

The Trajectory of Cerebrospinal Fluid Growth-Associated Protein 43 in the Alzheimer's Disease Continuum: A Longitudinal Study

Heng Zhang^{a,1}, Diyang Lyu^{a,1} and Jianping Jia^{a,b,c,d,e,*} for the Alzheimer's Disease Neuroimaging Initiative²

^a*Innovation Center for Neurological Disorders and Department of Neurology, Xuanwu Hospital, Capital Medical University, Beijing, China*

^b*Beijing Key Laboratory of Geriatric Cognitive Disorders, Beijing, China*

^c*Clinical Center for Neurodegenerative Disease and Memory Impairment, Capital Medical University, Beijing, China*

^d*Center of Alzheimer's Disease, Beijing Institute of Brain Disorders, Collaborative Innovation Center for Brain Disorders, Capital Medical University, Beijing, China*

^e*Key Laboratory of Neurodegenerative Diseases, Ministry of Education, Beijing, China*

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Abstract.

Background: Synaptic degeneration has been suggested as an early pathological event that strongly correlates with severity of dementia in Alzheimer's disease (AD). However, changes in longitudinal cerebrospinal fluid (CSF) growth-associated protein 43 (GAP-43) as a synaptic biomarker in the AD continuum remain unclear.

Objective: To assess the trajectory of CSF GAP-43 with AD progression and its association with other AD hallmarks.

Methods: CSF GAP-43 was analyzed in 788 participants from the Alzheimer's Disease Neuroimaging Initiative (ADNI), including 246 cognitively normal (CN) individuals, 415 individuals with mild cognitive impairment (MCI), and 127 with AD dementia based on cognitive assessments. The associations between a multimodal classification scheme with amyloid- β (A β), tau, and neurodegeneration, and changes in CSF GAP-43 over time were also analyzed.

Results: CSF GAP-43 levels were increased at baseline in MCI and dementia patients, and increased significantly over time in the preclinical (A β -positive CN), prodromal (A β -positive MCI), and dementia (A β -positive dementia) stages of AD. Higher levels of CSF GAP-43 were also associated with higher CSF phosphorylated tau (p-tau) and total tau (t-tau), cerebral amyloid deposition and hypometabolism on positron emission tomography, the hippocampus and middle temporal atrophy, and cognitive performance deterioration at baseline and follow-up. Furthermore, CSF GAP-43 may assist in effectively predicting the probability of dementia onset at 2- or 4-year follow-up.

¹These authors contributed equally to this work.

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

at: http://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf

*Correspondence to: Prof Jianping Jia, Innovation Center for Neurological Disorders and Department of Neurology, Xuanwu Hospital, Capital Medical University, 100053, Beijing, China. E-mail: jjp@ccmu.edu.cn.

Conclusion: CSF GAP-43 can be used as a potential biomarker associated with synaptic degeneration in subjects with AD; it may also be useful for tracking the disease progression and for monitoring the effects of clinical trials.

Keywords: Alzheimer's disease, biomarker, growth-associated protein 43, synaptic dysfunction

INTRODUCTION

Alzheimer's disease (AD), characterized by progressive cognitive decline, is the most common form of dementia [1]. The pathological changes of AD occur in clinically normal individuals and develop gradually over decades before clinical manifestations appear [2, 3]. The concealed and heterogeneous pathogenesis of AD may drive drug clinical trial failure. Therefore, accurate biomarkers are used to identify AD patients at the preclinical stage; it is particularly important to predict the disease progression of AD.

Based on their proximity to the brain, cerebrospinal fluid (CSF) biomarkers can provide reliable and clinically relevant diagnostic information in AD patients. CSF total tau (t-tau), phosphorylated tau (p-tau), and amyloid- β_{42} ($A\beta_{42}$) are often referred to as core AD features [4]. The National Institute on Aging and Alzheimer's Association (NIA-AA) proposed a new biomarker-oriented framework that classifies $A\beta$, p-tau, and t-tau into the $A\beta$ /tau/neurodegeneration (ATN) system across the AD continuum [5]. The ATN system can potentially help screen, diagnose, predict prognoses, and make appropriate therapeutic decisions. Importantly, incorporating novel candidate biomarkers that represent additional pathophysiological mechanisms of AD to the ATN framework will add further depth and precision to identify relatively homogeneous individuals in the early stage. Substantial studies have shown that synaptic loss and dysfunction are strongly correlated with memory decline and severity of dementia. In addition, synaptic dysfunction occurs before the occurrence of obvious morphological abnormalities and neuronal degeneration [6, 7]. Therefore, synaptic biomarkers in CSF should be investigated in order to expand the ATN system and for clinical practice.

Growth-associated protein 43 (GAP-43) is a presynaptic protein that is mainly distributed in the axon and presynaptic terminals and promotes neurodevelopment, synaptogenesis, and nerve regeneration through regulation of actin dynamics and presynaptic vesicle cycling [8–13]. In postmortem AD brains, there was a significant decrease of GAP-43 in the

frontal cortex and in some areas of the hippocampus [14, 15], while a consistent increase in GAP-43 staining in the stratum lacunosum moleculare, a subfield of the hippocampus, has also been reported [16]. A recent study indicated increased levels of CSF GAP-43 in AD patients compared to controls, while no significant changes were noted in other neurodegenerative disorders [17, 18]. Additionally, previous studies found that GAP-43 increases in line with the distribution of amyloid plaques and tau neurofibrillary tangles as well as with the progression of cognitive decline [18]. Therefore, GAP-43 has the capacity to sensitively and specifically identify AD patients in clinical research. Nevertheless, studies investigating longitudinal CSF GAP-43 changes are lacking.

This study involved 788 individuals from the Alzheimer's Disease Neuroimaging Initiative (ADNI) who were cognitively normal (CN) or were diagnosed with mild cognitive impairment (MCI) or AD dementia. We hypothesized that GAP-43 levels in CSF increases with disease progression and are longitudinally correlated with biochemical, imaging, and cognitive measurements during the disease process. We aimed to determine the potential of CSF GAP-43 in tracking the symptom-based or pathology-oriented progression of AD and evaluate the efficiency of CSF GAP-43 in predicting the onset of dementia using longitudinal data.

METHODS

Alzheimer's disease neuroimaging initiative

The data used in this study were obtained from the ADNI database (<http://adni.loni.usc.edu>), which is a longitudinal multisite study launched in 2003 by the National Institute of Biomedical Imaging and Bioengineering, the Food and Drug Administration, private pharmaceutical companies, and nonprofit organizations in order to develop clinical, imaging, genetic, and biochemical biomarkers for the early detection and tracking of AD. Individuals for ADNI were recruited from over 50 sites across the US and

Canada. The ADNI was approved by the medical ethics committees of all participating institutions.

Participants

We selected 788 participants with available CSF GAP-43 data at baseline from the ADNI-GO and ADNI-2 databases. The diagnoses of CN, MCI, or AD dementia were based on cognitive assessments. Inclusion and exclusion criteria have been described [19]. CN participants were included if their Mini-Mental State Examination (MMSE) scores were between 24 and 30 and if their Clinical Dementia Rating Scale (CDR) scores were zero. MCI participants were included if they had MMSE scores between 24 and 30, abnormal memory function documented with scores within the education-adjusted ranges on the Logical Memory II subscale from the Wechsler Memory Scale, CDR scores of 0.5, preserved activities of daily living, and absence of dementia. If individuals fulfilled the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer Disease and Related Disorders Association criteria for probable AD, reported an MMSE score between 20 and 26, and had a CDR score from 0.5 to 1.0, they were considered to have AD dementia.

Cerebrospinal fluid GAP-43

CSF GAP-43 was analyzed using an in-house enzyme-linked immunoassay (ELISA) method described previously in detail [18]. The ELISA was developed by combining the mouse monoclonal GAP-43 antibody NM4 (coating antibody) and a polyclonal GAP-43 antibody (detector antibody) that recognizes the C-terminal of GAP-43. The analyses were performed by board-certified laboratory technicians. The assay range was 312–20,000 pg/mL. During sample runs in the clinical evaluation study, the repeatability coefficient of variation (CV) % of quality controls (QC1 and QC2) was 5.5% versus 11%, and the inter-assay CV% was 6.9% versus 15.6%.

Biomarkers in the CSF

CSF samples were collected and shipped on dry ice to the ADNI Biomarker core laboratory and stored in polypropylene tubes at -80°C . All CSF concentrations were measured using automated Roche Elecsys and Cobas e 601 immunoassay analyzer systems. According to published and validated cut-off point [20], CSF $\text{A}\beta_{42}$ (<977 pg/mL), p-tau181

(>27 pg/mL), and t-tau (>300 pg/mL) were used to define biomarker (A/T/N) positivity and stratify participants according to the ATN framework [5] in this study. CSF $\text{A}\beta_{42}$ was also used to determine amyloid status.

Neuroimage acquisition and analysis

The imaging data obtained from the ADNI dataset were fully preprocessed using a standardized pipeline; the image acquisition details are provided elsewhere (<http://adni.loni.usc.edu/>) and are summarized briefly below.

Structural imaging was performed using a 3.0-Tesla magnetic resonance imaging (MRI) scanner with T1-weighted imaging parameters. Details of the parameters are provided on the ADNI website (<http://adni.loni.usc.edu/>). FreeSurfer (version 5.1) was used to quantify the regional volumes. Data of hippocampal, entorhinal, middle temporal, and whole brain volume were used and adjusted for total intracranial volume [21].

Florbetapir was used for amyloid PET images, and these data were acquired 50 to 70 min post-injection; images were averaged, spatially aligned, interpolated to a standard voxel size, and smoothed to a common resolution of 8 mm full width at half maximum [22]. The MRI T1-weighted magnetization-prepared rapid acquisition gradient echo (MP-RAGE) image of each participant from the nearest available visit was segmented and parcellated using FreeSurfer (version 5.3.0) to define regions of interest (ROIs) in the native space. The PET images were then co-registered to the corresponding MP-RAGE using SPM (version 5). The intensity-normalized standard uptake value ratio (SUVR) value for each ROI was obtained by dividing the tracer uptake in these regions by the value in the whole cerebellum. To estimate the global florbetapir SUVR, values from the frontal, cingulate, parietal, and temporal regions were averaged. Florbetapir-PET results were considered positive if global SUVRs were at least 1.11 as recommended by the ADNI [23].

Fluorodeoxyglucose (FDG)-PET data were acquired 30 to 60 min post-injection; the frames were then averaged, spatially aligned, interpolated to a standard voxel size, and smoothed to a common resolution of 8 mm full width at half maximum. Each subject's summary FDG index was the mean uptake in the right and left angular, temporal, and bilateral posterior cingulate regions relative to the mean of a pons/vermis reference region.

APOE ε4 genotyping

APOE genotyping was performed during participant enrollment and included in the ADNI database. DNA was extracted from 3 mL blood samples. *APOE* genotyping of these samples, was performed using polymerase chain reaction amplification, HhaI restriction enzyme digestion, resolution on 4% MetaPhor gel, and visualization by ethidium bromide staining [24], which were described in <http://adni.loni.usc.edu> in detail.

Cognitive assessment

Global cognition was assessed using the MMSE, the Alzheimer Disease Assessment Scale-Cognitive Subscale (ADAS-Cog), and the CDR Scale Sum of Boxes (CDR-SB). We also obtained composite scores reflecting memory and executive functions (EF), language, and visuospatial (VS) functions [25, 26].

Statistical analysis

Demographic variables (age, sex, education, and *APOE* ε4 status) and other characteristics at baseline were compared among groups using chi-square, Fisher's exact, or Kruskal-Wallis tests, where appropriate. We also used linear mixed effect (LME) model to evaluate longitudinal changes of CSF GAP-43 levels in groups with different clinical diagnoses (stratified by Aβ status or not) and different ATN classifications. All LME models were adjusted for age, sex, *APOE* ε4 counts, and education for comparison among the groups. The same models were applied for the association between CSF GAP-43 and levels of CSF core biomarkers, neuroimaging measures, and cognitive measures at baseline and follow-up. Baseline and longitudinal data of all variables except for CSF GAP-43 were z-scale transformed to ensure that the effect sizes could be directly comparable between association analyses. *p* values corrected for multiple comparisons were performed using the Benjamini-Hochberg procedure.

To evaluate the diagnostic effectiveness of CSF GAP-43 between different groups, we obtained the receiver operating characteristic (ROC) curves. We also generated a nomogram to predict the probability of dementia or being free from dementia at 2 and 4 years. The performance evaluation of the nomogram included calibration, a time-dependent ROC curve, and the Harrell concordance index. Internal validation was performed using bootstrapping with a 1000 resampling method.

All statistical analyses were performed using R statistical software (version 4.0.3) and the R package "lmerTest" was used for LME model, "timeROC", "survivalROC", "pROC", "survival" and "rms" for establishing diagnosis and prediction models. Two-sided *P* values <0.05 were considered statistically significant.

RESULTS

Participant characteristics

We included 788 participants at baseline, of which 246 (31.2%) were CN participants, 415 (52.7%) had MCI, and 127 (16.1%) had AD dementia. The mean (standard deviation, SD) age and education of all included individuals was 72.5 (7.30) and 16.3 (2.60) years, respectively; 47.1% of the participants were women, and 45.4% had at least one *APOE* ε4 allele. The demographics, CSF core biomarkers, neuroimaging, and cognition characteristics of CN, MCI, and AD dementia at baseline are shown in Table 1. The mean MMSE scores were 29.1, 28.1, and 23.2, respectively for the three clinically diagnostic groups. The baseline level of CSF GAP-43 was increased across the AD continuum (CN, 4,990.0 pg/mL; MCI, 5,118.8 pg/mL; dementia, 6,331.1 pg/mL). There was a distinct increase in CSF GAP-43 in dementia versus CN ($p < 0.001$) and dementia versus MCI ($p < 0.001$) patients. The baseline characteristics of participants grouped by diagnosis and Aβ status and by ATN frameworks are presented in Supplementary Tables 1 and 2.

Longitudinal CSF GAP-43 and baseline diagnosis

The results from LME models showed that the GAP-43 level increased significantly over time in all groups (CN, 9.42 pg/mL per month; MCI, 9.85 pg/mL per month; dementia 9.82 pg/mL per month), with greater rates among patients with MCI compared to CN controls and patients with AD dementia (Fig. 1A, B). Although there was no significant difference in the increase rate between the diagnostic groups, there was a significant difference in the baseline GAP-43 levels between the two groups (Supplementary Table 3).

Similar results were found when the diagnostic groups were stratified by Aβ status (Fig. 1C, Supplementary Tables 4 and 5). Patients with Aβ-positive MCI had increased baseline levels ($p < 0.001$) and

Table 1
Baseline participant demographics

	CN (N = 246)	MCI (N = 415)	Dementia (N = 127)
Age	73.0 (5.99)	71.5 (7.47)	74.6 (8.48)
Sex (Female)	134 (54.5)	187 (45.1)	50 (23.0)
Education	16.7 (2.48)	16.2 (2.63)	15.7 (2.67)
<i>APOE</i>			
<i>APOE</i> ϵ 4 ^{-/-} No. (%)	174 (40.5)	214 (49.8)	42 (9.8)
<i>APOE</i> ϵ 4 ^{-/+} No. (%)	65 (23.2)	156 (55.7)	59 (21.1)
<i>APOE</i> ϵ 4 ^{+/+} No. (%)	7 (9.0)	45 (57.7)	26 (33.3)
CSF GAP-43(pg/mL)	4990.0 (2706.19)	5118.8 (2826.12)	6331.1 (3126.55)
CSF core biomarkers (pg/mL)			
A β ₄₂	1384.5 (646.99)	1094.8 (569.09)	724.0 (451.35)
p-tau	21.7 (9.43)	26.4 (14.38)	36.6 (16.18)
t-tau	236.8 (92.75)	274.9 (128.71)	371.4 (154.61)
PET imaging			
A β -PET (AV45)	1.12 (0.18)	1.22 (0.23)	1.40 (0.22)
FDG-PET	1.32 (0.11)	1.26 (0.13)	1.06 (0.15)
Structure imaging (volume)*			
Hippocampus	7545.1 (882.8)	7063.5 (1111.5)	5924.9 (968.3)
Entorhinal	3870.1 (591.8)	3635.4 (722.0)	2824.8 (666.8)
Mid temporal	20752.3 (2504.4)	20393.5 (2703.9)	17770.6 (3148.3)
Whole brain	1055409.7 (103137.9)	1057948.9 (104526.2)	1011565.0 (113117.4)
Cognitive measures			
MMSE	29.1 (1.17)	28.1 (1.71)	23.2 (2.03)
CDR-SB	0.05 (0.15)	1.44 (0.87)	4.59 (1.70)
ADAS-Cog	5.7 (2.94)	9.2 (4.46)	20.7 (6.86)
Memory	0.88 (0.47)	0.27 (0.54)	-0.78 (0.34)
EF	0.80 (1.5)	0.34 (1.77)	-0.38 (2.35)
Language	0.80 (0.48)	0.46 (0.49)	-0.21 (0.54)
VS	-0.24 (1.97)	-0.20 (2.22)	-1.16 (2.78)
CSF GAP-43 No. of samples			
Month			
0	246	415	127
24	126	160	51
48	50	54	22

Continuous variables were expressed as mean (SD) and categorical variables as number (%). *Structure imaging measures reported here are unadjusted by total intracranial volume. CN, cognitively normal; CSF, cerebrospinal fluid; EF, executive function; FDG, fluorodeoxyglucose; MCI, mild cognitive impairment; PET, positron emission tomography; p-tau, phosphorylated tau; t-tau, total tau; MMSE, Mini-Mental State Examination; VS, visuospatial.

309 higher rates ($p=0.607$) compared to patients with
310 A β -negative MCI. However, there were no significant
311 differences between the A β -negative and A β -positive
312 AD groups (Supplementary Table 5).

313 *Longitudinal CSF GAP-43 in groups stratified by* 314 *A β , tau, and neurodegeneration*

315 According to the ATN system, a significant
316 increase in CSF GAP-43 levels over time was
317 found in participants with normal AD biomarkers
318 (A-T-N-), AD pathological changes (A+T-N-),
319 and AD (A+T+N- or A+T+N+) (Fig. 1D, Sup-
320 plementary Table 6). Baseline CSF GAP-43 levels
321 were higher in participants with AD (A+T+N- or
322 A+T+N+) compared to participants with normal AD
323 biomarkers (A-T-N-) ($p<0.001$) and those with
324 pathological AD changes (A+T-N-) ($p<0.001$).

325 Moreover, accompanying the deteriorating pathology
326 of AD, CSF GAP-43 levels in A+T+N+ partici-
327 pants were significantly higher than in A+T+N-
328 patients ($p<0.001$) (Supplementary Table 7). How-
329 ever, no differences in the change rates were identified
330 between the ATN groups. Surprisingly, the baseline
331 levels of GAP-43 in A+T-N- patients were lower
332 than in A-T-N- patients (Supplementary Table 7).

333 In addition, compared with the A-, T-, and N-
334 groups, subjects in the A+ ($p<0.001$), T+ ($p<0.001$),
335 and N+ ($p<0.001$) groups had significantly higher
336 CSF GAP-43 levels at baseline, respectively. There
337 was a large difference in GAP-43 levels between T+
338 and T- cases as well as between N+ and N- cases,
339 but the difference between A+ and A- patients was
340 relatively small. Similarly, there was no slope dif-
341 ference between the positive and negative biomarker
342 groups (Fig. 1E, G, Supplementary Table 8).

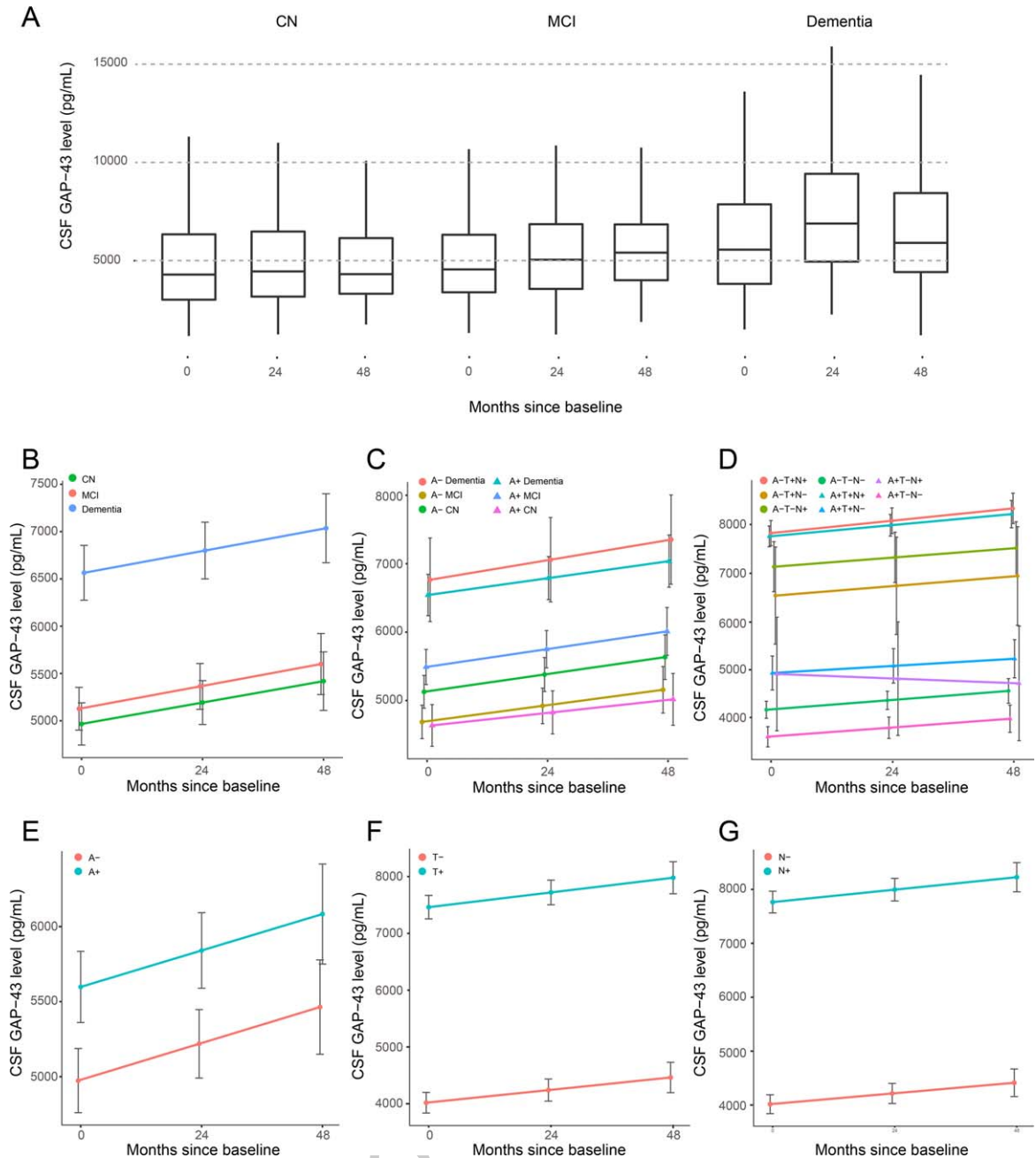


Fig. 1. CSF GAP-43 levels by diagnostic group, A β status, and ATN classification. A) Observed data in different diagnostic groups; estimated CSF GAP-43 trajectories by diagnosis (B), by diagnosis and A β status (C), by ATN classification (D); estimated CSF GAP-43 trajectories by A status (E), by T status (F), and by N status (G). A, amyloid- β ; T, tau pathology; N, neurodegeneration; CN, cognitive normal; MCI, mild cognitive impairment; AD, Alzheimer's disease.

342 Association of CSF GAP-43 with CSF
343 biomarkers, neuroimaging, and cognition

344 Table 2 shows the associations between base-
345 line and longitudinal CSF GAP-43 levels with CSF

core biomarker (A β ₄₂, p-tau, and t-tau) levels;
346 structure volume (hippocampal, entorhinal, middle
347 temporal, and whole brain) measured by MRI; AV45-
348 PET; FDG-PET; and cognitive measures (MMSE,
349 CDR-SB, and ADAS-Cog score; memory, executive
350

Table 2
Associations of AD-related hallmarks with baseline and longitudinal levels of CSF GAP-43

	Baseline CSF GAP-43			Longitudinal CSF GAP-43		
	β	s.e.	<i>p</i>	β	s.e.	<i>p</i>
CSF core biomarkers						
A β ₄₂	0.014	0.0352	0.683 (0.785)	0.012	0.0285	0.685 (0.685)
p-tau	0.731	0.0240	<0.001 (<0.001)	0.728	0.0197	<0.001 (<0.001)
t-tau	0.710	0.0247	<0.001 (<0.001)	0.703	0.0203	<0.001 (<0.001)
PET imaging						
A β -PET (AV45)	0.259	0.0345	<0.001 (<0.001)	0.263	0.0279	<0.001 (<0.001)
FDG-PET	-0.110	0.0347	0.0016 (0.0027)	-0.117	0.0316	<0.001 (<0.001)
Structure imaging						
Hippocampal	-0.104	0.0403	0.0102 (0.0136)	-0.116	0.0372	0.0019 (0.0030)
Entorhinal	-0.103	0.0377	0.0063 (0.0095)	-0.091	0.0348	0.0088 (0.0127)
Mid-Temporal	-0.075	0.0367	0.0426 (0.0511)	-0.087	0.0349	0.0129 (0.0165)
Whole brain	0.045	0.0430	0.297 (0.324)	0.063	0.0364	0.083 (0.093)
Cognitive assessment						
MMSE	-0.193	0.0402	<0.001 (<0.001)	-0.166	0.0290	<0.001 (<0.001)
CDR-SB	0.173	0.0412	<0.001 (<0.001)	0.160	0.0293	<0.001 (<0.001)
ADAS-Cog	0.157	0.0376	<0.001 (<0.001)	0.161	0.0288	<0.001 (<0.001)
Memory composite	-0.189	0.0378	<0.001 (<0.001)	-0.184	0.0290	<0.001 (<0.001)
EF composite	-0.084	0.0367	0.0222 (0.0275)	-0.10	0.0293	<0.001 (0.0012)
Language composite	-0.096	0.0372	0.010 (0.0136)	-0.121	0.0289	<0.001 (<0.001)
VS composite	-0.046	0.0451	0.306 (0.324)	-0.067	0.0383	0.082 (0.093)

Levels of all AD-related hallmarks were z-transformed so that effect sizes were directly comparable. Linear mixed-effects models were adjusted for age and sex. *p* values in parentheses were corrected for multiple comparisons by Benjamini–Hochberg procedure. A β , amyloid- β ; AD, Alzheimer's disease; ADAS-Cog, Alzheimer Disease Assessment Scale-Cognitive Subscale; CDR-SB, Clinical Dementia Rating Scale Sum of Boxes; CSF, cerebrospinal fluid; EF, executive function; FDG, fluorodeoxyglucose; MCI, mild cognitive impairment; PET, positron emission tomography; p-tau, phosphorylated tau; t-tau, total tau; MMSE, mini-mental state examination; VS, visuospatial. *p* values in parentheses are corrected for multiple comparisons using the Benjamini-Hochberg procedure.

function, language, and visual-spatial composite) irrespective of the diagnostic groups.

Except for CSF A β ₄₂, whole brain volume, and VS composite, all the variables were cross-sectionally correlated with CSF GAP-43 levels. After correcting for multiple comparisons, all the above-mentioned associations remained significant, with the exception of the mid-temporal volume. Of all the variables, CSF p-tau has the strongest association with GAP-43 positivity, followed by CSF t-tau. Of the imaging measures, A β -PET had the largest association with CSF GAP-43. MMSE, CDR-SB, ADAS-Cog, and memory composite had the largest associations among the cognitive measures. During follow-up, all variables were significantly associated with longitudinal GAP-43, except for CSF A β ₄₂, whole brain volume, and VS composite. We also reported an association in each diagnostic group at baseline (Supplementary Table 9). Among the CN participants, CSF p-tau and t-tau were most correlated with CSF GAP-43 after *p*-value correction. In the MCI group, higher levels of GAP-43 were associated with higher CSF p-tau and t-tau levels, greater cerebral A β deposition (A β -PET), lower MMSE score, higher ADAS-cog score, and lower memory and

executive function composites. Among participants with dementia, the association remained significant only for CSF p-tau and t-tau levels.

The diagnostic and predictive effectiveness of CSF GAP-43

Given the significant increase in CSF GAP-43 over time, we assessed the ability of CSF GAP-43 to predict the probability of dementia onset using a prediction model. A nomogram (concordance index = 0.710), including clinical features and risk scores, was constructed (Fig. 2A). The predictive accuracy of the nomogram was evaluated using a time-dependent ROC curve analysis. The results showed that the area under the curve (AUC) of the nomogram was 0.740 (95% confidence interval [CI]: 0.662–0.818) and 0.808 (95% CI: 0.704–0.912) in predicting 2- and 4-year dementia-free onset, respectively (Fig. 2B). A better estimation of this prediction model was also verified by calibration curves, which showed that the observed nomogram was closer to the ideal nomogram (Supplementary Figure 1). In addition, the capacity of CSF GAP-43 to differentiate between different diagnostic groups was also

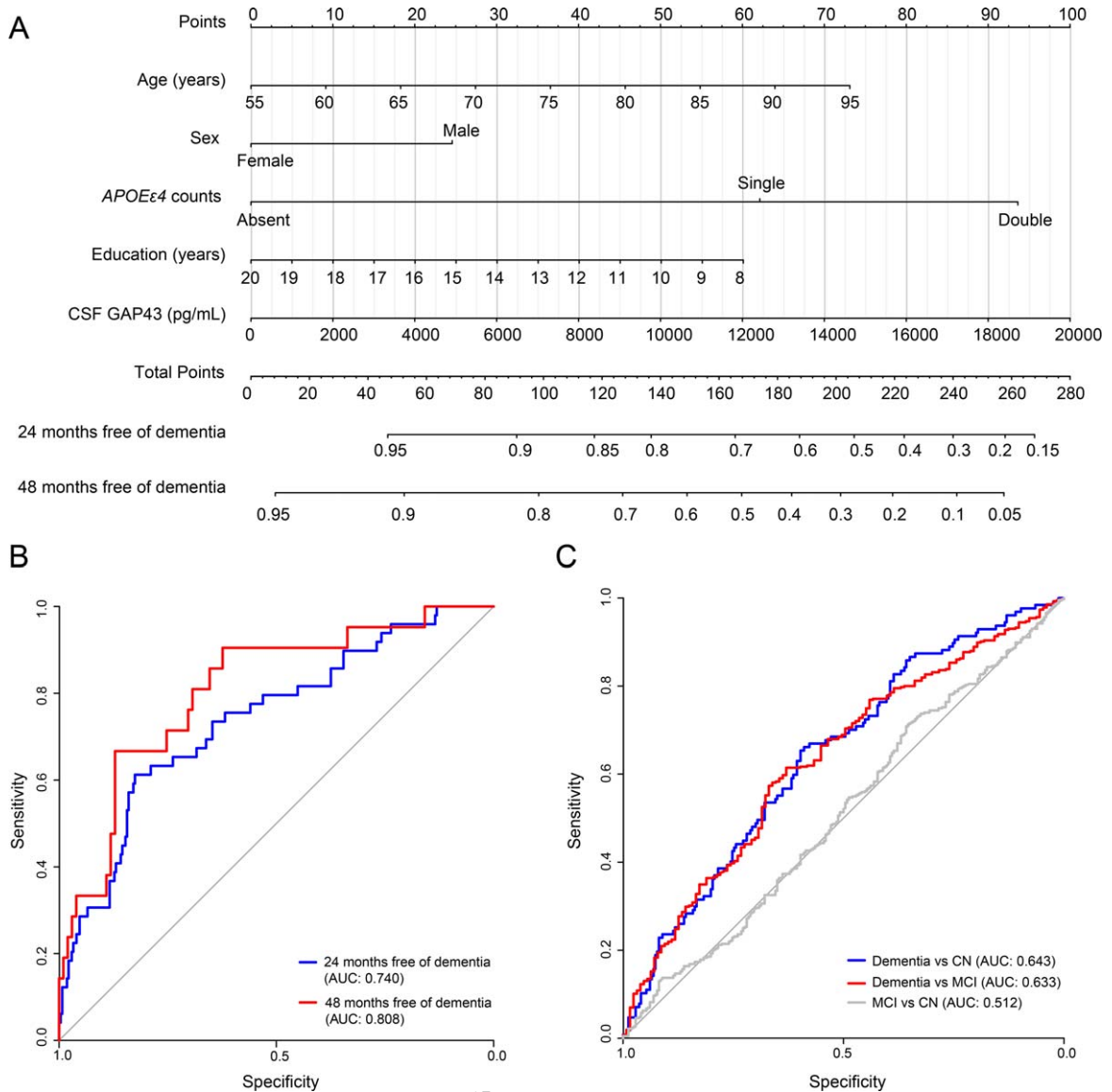


Fig. 2. The prediction and diagnostic model of GAP-43 for Alzheimer's disease. A) Nomogram based on the results of the multivariable cox analysis. Points were assigned for age, sex, APOE ϵ 4 counts, education, and CSF GAP-43 level by drawing a line upward from the corresponding values to the point line. The sum of these five points, plotted on the "total points" line, corresponds to estimates of the overall dementia-free subjects at 2 years and 4 years. B) The time-dependent ROC curves for verifying the accuracy of the nomogram. C) Receiver operating characteristic curves for differentiating CN, MCI, and AD dementia. CN, cognitive normal; MCI, mild cognitive impairment; AD, Alzheimer's disease.

399 estimated using ROC curve analysis. The AUC for
 400 CN versus MCI, CN versus dementia, and MCI versus
 401 dementia was 0.512, 0.634, and 0.633, respectively
 402 (Fig. 2C).

403 DISCUSSION

404 In this study, we found that CSF GAP-43 lev-
 405 els increased over time with disease progression.

406 Compared with the CN group, CSF GAP-43 levels
 407 were increased in patients with MCI and AD demen-
 408 tia at baseline. In addition, increasing levels over
 409 time were also identified in preclinical AD, prodromal
 410 AD, and dementia stages of AD. Besides, there
 411 was a high concordance between CSF GAP-43 lev-
 412 els and other AD pathologies (particularly CSF p-tau,
 413 t-tau, and A β -PET) in all diagnostic groups at the
 414 baseline and longitudinal stages. When stratified by

415 ATN class, the baseline GAP-43 level was mainly
416 increased in T+ or N+ cases, especially in A-T+N-,
417 A-T+N+, A+ T+N-, and A+T+N+ profiles, and
418 rates were most obviously increased over time in
419 A+T+N+ patients. Taken together, these findings sug-
420 gest that CSF GAP-43 level is a dynamic biomarker
421 that changes throughout the process of AD and is
422 sensitive to progressive neurodegeneration. Incorpor-
423 ating CSF GAP-43 into the ATN framework may
424 contribute to the diagnosis, prediction of disease pro-
425 gression, and staging of AD, even in its preclinical
426 stage.

427 Previous studies have indicated that synaptic
428 decline in brains with early AD or MCI is closely
429 associated with cognitive function long before
430 symptoms appear [27–30], which supports monitor-
431 ing biomarkers reflecting synaptic pathology, such
432 as presynaptic proteins synaptosomal-associated
433 protein-25 (SNAP-25) [31], synaptotagmin-1 [32],
434 and GAP-43 [18, 33], which are helpful in iden-
435 tifying AD as early as possible. However, to our
436 knowledge, CSF GAP-43 in the AD continuum
437 has been less investigated, especially in terms of
438 longitudinal observations of the trajectory of CSF
439 GAP-43 changes across AD progression. Our study
440 replicated some previous findings on CSF GAP-
441 43 cross-sectionally [18, 34] and comprehensively
442 reported its association with other AD-related neu-
443 roimaging parameters and cognitive measures for the
444 first time. There was a stable significant correlation
445 between CSF GAP-43 concentration and CSF p-tau,
446 CSF t-tau, and cerebral amyloid deposition measured
447 by AV45-PET in all subjects, but not with CSF A β ₄₂
448 [35–37]. The reason for this phenomenon may be
449 that CSF A β ₄₂ reflects mainly soluble A β forms, and
450 represents temporary state, in contrast to A β PET,
451 which most likely reflects the continuous A β depo-
452 sition forming the plaque, correlating strongly with
453 synaptic injury. We also noticed that CSF GAP-43
454 levels were positively correlated with CSF A β ₄₂ lev-
455 els in the CN group. The potential explanation for this
456 is that the transportation of A β ₄₂ from the brain to
457 the periphery is active with enhanced compensatory
458 function of the blood-brain barrier in CN participants.

459 We found that CSF levels of GAP-43 were sig-
460 nificantly increased in subjects with AD dementia
461 compared to subjects with MCI and CN. These results
462 are consistent with previous research in other cohorts
463 [38]. In addition, we found no significant difference in
464 the rate of change of CSF GAP-43 levels between the
465 different diagnostic groups. It seemed that CSF GAP-
466 43 levels increased linearly from the asymptomatic

467 stage to mild dementia. One explanation is that the
468 sample size or follow-up time was insufficient to
469 detect a small CSF GAP-43 change per unit time.
470 We hypothesized that the heterogeneous pathologies
471 presented in the different diagnostic groups mainly
472 resulted in this phenomenon. Further, we explored
473 the hidden association between the longitudinal tra-
474 jectory of CSF GAP-43 and the specific pathology
475 of A β , tau, and neurodegeneration defined by ATN
476 profiles. Surprisingly, participants with A-negative
477 CN and A-T-N- profiles showed elevated levels
478 of CSF GAP-43, which may indicate that the ear-
479 liest elevations of CSF GAP-43 levels may occur
480 before CSF biomarkers of amyloid pathology reach
481 their abnormal thresholds. The level of CSF GAP-
482 43 increased in participants with exclusively T+ or
483 N+ATN profiles and increased to a maximum in
484 participants with both T+ and N+ generally (e.g.,
485 A-T+N+ or A+T+N+). This result is similar to find-
486 ings from other neurodegenerative conditions and
487 brain injuries due to other causes [12, 39]. There is
488 evidence that tau pathology is involved in synaptic
489 degeneration and contributes to cognitive decline [40,
490 41]. One interpretation of this is that GAP-43 reflects
491 the synaptic loss or dysfunction that occurs indepen-
492 dent of A β pathology. When all participants were
493 stratified into A-negative or A-positive subgroups,
494 GAP-43 levels in the A-positive group were signif-
495 icantly higher than in the A-negative subgroup. Of
496 note, dichotomous categories of A β , irrespective of
497 tau (T) or neurodegeneration (N) in previous studies,
498 may imperceptibly increase the effect of A β on GAP-
499 43 levels. In addition, small sample sizes of certain
500 groups (e.g., $n = 11$ in the A-T-N+ group, $n = 2$ in
501 the A+T-N+ group) might also have impacted the
502 analytical results, resulting in an inaccurate estimate.

503 This study had some limitations. First, the enrolled
504 subjects in the dementia group had mild clinical
505 severity, which may not represent all AD patients,
506 especially AD subjects with moderate to severe
507 dementia. Moreover, during the long-term follow-
508 up, many participants, especially those with AD
509 dementia, dropped out, resulting in the estimated tra-
510 jectory of CSF GAP-43 being uncertain. Previous
511 studies have reported that tau-PET imaging is more
512 closely associated with neurodegeneration than CSF
513 tau biomarkers. However, there also was a lack of
514 tau-PET imaging data, which was used to investigate
515 the association between CSF GAP-43 and tau pathol-
516 ogy in the present study. A tau-PET program has been
517 available in the ADNI-3 since 2015. It would be valu-
518 able to investigate the association between GAP-43

519 and the distribution pattern of tau pathology. In addition, we used the prediction model to estimate the probability of dementia between CN and AD participants and not for other types of dementia. Finally, the participants participating in the ADNI were mainly recruited in the United States and Canada, and thus our research results may not be universal to other races in the world. Future studies should validate this trajectory in more cohorts.

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528 Taken together, these findings suggest that CSF GAP-43 can be used as a synaptic pathology biomarker to track disease progression across the AD continuum, which supports the incorporation of CSF GAP-43 into the ATN system to increase the accuracy of future classification algorithms, evaluate prognosis, and make appropriate clinical therapeutic decisions.

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