



## Featured Article

# Elevated CSF GAP-43 is Alzheimer's disease specific and associated with tau and amyloid pathology

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**Abstract**

**Introduction:** The level of the presynaptic protein growth-associated protein 43 (GAP-43) in cerebrospinal fluid (CSF) has previously been shown to be increased in Alzheimer's disease (AD) and thus may serve as an outcome measure in clinical trials and facilitate earlier disease detection.

**Methods:** We developed an enzyme-linked immunosorbent assay for CSF GAP-43 and measured healthy controls (n = 43), patients with AD (n = 275), or patients with other neurodegenerative diseases (n = 344). In a subpopulation (n = 93), CSF GAP-43 concentrations from neuropathologically confirmed cases were related to A $\beta$  plaques, tau,  $\alpha$ -synuclein, and TDP-43 pathologies.

**Results:** GAP-43 was significantly increased in AD compared to controls and most neurodegenerative diseases and correlated with the magnitude of neurofibrillary tangles and A $\beta$  plaques in the hippocampus, amygdala, and cortex. GAP-43 was not associated to  $\alpha$ -synuclein or TDP-43 pathology.

**Discussion:** The presynaptic marker GAP-43 is associated with both diagnosis and neuropathology of AD and thus may be useful as a sensitive and specific biomarker for clinical research.

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**Keywords:**

GAP-43; CSF biomarker; Alzheimer's disease; Differential diagnosis; Enzyme-linked immunosorbent assay

**1. Background**

Alzheimer's disease (AD) patients constitute a heterogeneous group, and patients with other neurodegenerative diseases such as frontotemporal lobar degeneration (FTD), dementia with Lewy bodies (DLB), and Parkinson's disease

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(PD) often display similar symptoms and mixed pathologies, which complicates the diagnosis [1,2]. In addition to clinical evaluation, three core cerebrospinal fluid (CSF) biomarkers are currently used for diagnosis of AD; the 42 amino acid form of amyloid  $\beta$  ( $A\beta_{42}$ ), total-tau (T-tau), and phosphorylated tau (P-tau), reflecting amyloid pathology, neurodegeneration, and tangle pathology, respectively, are included in the diagnostic criteria for AD [3,4]. Recently, disease-modifying drugs targeting  $A\beta$  pathology, such as  $\beta$ -secretase inhibitors and the anti- $A\beta$  antibody aducanumab, have shown promising results [5,6] highlighting an increasing need to correctly identify patients with AD pathology. Furthermore, such therapeutics is expected to be most effective at the early stages of AD before neuronal damage is too widespread.

It is known that pathological changes due to AD occur long before clinical symptoms appear. Therefore, it is important to diagnose patients as early as possible. It is widely recognized that synaptic dysfunction is an early core feature of AD and synaptic dysfunction and loss is highly correlated to cognitive decline [7–10]. Thus, additional biomarkers reflecting synaptic dysfunction and loss may further improve differential diagnosis, be useful as outcome markers in clinical trials, provide novel disease stage information, and enable earlier diagnosis in combination with the core biomarkers.

Growth-associated protein 43 (GAP-43) is a presynaptic protein highly expressed during neuronal development and synaptogenesis and thereafter in the hippocampus and association cortices in the adult human brain [11–13]. Furthermore, GAP-43 is involved in the regulation of axonal outgrowth, synaptic plasticity, and learning and memory functions [14–19], and previous postmortem brain studies of GAP-43 reported decreased protein expression in frontal cortex and regional increases in the hippocampus of patients with AD [20–22]. In a recent explorative study including 280 proteins, it was shown that the fluorescence intensity per bead identity and sample for CSF GAP-43 was increased in preclinical and clinical AD compared to controls, while there were no significant changes in PD or DLB patients compared to controls [23]. However, assay characterization was not provided. Previous CSF quantification of GAP-43 in the early 2000s showed a decrease in PD but normal or only slightly increased concentration in AD compared to neurological controls [24,25]. Discrepancies between the studies may be due to assay differences or the lack of knowledge at the time regarding differential diagnosis leading to mixed patient cohorts. Current knowledge on differential diagnosis allows for more homogeneous patient cohorts, which increases the chances of finding actual AD-specific biomarkers. Therefore, we developed and validated a novel enzyme-linked immunosorbent assay (ELISA) for quantification of GAP-43 in CSF and analyzed a large, well-characterized cohort including controls and clinically diagnosed AD patients and other dementia patients. A sub-population of patients was neuropathologically assessed for

amyloid, tau,  $\alpha$ -synuclein, TDP pathology, and neuron loss in several different brain regions allowing for the first time an evaluation of associations between CSF GAP-43 concentration and several dementia pathologies. Furthermore, GAP-43 CSF concentration was tested for association with global cognition and decline over a 2-year period using the Mini-Mental State Examination (MMSE) score. The following hypotheses were tested: (1) CSF GAP-43 is especially affected in AD and useful for differential diagnosis; (2) CSF GAP-43 quantification is associated to cognitive decline; and (3) CSF GAP-43 is associated with AD pathology.

## 2. Methods

### 2.1. Participants

#### 2.1.1. Pilot study

The pilot study included 15 AD patients and 12 controls. Demographics are summarized in [Supplementary Table 1](#).  $A\beta_{42}$ , T-tau, and P-tau were used for neurochemical classification of the patients into AD or control groups.

#### 2.1.2. Clinical validation study

The clinical validation study included 662 participants from the University of Pennsylvania Integrated NeuroDegenerative Disease Database [26], comprising participants without signs of neurodegenerative diseases serving as controls ( $n = 43$ ), mild cognitive impairment (MCI) ( $n = 84$ ), AD ( $n = 275$ ), amyotrophic lateral sclerosis (ALS) ( $n = 53$ , of which ALS with MCI  $n = 3$ , ALS with FTD  $n = 9$ ), behavioral variant FTD (bvFTD,  $n = 39$ ), noncognitively impaired PD ( $n = 28$ ), PD with MCI ( $n = 14$ ), PD with dementia ( $n = 15$ ), DLB ( $n = 27$ ), logopenic variant of primary progressive aphasia (lvPPA,  $n = 10$ ), semantic dementia variant of primary progressive aphasia (svPPA,  $n = 16$ ), progressive nonfluent aphasia variant of primary progressive aphasia (naPPA,  $n = 15$ ), progressive supranuclear palsy ( $n = 18$ ), corticobasal syndrome ( $n = 19$ ), and posterior cortical atrophy (PCA,  $n = 6$ ) patients. CSF sampling was performed according to standard operating procedures of the Alzheimer's Disease Neuroimaging Initiative (<http://www.adni-info.org/>).

MMSE score was available for 593 subjects. Two-year follow-up MMSE scores were available for 305 subjects, and the decline in MMSE score per year was used to analyze the association between GAP-43 and cognitive decline.

In 93 patients, Braak neurofibrillary tau staging and Consortium to Establish a Registry for Alzheimer's Disease (CERAD) score was performed as previously described [26]. Furthermore, postmortem examination and scoring of tau, thioflavin/amyloid,  $\alpha$ -synuclein, and TDP-43 pathology, as well as amyloid angiopathy, gliosis, and neuron loss, was carried out on brain sections from 17 brain regions: amygdala, cornu ammonis/subiculum-hippocampus, entorhinal cortex-hippocampus, midfrontal cortex, angular gyrus,

superior-midtemporal cortex, anterior cingulate cortex, occipital (visual, calcarine) cortex, caudate/putamen, globus pallidus, thalamus, midbrain, substantia nigra–midbrain, pons, locus coeruleus-pons, medulla, and dentate nucleus-cerebellum. Sections were processed and histologically (thioflavin S) or immunohistochemically stained (phosphorylated tau,  $\alpha$ -synuclein, TDP-43) and graded using a five-point scale (0 = none, 0.5 = rare, 1 = mild, 2 = moderate, 3 = severe) as previously described [26,27]. GAP-43 concentrations across this scale were compared for the mentioned stains across the 17 brain regions, after merging data from points 0 and 0.5 due to low numbers. Genomic DNA was extracted as described elsewhere [26], for determination of *APOE* allele status using two SNPs (rs7412 and rs429358), genotyped by TaqMan allelic discrimination assays (Thermo-Fisher, USA).

## 2.2. Ethics

The pilot study on leftover aliquots from clinical routine analyses followed a procedure approved by the Ethics Committee at University of Gothenburg (EPN140811). The clinical validation study was conducted in accordance with the provisions of the Helsinki declaration and approved by regional ethics committee.

## 2.3. AD biomarker quantification

In the clinical validation study, quantification of CSF T-tau, P-tau, and A $\beta$ 42 was performed on the Luminex platform (Luminex Corporation, Austin, TX) using the multiplexed AlzBio3 (Fujirebio, Ghent, Belgium) kit as instructed in kit inserts. For biomarker correlation analysis, ALS patients were merged, due to the low number of patients per group. Patients with variants of primary progressive aphasia were similarly merged for correlation analysis.

## 2.4. Brain homogenization, western blotting, and immunoprecipitation

Brain samples were taken from the superior parietal gyrus of AD subjects. Homogenization of brain tissue, western blotting, and immunoprecipitation of CSF was performed as previously described [28], with minor modifications (see [Supplementary Material](#) for a detailed description). Full-length recombinant GAP-43 protein was produced in house (see [Supplementary Material](#)) and compared to endogenous GAP-43 protein by western blotting in the brain and CSF.

## 2.5. In-house GAP-43 sandwich ELISA

The ELISA was developed in house combining the mouse monoclonal GAP-43 antibody NM4 (Fujirebio) and a polyclonal GAP-43 antibody (ABB-135, Nordic Biosite, Täby, Sweden) with a C-terminal epitope. For detailed description, see [Supplementary Material](#). The analyses were performed by board-certified laboratory technicians blinded to clinical

information. During sample runs in the clinical evaluation study, the repeatability CV% of control samples, with the concentrations 3157 pg/mL and 857 pg/mL, was 5.5% versus 11% and the interassay CV% was 6.9% versus 15.6% across 18 ELISA plates.

## 2.6. In-house GAP-43 ELISA validation

The performance of the novel ELISA was evaluated using leftover CSF samples from clinical routine of the Neurochemistry laboratory, Sahlgrenska University Hospital, Mölndal, Sweden (see [Supplementary Material](#) for an evaluation of precision, LLOQ, recovery, and sample dilution linearity).

## 2.7. Statistical analysis

Statistical analysis was performed using SPSS version 23.00 (IBM, NY, USA) and GraphPad Prism 7.0 (GraphPad Inc., CA, USA). As the biomarker data were not normally distributed, nonparametric statistics were used. Unless otherwise stated, demographics and clinical characteristics were summarized using medians and interquartile ranges for biomarkers and analyzed by Mann-Whitney *U*-test, Kruskal-Wallis test, and chi-square test. Comparisons of biomarker concentrations were performed using Kruskal-Wallis test and Spearman rank correlations. Statistical tests were two sided with significance threshold value of  $P < .05$ . The significance level was adjusted to  $P < .003$  to compensate for multiple comparisons for associations of CSF GAP-43 and pathology severity across 17 brain regions.

## 3. Results

### 3.1. GAP-43 detection in brain and CSF by western blotting

To ensure specificity of the antibodies used for quantification of GAP-43 by ELISA, brain and CSF samples were processed for western blotting and GAP-43 was detected as a single band around 43 kDa in soluble and insoluble protein fractions of brain extracts by the antibodies NM4 and ABB-135 ([Supplementary Fig. 1A](#)). After immunoprecipitation of CSF with the same antibodies, a band corresponding to GAP-43 was detected aligning with that of recombinant GAP-43 ([Supplementary Fig. 1B](#)).

### 3.2. In-house ELISA characterization

Results from assay characterization are summarized in [Supplementary Table 2](#). CSF GAP-43 quantification with NM4-ABB135 immunoassay was stable after one to four freeze thaw cycles ( $n = 9$ ; 100.9%–107.9%), and various storage temperatures ( $n = 7$ ; 94.8%–103.5%, percentage of  $-80^{\circ}\text{C}$  stored aliquot concentration).

### 3.3. Two-fold increase of GAP-43 in AD pilot study

The novel GAP-43 ELISA was tested in a pilot study including 12 AD patients and 15 control subjects. Control subjects were significantly younger than AD subjects; however, no correlation of GAP-43 with age was detected. CSF GAP-43 concentration was twofold higher in the AD patients compared to controls ( $P = .001$ , [Supplementary Fig. 2](#)). Based on these results, a clinical follow-up study was performed to validate the findings.

### 3.4. Demographics of clinical validation study

Demographics of participants in the validation study are provided in [Table 1](#). The clinical AD group was age and gender matched to controls and MCI patients. Age correlated weakly with CSF GAP-43 across the study population ( $r_s = 0.11$ ,  $P = .004$ ), but not in separate clinical diagnostic groups. Across all diagnostic groups, GAP-43 concentrations were higher in females than in males (median female: 2880 pg/mL, median male: 2547 pg/mL,  $P = .001$ ) and remained significant only in the AD group after division by clinical diagnosis (median AD female: 3489 pg/mL, median AD male: 3023 pg/mL,  $P = .017$ ). No statistically significant differences in GAP-43 concentrations were detected based on ethnicity, education years, handedness, or *MAPT* H1/H2 haplotypes.

### 3.5. CSF GAP-43 concentration in AD and MCI patients

CSF GAP-43 concentrations were significantly increased in clinical AD patients compared to controls ( $P < .0001$ ), but not in clinical MCI patients ([Fig. 1A](#)). Of all the patients included

in the study, 56 had a definitive Alzheimer's neuropathological diagnosis, and there was a trend toward increased CSF GAP-43 concentrations in this group compared to clinical AD. To identify possible clinical misdiagnosed patients, a neurochemical cutoff based on the INNO-BIA AlzBio3 T-tau/A $\beta_{42}$  ratio (AD positive: ratio  $>0.48$ , T-tau: 93 pg/mL and A $\beta_{42}$ : 192 pg/mL) suggested by Shaw et al. [29] was applied to the clinical AD group. The ratio identified 209 biomarker-defined AD biomarker-positive patients out of the 275 clinically diagnosed patients and CSF GAP-43 concentrations were significantly higher in AD biomarker-positive compared to biomarker-negative clinical AD patients ( $P < .001$ , [Fig. 1B](#)). Furthermore, patients without a positive T-tau/A $\beta_{42}$  ratio displayed GAP-43 concentrations equal to controls ([Fig. 1B](#)). MCI patients were similarly divided by the T-tau/A $\beta_{42}$  cutoff, and the biomarker-positive MCI group had significantly increased CSF GAP-43 concentrations compared to the biomarker-positive MCI group ( $P < .0001$ , [Fig. 1C](#)).

### 3.6. CSF GAP-43 concentration in AD differential diagnoses

Apart from AD patients, only lvPPA patients had altered CSF GAP-43 concentration compared to controls ( $P = .01$ , [Fig. 1F](#)). The CSF GAP-43 concentrations in AD patients were compared in three subgroups of differential diagnoses: (1) Parkinsonian disorders and Parkinson's disease; (2) ALS; and (3) atypical AD and subgroups of FTD diagnoses.

CSF GAP-43 concentrations in clinical AD patients were significantly increased compared to both Parkinson's disease and Parkinsonian disorders (nondemented  $P = .0002$ , demented  $P = .027$ , with MCI;  $P = .0002$ ), DLB

Table 1  
Demographics of participants in the clinical evaluation study

Patient group	No.	Age (mean, SD)	Gender (F%)	APOE $\epsilon 4$ alleles (%)			MMSE score (% , mean, SD)	GAP-43 (pg/mL)	T-tau (pg/mL)	A $\beta_{1-42}$ (pg/mL)	P-tau (pg/mL)
				0	1	2					
Control	43	69 (9.1)	69.8	70.7	26.8	2.3	28.9 (2.4)	2334 $\pm$ (1104)	52 $\pm$ (25)	250 $\pm$ (111)	17 $\pm$ (10)
MCI	84	72 (8.9)	46.4*	51.2	27.4	3.6	26.1 (3.5)	2372 $\pm$ (1972)	58 $\pm$ (54)	176 $\pm$ (104)	16 $\pm$ (25)
AD	275	71.2 (9.2)	58.2	34.2	38.5	13.1	21.2 (5.8)	3202 $\pm$ (1800)	106 $\pm$ (81)	135 $\pm$ (57)	37 $\pm$ (29)
ALS	53	58 (11.5)*,†††	26.4***,†††	73.6	20.8	1.9	26.8 (4)	2078 $\pm$ (1228)	49 $\pm$ (33)	272 $\pm$ (104)	11 $\pm$ (6)
FTD	39	61 (7.7)*,†††	30.8**,††	66.7	25.6	2.6	25.4 (3.6)	2319 $\pm$ (1274)	62 $\pm$ (38)	243 $\pm$ (145)	14 $\pm$ (10)
PD	28	65.9 (7.2)	32.1**,†	85.7	10.7	/	28.7 (1.6)	2349 $\pm$ (1033)	45 $\pm$ (21)	283 $\pm$ (64)	18 $\pm$ (15)
PD-MCI	14	63.4 (5.7)	14.3**,†	61.5	38.5	/	28.4 (1.3)	2022 $\pm$ (812)	39 $\pm$ (19)	214 $\pm$ (71)	18 $\pm$ (20)
PD-demented	15	74.3 (6.9)	20*,†	66.7	33.3	/	25.6 (4.6)	2550 $\pm$ (1038)	47 $\pm$ (32)	262 $\pm$ (72)	22 $\pm$ (14)
DLB	27	73 (9.2)	44.4	44.4	29.6	7.4	19.4 (6.6)	2195 $\pm$ (2303)	51 $\pm$ (50)	414 $\pm$ (38)	15 $\pm$ (22)
PPA (logopenic)	10	62.6 (6.1)	60	33.3	66.7	/	22.5 (5.7)	3350 $\pm$ (2346)	139 $\pm$ (64)	131 $\pm$ (31)	39 $\pm$ (16)
PPA (PNFA)	16	63.7 (9.4)	43.3	43.8	43.8	12.5	22.5 (4.9)	2350 $\pm$ (1851)	59 $\pm$ (45)	224 $\pm$ (110)	17 $\pm$ (17)
PPA (SD)	15	62.5 (8.1)	66.7	80	13.3	6.7	22.9 (5.3)	3040 $\pm$ (2212)	94 $\pm$ (62)	289 $\pm$ (101)	15 $\pm$ (13)
PSP	18	70.2 (9.2)	55.6	88.9	11.1	0	26.4 (4.3)	1772 $\pm$ (1811)	50 $\pm$ (39)	210 $\pm$ (144)	12 $\pm$ (8)
CBS	19	66.2 (7.2)	57.9	73.7	21.1	5.3	24.5 (4)	3200 $\pm$ (2103)	76 $\pm$ (45)	225 $\pm$ (109)	21 $\pm$ (13)
PCA	6	60 (6.2)	83.3	50	33.3	0	22.8 (5.5)	2264 $\pm$ (1859)	53 $\pm$ (82)	206 $\pm$ (184)	26 $\pm$ (26)

Abbreviations: SD, standard deviation; F, female; MMSE, Mini-Mental State Examination; GAP-43, growth-associated protein 43; MCI, mild cognitive impairment; AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; FTD, frontotemporal dementia; PD, Parkinson's disease; DLB, dementia with Lewy bodies; PPA, primary progressive aphasia; PSP, progressive supranuclear palsy; CBS, corticobasal syndrome; PCA, posterior cortical atrophy.

NOTE. Biomarker concentrations are median and interquartile range.

NOTE. \*Indicates differences compared to control group;  $P < .05$ . †Indicates differences compared to Alzheimer's disease group; † $P < .05$ , †† $P < .001$ , ††† $P < .0001$ .

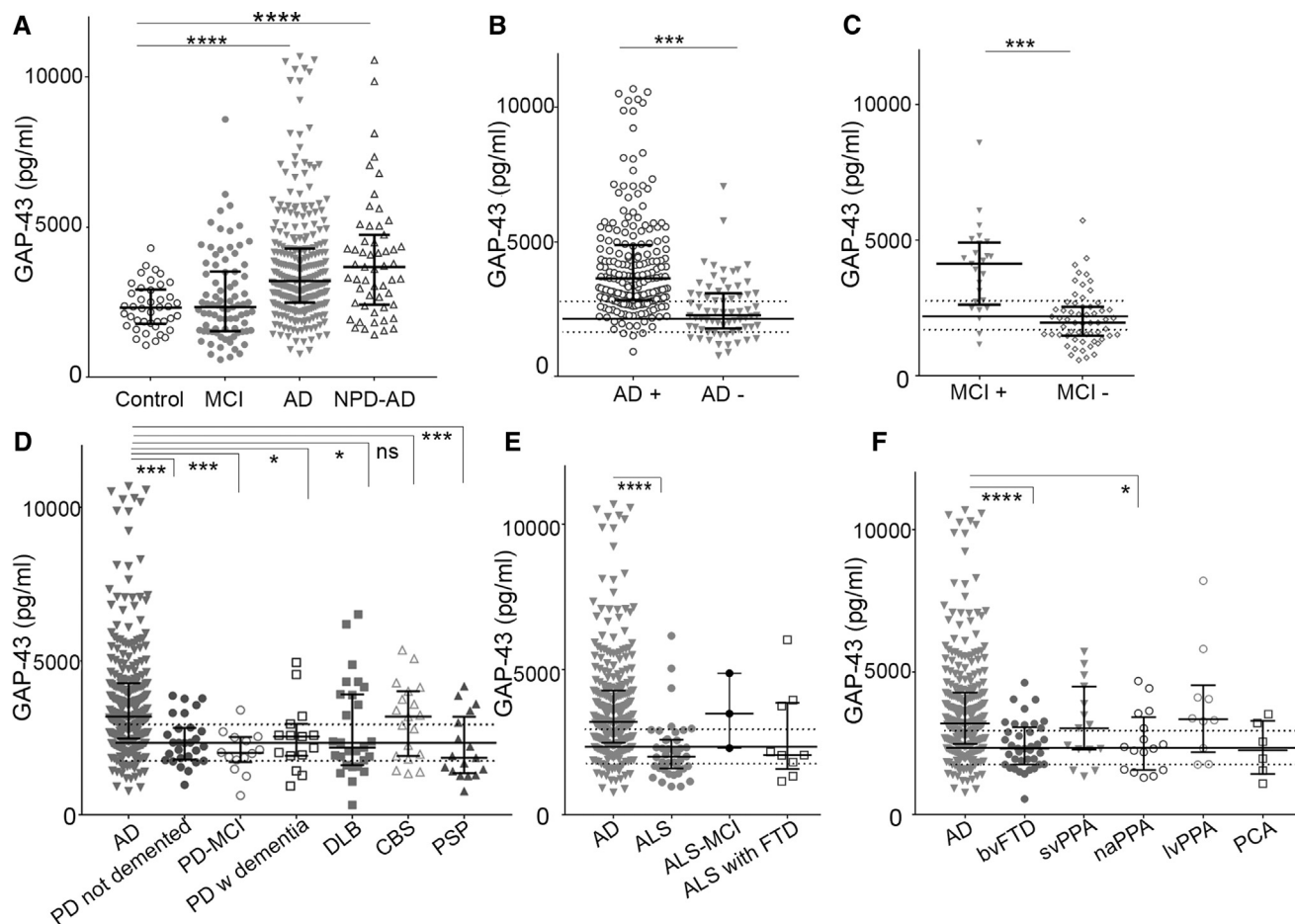


Fig. 1. Clinical evaluation of CSF GAP-43 quantification in control, MCI and Alzheimer's disease and differential diagnoses to Alzheimer's disease. (A) CSF GAP-43 concentration was increased in patients with a clinical AD diagnosis, whereas clinical MCI patients were similar to control level. The median CSF GAP-43 concentration was particularly increased in a group of AD patients with a neuropathologically confirmed AD diagnosis (NPD-AD). (B) Applying a neurochemical cut off (IB AlzBio3 T-Tau/IB AlzBio3 A $\beta$ <sub>42</sub> ratio [ $>0.48$  = AD]), identified a large proportion of clinical AD patients with lower GAP-43 concentrations which were biomarker negative. (C) MCI patients with a T-tau/A $\beta$ <sub>42</sub> ratio above the cut off had increased GAP-43 concentration whereas MCI patients below the cut off were similar to control. (D) CSF GAP-43 concentration was increased in clinical AD patients compared to PD patients without dementia, with MCI or demented, DLB patients and PSP, whereas patients with CBS displayed comparable levels. (E) CSF GAP-43 concentration was increased in clinical AD patients compared to ALS, whereas there was no significant difference to ALS with MCI or ALS with FTD. (F) CSF GAP-43 concentration was increased in clinical AD patients compared to bvFTD and naPPA, whereas lvPPA and svPPA variants and PCA patients displayed comparable levels. The solid and dotted lines indicate the median and IQR of the neurochemical control group. Concentrations are medians with interquartile range. \* $P < .05$ ; \*\*\* $P < .001$ ; \*\*\*\* $P < .0001$ . Abbreviations: AD, Alzheimer's disease; ns, not significant; NPD-AD, neuropathologically confirmed Alzheimer's disease; MCI, mild cognitive impairment; AD +, clinical Alzheimer's disease patients with a positive T-Tau/A $\beta$ <sub>42</sub> ratio; AD -, clinical Alzheimer's disease patients with a negative T-Tau/A $\beta$ <sub>42</sub> ratio; MCI +, clinical MCI patients with a positive T-Tau/A $\beta$ <sub>42</sub> ratio; MCI -, clinical MCI patients with a negative T-Tau/A $\beta$ <sub>42</sub> ratio; PD, Parkinson disease; PSP, progressive supranuclear palsy; CBS, corticobasal syndrome; ALS, amyotrophic lateral sclerosis; bvFTD, behavioural variant frontotemporal lobar degeneration; lvPPA, logopenic variant of primary progressive aphasia; svPPA, semantic dementia variant of primary progressive aphasia; naPPA, progressive nonfluent aphasia variant of primary progressive aphasia; PCA, primary cortical atrophy.

( $P = .018$ ), and progressive supranuclear palsy ( $P = .0004$ ), while there were no differences in the concentrations between AD and corticobasal syndrome patients ( $P > .99$ , Fig. 1D). Eighteen percent of corticobasal syndrome patients had a T-tau/A $\beta$ <sub>42</sub> ratio indicative of AD.

ALS ( $P < .0001$ ) and bvFTD ( $P < .0001$ ) both had significantly lower GAP-43 concentrations compared to AD (Fig. 1E and F). The ALS patients with additional MCI or additional FTD were not significantly different from AD patients ( $P > .999$  and  $P = .138$ , respectively, Fig. 1E).

The FTD spectrum includes several related disorders in which progressive degeneration of the frontal and temporal lobes is common [30,31]. On the basis of clinical phenotypes, we compared AD to FTD subgroups and atypical AD; PCA, bvFTD, and PPA of which the later was further divided into naPPA, lvPPA, and svPPA [30]. naPPA had significantly lower GAP-43 concentrations ( $P = .022$ , Fig. 1F), whereas PCA ( $P = .182$ ), lvPPA, and svPPA patients had similar CSF GAP-43 concentrations as AD patients (both  $P > .99$ , Fig. 1F). Seventy percent of

Table 2  
Spearman's correlation analysis of GAP-43 concentration with CSF biomarkers of Alzheimer's disease pathology

Diagnostic group	IB AlzBio3 T-tau (pg/mL)			IB AlzBio3 A $\beta$ 42 (pg/mL)			IB AlzBio3 PTau (pg/mL)		
	$r_s$	$P$	n	$r_s$	$P$	n	$r_s$	$P$	n
Control	0.841	<.0001	42	-0.083	.595	43	0.356	.019	43
All MCI	0.908	<.0001	83	-0.066	.551	83	0.649	<.0001	83
AD	0.818	<.0001	261	-0.131	.034	262	0.516	<.0001	262
ALS	0.806	<.0001	52	0.531	<.0001	53	0.456	.001	52
FTD	0.752	<.0001	32	0.149	.408	33	0.384	.03	32
PD not demented	0.639	<.0001	26	0.346	.083	26	0.055	.791	26
PD-MCI	0.7	.005	14	-0.407	.149	14	0.569	.034	14
PD with dementia	0.688	.007	14	0.368	.177	15	0.45	.092	15
DLB	0.892	<.0001	18	-0.077	.753	19	0.522	.022	19
PPA	0.782	<.0001	31	0.092	.621	31	0.207	.265	31
PSP	0.829	<.0001	17	0.437	.08	17	0.692	.002	17
Corticobasal syndrome	0.676	.011	13	0.407	.168	13	-0.58	.851	13

Abbreviations: MCI, mild cognitive impairment; AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; FTD, frontotemporal dementia; PD, Parkinson's disease; DLB, dementia with Lewy bodies; PPA, primary progressive aphasia; PSP, progressive supranuclear palsy.

patients with lvPPA and 13% of patients with svPPA had T-tau/A $\beta$ <sub>42</sub> ratios indicative of AD.

### 3.7. CSF GAP-43 concentration correlates with core AD biomarkers

Biomarker correlations were performed in patient groups with at least 10 patients and are presented in detail in Table 2. Strong correlations were found between GAP-43 and T-tau in all clinical groups. Furthermore, GAP-43 correlated moderately with P-tau in MCI patients, AD, ALS, DLB, progressive supranuclear palsy, PD with MCI, and weakly in FTD patients and controls. A weak negative correlation was found between GAP-43 concentration and A $\beta$ <sub>42</sub> in clinical AD and surprisingly a moderate positive correlation with A $\beta$ <sub>42</sub> in ALS patients (Table 2).

### 3.8. CSF GAP-43 concentration correlates with cognitive decline and AD pathology

CSF GAP-43 concentrations correlated weakly with MMSE scores when including all diagnostic groups ( $r_s = -0.21$ ,  $P < .0001$ ,  $n = 593$ , Fig. 2A), but no significant

correlations were detected in clinical subgroups. In addition, the longitudinal decrease in MMSE score per year correlated weakly with increasing GAP-43 concentration ( $r_s = -0.26$ ,  $P = .0001$ ,  $n = 305$ , Fig. 2B). Similar to MMSE scores, there were no correlations of GAP-43 with MMSE change per year when dividing the cohort by clinical diagnosis. CSF GAP-43 correlated with Braak neurofibrillary tau staging ( $r_s = 0.41$ ,  $P = .0001$ ) and was significantly higher in stage V–VI compared to 0 Braak scores ( $P = .001$ ,  $n = 91$ , Fig. 3A). Furthermore, GAP-43 correlated with CERAD score of A $\beta$  deposition across all diagnostic groups ( $r_s = 0.37$ ,  $P < .0001$ ) and was significantly higher for score C compared to 0 ( $P = .001$ ,  $n = 92$ , Fig. 3B). GAP-43 concentration was negatively correlated to brain weight ( $r_s = -0.30$ ,  $P = .003$ ,  $n = 94$ ) but not associated to hippocampal sclerosis.

Examination and scoring of tau, thioflavin/amyloid,  $\alpha$ -synuclein and TDP-43 pathology, amyloid angiopathy, gliosis, and neuron loss carried out in 17 brain regions revealed that CSF GAP-43 concentration was specifically associated with AD pathology. GAP-43 concentration increased significantly with severity scores of amyloid pathology in the CA/subiculum hippocampus region and amygdala (Fig. 3C and D), and severity of both tau and amyloid pathology in the angular gyrus, superior-midtemporal cortex, and anterior cingulate cortex (Fig. 3E–H, J and K). In the occipital cortex, GAP-43 was increased with tau pathology (Fig. 3I). In the midfrontal cortex, GAP-43 increased with only tau pathology and gliosis (Fig. 3L and M). No difference was found in GAP-43 concentration across the severity grades of  $\alpha$ -synuclein, amyloid angiopathy, and TDP-43 pathology in any brain region.

### 3.9. CSF GAP-43 concentration in APOE $\epsilon$ 4 carriers

GAP-43 concentration was higher in subjects with one or two APOE  $\epsilon$ 4 alleles compared to subjects with no APOE  $\epsilon$ 4 alleles (0 vs. 1;  $P < .0001$ , vs. 2;  $P < .0001$ , Fig. 4A). There was also a statistically significant decrease in GAP-43

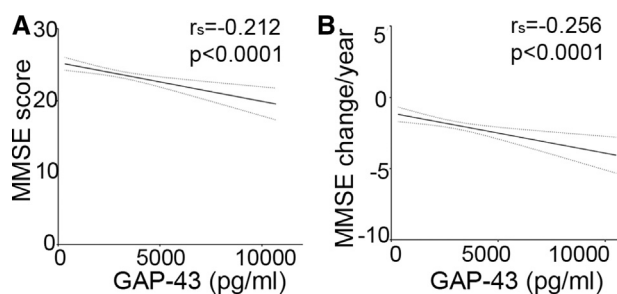


Fig. 2. CSF GAP-43 concentration is associated with cognitive decline. (A) Lower MMSE scores were correlated to higher CSF GAP-43 concentration across all diagnostic groups. (B) MMSE change per year across all diagnostic groups indicates increasing concentration of CSF GAP-43 with larger decline in MMSE. Abbreviation: MMSE, Mini-Mental State Examination.

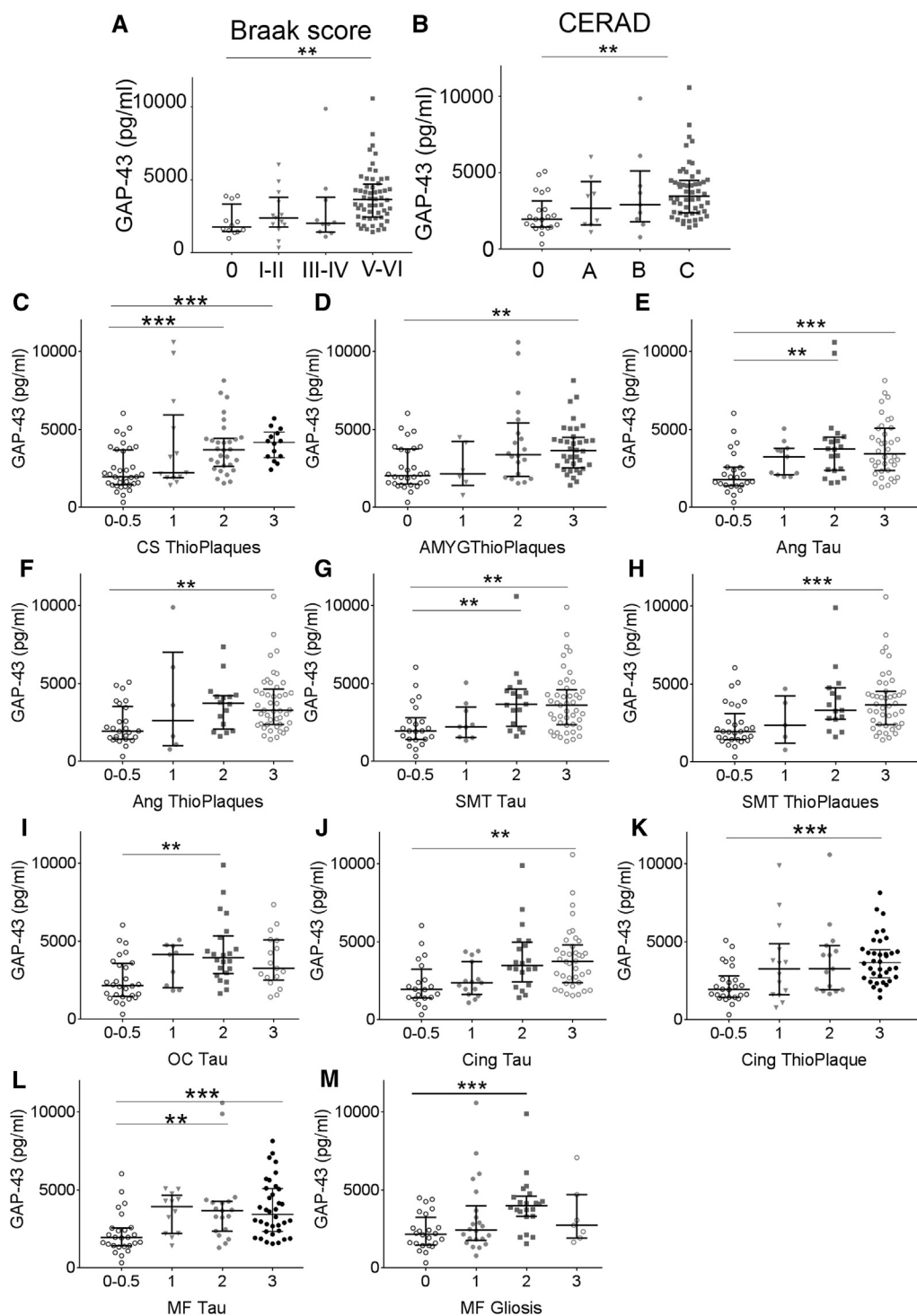


Fig. 3. CSF GAP-43 concentration is associated with disease severity. (A) CSF GAP-43 concentration was increased in patients with high Braak scores (0 to V-VI;  $P = .0027$ ). (B) CSF GAP-43 concentration was increased in patients with high CERAD scores (0 to C;  $P = .0012$ ). (C-M) GAP-43 concentration increased with amyloid plaque and/or Tau pathology (0 = none, 0.5 = rare, 1 = mild, 2 = moderate, 3 = severe) in CA/subiculum hippocampus region (CS), amygdala (Amyg), angular gyrus (Ang), superior-middle temporal cortex (SMT) occipital cortex (OC), anterior cingulate cortex (Cing), mid-frontal cortex (MF), and gliosis in mid-frontal cortex. Scores 0 and 0.5 were merged due to low numbers in the 0.5 core group.  $**P < .003$ ;  $***P < .001$ .

concentration in subjects with one or two *APOE*  $\epsilon 3$  alleles compared to subjects with no *APOE*  $\epsilon 3$  allele (0 vs. 2;  $P = .0001$ , 1 vs. 2;  $P < .0001$ , Fig. 4B). When stratified

based on clinical diagnosis, GAP-43 was only increasing with the number of *APOE*  $\epsilon 4$  alleles in AD patients ( $n = 236$ ,  $P = .022$ ,  $n(0) = 94$ ,  $n(1) = 106$ ,  $n(2) = 36$ ;

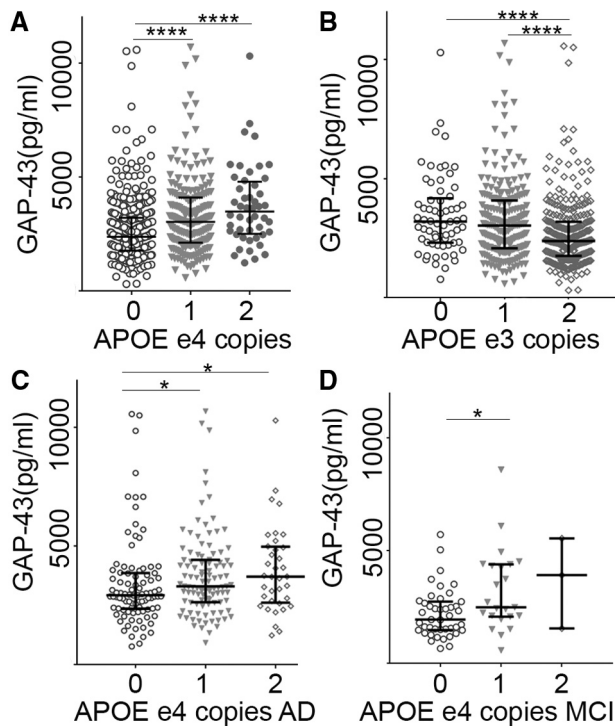


Fig. 4. GAP-43 concentration increased with the number of *APOE*  $\epsilon 4$  alleles. (A) GAP-43 concentration increased with the number of *APOE*  $\epsilon 4$  alleles across all diagnostic groups. (B) GAP-43 concentration decreased with the number of *APOE*  $\epsilon 3$  alleles across all diagnostic groups. (C) GAP-43 concentration increased with the number of *APOE*  $\epsilon 4$  alleles in AD patients and (D) MCI patients. \* $P < .05$ ; \*\*\*\* $P < .0001$ . Abbreviations: AD, Alzheimer's disease; MCI, mild cognitive impairment.

pairwise comparison: 0 vs. 1;  $P = .028$ , vs. 2;  $P = .046$ , Fig. 4C) and MCI patients ( $P = .038$ ,  $n(0) = 43$ ,  $n(1) = 23$ ,  $n(2) = 3$ , pairwise comparison 0 vs. 1;  $P = .03$ , vs. 2;  $P = .242$ , Fig. 4D), but not in the other neurodegenerative diseases or controls.

#### 4. Discussion

In this study, we quantified CSF GAP-43 in patients with different neurodegenerative dementias, including for the first time a large number of neuropathologically confirmed AD patients and important differential diagnoses such as several frontotemporal dementia diagnoses and PD and Parkinsonian disorders. Our results are in line with previous findings reporting increased CSF GAP-43 levels in AD [23,24], and patients with MCI due to AD [23]. However, the GAP-43 increase was more pronounced in our study compared to two previous studies [24,25], which may be due to assay differences or mixed patient cohorts at the time. We showed that GAP-43 can be reliably quantified in CSF by a novel in-house sandwich ELISA. Importantly, using this cohort, we show that CSF GAP-43 is associated with AD pathology, specifically increased in patients with high Braak neurofibrillary tangle stages and CERAD scores and in patients with moderate to severe tau and  $A\beta$  neuropathology,

particularly in the hippocampus, amygdala, temporal and occipital lobe. As there was only one association to gliosis and no associations to the extent of TDP-43,  $\alpha$ -synuclein, or amyloid angiopathy, we conclude that these pathologies have no major influence on GAP-43 CSF concentration. These findings suggest that CSF GAP-43 could be an AD-specific biomarker as the concentrations follow amyloid and tau deposition in the brain and is not increased to the same extremes in any other neurological disease tested here. The positive correlation of GAP-43 with biomarker levels of T-tau and P-tau and the negative correlation with  $A\beta_{42}$  are in line with the findings of increased GAP-43 concentration with increased severity of tau and amyloid deposition in the brain. This indicates that there is a specific association between synaptic, tau, and amyloid pathology in AD; however, the biological connection between the three is yet to be demonstrated. Furthermore, low MMSE score and decline in MMSE score over time were weakly associated to higher GAP-43 concentration when analyzed in all the groups. The lack of association in subgroups may be due to several converging factors. GAP-43 concentrations could be saturated in AD patients resulting in a lack of correlation to MMSE change over time. As GAP-43 is a regulator of synaptic plasticity and regenerative axonal growth and induced by both injury and cognitive stimulation [18,19,32], there is also a possibility that GAP-43 increases with improvement in MMSE. Further studies are necessary to clarify this. GAP-43 was specifically increased in AD when compared to most other dementia disorders analyzed, and therefore, it could become useful for differential diagnosis. CSF GAP-43 concentrations in patients with corticobasal syndrome, PCA, lvPPA and svPPA were not significantly different from AD patients, which is expected as these clinical syndromes often have underlying AD pathology [1,33–35]. Several of these patients had a neurochemical T-tau/ $A\beta_{42}$  ratio indicative of AD pathology which may partly explain the elevated CSF GAP-43 concentrations. For comparison, a study by Lee et al. reported presence of AD pathology in 32% of clinical corticobasal syndrome patients, whereas 17% of these patients in our study had a positive AD biomarker profile [35]. A substantial proportion of patients with variants of primary progressive aphasia are known to display AD pathology [36,37], and CSF biomarker profile, based on  $A\beta_{42}$ / $A\beta_{40}$ , PIB PET, T-tau, and P-tau analysis [38–40]. The low number of patients in the PCA group makes it inappropriate to draw definite conclusions regarding this group.

It has been reported that evaluations of novel biomarkers may underestimate their potential due to a proportion of clinically misdiagnosed patients. Several studies suggest an increased accuracy of the diagnosis, based on simultaneous neuropathological assessment or when using neurochemical cutoffs based on tau and  $A\beta_{42}$  quantification [1,29]. We applied a neurochemical T-tau/ $A\beta_{42}$  ratio cutoff described in the study by Shaw et al., to reclassify the clinical AD



patients. The biomarker-positive AD group had GAP-43 concentrations similar to the neuropathologically confirmed AD group indicating a valid classification. The neurochemical division of the MCI patients indicates that GAP-43 may be useful to predict conversion from MCI to AD; future longitudinal studies should address this further.

Unexpectedly, there was a strong positive correlation of GAP-43 concentration with  $A\beta_{42}$  selectively in ALS patients. Peripheral levels of amyloid precursor protein and  $A\beta$  species have been reported to increase with disease onset in both ALS mouse models and patients and suggested to be directly involved in nerve degeneration [41–43]. This finding could also reflect that this is the only disease group included which does not have significant  $A\beta$  pathology (only 7.5% biomarker-positive ALS patients) and CSF concentrations of  $A\beta_{42}$  and GAP-43 are the result of secretion in normally functioning synapses. Possibly, the GAP-43 concentration reflects a concurrent increase with  $A\beta_{42}$  concentration in ALS patients due to neurodegeneration; however, neither biomarker reaches the level of AD patients.

Furthermore, GAP-43 was especially high in AD and MCI patients who were also *APOE*  $\epsilon 4$  carriers, suggesting that this phenotype is connected to an early synaptic vulnerability leading to the early cognitive decline.

In conclusion, CSF concentration of the presynaptic protein GAP-43 is specifically increased in tau- and amyloid-positive individuals and could therefore be used as a marker of synaptic dysfunction in AD, provide an additional outcome measure in clinical trials, and help to separate patients with AD from patients with other forms of neurodegenerative diseases.

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the submitted work. The remaining authors have no conflicts to declare.

### Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jalz.2018.08.006>.

### RESEARCH IN CONTEXT

1. Systematic review: We reviewed studies investigating the presynaptic protein growth-associated protein 43 (GAP-43) in cerebrospinal fluid (CSF) as a biomarker for Alzheimer's disease, identifying five research articles using the search terms "GAP-43," "cerebrospinal fluid," and "Alzheimer's" in the PubMed database. One exploratory study indicated that CSF GAP-43 may be increased in preclinical Alzheimer's disease and another suggested a decrease in Parkinson's disease although no larger biomarker study was conducted.
2. Interpretation: Our findings suggest that CSF GAP-43 concentration is specifically associated to Alzheimer's disease pathology and correlated with cognitive decline, whereas no connection was found to the extent of  $\alpha$ -synuclein and TDP-43 pathology.
3. Future directions: CSF GAP-43 may be useful as a synaptic outcome marker in clinical trials of Alzheimer's disease drugs and could facilitate early and differential diagnosis. The results need to be replicated in other cohorts, and longitudinal studies would be highly interesting to determine how early GAP-43 is affected.

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