curve

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# Tables

Table 1. Main demographics of subjects at baseline.

| Characteristics | CN □4- (n=48) | CN □4+ (n=17) | MCI □4- (n=50) | MCI □4+ (n=72) | AD □4- (n=17) | AD □4+ (n=46) |
|---|---|---|---|---|---|---|
| Age (years) | 74.9 (0.7) | 75.7 (1.4) | 72.9 (1.2) | 73.0 (0.8) | 73.1 (2.3) | 74.2 (1.1) |
| Sex (F %) | 26 (54.2%)[c] | 6 (35.3%) | 14 (28.0%)[a,e] | 31 (43.1%) | 10 (58.8%)[c] | 21 (45.7%) |
| Education (years) | 15.7 (0.4) | 15.7 (0.9) | 15.5 (0.5) | 15.7 (0.3) | 15.7 (0.7) | 14.7 (0.4) |
| Aβ42 (pg/ml) | 1128.4 (53.8)[b,c,d,e,f] | 768.2 (70.0)[a,f] | 902.2 (57.2)[a,d,f] | 632.5 (30.3)[a,c] | 729.5 (67.7)[a,f] | 538.7 (20.7)[a,b,c,e] |
| T-tau (pg/ml) | 215.6 (8.0)[d,e,f] | 267.8 (25.5) [d,e,f] | 273.1 (16.8) [d,e] | 351.9 (14.3)[a,b,c] | 391.2 (42.1)[a,b,c] | 344.1 (15.3)[a,b] |
| P-tau (pg/ml) | 19.9 (0.9)[d,e,f] | 26.4 (2.8)[d,e] | 26.4 (1.9)[d,e] | 36.1 (1.7)[a,b,c] | 39.4 (4.8)[a,b,c] | 34.7 (1.6)[a] |
| MMSE | 29.3 (0.1)[c,d,e,f] | 29.0 (0.2)[c,d,e,f] | 26.8 (0.3)[a,b,e,f] | 26.9 (0.2)[a,b,e,f] | 23.9 (0.4)[a,b,c,d] | 23.1 (0.3)[a,b,c,d] |
| ADAS-cog 11 | 6.4 (0.4)[c,d,e,f] | 7.5 (0.8)[c,d,e,f] | 10.9 (0.7)[a,b,e,f] | 12.4 (0.6)[a,b,e,f] | 18.8 (1.7)[a,b,c,d] | 18.4 (0.8)[a,b,c,d] |

Measurement data are expressed by mean and standard error. P values indicate the values assessed with analyses of variance for each variable except gender, where a contingency chi-square was performed. Post hoc analysis provided significant differences between groups: [a]from CN □4-; [b]from CN □4+; [c]from MCI □4-; [d]from MCI □4+; [e]from AD □4-; [f]from AD □4+. Abbreviations: MMSE, Mini–Mental State Examination; ADAS-cog 11, Alzheimer's disease assessment scale-cog 11; CN, cognitively normal; MCI, mild cognitive impairment; AD, Alzheimer's disease.

Table 2. AUC of CSF biomarkers.

| | Ng | T-tau | P-tau | Ng+T-tau | Ng+P-tau | Ng+T-tau+P-tau |
|---|---|---|---|---|---|---|
| MCI □4- | 0.585 (0.469-0.700) (p=0.148) | 0.606 (0.493-0.720) (p=0.070) | 0.613 (0.498-0.724) (p=0.058) | 0.600 (0.486-0.715) (p=0.087) | 0.612 (0.500-0.725) (p=0.055) | 0.603 (0.489-0.717) (p=0.078) |
| MCI □4+ | 0.808 (0.730-0.887) (p<0.001) | 0.875 (0.812-0.937) (p<0.001) | 0.881 (0.819-0.943) (p<0.001) | 0.876 (0.814-0.938) (p<0.001) | 0.881 (0.820-0.942) (p<0.001) | 0.881 (0.820-0.942) (p<0.001) |
| AD □4- | 0.809 (0.676-0.941) (p<0.001) | 0.827 (0.686-0.968) (p<0.001) | 0.824 (0.677-0.970) (p<0.001) | 0.825 (0.683-0.967) (p<0.001) | 0.819 (0.672-0.965) (p<0.001) | 0.833 (0.699-0.968) (p<0.001) |
| AD □4+ | 0.783 (0.687-0.878) (p<0.001) | 0.879 (0.809-0.949) (p<0.001) | 0.888 (0.821-0.955) (p<0.001) | 0.880 (0.811-0.949) (p<0.001) | 0.889 (0.822-0.956) (p<0.001) | 0.888 (0.821-0.956) (p<0.001) |

Abbreviations: AUC, area under the receiver operator characteristics curve; Ng, neurogranin; MCI, mild cognitive impairment; AD, Alzheimer's

# Figures

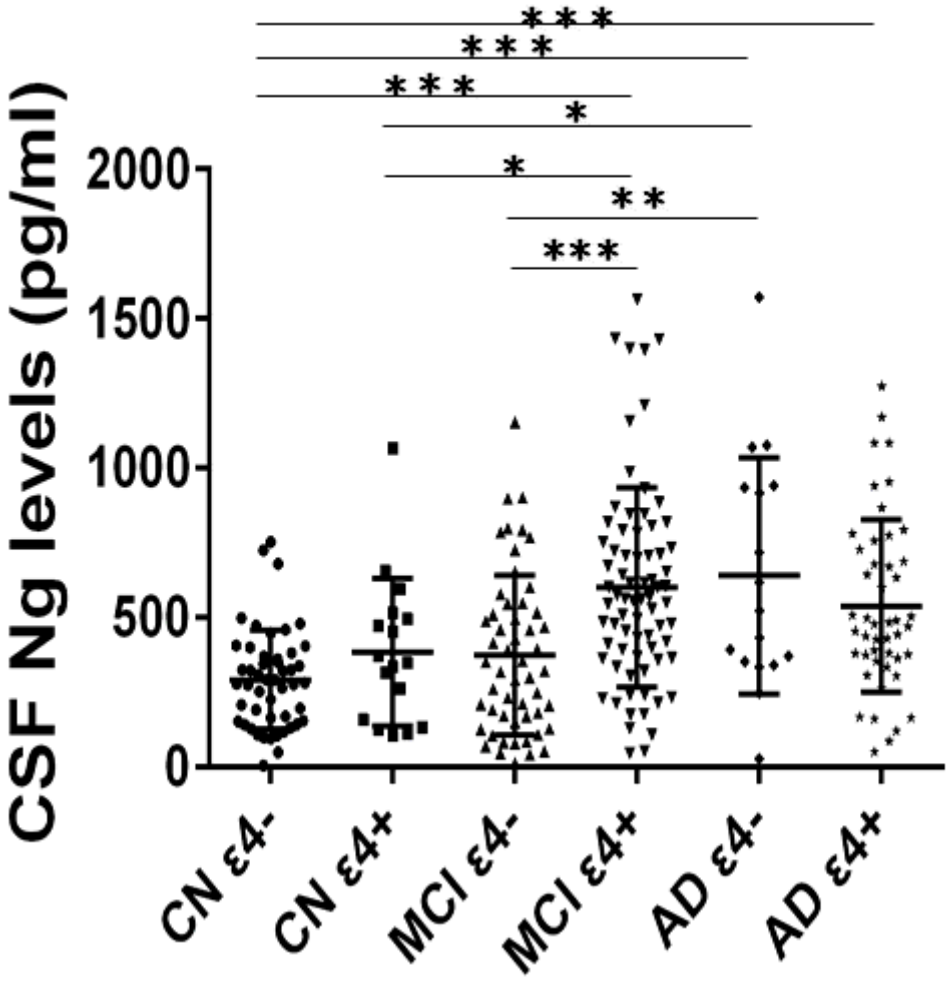



**Figure 1**

CSF Ng levels in different diagnostic groups. CSF Ng levels in different diagnostic groups. Differences between groups were tested by multiple-variable linear regression, adjusted for age and sex. *P<0.05; **P<0.01; ***P<0.0001. Abbreviations: Ng, neurogranin; CN, healthy controls; MCI, mild cognitive impairment; AD, Alzheimer's disease.

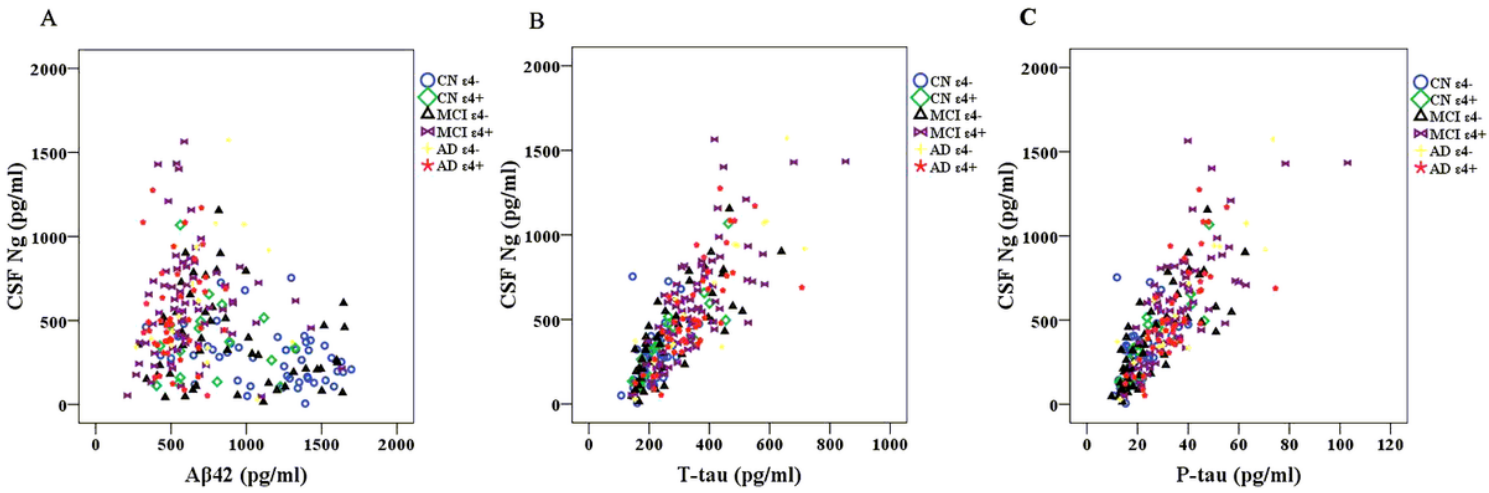
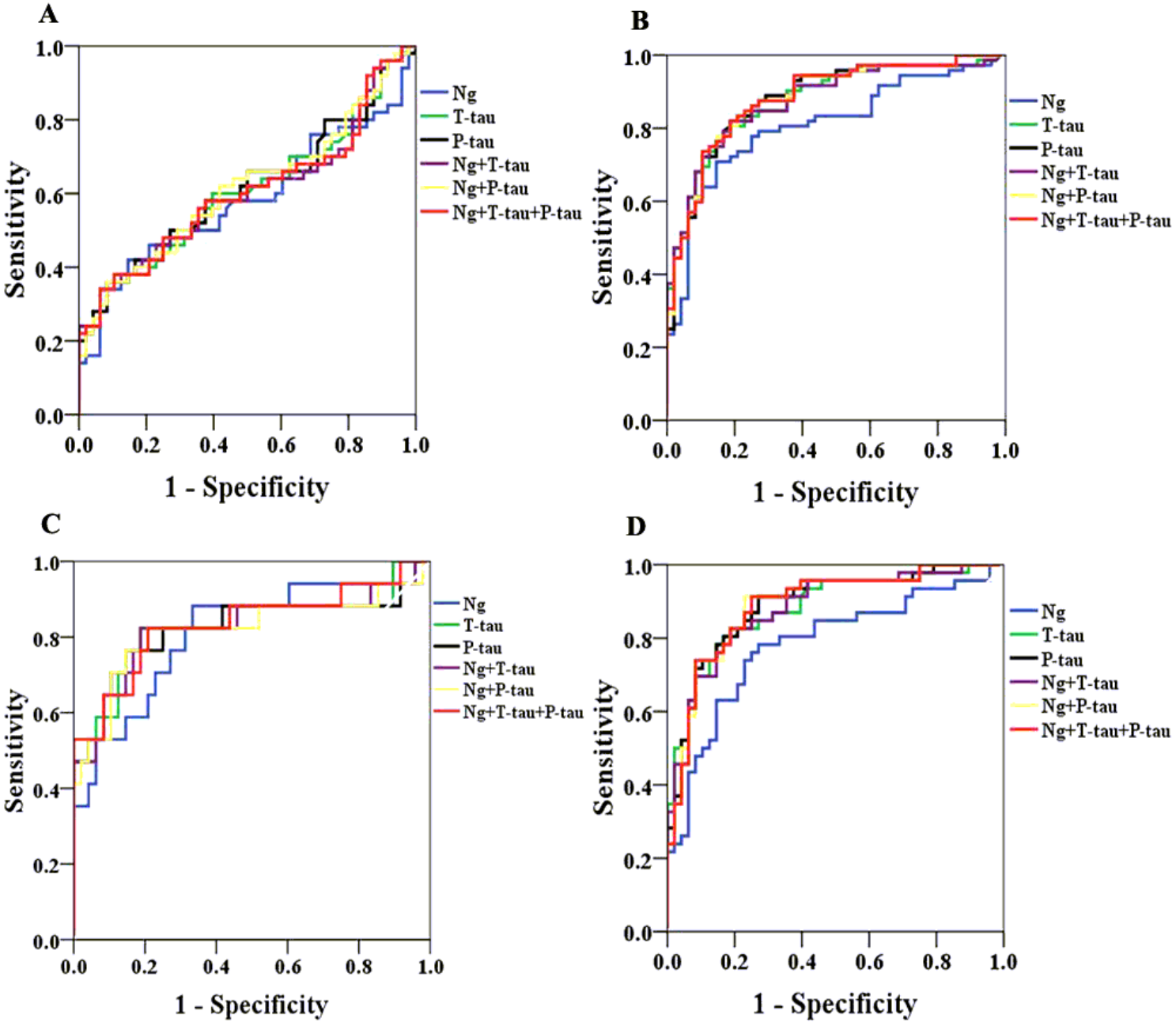


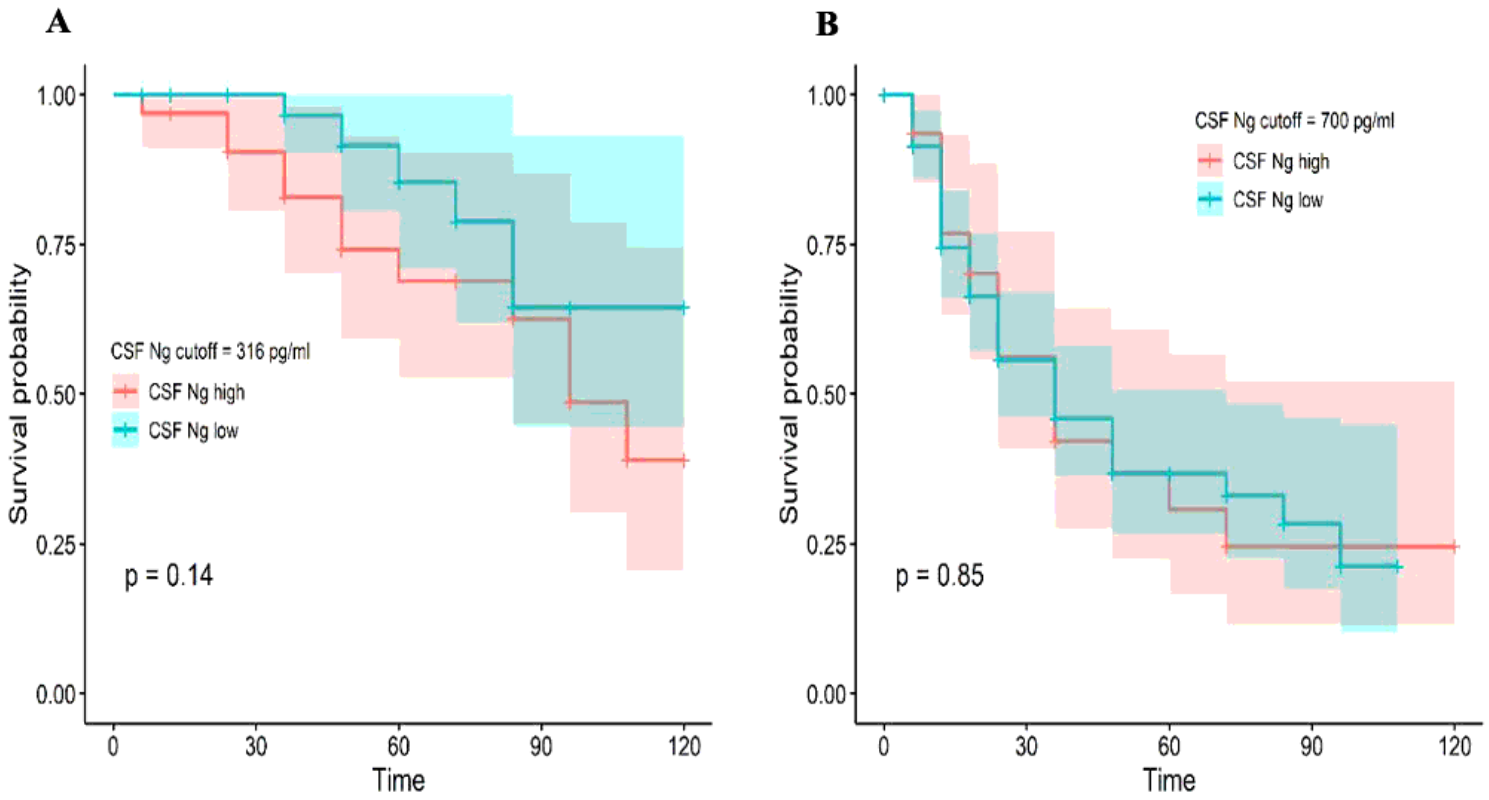

**Figure 2**

CSF Ng in relation to Aβ42 and tau biomarkers. Correlations between CSF Ng and Aβ42 (A) and tau biomarkers (B and C) in different diagnostic groups. Abbreviations: Ng, neurogranin; CN, healthy controls; MCI, mild cognitive impairment; AD, Alzheimer's disease.



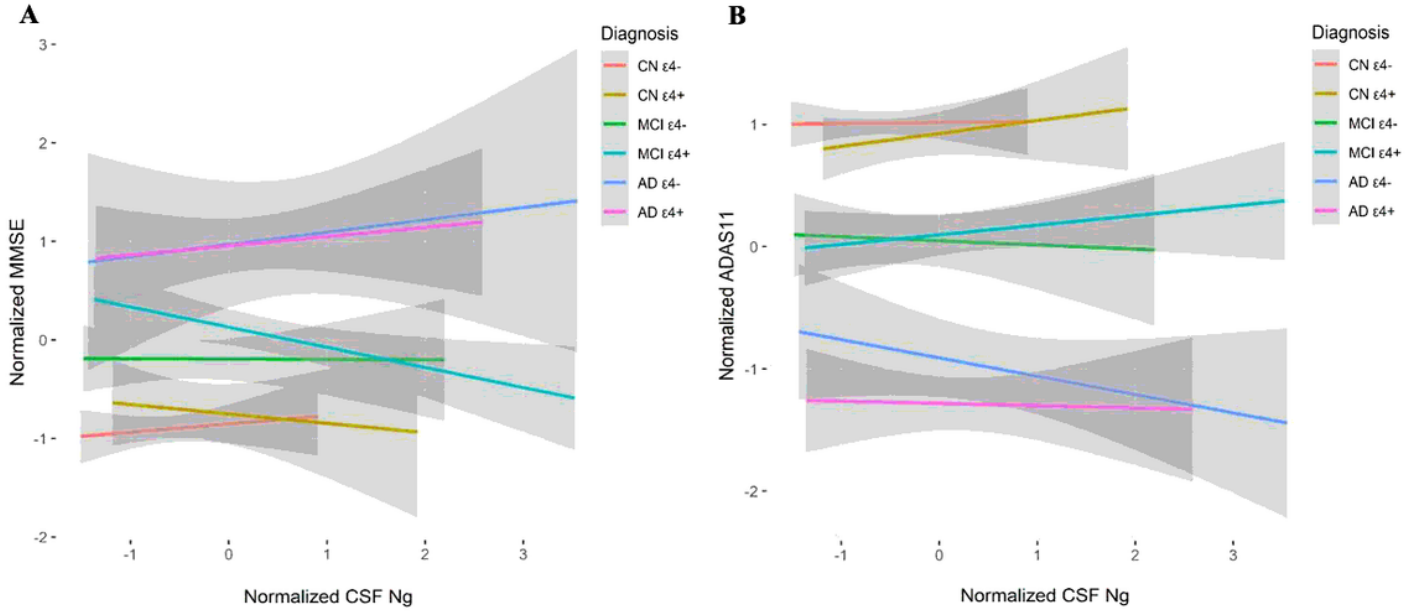

## Figure 3

ROC analyses. ROC analyses were performed to test the CSF biomarkers in relation to clinical diagnoses for MCI □4- (A), MCI □4+ (B), AD □4- (C), and AD □4+ (D). Abbreviations: Ng, neurogranin.



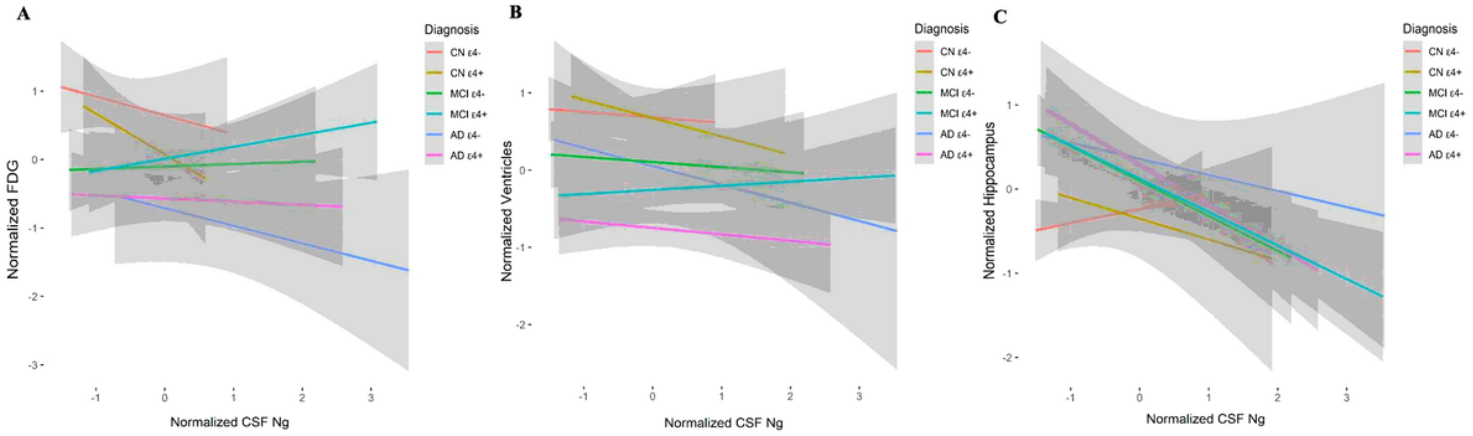

## Figure 4

Baseline CSF measures of Ng as predictors of conversion from CN to MCI or AD and MCI to AD. Conversion from CN to MCI or AD (A) and MCI to AD (B) as a function of CSF Ng measures (dichotomized at the median values) are shown. Analyses were adjusted for age, education, and gender. Cutoff values were 316 pg/ml (CN) and 700 pg/ml (MCI) for Ng. Abbreviations: Ng, neurogranin.




## Figure 5

CSF Ng in relation to cognition. MMSE and ADAS-Cog 11 at baseline (A and B) as a function of baseline CSF Ng in different diagnostic groups. CSF Ng levels are normalized. Abbreviations: CSF, cerebrospinal fluid; Ng, neurogranin; CN, cognitively normal; MCI, mild cognitive impairment; AD, Alzheimer's disease.




## Figure 6

CSF Ng in relation to brain structure and metabolism. FDG, ventricular volumes, and hippocampal volumes at baseline (A, B, and C) as a function of baseline CSF Ng in different diagnostic groups. CSF Ng levels are normalized. Abbreviations: CSF, cerebrospinal fluid; Ng, neurogranin; FDG, 18F-Fluorodeoxyglucose; CN, cognitively normal; MCI, mild cognitive impairment; AD, Alzheimer's disease.