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Level of CSF GAP-43 and white matter microstructural changes in Alzheimer's disease

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Abstract

Objectives: Several studies have reported altered cerebrospinal fluid (CSF) concentrations of presynaptic proteins, such as growth-associated protein 43 (GAP-43) in Alzheimer's disease (AD) patients. Given the potential predictive role of CSF GAP-43 for AD, the current study aimed to investigate the relationship between CSF GAP-43 levels and DTI-detected microstructural changes in the white matter (WM).

Methods: Data from three groups of participants including 39 control normals (CN), 138 MCI, and 39 AD subjects were obtained from the Alzheimer's disease Neuroimaging Initiative (ADNI). Linear regression was used to the association of CSF-GAP43 and DTI values (including MD, RD, AxD, and FA) in the brain.

Results: We found a significant association between CSF-GAP43 and FA (p-value = 0.011). Also, a negative association was found between CSF-GAP43 concentration and AD, MD, and RD values in MCI (p-value = 0.013, p-value = 0.004, p-value = 0.017). The regression models also revealed a positive association between CSF-GAP43 and FA value in AD subjects (p-value = 0.028). Furthermore, increased CSF-GAP43 level was associated with lower AD, MD, and RD values in brain WM of AD patients (p-value = 0.022, p-value = 0.033, p-value = 0.041).

Conclusion: Our study provides a better understanding of the link between CSF GAP-43 and WM changes in patients with MCI and AD. Our findings support the application of CSF GAP-43 as an effective biomarker for monitoring individuals at high risk of AD in the early stages.

Keywords: Alzheimer's Disease, white matter, GAP-43, synaptic, DTI

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Introduction

Alzheimer's disease (AD) is one of the most common neurodegenerative disorders and the leading cause of dementia worldwide. The neuropathological hallmarks of AD include the extracellular aggregation of amyloid-β (Aβ) plaques, intracellular deposition of neurofibrillary tangles containing phosphorylated tau protein, and synaptic loss (1). Synapses are crucial for cognitive function, and synaptic impairment is a pathological feature of AD. Quantitative morphometric studies have demonstrated a 25-35% decline in synaptic density in the temporal and frontal cortical biopsies of patients within 2-4 years of AD onset (2). In AD patients, synaptic loss primarily occurs in the neocortex and hippocampus (3). Synaptic damage and cell loss lead to brain atrophy, and synaptic dysfunction is associated with cognitive impairment in AD (4).
Evaluating synaptic dysfunction in vivo could guide AD clinical research and provide biomarkers for outcome

Table1. Participants characteristic

Demographic and health characteristics	CN (39)	MCI(138)	AD(39)	*P-value*
Age, years	73.1(±6.3)	72.8(±6.8)	74.4 (±8.1)	0.655
Education, years	16.4(±2.6)	16.0(±2.6)	15.2(±2.9)	0.084
MMSE score	28.8(±1.4)	27.9(±1.9)	23.4(±1.8)	<0.001
CSF GAP-43 level, pg/mL	4586.9 (±1240.4)	5322.7(±1539.6)	6083.3(±1136.3)	<0.001

Values are shown as mean(±SD), Mini-Mental State Examination(MMSE), Control normal (CN), Mild cognitive impairment (MCI), and Alzheimer's disease (AD); results of ANOVA analysis between groups noted as p-value

assessment in AD clinical trials. In recent years, progress has been made in the evaluation of synaptic biomarkers in the cerebrospinal fluid (CSF). Depending on the localization of the synaptic protein, synaptic biomarkers can be divided into pre- and postsynaptic biomarkers. Several studies have reported altered CSF concentrations of presynaptic proteins, such as growth-associated protein 43 (GAP-43), synaptosomal-associated protein 25 (SNAP-25), and synaptotagmin-1, as well as the postsynaptic protein neurogranin, in AD patients (5-8). Among these synaptic proteins, GAP-43 plays an important role in the learning and memory storage process (9, 10).

Diffusion Tensor Imaging (DTI) enables the in vivo, non-invasive assessment of neurodegeneration, white matter (WM) disruption, and synaptic damage in patients with Alzheimer's disease (AD) (10). Emerging research suggests that WM changes may serve as a marker for pathological significance, potentially offering a promising target for the early diagnosis of dementia (11). The evidence regarding the association between CSF GAP-43 and WM microstructure in the AD continuum is currently insufficient. Given the potential predictive role of CSF GAP-43 for AD, the current study aimed to investigate the relationship between CSF GAP-43 levels and DTI-detected microstructural changes in the WM.

Materials and Methods

Data Acquisition

The data for this investigation was obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). ADNI was established in 2003 as a public-private partnership directed by Principal Investigator Michael W. Weiner, MD. The primary objective of ADNI is to assess the progression of mild cognitive impairment (MCI) and early Alzheimer's disease (AD) by combining serial PET, MRI, biological markers, and clinical and neuropsychological measures. The most up-to-date information is available at www.adni-info.org.

Participants

The study included baseline data from participants in the ADNI-2 and ADNI-GO cohorts who had available CSF GAP-43 and DTI statistical results. The sample comprised 39 control normals (CN), 138 MCI, and 39 AD subjects. All MCI subjects were diagnosed based on the following criteria: Mini-Mental State Examination (MMSE) scores between 24 and 30, a memory complaint, objective memory loss measured by education-adjusted scores on the Wechsler Memory Scale Logical Memory II, a Clinical Dementia Rating (CDR) of 0.5, absence of significant impairment in other cognitive domains, essentially preserved activities of daily living, and absence of dementia.

CSF GAP-43 Measurement

The cerebrospinal fluid (CSF) levels of growth-associated protein 43 (GAP-43) were measured using an in-house enzyme-linked immunosorbent assay (ELISA) method developed at the Clinical Neurochemistry Lab, University of Gothenburg, Sweden. The ELISA was developed by using a combination of the monoclonal GAP-43 antibody NM4 as the coating antibody and the polyclonal GAP-43 antibody ABB-135 as the detector antibody, which recognizes the C-terminal of GAP-43. The ELISA assay range for CSF-GAP43 was from 312 to 20,000 pg/ml. Quality control CSF samples were provided by the Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden. The details of the ELISA assay for the measurement of CSF GAP-43 have been described previously.

DTI Processing and Image Analysis

The results of the DTI regions of interest (ROI) analysis were downloaded from the ADNI cohort. DTI scans underwent normalization using the Montreal Neurological Institute and Hospital (MNI) nu_correct tool (www.bic.mni.mcgill.ca/software/). Non-brain tissues were removed using the Brain Extraction Tool (BET) from FSL (12). The T1-weighted image was aligned to a version of the Colins27 brain template (13) using FSL's flirt (14). A single diffusion tensor was modeled at each voxel in the brain [19]. Scalar anisotropy and diffusivity maps were obtained from the resulting diffusion tensor eigenvalues (λ1, λ2, λ3). Fractional anisotropy (FA), mean diffusivity (MD), radial diffusivity (RD), and axial diffusivity (AxD) were calculated. Lower FA and higher RD, AxD, and MD are related to demyelination and degeneration in white matter. The FA image from the Johns Hopkins University (JHU) DTI atlas was registered to each subject using a shared information-based elastic registration algorithm. To prevent label intermixing, nearest-neighbor interpolation was used to apply the deformation to the stereotaxic JHU "Eve" white matter atlas labels. This placed the

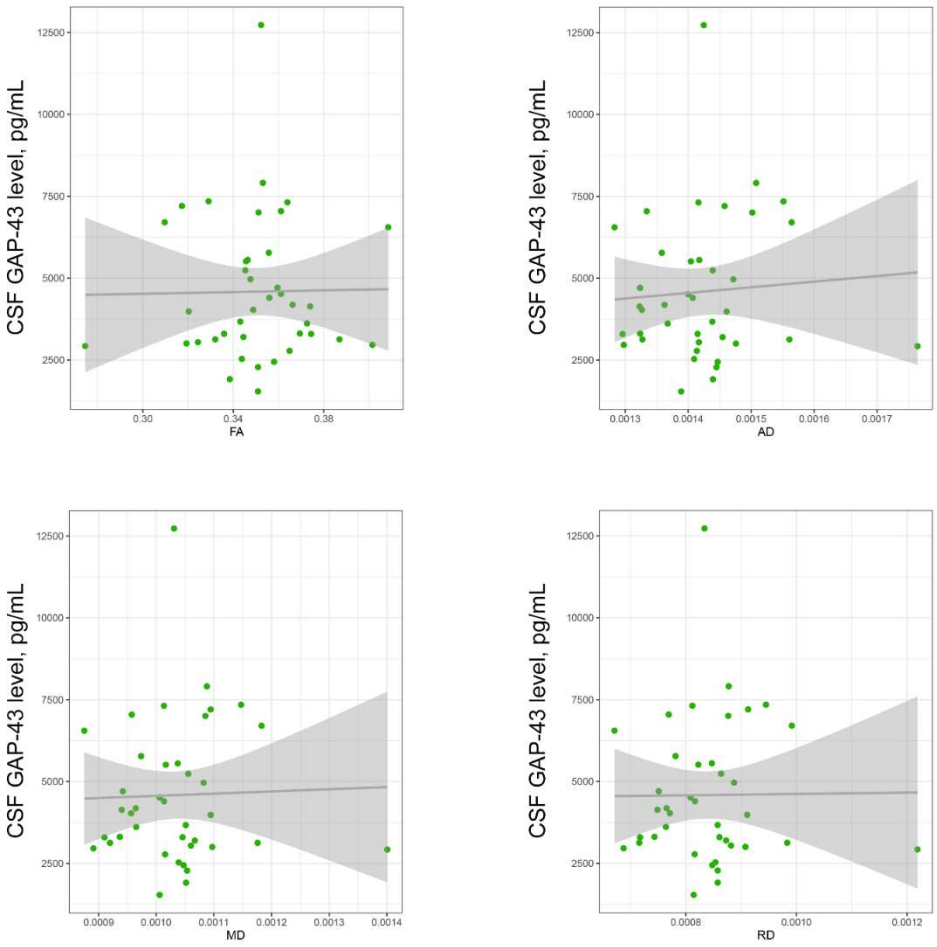

Figure1. Association between CSF-GAP43 and DTI measures in CN subjects.

atlas ROIs in the same coordinate space as the DTI maps. The average FA and MD were then calculated within the boundaries of each ROI mask for each subject. Tensor-based spatial statistics and tract-based spatial statistics (TBSS) were also performed to extract the mean FA in the ROIs along with the skeleton.

Statistical Analysis

The SPSS software (Statistical Package for the Social Sciences, version 16, USA) was used for data analysis. First, simple linear regression models were conducted to assess the association of CSF-GAP43 with clinical and demographic. Next, the association of CSF-GAP43 and DTI values (including MD, RD, AxD, and FA) in the brain was measured using simple linear regression models. To address multiple comparisons and type I errors, the Benjamini-Hochberg method was utilized. Results with a p-value ≤ 0.05 were considered statistically significant.

Results

Patient demographic

The mean age of the studied population was 73.35 ± 6.81 years, and the mean Mini-Mental State Examination (MMSE) score was 27.33. The details of the demographic characteristics are described in Table 1.

Associations between Baseline Characteristics and CSF-GAP43 Levels

By investigating the associations between relevant baseline characteristics and CSF-GAP43 levels, stratified by clinical groups we observed that age was significantly associated with CSF-GAP43 levels ($\beta = 0.44$, p-value < 0.001) and MMSE ($\beta = -0.34$, p-value < 0.001) in all participants.

CSF-GAP43 and DTI

Linear regression analysis in CN subjects revealed no association between CSF-GAP43 and DTI values (including MD, RD, AxD, and FA) (Figure 1). However, the same analysis in MCI participants showed a significant association for all DTI values. We found a significant association between CSF-GAP43 and FA ($\beta = 0.31$, p-value = 0.011). Also, a negative association was found between CSF-GAP43 concentration and AD, MD, and

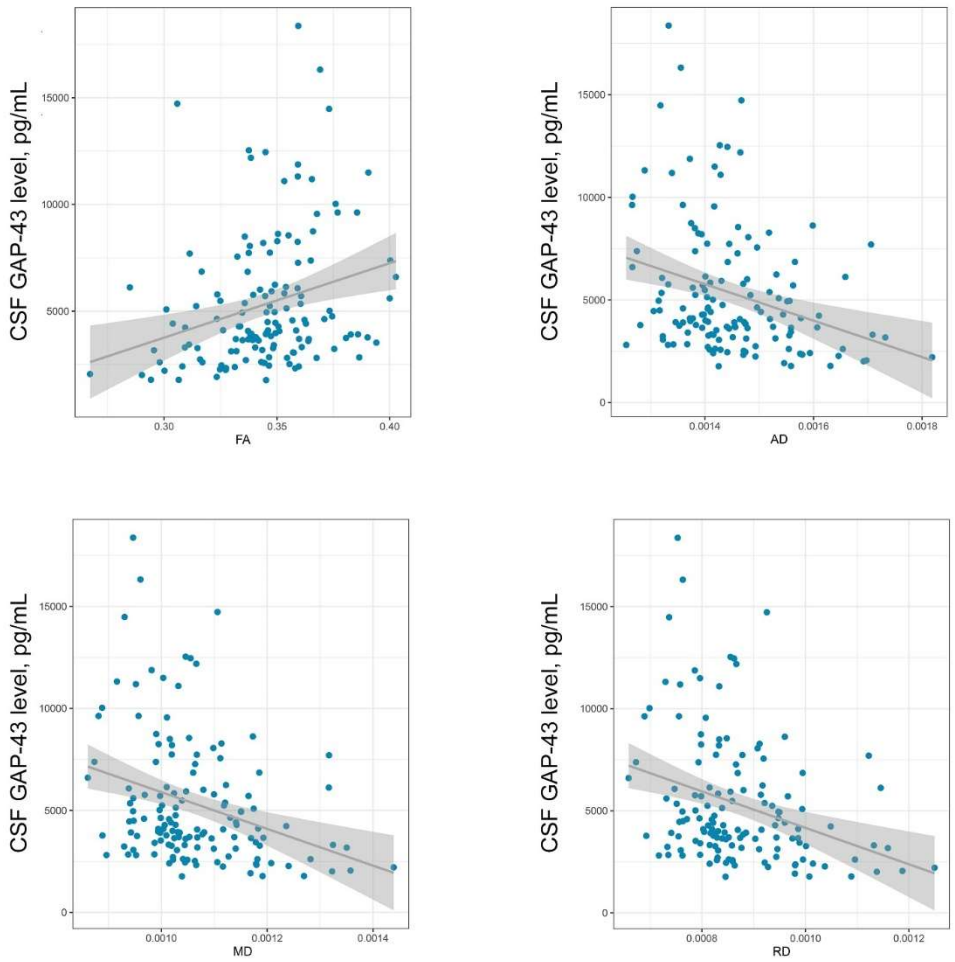

Figure2. Association between CSF-GAP43 and DTI measures in MCI subjects.

RD values in MCI (β = -0.32, p-value = 0.013, β = -0.35, p-value = 0.004, β = -0.28, p-value = 0.017) (Figure 2).

The regression models also revealed a positive association between CSF-GAP43 and FA value in AD subjects (β = 0.34, p-value = 0.028) (Figure 3). Furthermore, increased CSF-GAP43 level was associated with lower AD, MD, and RD values in brain WM of AD patients (β = -0.36, p-value = 0.022, β = -0.30, p-value = 0.033, β = -0.25, p-value = 0.041).

Discussion

There are limited reports on the association between CSF-GAP43 and WM structures across the AD continuum. Our results showed significant associations between CSF-GAP43 and abnormal WM microstructure in various brain regions in MCI and AD participants. The results suggest that the associations of CSF-GAP43 with WM might be a biomarker to detect neurodegeneration, which also tracks well with the observed changes.

There is substantial evidence that synaptic loss is correlated with cognitive decline in AD, and synaptic dysfunction is one of the earliest detectable changes in many neurodegenerative diseases, which may appear even before neuronal loss (15). The significant role of synaptic dysfunction in the pathology of AD promotes the analysis and quantification of synaptic proteins.

GAP-43 is a synaptic membrane protein that plays an important role in the regulation of synaptic plasticity, learning, and memory functionality (16, 17). Previous studies have reported that the concentration of CSF GAP-43 was increased in AD. Also, it was demonstrated that CSF GAP-43 was correlated with CSF phosphorylated tau (p-tau), CSF total tau (t-tau), and plasma p-tau, which might reflect a common pathogenic process between GAP-43 and tau pathology. It has been suggested that high concentrations of CSF t-tau represent axonal degeneration and high concentrations of CSF p-tau represent the increased formation of neurofibrillary tangles, and that these two events are associated (18, 19). As CSF GAP-43 was highly correlated with CSF p-tau and CSF t-tau, this may indicate that increased CSF GAP-43 concentration is associated with the degeneration of axons or presynaptic terminals or the regeneration of axons and/or synapses (20). Moreover, GAP-43-related synaptic changes are linked to faster Aβ-related tau spread across connected regions and synapses could be key targets for preventing tau spreading in AD (21, 22).

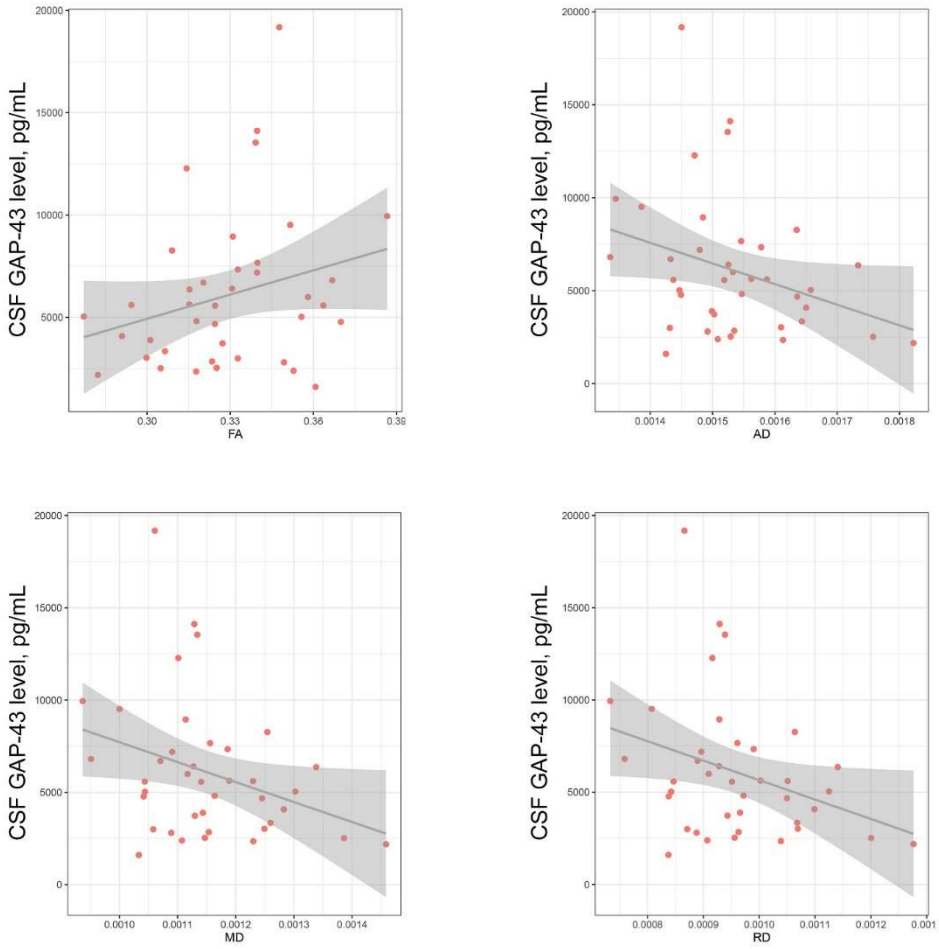

Figure3. Association between CSF-GAP43 and DTI measures in AD subjects.

DTI metrics can provide information about the different specifications of WM. However, the exact relationship between the four DTI values - FA, RD, AxD, and MD - and the pathophysiological mechanisms of AD has not been completely understood. Commonly, lower FA and higher RD, AxD, and MD have been reported in relation to demyelination and degeneration in WM (21). Several studies have reported the association between CSF levels of GAP-43 and WM damage in both MCI and AD (22, 23).

Conclusion

Our study provides a better understanding of the link between CSF GAP-43 and WM changes in patients with MCI and AD. Our findings support the application of CSF GAP-43 as an effective biomarker for monitoring individuals at high risk of AD in the early stages. Recent studies have shown that CSF GAP-43 is a reliable biomarker for the conversion from MCI to AD, and our research reflects the association of CSF GAP-43 with WM damage as measured by neuroimaging. However, further longitudinal studies are necessary to validate the predictive role of CSF GAP-43in cognitive decline.

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Declarations

Funding

We do not have any financial support for this study.

Conflict of interest

The authors have no conflicts of interest to disclose.

Availability of data

The datasets analyzed during the current study are available upon request with no restriction.

Code availability

Not applicable

Ethical approval

The data in this paper were obtained from the ADNI database (adni.loni.usc.edu). It does not include any examination of human or animal subjects.

Consent for publication

This manuscript has been approved for publication by all authors.

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