HHS Public Access

Author manuscript

JAMA Neurol. Author manuscript; available in PMC 2017 May 01.

Published in final edited form as:

JAMA Neurol. 2016 May 1; 73(5): 561–571. doi:10.1001/jamaneurol.2016.0086.

Diagnostic and Prognostic Utility of the Synaptic Marker Neurogranin in Alzheimer Disease

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Abstract

IMPORTANCE—Synaptic loss is an early pathologic substrate of Alzheimer disease (AD). Neurogranin is a postsynaptic neuronal protein that has demonstrated utility as a cerebrospinal fluid (CSF) marker of synaptic loss in AD.

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Author Contributions: Dr Tarawneh had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
Study concept and design: Tarawneh, D'Angelo, Ladenson, Morris, Holtzman.
Acquisition, analysis, or interpretation of data: All authors.
Drafting of the manuscript: Tarawneh, Holtzman.
Critical revision of the manuscript for important intellectual content: All authors.
Statistical analysis: Tarawneh, D'Angelo.
Obtained funding: Ladenson, Morris, Holtzman.
Administrative, technical, or material support: Crimmins, Herries, Griest, Fagan, Zipfel, Ladenson, Morris, Holtzman.
Study supervision: Tarawneh, Morris, Holtzman.

Conflict of Interest Disclosures: Dr Holtzman reported being a cofounder of and serving on the scientific advisory board of C2N Diagnostics and reported serving as a consultant for Genentech, AstraZeneca, AbbVie, Denali, and NeuroPhage. Dr Fagan reported serving on the scientific advisory boards of IBL International and Roche and working as a consultant for AbbVie and DiamR. Dr Ladenson reported being a coinventor of patents filed by Washington University concerning brain biomarkers. These patents are being managed by Washington University in accordance with university policy. Dr Morris reported previously or currently participating in the following clinical trials of antidementia drugs: SNIFF (Study of Nasal Insulin to Fight Forgetfullness) and A4 (Anti-Amyloid Treatment in Asymptomatic Alzheimer's Disease). Dr Morris reported serving as a consultant for Lilly USA and Takeda Pharmaceuticals. No other disclosures were reported.

OBJECTIVE—To investigate the diagnostic and prognostic utility of CSF neurogranin levels in a large, well-characterized cohort of individuals with symptomatic AD and cognitively normal controls.

DESIGN, SETTING, AND PARTICIPANTS—A cross-sectional and longitudinal observational study of cognitive decline in patients with symptomatic AD and cognitively normal controls was performed. Participants were individuals with a clinical diagnosis of early symptomatic AD and cognitively normal controls who were enrolled in longitudinal studies of aging and dementia at the Charles F. and Joanne Knight Alzheimer Disease Research Center, Washington University School of Medicine, from January 21, 2000, through March 21, 2011. Data analysis was performed from November 1, 2013, to March 31, 2015.

MAIN OUTCOMES AND MEASURES—Correlations between baseline CSF biomarker levels and future cognitive decline in patients with symptomatic AD and cognitively normal controls overtime.

RESULTS—A total of 302 individuals (mean [SE] age, 73.1 [0.4] years) were included in this study (95 patients [52 women and 43 men] with AD and 207 controls [125 women and 82 men]). The CSF neurogranin levels differentiated patients with early symptomatic AD from controls with comparable diagnostic utility (mean [SE] area under the receiver operating characteristic curve, 0.71 [0.03]; 95% CI, 0.64–0.77) to the other CSF biomarkers. The CSF neurogranin levels correlated with brain atrophy (normalized whole-brain volumes: adjusted $r = -0.38$, $P = .02$; hippocampal volumes: adjusted $r = -0.36$, $P = .03$; entorhinal volumes: adjusted $r = -0.46$, $P = .006$; and parahippocampal volumes: adjusted $r = -0.47$, $P = .005$, n = 38) in AD and with amyloid load ($r = 0.39$, $P = .02$, n = 36) in preclinical AD. The CSF neurogranin levels predicted future cognitive impairment (adjusted hazard ratio, 1.89; 95% CI, 1.29–2.78; $P = .001$ as a continuous measure, and adjusted hazard ratio, 2.78; 95% CI, 1.13–5.99; $P = .02$ as a categorical measure using the 85th percentile cutoff value) in controls and rates of cognitive decline (Clinical Dementia Rating sum of boxes score: β estimate, 0.29; $P = .001$; global composite scores: β estimate, −0.11; $P = .001$; episodic memory scores: β estimate, −0.18; $P < .001$; and semantic memory scores: β estimate, −0.06; $P = .04$, n = 57) in patients with symptomatic AD over time, similarly to the CSF proteins VILIP-1, tau, and p-tau181.

CONCLUSIONS AND RELEVANCE—The CSF levels of the synaptic marker neurogranin offer diagnostic and prognostic utility for early symptomatic AD that is comparable to other CSF markers of AD. Importantly, CSF neurogranin complements the collective ability of these markers to predict future cognitive decline in cognitively normal individuals and, therefore, will be a useful addition to the current panel of AD biomarkers.

The aggregation and deposition of amyloid-β (Aβ) and tau, the 2 key proteins involved in Alzheimer disease (AD) pathogenesis, are estimated to begin years before the onset of cognitive impairment[1,2] However, the first signs of cognitive impairment only appear after significant neuronal and synaptic loss has occurred in vulnerable brain regions[3] Neuronal and synaptic loss reflects the cumulative outcome of different pathologic substrates in AD and, therefore, may provide the best surrogate for clinical and radiologic disease progression[2,4–7]

Synaptic dysfunction is an early and prominent pathologic feature of AD that precedes frank neuronal loss in several brain regions[8–12] Cortical synaptic density is reduced by 25% to 30% and synaptic density per neuron by 15% to 35% in even the earliest symptomatic stages of the disease[5,7] Presynaptic, synaptic, and postsynaptic protein expression levels are reduced in postmortem AD brains compared with controls[13,14]

Neurogranin is a calmodulin-binding postsynaptic[15] neuronal protein[16] that is abundantly expressed in perikaryal and dendritic cytoplasm[15] Neurogranin is thought to be involved in activity-dependent synaptic plasticity and long-term potentiation through the modulation of calcium-mediated signaling pathways[17–19] Because of its abundant and preferential neuronal expression, neurogranin has been identified as a potential marker of neurodegeneration in large-scale gene arrays,[20] along with other candidate markers, such as visinin-like protein-1 (VILIP-1)[21–23] Previous studies suggest that cerebrospinal fluid (CSF) neurogranin levels are elevated in AD[24] and predict conversion from mild cognitive impairment (MCI) to AD dementia[25–27]

We investigate the diagnostic and prognostic utility of CSF neurogranin levels in a large cohort of well-characterized individuals with early AD and controls who were followed up for 2 to 3 years. Our results are consistent with previous reports[25,26] of increased CSF neurogranin levels in AD. Furthermore, we found that CSF neurogranin levels correlate with whole-brain and regional atrophy in AD and with amyloid load in preclinical AD. Importantly, in our cohort, CSF neurogranin levels predicted rates of cognitive decline in patients with early symptomatic AD and future cognitive impairment in cognitively normal controls similarly to the CSF proteins VILIP-1, tau, and p-tau181 over time.

Methods

Participants

Participants were community-dwelling volunteers enrolled in longitudinal studies of healthy aging and dementia through the Charles F. and Joanne Knight Alzheimer Disease Research Center, Washington University School of Medicine, from January 21, 2000, through March 21, 2011. Data analysis was performed from November 1, 2013, to March 31, 2015. All participants in this study were included in a previous study of CSF VILIP-1 in AD to allow comparison of markers (eMethods in the Supplement)[21,22] Participants were in good general health with no other medical illness that could contribute to dementia and no contraindication to lumbar puncture (LP) or magnetic resonance imaging (MRI). *APOE* genotypes were obtained as previously described[28]

The Clinical Dementia Rating (CDR) was used to denote the presence or absence of symptomatic AD and, when present, its severity[29,30] A CDR score of 0, indicating no dementia, characterizes individuals who are cognitively normal. In the cohort being studied, a CDR score of 0.5 denotes very mild symptomatic AD (encompassing MCI caused by AD[31]), whereas a CDR score of 1 and a CDR score of 2 denote mild and moderate symptomatic AD,[32] respectively. Annual clinical assessments included assignment of CDR, CDR sum of boxes (CDR-SB),[33] Mini-Mental State Examination,[34] and a 1.5-hour psychometric test battery (eMethods in the Supplement)[22,30] The CDR scores and clinical

diagnoses were based on the cognitive assessment closest to the time of the LP (median interval, 3.4 months).

For comparison, research participants with a clinical diagnosis of frontotemporal lobar degeneration, progressive supranuclear palsy, or Lewy body dementia at the University of California, San Francisco (UCSF) Memory and Aging Center were included in this study.

All clinical diagnoses were made in accordance with standard criteria[35–40] Studies were approved by the Human Research Protection Office at Washington University and the UCSF Committee on Human Research. Written informed consent was obtained from all participants. All data were deidentified.

CSF Collection and Processing

The CSF samples (20–30 mL) were collected from all participants and analyzed for total tau, p-tau181, and Aβ1–42 (Aβ42) by enzyme-linked immunosorbent assays (Innotest, Fujire-bio [formerly Innogenetics])[41] The CSF samples were analyzed for VILIP-1 by a microparticle-based immunoassay (Erenna, Singulex)[21,22]

The CSF neurogranin levels were measured using a 2-site immunoassay that uses an affinity-efficient trapping and purification technique for polyclonal antibodies developed in the Laboratory of Jack H. Ladenson, PhD, Department of Pathology and Immunology, Washington University School of Medicine, St Louis, Missouri (eMethods in the Supplement)[42]

Regional and Whole-Brain Volumetry

A subset of the control and AD cohorts underwent MRI within 1.1 years of their LP (median interval, 1.7 months) (eMethods in the Supplement)[43,44]

In Vivo Amyloid Imaging

A subset of the control and AD cohorts underwent amyloid imaging via positron emission tomography (PET) using Pittsburgh Compound B (PiB) within 1.1 years of their LP (median interval, 2.7 months) (eMethods in the Supplement)[45]

Statistical Analysis

Analysis of variance, *t* tests, Fisher exact tests, Wilcoxon rank sum tests, or χ^2 tests were used to assess differences in demographic, clinical, genotype, MRI, or CSF biomarker variables between the clinical groups. The Bonferroni correction was performed for all multiple comparisons. Receiver operating characteristic curve analyses assessed rates of agreement between CSF biomarkers and clinical diagnoses or PiB-positivity (SPSS, statistical software, version 15; SPSS Inc).

Pearson correlations examined associations among CSF biomarkers and between CSF biomarkers and mean cortical binding potential (MCBP) on PET-PiB (SPSS statistical software, version 15). Partial correlations examined associations between CSF and MRI measures, adjusting for age, sex, and scanner type (SPSS statistical software, version 15).

Cox proportional hazards regression models tested the effects of CSF biomarkers, individually or in combination (using principal components analyses), on the conversion rate from a CDR score of 0 to a CDR score of 0.5 or higher (SAS Institute, Inc). The CSF biomarker measures were analyzed as continuous and categorical (dichotomized at the 85th percentile value) variables, adjusting for age, sex, educational level, and *APOE* ε4 genotype. The bootstrap method was used to compare CSF biomarkers (individually or in combination) as predictors of conversion in nonnested models[46,47] (R Project for Statistical Computing, R Foundation).

Mixed linear models (PROC MIXED, SAS Institute Inc) tested the ability of CSF biomarkers to predict annual change in CDR-SB, global, episodic memory, semantic memory, working memory, or visual-spatial composite scores in AD over time (SAS statistical software, version 9.2). Analyses were adjusted for age, educational level, sex, *APOE* ε4 genotype, and baseline dementia severity (eMethods in the Supplement). Statistical significance was defined as $P < .05$ for all analyses.

Results

Participants

A total of 302 individuals (mean [SE] age, 73.1 [0.4] years) were included in this study (95 patients with AD and 207 controls). Of the 302 participants from the Charles F. and Joanne Knight Alzheimer Disease Research Center, Washington University School of Medicine, included in this study, 221 participants (164 controls and 57 patients with AD) had more than 1 annual cognitive assessment during follow-up. For comparison, 19 research participants with a clinical diagnosis of frontotemporal lobar degeneration (n = 11), progressive supranuclear palsy (n = 7),or Lewy body dementia(n = 1) at the UCSF Memory and Aging Center were included in this study.

The demographic, clinical, psychometric, genotype, and CSF biomarker characteristics of the study participants are summarized in the Table. Individuals with symptomatic AD were older than controls and included a higher percentage of individuals with the *APOE* ε4 genotype or with cortical amyloid binding on PET-PiB. Baseline Mini-Mental State Examination and psychometric composite scores were lower and baseline CDR-SB scores were higher in patients with AD than in controls. The CSF neurogranin levels did not differ by age or sex in this cohort (eResults in the Supplement).

Participants with very mild (CDR score of 0.5, n = 70),mild (CDR score of 1, n = 22), and moderate (CDR score of 2, n = 3) symptomatic AD exhibited the typical CSF biomarker phenotype of AD with higher mean levels of CSF tau, p-tau181, tau/Aβ42, and p-tau181/Aβ42 and lower mean levels of CSF Aβ42 compared with controls (Figure 1 and eFigure 1 in the Supplement). As previously reported in this cohort, mean CSF VILIP-1 and CSF VILIP-1/Aβ42 levels were higher in patients with AD than in controls.

Diagnostic Utility of CSF Neurogranin in AD

Mean (SE) CSF neurogranin levels were higher in those with CDR scores of 0.5 (2.04 [0.12] ng/mL, n = 70) and CDR scores of 1 or higher (1.98 [0.18] ng/mL, n = 25) compared with

those with CDR scores of 0 (1.47 [0.06] ng/mL, n = 207) ($P < .001$) or those with non-AD dementias (1.08 [0.23] ng/mL, n =19) ($P < .001$). Similarly, mean (SE) CSF neurogranin/Aβ42 levels were higher in those with CDR scores of 0.5 (0.006 [0.0005], n = 67) and CDR scores of 1 or higher (0.007 [0.001], n = 25) compared with those with CDR scores of 0 (0.003 [0.0002], n = 196) ($P < .001$) or those with non-AD dementias (0.0013 [0.0002], n = 19) ($P < .001$). No significant differences in mean neurogranin or neurogranin/Aβ42 levels were observed among the CDR categories in the AD cohort (Figure 1). The diagnostic accuracy (area under the receiver operating characteristic curve [AUC]) of CSF neurogranin in differentiating patients with AD from controls was comparable to that of the other markers (Figure 1E). The mean (SE) AUCs were 0.85 (0.02) for tau, 0.81 (0.03) for p-tau181, 0.77 (0.03) for Aβ42, 0.74 (0.03) for VILIP-1, and 0.71 (0.03) for neurogranin. The mean (SE) AUCs for the CSF marker ratios to Aβ42 were 0.88 (0.02) for tau/Aβ42, 0.86 (0.02) for p-tau181/Aβ42, 0.85 (0.02) for VILIP-1/Aβ42, and 0.81 (0.03) for neurogranin/Aβ42 (eResults in the Supplement).

The CSF neurogranin levels predicted PiB status with comparable utility to that of the other CSF biomarkers, irrespective of clinical diagnoses (Figure 1F). The mean (SE) AUC was 0.86 (0.03) for tau, 0.81 (0.04) for p-tau181, 0.87 (0.03) for Aβ42, 0.77 (0.04) for VILIP-1, and 0.73 (0.04) for neurogranin. The mean (SE) AUCs for the CSF marker ratios to Aβ42 were 0.95 (0.02) for tau/Aβ42, 0.95 (0.02) for p-tau181/Aβ42, 0.93 (0.02) for VILIP-1/Aβ42, and 0.89 (0.03) for neurogranin/Aβ42. The CSF neurogranin differentiated PiB-positive from PiB-negative individuals with a sensitivity of 79% and a specificity of 60%. The ratios of CSF tau, p-tau181, VILIP-1, and neurogranin to CSF Aβ42 levels provided higher diagnostic accuracy than each marker alone (respectively) and higher diagnostic accuracy for PiB status than for clinical diagnoses (Figure 1 and eTable 1 in the Supplement).

Correlation of CSF Neurogranin With CSF and Imaging Markers of AD

The CSF neurogranin levels correlated with CSF VILIP-1 ($r = 0.76$ and $r = 0.83$), tau ($r = 0.81$ and $r = 0.77$), and p-tau181 ($r = 0.80$ and $r = 0.77$) levels in patients with AD and controls, respectively ($P < .001$). No correlations were observed between CSF neurogranin levels and CSF Aβ42 levels in patients with AD ($r = −0.03$, $P = .77$) or controls ($r = 0.12$, $P = .10$) (Figure 2 and eFigure 2 in the Supplement). The CSF neurogranin levels negatively correlated with baseline normalized whole-brain ($r = −0.38$, $P = .02$), hippocampal ($r = −0.36$, $P = .03$), entorhinal ($r = −0.46$, $P = .006$), and parahippocampal volumes ($r = −0.47$, $P = .005$) in AD (n = 38), adjusting for age, sex, and scanner type (Figure 2 and eTable 2 in the Supplement). No correlations between the CSF neurogranin levels and brain volumes were observed in controls (n = 144) (eResults in the Supplement).

The CSF neurogranin levels correlated with MCBP on PET-PiB in the combined ($r = 0.28$, $P < .001$, n = 152) (Figure 2) and control cohorts ($r = 0.29$, $P = .001$, n = 128) but not in the AD cohort ($r = −0.1$, $P = .68$, n = 24). The CSF neurogranin/Aβ42 levels correlated with MCBP in the AD ($r = 0.54$, $P = .01$) and control ($r = 0.65$, $P < .001$) cohorts. The CSF neurogranin levels correlated with MCBP ($r = 0.39$, $P = .02$) in PiB- positive, cognitively normal controls (ie, MCBP ≥0.18, n = 36) (Figure 2).

Ability of CSF Neurogranin Levels to Predict Future Cognitive Impairment in Controls

Cox proportional hazards regression models assessed the ability of CSF biomarkers (as continuous or categorical variables) to predict future cognitive impairment in cognitively normal controls over time (eTable 3 in the Supplement), adjusting for age, sex, educational level, and *APOE* ε4 genotype (eTable 4 in the Supplement and Figure 3). Analyses included cognitively normal controls who had at least 1 follow-up annual cognitive assessment (n=164). Of these, 26 participants (15.9%) progressed from CDR scores of 0 to a CDR score of 0.5 or higher during follow-up. With the exception of CSF Aβ42, all CSF biomarkers predicted conversion from a CDR score of 0 to a CDR score of 0.5 or higher during follow-up (eTable 4 in the Supplement). The CSF neurogranin (adjusted hazard ratio, 1.89; 95% CI, 1.29–2.78; $P = .001$) and neurogranin/Aβ42 (adjusted hazard ratio, 27.9; 95% CI, 6.93–112.1; $P < .001$) levels predicted conversion from a CDR score of 0 to a CDR score of 0.5 or higher over time. Individuals whose neurogranin or neurogranin/Aβ42 levels were in the upper 15th percentile of values progressed more rapidly to cognitive impairment than individuals whose levels were in the lower 85th percentile (Figure 3).

Results from the bootstrap analyses indicate that the predictive ability for future cognitive impairment was 0.890 ($P = .001$) for neurogranin, 0.892 ($P = .001$) for VILIP-1,0.866 ($P = .002$) for tau, 0.452 ($P = .04$) for p-tau181, 0.328 ($P = .11$) for Aβ42, 0.993 ($P < .001$) for neurogranin/Aβ42, 0.998 ($P < .001$) for VILIP-1/Aβ42,0.974 ($P < .001$) for tau/Aβ42,and 0.902 ($P = .002$) for p-tau181/Aβ42. The combinations of CSF neurogranin and tau (0.885, $P = .001$) and of CSF neurogranin and p-tau181 (0.758, $P = .007$) were stronger predictors of conversion than tau (0.866, $P = .002$) or p-tau181 (0.452, $P = .04$) alone, respectively. When neurogranin was added to the combination of VILIP-1, tau, and p-tau181, the 4 markers together were stronger predictors of conversion (0.869, $P = .002$) than the combination of VILIP-1, tau, and p-tau181 (0.844, $P = .002$). When neurogranin was added to the combination of VILIP-1, tau, p-tau181, and Aβ42, the combination of all 5 markers (0.859, $P = .002$) was a stronger predictor of conversion than the combination of VILIP-1, tau, p-tau181, and Aβ42 (0.826, $P = .002$).

Ability of CSF Neurogranin Levels to Predict Rates of Cognitive Decline in AD

All CSF biomarkers except CSF Aβ42 predicted annual change in CDR-SB, global, episodic, and semantic memory scores in patients with symptomatic AD (n = 57) during follow-up (eTable 5 in the Supplement and Figure 4). Baseline CSF neurogranin levels (as continuous measures) predicted annual change in CDR-SB (β estimate, 0.29, $P = .001$), global (β estimate, −0.11, $P = .001$), episodic memory (β estimate, −0.18, $P < .001$), and semantic memory (β estimate, −0.06, $P = .04$) scores. Baseline CSF neurogranin/Aβ42 levels predicted annual change in CDR-SB (β estimate, 0.27, $P = .001$), global (β estimate, −0.13, $P < .001$), episodic memory (β estimate, −0.16, $P < .001$), and semantic memory (β estimate, −0.06, $P = .02$) scores. Individuals with AD whose CSF neurogranin or neurogranin/Aβ42 levels were in the upper tercile (corresponding to a CSF neurogranin level ≥2.0 ng/mL and a neurogranin/Aβ42 level >0.007) progressed more rapidly in CDR-SB($P = .03$ and $P = .02$, respectively), global ($P = .02$ and $P < .001$, respectively), and episodic memory ($P < .001$ and $P = .001$,respectively) scores than those in the lower 2 terciles (eTable 5 in the Supplement).

Discussion

Neurogranin is a calmodulin-binding[16] postsynaptic neuronal protein[15] that is abundantly expressed in neuronal perikarya and dendritic spines[15,16] Studies suggest that neurogranin is involved in synaptic plasticity, synaptic regeneration, and long-term potentiation through the modulation of calcium- and calmodulin-signaling pathways[17–19] and plays an important role in memory and learning[16,48–51]

Neurogranin has been proposed as a potential marker of synaptic injury in large-scale gene arrays[20] because of its preferential neuronal expression and widespread distribution in different brain regions[52] Pathologic studies[13,14,53] indicate that neurogranin immunoreactivity is reduced in patients with early symptomatic AD compared with controls. Because expression levels of other synaptic proteins are also decreased in AD[13,14] and correlate with dementia severity,[6,53–55] reduced tissue neurogranin levels in AD are thought to reflect synaptic degeneration and loss of whole synaptic elements in the presence of AD[13,14] The extracellular release of synaptic elements as a result of AD-associated synaptic degeneration likely explains previous reports[25,26] of increased CSF neurogranin levels in AD.

We confirm the diagnostic utility of CSF neurogranin in a large, well-characterized cohort of AD and controls using a highly sensitive immunoassay developed in the Laboratory of Jack H. Ladenson, PhD, Department of Pathology and Immunology, Washington University School of Medicine in St Louis, St Louis, Missouri. Furthermore, we found that CSF neurogranin levels correlate with brain atrophy in AD, with amyloid load in preclinical AD, and with other CSF markers of AD in patients with AD and controls. Importantly, we report for the first time, to our knowledge, that CSF neurogranin predicts future cognitive impairment in cognitively normal controls as well as the other CSF biomarkers and complements their predictive ability (collectively) for future cognitive impairment during a 2- to 3-year follow-up period.

In our cohort, the diagnostic utility of CSF neurogranin in differentiating patients with AD from controls was comparable to that of other CSF markers. Because most of our AD cohort includes individuals with very mild dementia (CDR score of 0.5), some of whom may elsewhere be classified as having MCI or pre-MCI, neurogranin may be a useful diagnostic marker for even the earliest symptomatic stages of the disease. The diagnostic accuracy of all CSF biomarkers and ratios was higher in relation to PiB status than in relation to clinical diagnoses, supporting the potential value of CSF biomarkers in identifying AD irrespective of clinical status.

Synaptic loss or dysfunction is an early and primary pathologic substrate of AD[8–12] Cognitive deficits attributed to synaptic dysfunction occur in the absence of, or even before, neuronal loss in AD[55] Cortical synaptic density is reduced by as much as 35% in even the earliest stages of AD[56] and reflects neuronal loss and reduced synaptic density of viable neurons[57] Synaptic loss is a good surrogate for cognitive decline and disease progression in AD[5,7,10,58] because it appears to be more closely correlated with cognitive deficits than the

numbers of plaques or tangles or extent of cortical gliosis in pathologic studies[5,7] of postmortem AD brains.

We found that baseline CSF neurogranin, VILIP-1,[21,22] tau, and p-tau181,[59–62] but not CSF Aβ42,[61,63,64] levels predict future cognitive impairment in cognitively normal controls and rates of cognitive decline in patients with symptomatic AD over time. Importantly, CSF neurogranin levels predicted future cognitive impairment in cognitively normal controls similarly to other CSF markers of AD in this cohort and complemented their collective predictive ability for future cognitive decline. These findings are consistent with previous reports[65–67] and proposed models of disease progression that suggest the presence of significant synaptic disease years before symptom onset[2,68,69] Although CSF Aβ42 levels begin to decrease a decade or more before cognitive impairment,[1,2,41,70] they do not change substantially over time once this low set point has been reached[71,72] On the other hand, ongoing deposition of neurofibrillary tangles and progressive neuronal or synaptic loss accompany disease progression into the symptomatic stages[2,68–70] Associations of neurofibrillary tangle load,[73–75] synaptic disease,[6,9] and neuronal loss,[76,77] but not cortical amyloid burden,[63,78] with rates of cognitive decline have previously been reported. Therefore, CSF markers of neuronal or synaptic loss or tau disease are more closely associated with future cognitive decline than CSF markers of amyloid disease during short follow-up periods.

The CSF neurogranin levels correlate with brain atrophy in our AD cohort, with higher CSF neurogranin levels indicating more severe synaptic disease that accompanies brain volume loss in AD[79] The correlation of CSF neurogranin, VILIP-1, tau, and p-tau181[21,80] with amyloid load in preclinical AD is consistent with the notion that increasing amyloid deposition[2,81] is associated with ongoing neuronal or synaptic loss before symptom onset. Because cortical amyloid deposition likely plateaus near the time of symptom onset,[70,82] no correlations were observed between these CSF biomarkers and amyloid load in AD.

Synaptic disease in patients with AD is predominantly observed in the neuropil with no clear relation to amyloid plaques[10,83,84] or neurofibrillary tangles[14,58,84,85] Our findings that neurogranin immunoreactivity is detected in neuronal perikarya independently of neurofibrillary tangles and amyloid deposition are consistent with this notion. Associations between CSF neurogranin and CSF VILIP-1, tau, or p-tau181 levels in our cohort are likely caused by the ability of these markers to measure neurodegeneration because neither VILIP-1[21] nor neurogranin appears to be a component of neurofibrillary tangles. Furthermore, chronic alterations in synaptic input may influence the degree of phosphorylation of cytoskeletal proteins, including tau[86] None of the CSF biomarkers correlated with CSF Aβ42 levels, which first decrease years before symptom onset and remain relatively stable with further disease progression[41,71,72,80] Synaptic dysfunction is thought to be in part mediated by soluble Aβ oligomers in AD,[87,88] with significant synaptic disease observed in areas devoid of or distant from insoluble amyloid deposits[12,83,88,89]

Our study is limited by the short duration of follow-up. It will be important to validate these findings across different centers. Because synaptic dysfunction may occur in the absence of synaptic loss,[57] the identification of imaging markers of synaptic function may provide

further insight into synaptic disease in AD and complement information provided by the CSF.

Conclusions

Our findings highlight the potential utility of CSF neurogranin as a biomarker surrogate for synaptic loss in AD. Markers of synaptic and neuronal injury, such as neurogranin and VILIP-1, may assist in monitoring response to potential therapies independently of effects on tau or amyloid disease. The CSF neurogranin levels may complement information provided by other CSF and imaging markers to guide diagnostic and prognostic decisions in clinical trials of disease-modifying therapies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding/Support: This work was supported by grants P50-AG05681, P01-AG03991, and P01-AG026276 from the National Institutes of Health (Dr Morris), a grant from Eli Lilly (Dr Holtzman), the JPB Foundation (Dr Holtzman), Siemens Health Care Diagnostics (Dr Ladenson), and the Charles F. and Joanne Knight Alzheimer Research Initiative. The frontotemporal lobar degeneration, progressive supranuclear palsy, and Lewy body dementia cerebrospinal fluid was generously provided by the University of California, San Francisco (UCSF) Memory and Aging Center (work supported by grants R01AG031278, K23-AG031861, P01AG019724, and P50 AG023501 from the National Institutes of Health, National Institute on Aging; grant P50 AG023501 from the UCSF Alzheimer's Disease Research Centers; CurePSP; and Association for Frontotemporal Dementias).

Role of the Funder/Sponsor: The funding sources had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and the decision to submit the manuscript for publication.

Additional Contributions: We acknowledge the contributions of the clinical, genetic, biomarker, imaging, and biostatistics cores of the Charles F. and Joanne Knight Alzheimer's Disease Research Center, Washington University School of Medicine. We also acknowledge the altruism of all Alzheimer Disease Research Center research participants and their families.

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Key Points

Question

What is the diagnostic and prognostic utility of cerebrospinal fluid (CSF) levels of the synaptic marker neurogranin in Alzheimer disease (AD)?

Findings

In this study comparing patients with symptomatic AD to a group of cognitively normal individuals, the CSF neurogranin levels differentiated patients with early symptomatic AD from controls with comparable diagnostic utility to other CSF markers of AD (tau, p-tau181, amyloid-β1–42, and VILIP-1).

Meaning

Cerebrospinal fluid neurogranin shows promise as a CSF biomarker for synaptic loss in AD.

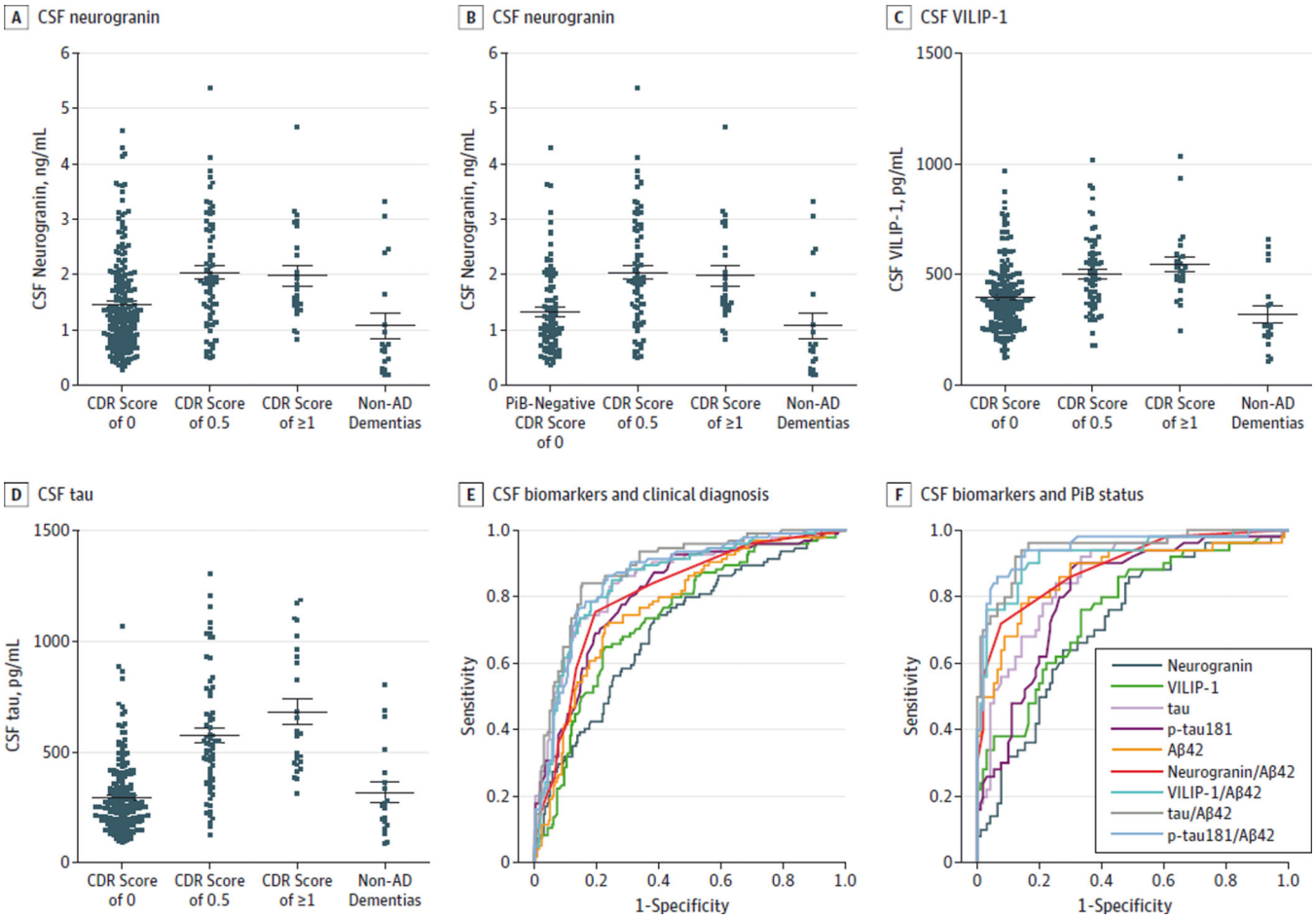

Figure 1. Scatterplots of Cerebrospinal Fluid (CSF) Biomarker Levels by Clinical Diagnosis and Clinical Dementia Rating (CDR) Scores

A, Mean CSF neurogranin levels were higher in those with CDR scores of 0.5 and those with CDR scores of 1 or higher compared with those with CDR scores of 0 ($P < .001$) or non-Alzheimer disease (AD) dementias ($P < .001$). B, Mean CSF neurogranin levels were higher in those with CDR scores of 0.5 and those with CDR scores of 1 or higher compared with those with negative Pittsburgh Compound B (PiB) test results and CDR scores of 0 ($P < .001$). C, Mean (SE) CSF VILIP-1 levels were higher in those with CDR scores of 0.5 (503 [20] pg/mL, n = 70) and those with CDR scores of 1 or higher (545 [33] pg/mL, n = 25) compared with those with CDR scores of 0 (397 [10] pg/mL, n = 207) ($P < .001$) and those with non-AD dementias (323 [40] pg/mL, n = 19) ($P < .001$). D, Mean (SE) CSF tau levels were higher in those with CDR scores of 0.5 (573 [34] pg/mL, n = 67) and those with CDR scores of 1 or higher (680 [57] pg/mL, n = 25) compared with those with CDR scores of 0 (296 [11] pg/mL, n = 197) ($P < .001$) and non-AD dementias (319 [48] pg/mL, n = 19) ($P < .001$). One-way analysis of variance with Welch correction for unequal variances and the Tukey post hoc test were used for all group comparisons. Similar results were obtained when Bonferroni corrections were used for all group comparisons. E and F, Receiver operating characteristic curves for the diagnostic utility of CSF biomarkers in differentiating AD from controls by clinical diagnosis and PiB status. Figure panels C and D are reproduced from Tarawneh et al[21] with permission from John Wiley and Sons, Inc.

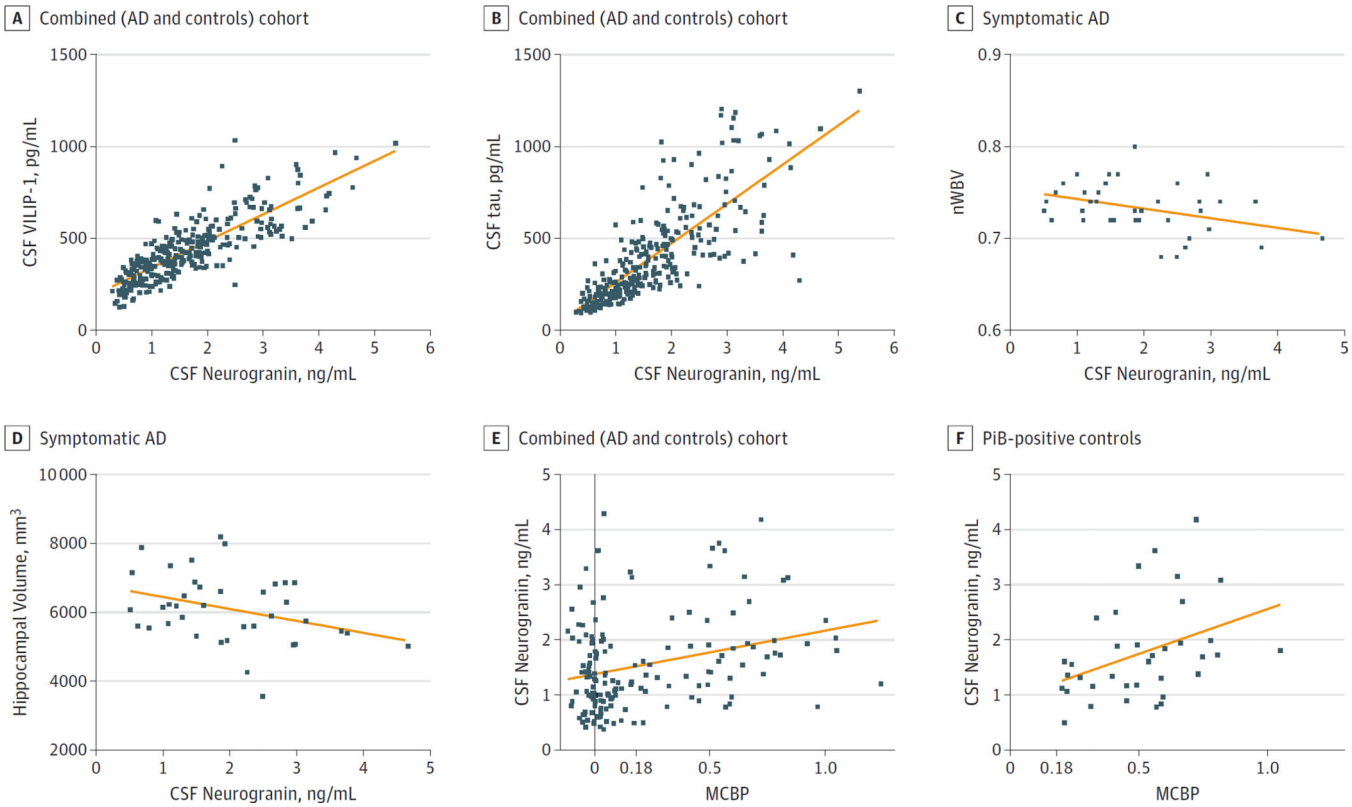

Figure 2. Correlations Between Cerebrospinal Fluid (CSF) Neurogranin Levels and CSF, Magnetic Resonance Imaging, or Amyloid Markers

The CSF neurogranin levels correlated with CSF VILIP-1 ($r = 0.82$, $P < .001$) (A) and CSF tau levels ($r = 0.78$, $P < .001$) (B) in the combined cohort of patients with Alzheimer disease (AD) and controls. The CSF neurogranin levels negatively correlated with normalized whole-brain volume (nWBV) (unadjusted $r = -0.38$, $P = .02$; adjusted $r = -0.38$, $P = .02$) (C) and hippocampal volumes (unadjusted $r = -0.34$, $P = .04$; adjusted $r = -0.36$, $P = .03$) in patients with AD (n = 38) (D). Unadjusted linear regression lines are shown. The CSF neurogranin levels correlated with mean cortical binding potential (MCBP) on positron emission tomography with Pittsburgh Compound B (PiB) in the combined (patients with AD and controls) cohort (E) and cognitively normal controls who are PiB–positive (preclinical AD; ie, MCBP ≥0.18, n = 36) (F).

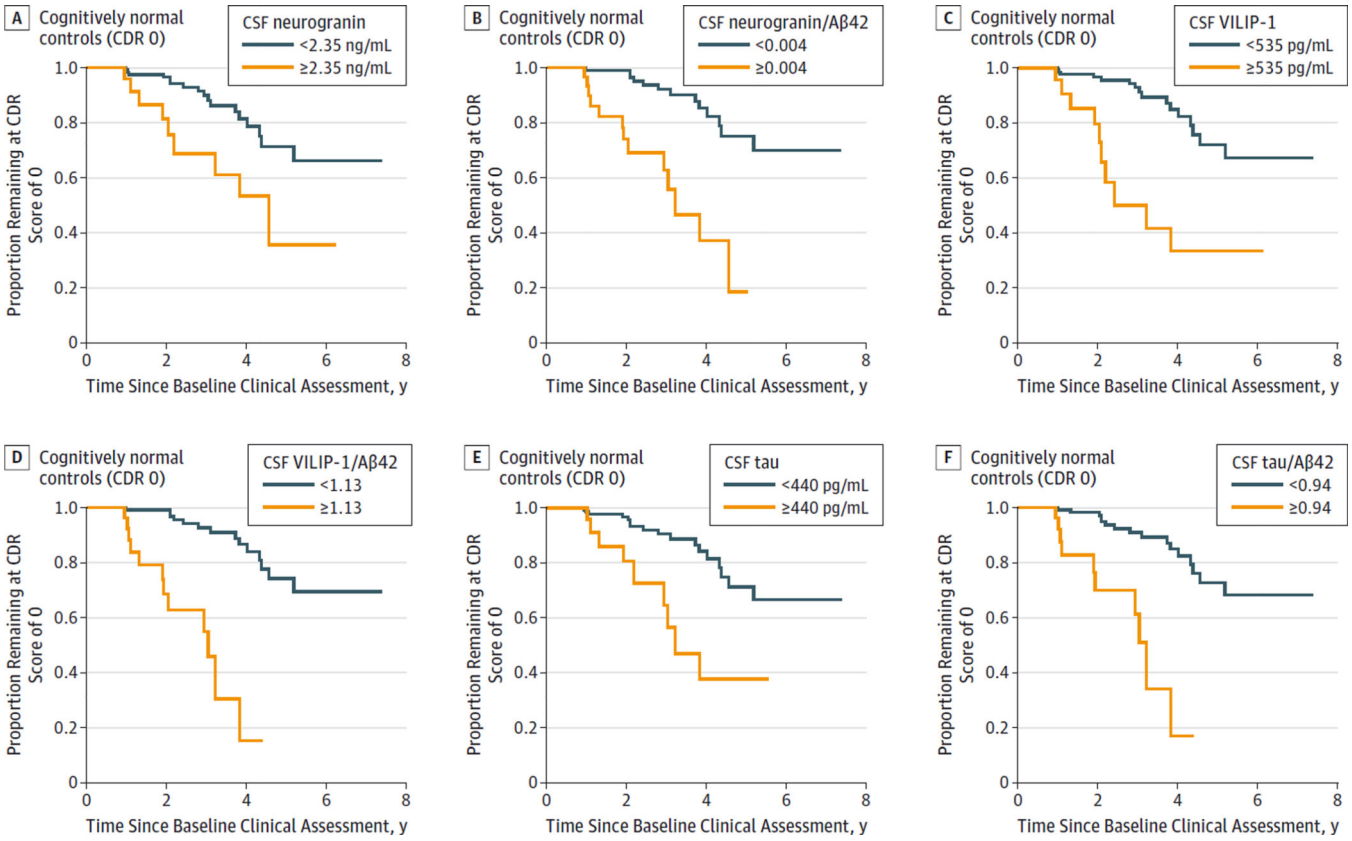

Figure 3. Baseline Cerebrospinal Fluid (CSF) Biomarker Levels as Predictors of Conversion From Clinical Dementia Rating (CDR) of 0 to 0.5 or Greater

Kaplan-Meier estimates of the rates of conversion from CDR scores of 0 to 0.5 or greater over time as a function of CSF biomarker measures are shown. The CSF biomarkers or ratios were analyzed as categorical variables (dichotomized at the 85th percentile value), and analyses were adjusted for age, sex, educational level, and *APOE* ε4 genotype. The adjusted hazard ratios for the CSF biomarkers or ratios (dichotomized at the 85th percentile value) as predictors of future cognitive decline in cognitively normal individuals were 2.78 for neurogranin (95%CI, 1.13–5.99; $P = .02$), 3.74 for VILIP-1 (95%CI, 1.98–9.57; $P = .002$), 2.57 for tau (95%CI, 1.31–6.97; $P = .03$), 1.72 for p-tau181 (95%CI, 0.97–5.38; $P = .06$), 11.00 for neurogranin/amyloid-β (Aβ42) (95%CI, 4.41–27.39; $P < .001$), 13.00 for VILIP-1/Aβ42 (95%CI, 4.38–30.90; $P < .001$), 9.82 for tau/Aβ42 (95%CI, 3.11–21.28; $P < .001$), and 7.83 for p-tau181/Aβ42 (95%CI, 2.65–16.34; $P < .001$). The Kaplan-Meier curves for p-tau181 and p-tau181/Aβ42 are not shown in the figure. Figure panels C through F are from Tarawneh et al[21] with permission from John Wiley and Sons, Inc.

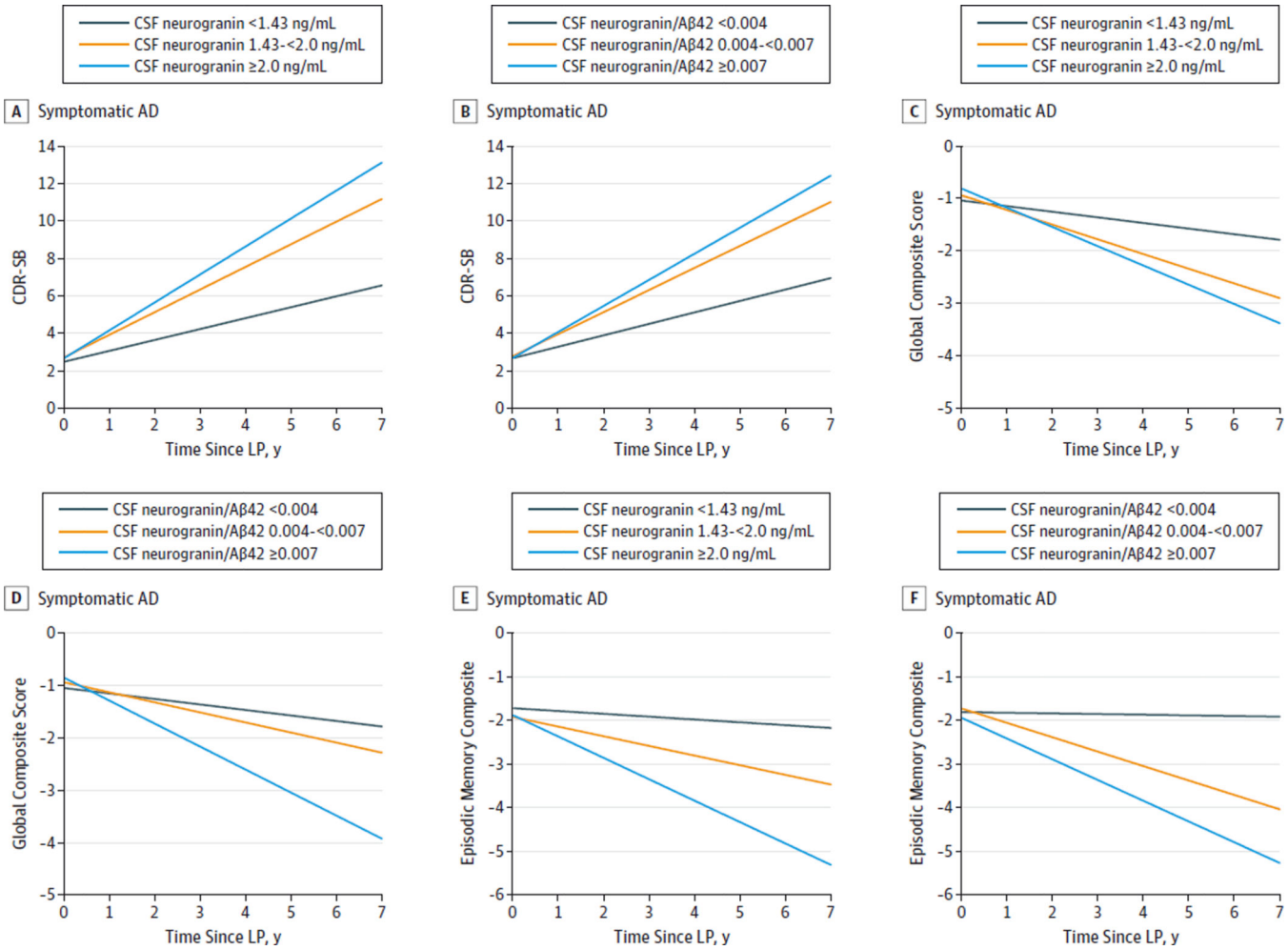

Figure 4. Rates of Cognitive Decline as a Function of Cerebrospinal Fluid (CSF) Neurogranin and Neurogranin/Amyloid-β 42 (Aβ42) Terciles in Alzheimer Disease (AD)

Mixed linear models were used to estimate rates of decline in Clinical Dementia Rating sum of boxes (CDR-SB) (A and B), global composite (C and D), and episodic memory (E and F) scores over time in the symptomatic AD cohort as a function of CSF neurogranin and neurogranin/Aβ42 levels. The slope and intercept for each of the 3 terciles of CSF neurogranin and CSF neurogranin/Aβ42 are plotted. Adjusted rates of cognitive decline in the upper, middle, and lower terciles of neurogranin values were 1.40, 1.21, and 0.58 boxes per year, respectively, for CDR-SB; −0.37, −0.28, and −0.11 points per year, respectively, for global composite scores; and −0.49, −0.22, and −0.06 points per year, respectively, in episodic memory scores. Adjusted rates of cognitive decline in the upper, middle, and lower terciles of neurogranin/Aβ42 values were 1.39, 1.18, and 0.61 boxes per year, respectively, in CDR-SB; −0.44, −0.19, and −0.11 points per year, respectively, in global composite scores; and −0.47, −0.33, and −0.02 points per year, respectively, in episodic memory scores. LP indicates lumbar puncture.

Table

Demographic, Clinical, Psychometric, Genotype, and CSF Biomarker Characteristics of Study Participants[a]

Characteristic	Controls (n = 207)	AD (n = 95)	*P* Value
Age at LP, y	72.3 (0.5)	75.0 (0.8)	.003
Female/male sex, No. (% female)[b,c]	125/82 (60)	52/43 (55)	.38
Educational level, y	15.6 (0.2)	14.4 (0.3)	.001
APOE genotype (ε4+/ε4−), No. (% ε4+)[c,d]	59/148 (29)	56/39 (59)	<.001
PiB+/PiB−, No. (% PiB+)[c,e]	36/92 (28)	17/7 (71)	<.001
Baseline scores			
CDR-SB	0.03 (0.01)	3.4 (0.25)	<.001
MMSE	28.9 (0.09)	25.3 (0.40)	<.001
Global composite	0.19 (0.04)	−0.95 (0.09)	<.001
Memory composite			
Episodic	0.18 (0.06)	−1.75 (0.10)	<.001
Semantic	0.10 (0.05)	−1.13 (0.13)	<.001
Working	0.14 (0.04)	−0.52 (0.09)	<.001
Visual spatial composite	0.32 (0.06)	−0.72 (0.12)	<.001
CSF levels			
Neurogranin, ng/mL	1.47 (0.06)	2.02 (0.10)	<.001
VILIP-1, pg/mL	397 (10)	514 (17)	<.001
tau, pg/mL[f]	296 (11)	602 (29)	<.001
p-tau181, pg/mL[f]	54 (2)	90 (4)	<.001
Aβ42, pg/mL[g]	613 (18)	390 (18)	<.001
Neurogranin/Aβ42[g]	0.003 (0.0002)	0.006 (0.0004)	<.001
VILIP-1/Aβ42[g]	0.75 (0.03)	1.54 (0.08)	<.001
tau/Aβ42[g]	0.60 (0.04)	1.87 (0.13)	<.001
p-tau181/Aβ42[g]	0.11 (0.01)	0.28 (0.02)	<.001

Abbreviations: Aβ42, amyloid-β 42; AD, Alzheimer disease; CDR-SB, Clinical Dementia Rating sum of boxes; CSF, cerebrospinal fluid; LP, lumbar puncture; MMSE, Mini-Mental State Examination; PiB, Pittsburgh Compound B.

[a] Data are presented as mean (SE) unless otherwise indicated.

[b] Mean CSF neurogranin levels were 1.6 and 1.7 ng/mL for men and women, respectively.

[c] χ^2 Tests were used for group comparisons. All other group comparisons were performed using *t* tests.

[d] *APOE* ε4+ genotype was defined by the presence of at least one *APOE* ε4 allele.

[e] Individuals who underwent positron emission tomography with PiB (n = 152) included cognitively normal controls (n = 128) and patients with AD (n = 24).

[f] n = 289.

$g_{n} = 288$.