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Journal

Neurology, 100(3)

ISSN

0028-3878

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Publication Date

2023-01-17

DOI

10.1212/wnl.0000000000201417

Peer reviewed

RESEARCH ARTICLE

Association of CSF GAP-43 With the Rate of Cognitive Decline and Progression to Dementia in Amyloid-Positive Individuals

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Neurology® 2023;100:e275-e285. doi:10.1212/WNL.0000000000201417

Abstract

Background and Objectives

To test the associations between the presynaptic growth-associated protein 43 (GAP-43), quantified in CSF, and biomarkers of Alzheimer disease (AD) pathophysiology, cross-sectionally and longitudinally.

Methods

In this retrospective study, GAP-43 was measured in participants from the AD Neuroimaging Initiative (ADNI) cohort using an in-house ELISA method, and levels were compared between groups, both cross-sectionally and longitudinally. Linear regression models tested the associations between biomarkers of AD (amyloid beta [Aβ] and tau pathologies, neurodegeneration, and cognition) adjusted by age, sex, and diagnosis. Linear mixed-effect models evaluated how baseline GAP-43 predicts brain hypometabolism, atrophy, and cognitive decline over time. Cox proportional hazard regression models tested how GAP-43 levels and Aβ status, at baseline, increased the risk of progression to AD dementia over time.

Results

This study included 786 participants from the ADNI cohort, which were further classified in cognitively unimpaired (CU) Aβ-negative (n_{CU-} = 197); CU Aβ-positive (n_{CU+} = 55), mild cognitively impaired (MCI) Aβ-negative (n_{MCI-} = 228), MCI Aβ-positive (n_{MCI+} = 193), and AD dementia Aβ-positive (n_{AD} = 113). CSF GAP-43 levels were increased in Aβ-positive compared with Aβ-negative participants, independent of the cognitive status. In Aβ-positive participants, high baseline GAP-43 levels led to worse brain metabolic decline ($p = 0.01$), worse brain atrophy ($p = 8.8 \times 10^{-27}$), and worse MMSE scores ($p = 0.03$) over time, as compared with those with low GAP-43 levels. Similarly, Aβ-positive participants with high baseline GAP-43 had the highest risk to convert to AD dementia (hazard ratio [HR = 8.56, 95% CI 4.94–14.80, $p = 1.5 \times 10^{-14}$]). Despite the significant association with Aβ pathology ($\eta^2_{A\beta\ PET}$ = 0.09, $P_{A\beta\ PET} < 0.001$), CSF total tau (tTau) and phosphorylated tau (pTau) had a larger effect size on GAP43 than Aβ PET ($\eta^2_{pTau\text{-}181} = 0.53$, $P_{pTau\text{-}181} < 0.001$; $\eta^2_{tTau} = 0.59$, $P_{tTau} < 0.001$).

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Go to Neurology.org/N for full disclosures. Funding information and disclosures deemed relevant by the authors, if any, are provided at the end of the article.

Data used in preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). As such, the investigators within the ADNI contributed to the design and implementation of ADNI and/or provided data but did not participate in analysis or writing of this report. A complete listing of ADNI investigators can be found in Appendix 2 at links.lww.com/WNL/C409.

Glossary

AD = Alzheimer disease; **ADNI** = AD Neuroimaging Initiative; **Aβ** = amyloid beta; **CDR** = Clinical Dementia Rating; **CU** = cognitively unimpaired; **CV** = coefficients of variation; **FDG** = fluorodeoxyglucose; **GAP43** = growth-associated protein 43; **HR** = hazard ratios; **LM** = linear regression models; **LME** = linear mixed effect; **QC** = quality control; **MCI** = mild cognitive impairment; **MMSE** = Mini-Mental State Examination; **NFT** = neurofibrillary tangles; **NINCDS-ADRDA** = National Institute of Neurologic and Communicative Disorders and Stroke–Alzheimer's Disease and Related Disorders Association; **pTau** = phosphorylated tau; **SUVR** = standardized uptake value ratio; **tTau** = total tau.

Discussion

High baseline levels of CSF GAP-43 are associated with progression in Aβ-positive individuals, with a more aggressive neurodegenerative process, faster rate of cognitive decline, and increased risk of converting to dementia.

Accumulation of amyloid-β (Aβ) plaques and neurofibrillary tangles (NFT) together with synaptic loss and neurodegeneration are fundamental features of the Alzheimer disease (AD) pathophysiology. It is known that both tau and Aβ aggregation exert vulnerable effects on synapse integrity[1] while synaptic loss and/or synaptic degeneration are suggested to be much closer related to cognitive decline than the other pathologic hallmarks of AD.[2-4]

Although synaptic degeneration and loss are core characteristics of the AD pathophysiologic process, it is not evident how early during disease progression synaptic dysfunction appears. Together with the synaptic loss reported in AD,[2,3,5-7] many synaptic proteins have also been found at reduced levels in hippocampus and neocortices, regions affected by AD pathophysiology.[4,8,9] In recent years, CSF synaptic biomarkers, such as neurogranin, growth-associated protein 43 (GAP-43), synaptosomal-associated protein 25, and synaptotagmin proteins, were reported to be markedly increased in patients with AD and prodromal AD.[10-15] Furthermore, high levels of the postsynaptic marker neurogranin correlates with future cognitive decline in mild cognitive impaired (MCI) patients,[10,14] further suggesting that CSF synaptic biomarkers indicate the synaptic loss and degeneration that is known to occur in AD.[3,6]

GAP-43, or neuromodulin, is a presynaptic protein vastly linked to neurite outgrowth, axonal guidance, and synaptic plasticity.[16-18] GAP-43 is highly expressed during synaptogenesis and neuronal development[19] and then later on in the hippocampus and association cortices in the adult human brain.[20] Specifically in relation to AD pathology, quantitative neuropathologic analyses have shown decreased GAP-43 concentration in the frontal cortex and altered in the subfield regions of the hippocampus,[21,22] known brain regions affected by Aβ plaques, NFT, and neuronal and synaptic degeneration early in AD.[3,23,24] Earlier studies have shown increased CSF levels of GAP-43 in MCI and AD dementia,[13,25] which was also associated with both tau pathology and amyloid pathologies.[13] CSF GAP-43 has demonstrated potential as a candidate biomarker of synaptic dysfunction in AD,[13,25] although larger studies evaluating the prognostic potential of GAP-43 to predict cognitive decline and conversion to AD dementia are limited.

In this study, we further investigated the cross-sectional and longitudinal associations between CSF GAP-43 and biomarkers of AD pathophysiology, using data from the multicentric AD Neuroimaging Initiative (ADNI) cohort. In addition, we evaluated how CSF GAP-43 changes over time and how well it predicts cognitive decline and clinical progression to dementia.

Methods

Participants

This report used data obtained from the ADNI database,[26] which was launched in 2004 by the National Institute on Aging, the Food and Drug Administration, private pharmaceutical companies and nonprofit organizations as a highly innovative public-private partnership, led by Principal Investigator Michael W. Weiner, MD, VA Medical Center, and University of California, San Francisco. This study was performed in accordance with the transparent reporting of a multivariable prediction model for individual prognosis or diagnosis: reporting guideline.[27]

This study initially included 802 participants, ranging from clinically diagnosed cognitively unimpaired (CU), MCI, and AD dementia participants, which had available CSF GAP-43 measurements and paired baseline CSF Aβ42 and phosphorylated tau (pTau)-181 data (data accessed on June 2021). The AD participants met criteria for probable AD according to the National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA),[28] with a Mini-Mental State Examination (MMSE) ranging between 20 and 26 (inclusively) and Clinical Dementia Rating (CDR) ≥1. Participants were classified as MCI if MMSE ranged between 24 and 30, CDR of 0.5 (with the memory box score being 0.5 or greater), largely intact general cognition and functional performance, and could not meet criteria for dementia according to the NINCDS-ADRDA (for further details see Ref. 29). In addition, participants were classified according to the Aβ status, as further described, and AD dementia participants with no evidence of Aβ pathology were excluded from our analysis, leading to a final sample size of 786 participants. Longitudinal GAP-43 quantifications were available for 344 participants (227 with

Table 1 Demographic and Biomarker Summary Information of the Sample

	CU− (n = 197)	CU+ (n = 55)	MCI− (n = 228)	MCI+ (n = 193)	AD (n = 113)
Age, y	72.0 (5.78)	75.9 (5.61)[c]	70.1 (7.61)[b]	72.8 (6.93)	73.9 (8.39)[a]
Female, n (%)	104 (52)	37 (67)[d]	110 (48)	81 (44)[d]	50 (44)
Education, y	16.8 (2.49)	16.0 (2.33)[c]	16.2 (2.58)[a]	16.0 (2.72)[c]	15.6 (2.68)[c]
***APOE*-ε4 carriers, n (%)**	44 (22)	29 (52)[c]	63 (27)	143 (74)[c]	82 (72)[c]
MMSE	29.0 (1.16)	28.9 (1.20)	28.5 (1.47)[c]	27.4 (1.85)[c]	23.0 (2.05)[c]
CSF pTau-181/Aβ(1-42)	0.01 (0.004)	0.04 (0.01)[c]	0.01 (0.005)	0.05 (0.02)[c]	0.06 (0.03)[c]
CSF pTau-181, pg/mL	18.9 (6.26)	31.7 (11.7)[c]	18.0 (6.11)	36.3 (15.0)[c]	38.7 (16.1)[c]
CSF tTau, pg/mL	215.0 (72.0)	317.8 (110.8)[c]	204.2 (65.0)	358.7 (135.0)[c]	387.7 (156.4)[c]
CSF GAP-43, pg/mL	4,570 (2,200)	6,460 (3,600)[c]	4,040 (2,000)[a]	6,420 (3,120)[c]	6,430 (3,230)[c]
Aβ PET, SUVR	1.06 (0.11)	1.36 (0.20)[c]	1.05 (0.12)	1.40 (0.17)[c]	1.44 (0.18)[c]
FDG PET, SUVR	1.32 (0.10)	1.21 (0.09)	1.30 (0.11)[a]	1.21 (0.13)[c]	1.04 (0.13)[c]
Hippocampal vol, mm^3	7,633 (783)	7,391 (692)	7,368 (1,085)[c]	6,688 (1011)[c]	5,950 (801)[c]
Whole-brain vol, cm^3	1,070 (54.8)	1,050 (44.6)	1,070 (62.2)[a]	1,050 (58.6)[c]	1,010 (57)[c]

Abbreviations: Aβ(1-42) = amyloid-β 1-42; AD = Alzheimer disease; CU− = Aβ-negative cognitively unimpaired; CU+ = Aβ-positive cognitively unimpaired; FDG = [18F]fluorodeoxyglucose = GAP-43 = growth-associated protein 43 = MCI+ = Aβ-positive mild cognitive impairment; MMSE = Mini-Mental State Examination; p-tau181 = tau phosphorylated at threonine 181; tTau = total tau.
Data are shown as mean (SD) or n (%), as appropriate. One-way analysis of covariance was used to compare age, education y, and MMSE between groups (adjusting by sex) and Pearson χ^2 to compare sex and *APOE*-ε4 frequencies between groups. Imaging and fluid biomarkers were compared with a one-way ANCOVA adjusted by age and sex. Aβ status for group definition was based on CSF pTau-181/Aβ42 ratio. Hippocampal and whole-brain volumes are adjusted by intracranial volume.
[a] $p < 0.05$.
[b] $p < 0.01$.
[c] $p < 0.001$; for these, CU− was the reference group.
[d] $p < 0.05$ between these groups.

baseline plus one follow-up visit, 116 with baseline plus 2 follow-up visits, and 1 with baseline plus 3 follow-up visits).

Standard Protocol Approvals, Registrations, and Patient Consents

Participants have been recruited from over 50 sites across the United States and Canada (for up-to-date information, see Ref. 30), and ethical committees of all institutions have approved the study. All participants have provided informed consent. The study was performed in accordance with the provisions of the Declaration of Helsinki. The protocol was approved by the Institutional Review Board from each institute/site for the experiments using human participants described in this study.

CSF Biomarkers

The GAP-43 analysis was performed using an in-house ELISA method at the Clinical Neurochemistry Laboratory at the Sahlgrenska University Hospital (Mölndal, Sweden) by a board-certified laboratory technician blinded to clinical information as previously described.[13] All standards and control samples were analyzed in duplicate. The intermediate precision of the GAP-43 assay was determined using 2 quality control human CSF samples (quality control [QC 1] and QC 2), which had an intra-assay coefficient of variation (CV) of 5.5% and 11% and interassay CV of 6.9% and 15.6%, respectively. For this study, the first GAP-43 measurement was used to define the baseline visit in all analyses.

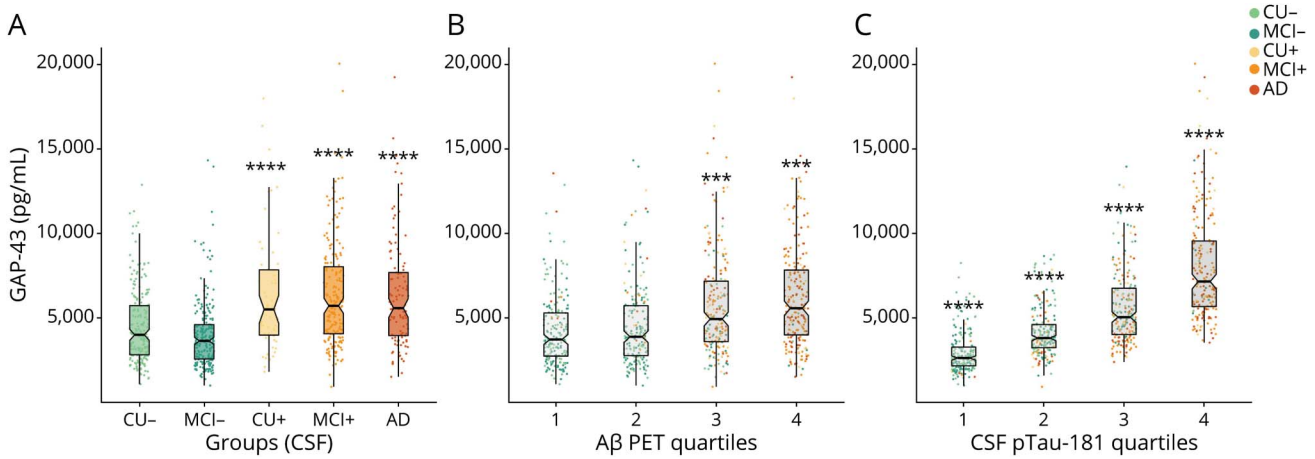
CSF Aβ42, total tau (tTau), and pTau-181 were quantified using the fully automated Elecsys assays (Roche Diagnostics) as reported elsewhere.[31] A positive Aβ status was given to participants who had CSF pTau-181/Aβ (1–42) ratio >0.028 at the baseline GAP-43 visit.[32] Only cross-sectional Aβ (1–42), tTau, and pTau-181 data were used in our analyses.

Neuroimaging Methods

MRI and PET summary measures were downloaded from the ADNI database, and scan acquisitions followed the reported protocols.[33]

Cross-sectional brain Aβ burden was estimated using [^{18}F] florbetapir PET, in which the global load is given based on the average standardized uptake value ratio (SUVR) of the precuneus, cingulate, inferior parietal, medial prefrontal, lateral temporal, and orbitofrontal cortices, and had the pons as reference region.[34] Glucose uptake was indexed by [^{18}F]fluorodeoxyglucose (FDG) PET, and the global SUVR was the average SUVR of the bilateral angular, posterior cingulate, and inferior temporal gyri, with the cerebellar vermis and the pons used as the reference regions.[35] Longitudinal FDG PET was

Figure 1 Cross-sectional GAP-43



Distribution of CSF GAP-43 concentrations across groups, showing Aβ-negative groups with lower levels of GAP-43 as compared with Aβ-positive groups (A; all Aβ-positive groups are significantly different from Aβ-negative groups, $p < 0.0001$). GAP-43 levels were also compared between Aβ PET (B; third and fourth quartiles are significantly higher than first and second quartiles, $p < 0.001$) and CSF pTau-181 (C; all groups are significantly different from each other, $p < 0.0001$) quartile groups. p values of group comparisons were corrected for multiple comparisons. Aβ = amyloid beta; GAP43 = growth-associated protein 43; pTau = phosphorylated tau.

used in this study, counting from baseline GAP-43, and 375 participants had data for more than one visit.

Brain atrophy was determined using hippocampal and whole-brain volumes. Automated volume measures were performed using FreeSurfer software package[36] and were adjusted for total intracranial volume using data from all cognitively impaired subjects as baseline, as previously described.[37] Longitudinal brain volume was used in this study, counting from baseline GAP-43, and 729 participants had data for more than one visit.

Statistical Analysis

Biomarker and demographic data were compared between groups using the χ^2 test for categorical variables and one-way analysis of variance, followed by the Tukey post hoc test when variables were continuous. Linear regression models (LM) tested the associations between GAP-43 concentrations and other variables at baseline, always adjusting for age, sex, and diagnostic group. Participants were also grouped according to baseline levels of GAP-43 in terciles (low, medium, and high) and according to baseline Aβ PET and CSF pTau-181 in quartiles (first, second, third, and fourth).

Linear mixed-effect (LME) models were used to evaluate longitudinal relationships, which always included random intercepts and were adjusted for age, sex, and baseline measures when needed. Adjusting for APOE4 did not affect the interpretation of findings and, therefore, was not included in this report. The models were fit using maximum likelihood estimation, and time was set as continuous variable, counting from baseline GAP-43. First, GAP-43 progression over time was compared between categorical groups. Then, participants were grouped according to baseline GAP-43 extreme terciles (low and high) and Aβ status, and biomarker longitudinal changes were assessed. These models had longitudinal FDG PET, longitudinal MMSE, and longitudinal brain atrophy as outcome measures (independently); time as continuous variable; random intercept and age, sex, education, and baseline measurements as covariates.

Cox proportional hazard regression models tested the association between groups (GAP-43 extreme terciles and Aβ status) and the risk of incident AD dementia or risk of diagnosis progression. The Cox proportional hazard regression analyses included only CU and MCI participants, with follow-up data up to 75 months. The outcome of the model was time to diagnosis change, and it was adjusted for age and sex. Participants were sensored at their last follow-up visit. Hazard ratios (HR) were reported. Schoenfield residuals tested the assumption of proportional hazards and Martingale residuals assessed nonlinearity.

To facilitate comparison and interpretation of findings, LM and LME were performed using standardized variables when indicated. GAP-43 was log transformed before standardization. All statistical analyses were performed in R statistical platform v.3.6.3.[38]

Data Availability

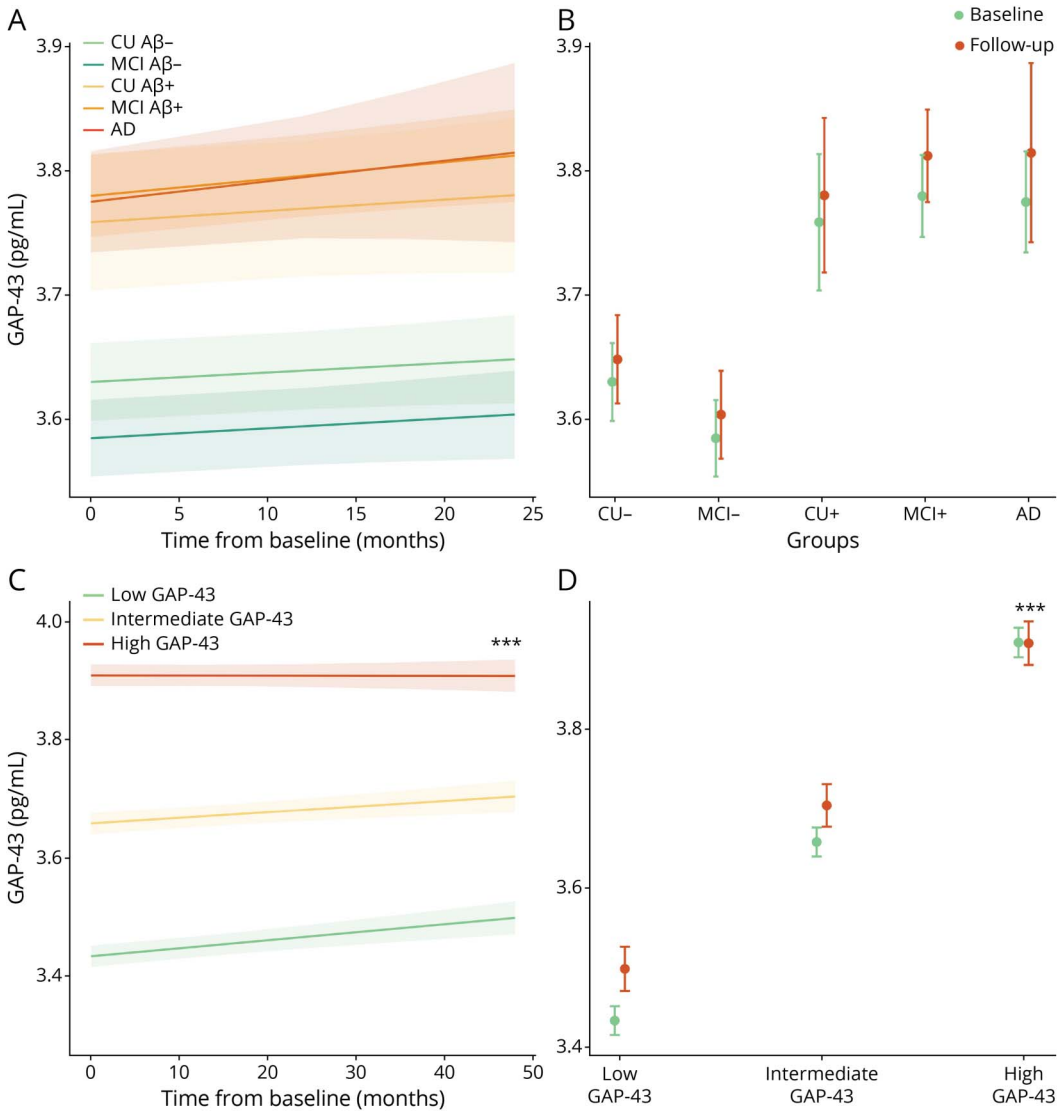
The data sets used and/or analyzed during this study are available from the corresponding author on reasonable request.

Results

Main Characteristics of the Study Sample

A total of 786 participants were included in the study: 197 Aβ-negative CU (CU–), 55 Aβ-positive CU (CU+), 228 Aβ-

Figure 2 Longitudinal Progression of GAP-43



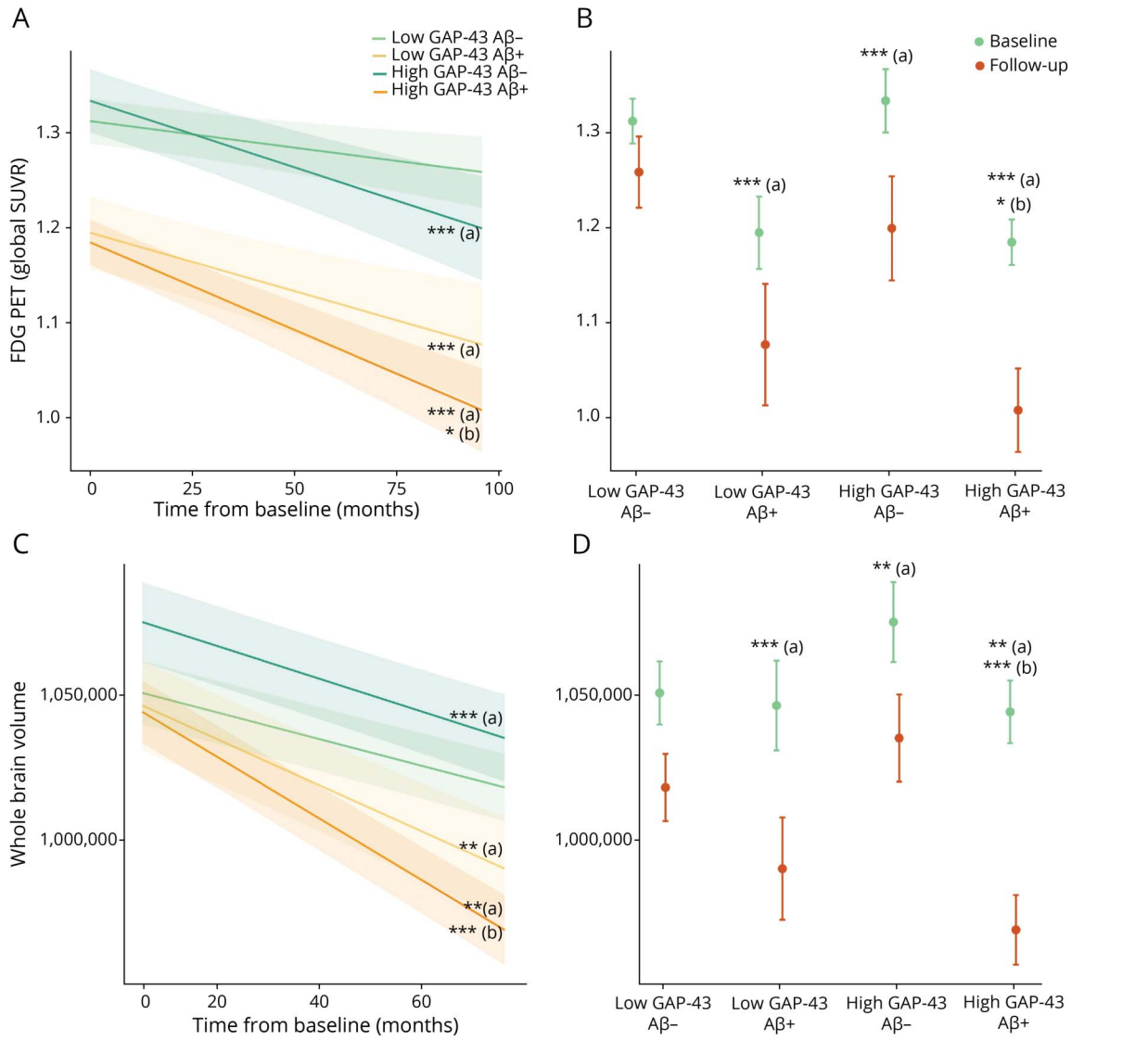
Linear mixed-effect models tested the evolution of CSF GAP-43 over time between groups. (A) We found no difference between the slopes of the groups (shaded areas represent CI), which is also represented in (B) forest plots. (C and D) When participants were grouped according to baseline GAP-43 levels (tercile groups), high GAP-43 at baseline showed no changes over time, which was significantly different from steeper biomarker progression when baseline GAP-43 levels were low (***$p = 3 \times 10^{-5}$; shaded areas represent CI). GAP43 = growth-associated protein 43.

negative MCI (MCI−), 193 Aβ-positive MCI (MCI+), and 113 Aβ-positive AD dementia (AD) participants. The average age of the population was 72.2 (±7.2) years, 48% were women, and the average years of formal education was 16.2 (±2.6) years. Specifics about groups characteristics can be found in Table 1, where we show that CU+ ($p < 0.0001$) and AD ($p = 0.02$) are in average older than CU−, whereas MCI− are younger ($p = 0.005$). As expected, MMSE scores are found to be lower in MCI−, MCI+, and AD groups in comparison with CU groups. In addition, AD and MCI+ participants have a larger proportion of *APOE*-ε4 carriers in comparison with CU− group. As expected, biomarkers profiles of Aβ and tau pathologies are abnormal in Aβ-positive groups as compared with CU− participants.

Baseline Levels of GAP-43 Better Reflects Tau Pathology Than Aβ Pathology

Cross-sectional GAP-43 levels were shown to be significantly increased in Aβ-positive groups as compared with CU− participants (average increase of 41% in CU+ and 40% in MCI+ and AD), whereas MCI− were unchanged from CU− (Figure 1A). We found no association between GAP-43 and age ($p = 0.25$; adjusting by sex and diagnosis), but a sex effect was found, where women had higher levels than men ($p = 0.02$; adjusting by age and diagnosis). Linear models tested the effect of Aβ PET, CSF pTau-181, and tTau on GAP-43, and despite all being significantly associated, CSF pTau-181 and tTau had a medium effect size on GAP-43, whereas Aβ PET had a small effect size ($\eta^2_{\text{Aβ PET}} = 0.09$, $P_{\text{Aβ PET}} < 0.001$; $\eta^2_{\text{pTau-181}} = 0.53$, $P_{\text{pTau-181}} < 0.001$;

Figure 3 GAP-43 Levels Predicting Longitudinal Metabolic Decline and Brain Atrophy



Linear mixed-effect models first compared FDG changes between GAP-43 and Aβ groups over time. (A and B) All groups have faster FDG decline in comparison with Aβ-negative (Aβ–) participants with low baseline GAP-43 (***$P_{\text{Low GAP-43 Aβ+}} = 1.1 \times 10^{-4}$; ***$P_{\text{High GAP-43 Aβ–}} = 7.5 \times 10^{-5}$; ***$P_{\text{High GAP-43 Aβ+}} = 2.2 \times 10^{-16}$). The results also showed that in Aβ-positive (Aβ+) participants, high GAP-43 levels led to worse FDG hypometabolism over time as compared with low GAP-43 levels (*$p = 0.01$; shaded areas represent CI). Similar models were also performed to compare changes in brain volume over time. (C and D) Rates of brain atrophy were greater in participants with low GAP-43 and Aβ+ (***$p = 1.2 \times 10^{-5}$), high GAP-43 and Aβ– (**$p = 0.008$), and high GAP-43 and Aβ+ groups (**$p = 0.001$) in contrast with low GAP-43 and Aβ– group. In addition, in Aβ+ individuals, longitudinal brain atrophy was worse in those who had high GAP-43 at baseline in comparison with those with low GAP-43 (***$p = 8.8 \times 10^{-27}$). Aβ = amyloid beta; FDG = fluorodeoxyglucose; GAP43 = growth-associated protein 43.

$\eta^2_{\text{tTau}} = 0.59$, $P_{\text{tTau}} < 0.001$). This relationship was clearly visualized when we compared GAP-43 levels between quartile groups (Figure 1, B and C).

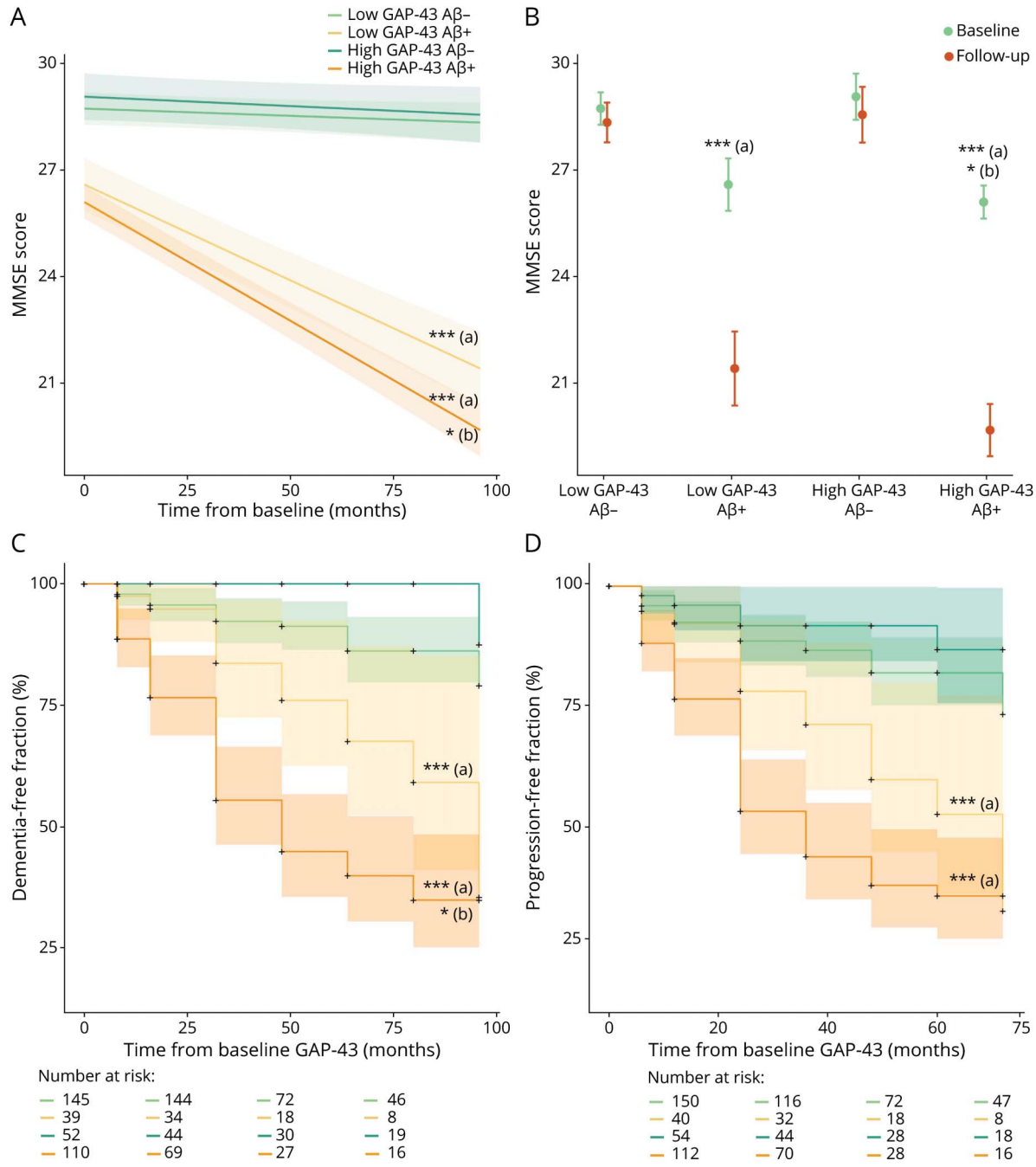
GAP-43 Has Steeper Increasing Levels in Participants With Low Baseline Measurements

When evaluating longitudinal changes, we did not observe differences on GAP-43 levels between pure clinically defined or "biomarker defined" diagnostic groups over time (Figure 2, A and B). However, when segregating participants based on GAP-43 terciles (first tercile GAP-43 < 3,681.3 pg/mL; 2nd tercile GAP-43 3681.3 pg/mL ≤ × < 5,760.7 pg/mL; and third tercile GAP-43 has concentrations above or equals to 5,760.7 pg/mL), we found that low baseline GAP-43 levels lead to a significantly steeper trajectory than does high baseline GAP-43, suggesting that GAP-43 plateaus over time (Figure 2, C and D).

Baseline Levels of GAP-43 Is Associated With More Rapid Rate of Hypometabolism and More Rapid Rate of Brain Atrophy Over Time

GAP-43 showed no association with baseline brain hypometabolism measured by FDG PET ($p = 0.57$). However, there was an association between longitudinal FDG PET and the

Figure 4 GAP-43 Levels Suggesting Cognitive Decline



(A and B) Linear mixed-effect models compared MMSE changes between GAP-43 and Aβ groups over time. Aβ-positive (Aβ+) groups had worse decline in MMSE scores when compared with participants Aβ-negative[a] (Aβ–) with low baseline GAP-43 levels (***$P_{\text{Low GAP-43 Aβ+}} = 1.8 \times 10^{-22}$; ***$P_{\text{High GAP-43 Aβ+}} = 3.3 \times 10^{-46}$). In Aβ+ participants, high GAP-43 at baseline also indicated worse MMSE scores over time as compared with those with low GAP-43[b] (*$p = 0.03$). Cox proportional hazard model (adjusted by age, sex, and education) showing that, in comparison with low GAP-43 Aβ-group[a], low levels of baseline GAP-43 and Aβ+ are associated with an increased risk to convert to AD dementia (HR = 4.17, 95% CI 2.04–8.49, $p = 8.3 \times 10^{-5}$), which the highest risk was found for high GAP-43 and Aβ+ group (HR = 8.56, 95% CI 4.94–14.80, $p = 1.5 \times 10^{-14}$), as evidenced by (C) the Kaplan-Meier curves. When comparing Aβ+ groups[b], high GAP-43 had highest conversion rate (HR = 2.05, 95% CI 1.13–3.07, $p = 0.01$). Similarly, when evaluating rates of diagnosis progression, as shown by (D) Kaplan-Meier curves, in comparison with low GAP-43 Aβ-group, low levels of baseline GAP-43 and Aβ+ are associated with an increased risk to progress clinically (HR = 3.67, 95% CI 1.98–6.78, $p = 3{,}3 \times 10^{-5}$), which the highest risk was found for high GAP-43 and Aβ+ group (HR = 5.80, 95% CI 3.61–9.33, $p = 3{,}8 \times 10^{-13}$). Aβ = amyloid beta; GAP43 = growth-associated protein 43; HR = hazard ratios; MMSE = Mini-Mental State Examination.

interaction between GAP-43 and time ($p = 1.24 \times 10^{-6}$). Similarly, when participants were grouped according to GAP-43 levels and their Aβ status, higher GAP-43 was associated with more rapid rate of hypometabolism over 96 months (Figure 3, A and B). In addition, GAP-43 showed no associations with cross-sectional hippocampal volume ($p = 0.83$), but it was associated with brain volume ($p < 0.001$; adjusted by age, sex, diagnosis, and education). High baseline GAP-43 was also linked to more rapid rate of brain atrophy over time when GAP-43 was evaluated as a continuous variable

($p = 1.04 \times 10^{-8}$) or when groups were considered (Figure 3, C and D).

High Baseline Levels of GAP-43 Predict Faster Cognitive Decline and Higher Risk of Dementia

Higher levels of GAP-43, cross-sectionally, were found to be associated with worse cognitive performance on the MMSE ($p = 0.01$) and associated with more rapid rate of cognitive decline over 96 months, when GAP-43 was considered a continuous variable ($p = 2.97 \times 10^{-12}$) or when GAP-43 groups were evaluated (Figure 4, A and B). Corroborating these findings, survival analysis showed that high baseline GAP-43 in combination with positivity for Aβ pathology was the profile that showed the greatest risk of converting to dementia (HR = 8.56, 95% CI 4.94–14.80; Figure 4C) or to clinically progress (HR = 5.80, 95% CI 3.61–9.33; Figure 4D) over the period of 6 years.

Discussion

In this study, we show that high CSF GAP-43 levels are associated with increased risk of dementia onset and with faster cognitive decline. Particularly in Aβ-positive individuals, a more rapid rate of decline in cognitive performance was observed in participants with high CSF levels in contrast to participants with lower GAP-43 levels. Similarly, in the presence of amyloid pathology, high CSF GAP-43 concentrations indicated an increased risk to convert to AD dementia. In addition, baseline CSF GAP-43 levels were associated with more rapid rates of hypometabolism and more rapid rates of brain atrophy over time.

In the current study, we showed that baseline GAP-43 levels were increased in Aβ-positive groups as compared with CU Aβ-negative group. Our results are in agreement with previous studies reporting elevated CSF levels of GAP-43 in AD[13,39,40] and in MCI because of AD compared with CU participants.[25,39] Interestingly, the observation that levels of GAP-43 already significantly increased in the CU+ group compared with CU− but not changed in MCI− indicates that synaptic alterations are related to amyloidosis, and it may occur even before clinical symptoms are manifested.[15,41] In addition, as previously described, CSF GAP-43 was linked to both Aβ and tau pathologies.[13,42] Interestingly, when we investigated the association between GAP-43 and core AD biomarkers, linear models showed that CSF tTau and pTau-181 had a medium effect on GAP-43 and Aβ PET had a small effect on GAP43, suggesting that CSF GAP-43 are more tightly associated with tau pathology and neurodegeneration than it is with Aβ pathology. In line with our results, previous studies showed a strong association between GAP-43 and tau pathology, indexed by Braak staging, as well as with tTau and pTau-181 in AD at a cross-sectional level.[13,25]

We showed that GAP-43 levels were associated with longitudinal cognitive performance, corroborating previous reports showing that synaptic loss is the pathologic change that most closely correlates with cognitive decline.[3] High baseline levels of GAP-43 predicted worse cognitive decline, indexed by MMSE, over time in the Aβ-positive group when these were compared with participants with initial low levels of GAP-43. In agreement with these findings, Aβ-positive individuals with high baseline GAP-43 had the highest risk to progress clinically and to convert to dementia. In alignment with those results, the levels of neurogranin, the postsynaptic counterpart of GAP-43, were previously associated with the severity of cognitive decline in AD.[10,43,44] These calmodulin-binding proteins seem to be inevitable for neuronal transmission and synaptic plasticity,[45,46] thereby their changes might reflect early signs of cognitive decline. Despite that amyloid-positive participants with high CSF GAP-43 had the worst cognitive performance, lower levels of GAP-43 in Aβ-positive participants were also associated with subsequent cognitive decline and clinical progression to AD. This suggests that the inclusion of GAP-43 to a diagnostic biomarker panel can increase the possibility to identify the patients who will have the most rapid rate of cognitive decline.

In the context of the A/T (N) framework, biomarkers of the Aβ pathology seem to change first in AD, followed by biomarkers of tau-related neuronal injury.[47] Studies based on CSF biomarkers have also shown that synaptic alterations precede and/or parallel neurodegeneration in preclinical AD.[48,49] In line with that, this study demonstrates that high baseline GAP-43 levels in Aβ-positive participants was associated with greater brain atrophy and worse metabolic decline over time, as proxied by longitudinal measures of brain volume and FDG-PET. As these biomarkers generally indicate neurodegeneration,[47] these findings further support the concept that synaptic abnormalities precede cell dysfunction and death, as previously suggested.[42,47,50,51] However, future studies with biomarkers that more specifically measure synaptopathies, e.g., synaptic vesicle glycoprotein 2A PET, are needed to closely examine the relationship between CSF GAP-43 and synaptic dysfunction in living individuals.[52]

The major strength of this study was its longitudinal design that made it possible to investigate how GAP-43 was associated with cognitive deterioration over time. Furthermore, CSF GAP-43 was assessed in a large multicentric cohort and quantified with a robust in-house assay.

There are some limitations to our study. First, although models were adjusted for them, demographic characteristics differed between groups. Second, the CSF biomarkers were used as an index of AD pathology; however, still autopsy is the golden standard for examination of AD pathology.

High baseline levels of GAP-43 were mostly linked to increased tau pathology and associated with future decline in brain metabolism, progressive brain atrophy, cognitive decline and higher risk to progress to dementia. Altogether, these results support the framework that synaptic changes stand in between AD pathologic changes and future neurodegeneration and cognitive symptoms. Furthermore, findings

point to GAP-43 as a potential marker of clinical progression particularly in participants with Aβ pathology, being a valuable tool for enrolling participants in clinical trials.

Acknowledgment

Coauthor J.Q. Trojanowski, MD, PhD, died in February 2022. Data collection and sharing was funded by ADNI (NIH #U01 AG024904) and DOD ADNI (#W81XWH-12-2-0012). ADNI is funded by the National Institute on Aging, the National Institute of Biomedical Imaging and Bioengineering, and through generous contributions from the following: AbbVie, Alzheimer's Association; Alzheimer's Drug Discovery Foundation; Araclon Biotech; BioClinica, Inc.; Biogen; Bristol-Myers Squibb Company; CereSpir, Inc.; Cogstate; Eisai Inc.; Elan Pharmaceuticals, Inc.; Eli Lilly and Company; EuroImmun; F. Hoffmann-La Roche Ltd and its affiliated company Genentech, Inc.; Fujirebio; GE Healthcare; IXICO Ltd.; Janssen Alzheimer Immunotherapy Research & Development, LLC.; Johnson & Johnson Pharmaceutical Research & Development LLC.; Lumosity; Lundbeck; Merck & Co., Inc.; Meso Scale Diagnostics, LLC.; NeuroRx Research; Neurotrack Technologies; Novartis Pharmaceuticals Corporation; Pfizer Inc.; Piramal Imaging; Servier; Takeda Pharmaceutical Company; and Transition Therapeutics. The Canadian Institutes of Health Research is providing funds to support ADNI clinical sites in Canada. Private sector contributions are facilitated by the Foundation for the NIH (fnih.org). The grantee organization is the Northern California Institute for Research and Education, and the study is coordinated by the Alzheimer's Therapeutic Research Institute at the University of Southern California. ADNI data are disseminated by the Laboratory for Neuro Imaging at the University of Southern California.

Study Funding

M.W. Weiner receives support for his research from the following funding sources: NIH: 5U19AG024904-14, 1R01AG053798-01A1, R01 MH098062, U24 AG057437-01, 1U2CA060426-01, 1R01AG058676-01A1, and 1RF1AG059009-01. Department of Defense (DOD): W81XWH-15-2-0070, 0W81XWH-12-2-0012, W81XWH-14-1-0462; and W81XWH-13-1-0259. Patient-Centered Outcomes Research Institute (PCORI): PPRN-1501-26817. California Department of Public Health (CDPH): 16-10054. University of Michigan: 18-PAF01312. Siemens: 444951-54249. Biogen: 174552; Hillblom Foundation: 2015-A-011-NET, Alzheimer's Association: BHR-16-459161. The State of California: 18-109929. He also receives support from Johnson & Johnson, Kevin and Connie Shanahan, GE, VUmc, Australian Catholic University (HBI-BHR), The Stroke Foundation, and the Veterans Administration. H. Zetterberg is a Wallenberg Scholar supported by grants from the Swedish Research Council (2018-02532), the European Research Council (681712), Swedish State Support for Clinical Research (ALFGBG-720931), the Alzheimer Drug Discovery Foundation (ADDF), USA (201809-2016862), the AD Strategic Fund and the Alzheimer's Association (ADSF-21-831376-C, ADSF-21-831381-C and ADSF-21-831377-C), the Olav Thon Foundation, the Erling-Persson Family Foundation, Stiftelsen för Gamla Tjänarinnor, Hjärnfonden, Sweden (FO2019-0228), the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 860197 (MIRIADE), and the UK Dementia Research Institute at UCL. K. Blennow is supported by the Swedish Research Council (2017-00915), the Alzheimer Drug Discovery Foundation (ADDF), USA (RDAPB-201809-2016615), the Swedish Alzheimer Foundation (AF-742881), Hjärnfonden, Sweden (FO2017-0243), the Swedish state under the agreement between the Swedish government and the County Councils, the ALF-agreement (ALFGBG-715986), the European Union Joint Program for Neurodegenerative Disorders (JPND2019-466-236), the National Institute of Health (NIH), USA, (grant 1R01AG068398-01), and the Alzheimer's Association 2021 Zenith Award (ZEN-21-848495). A. Öhrfelt is supported by grants from Demensfonden, Gun och Bertil Stohnes stiftelse, Stiftelsen gamla tjänarinnor, and Åhlén-stiftelsen. Industry-sponsored: Fujirebio supplied the antibodies for the in-house GAP43 assay. L. Shaw is supported by NIH grants: ADNI3 (U19 AG024904): PENN ADRC (P30 AG072979); R01MH117114 and a MJFox Fdn for Parkinson's Research grant 13637.01.

Disclosure

A. Öhrfelt, A.L. Benedet, H. Kvartsberg, N.J. Ashton, and J.Q. Trojanowski declare no disclosures. M. Vandijck is employee of Fujirebio Europe NV. M.W. Weiner has performed Paid Consulting on Advisory Boards for Alzheon, Inc., Biogen, Cerecin, Dolby Family Ventures, Eli Lilly, Merck Sharp & Dohme Corp., Nestle/Nestec, and Roche, University of Southern California (USC). He has provided Consulting to Baird Equity Capital, BioClinica, Cerecin, Inc., Cytox, Dolby Family Ventures, Duke University, FUJIFILM-Toyama Chemical (Japan), Garfield Weston, Genentech, Guidepoint Global, Indiana University, Japanese Organization for Medical Device Development, Inc. (JOMDD), Nestle/Nestec, NIH, Peerview Internal Medicine, Roche, T3D Therapeutics, University of Southern California (USC), and Vida Ventures. He has acted as a Speaker/Lecturer to The Buck Institute for Research on Aging; China Association for Alzheimer's Disease (CAAD), Japan Society for Dementia Research. He has traveled with the support of University of Southern California (USC), NervGen, ASFNR, CTAD Congres. He holds Stock options with: Alzeca, Alzheon, Inc., and Anven. He has served on Advisory Boards (paid and unpaid) for Acumen Pharmaceutical, ADNI, Alzheon, Inc., Biogen, Brain Health Registry, Cerecin, Dolby Family Ventures, Eli Lilly, Merck Sharp & Dohme Corp., National Institute on Aging (NIA), Nestle/Nestec, PCORI/PPRN, Roche, University of Southern California (USC), NervGen. He serves on Editorial Boards (unpaid) for Alzheimer's & Dementia, MRI and TMRI. L.M. Shaw has served on Advisory Boards for Biogen, Roche, Fujirebio, Siemens Healthineers. He has received honoraria for lecturing: Biogen teaching program on fluid biomarkers, Fujirebio teaching program on fluid biomarkers.; H. Zetterberg has served at scientific advisory boards and/or as a consultant for Abbvie, Alector, Annexon, Denali, Eisai, Roche, Wave, Samumed, Siemens Healthineers,

Pinteon Therapeutics, Nervgen, AZTherapies, CogRx, and Red Abbey Labs, has given lectures in symposia sponsored by Cellectricon, Fujirebio, Alzecure and Biogen, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program has served at scientific advisory boards for Denali, Roche Diagnostics, Wave, Samumed, Siemens Healthineers, Pinteon Therapeutics and CogRx, has given lectures in symposia sponsored by Fujirebio, Alzecure and Biogen, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program. K. Blennow has served as a consultant, at advisory boards, or at data monitoring committees for Abcam, Axon, BioArctic, Biogen, JOMDD/Shimadzu. Julius Clinical, Lilly, MagQu, Novartis, Pharmatrophix, Prothena, Roche Diagnostics, and Siemens Healthineers, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program, outside the work presented in this paper. Go to Neurology.org/N for full disclosures.

Publication History

Received by *Neurology* December 21, 2021. Accepted in final form August 31, 2022. Submitted and externally peer reviewed. The handling editor was Linda Hershey, MD, PhD, FAAN.

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Manu Vandijck, MS	Fujirebio Europe NV, Ghent, Belgium	Drafting/revision of the manuscript for content, including medical writing for content
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John Q. Trojanowski, MD, PhD	Department of Pathology and Laboratory Medicine, Institute on Aging, Center for Neurodegenerative Disease Research, University of Pennsylvania School of Medicine, Philadelphia, PA	Drafting/revision of the manuscript for content, including medical writing for content
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Appendix 1 *(continued)*

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Appendix 2 Coinvestigators

Coinvestigators are listed at links.lww.com/WNL/C409

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