Alzheimer's Research & Therapy

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Evaluation of cerebrospinal fluid levels of VAMP-2 and SNAP-25 in a dementia with Lewy bodies clinical cohort stratified by Alzheimer's pathophysiological biomarkers

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Abstract

Background Synaptic protein levels in cerebrospinal fluid (CSF) may represent much-needed objective biomarkers of cognitive impairment, disease progression and drug efficacy in patients with dementia with Lewy bodies (DLB). Soluble N-ethylmaleimide-sensitive factor attachment proteins receptors (SNARE) proteins, such as VAMP-2 and SNAP-25, are implicated in α-synuclein pathophysiology and CSF levels of these proteins are associated with pathophysiological biomarkers and cognitive decline in Alzheimer's disease (AD). The aim of the study was to compare CSF levels of VAMP-2 and SNAP-25 in patients with DLB to cognitively unimpaired controls and AD patients and study their association with cognitive performance and AD and neurodegeneration biomarkers.

Methods VAMP-2 and SNAP-25 were quantified in CSF from cognitively normal controls ($n=62$), DLB ($n=44$) and AD ($n=114$) patients from the Sant Pau Initiative for Neurodegeneration (SPIN) cohort using homebrew Single Molecule Array assays (Simoa). The DLB group was stratified into two groups with ("DLB + AD", $n=28$) or without AD co-pathology ("pure DLB", $n=16$) using our validated cut-off for the CSF phosphorylated tau (p-tau)/Aβ42 ratio. We used linear regression to test for group differences (adjusting for age) and association with AD biomarkers. We used standardized w-scores of the cognitive tests to analyze the association of the synaptic markers with cognitive performance.

Results CSF VAMP-2 and SNAP-25 levels correlated across all groups ($r=0.71$–0.9, $p<0.001$). Both proteins were decreased in pure DLB ($p<0.001$, $p=0.01$) but increased in DLB + AD ($p=0.01$, $p=0.02$) compared to controls and showed good accuracy to discriminate pure DLB from DLB + AD (AUC = 0.84, 0.85). Both proteins were associated

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with CSF p-tau and total tau (t-tau) across all groups ($r^2 = 0.49–0.88$, $p < 0.001$), with the Aβ42/40 ratio in DLB + AD ($r^2 = 0.29–0.36$, $p < 0.001$) and in AD ($r^2 = 0.12–0.23$, $p < 0.001$) and with CSF neurofilament-light chain (NfL) in controls (r^2=0.10–0.11, $p < 0.001–0.01$) and AD patients (r^2=0.01–0.08, $p = 0.01 – 0.001$). SNAP-25 was associated with CSF NfL in the DLB + AD group (r^2=0.15, $p = 0.02$). CSF VAMP-2 and SNAP-25 were associated with phonemic fluency in pure DLB ($r^2 = 0.39 – 0.28$, $p = 0.01–0.03$) and SNAP-25 with the Clock drawing test and the MMSE in DLB + AD (adj.$r^2 = 0.15 – 0.14$, $p = 0.03–0.03$) and DLB (adj.$r^2 = 0.12 – 0.08$, $p = 0.02–0.04$) groups.

Conclusions CSF VAMP-2 and SNAP-25 are promising surrogate markers of synapse degeneration in DLB. However, care should be taken when interpreting CSF levels of these synaptic markers in DLB in light of the confounding effect of AD pathophysiological markers.

Keywords VAMP-2, SNAP-25, Dementia with Lewy bodies, Synapse, Cerebrospinal fluid, Biomarker

Introduction

Dementia with Lewy bodies (DLB) is the second most common cause of neurodegenerative dementia after Alzheimer's disease (AD) [1]. Clinically, DLB causes predominantly cognitive fluctuations in attention, executive function, memory, and visuoperceptual ability [2]. There is however a lack of objective biofluid markers to measure these changes in DLB patients. The Lewy bodies and neurites found in DLB postmortem brains are mainly composed of aggregated forms of α-synuclein. The presynaptic accumulation of α-synuclein oligomers can lead to synaptic dysfunction early in DLB pathogenesis [3, 4]. Thus, synaptic proteins are emerging as potential biomarkers to detect and monitor α-synuclein-mediated synapse dysfunction as a surrogate of cognitive impairment in patients with DLB.

The vesicle-associated membrane protein 2 (VAMP-2) and the synaptosomal-associated protein of 25 kDa (SNAP-25) are principal components of the soluble n-ethylmaleimide sensitive factor attachment protein receptor (SNARE). Formation of the SNARE complex is regulated by α-synuclein [5]. Using digital immunoassays, we have previously shown that both proteins decrease in the cerebrospinal fluid (CSF) of individuals with preclinical AD and increase at later AD stages and were associated with core AD biomarkers and episodic, semantic, executive and visuospatial domains and global cognition in individuals on the AD continuum [6]. Another study has reported increased concentrations of SNAP-25 in patients with Lewy body disease (LBD) who have positive markers for amyloid (A+) and tau (T+) compared with LBD/A-T- subjects, thus suggesting a distinct CSF profile in LBD with concomitant AD morbidity [7].

The overall aim of this study was to perform a comprehensive evaluation of VAMP-2 and SNAP-25 as surrogate markers of synapse degeneration in CSF of DLB and AD patients and cognitively unimpaired controls from the Sant Pau Initiative on Neurodegeneration (SPIN) cohort [8]. Specifically, the aim was to compare the CSF profiles of these SNARE proteins in DLB patients with and without AD comorbidity, an important but often neglected factor in biomarker studies of DLB patients and to determine the relationship of the SNARE proteins in CSF to amyloid, tau and neurodegeneration biomarkers and cognitive impairment in these groups.

Materials and methods

Study design

This is a single-center, cross-sectional study of CSF levels of the synaptic proteins VAMP-2 and SNAP-25 in cognitively normal controls, DLB and AD participants selected from the Sant Pau Initiative on Neurodegeneration (SPIN) cohort at Hospital Sant Pau, Barcelona, Spain [8]. The study was approved by the local ethics committee and was conducted in accordance with the Declaration of Helsinki. All participants gave their written informed consent to participate in the study.

Clinical cohort

All participants from the SPIN cohort [8] were evaluated by neurologists with expertise in neurodegenerative diseases and by neuropsychologists using a previously published neuropsychological battery [8]. All participants were assessed for core AD biomarkers, namely brain amyloidosis (CSF $A\beta_{42}$, CSF $A\beta_{42:40}$ ratio using our in-house cutoffs or positive amyloid PET imaging), tau pathology and neurodegeneration (CSF phosphorylated tau (p-tau) and total tau (t-tau)) based on local cut-offs. Controls were recruited among patients' caregivers and healthy volunteers and did not include patients with subjective complaints of cognitive impairment. Inclusion criteria for controls required the absence of a cognitive or neurological disorders (MMSE 27–30, Clinical dementia rating = 0, FCSRT total immediate score > 7, absence of significant impairment in other domains or in daily living activities) and normal CSF core AD biomarker concentrations using our validated cut-offs for sporadic AD (CSF Aβ42: 916 pg/mL, Aβ42:40 ratio: 0.062, CSF p-tau: 63 pg/mL, CSF t-tau: 456 pg/mL). These cut-offs have high specificity and sensitivity to distinguish AD dementia patients from controls [9]. Inclusion criteria for AD included a diagnosis of prodromal AD or AD dementia

according to NIA-AA guidelines [10, 11] and positivity for AD biomarkers. All controls, were within the normal range following formal neuropsychological evaluation when accounting for age and education and were negative for AD biomarkers. The DLB group included all prodromal and dementia DLB cases that met probable diagnostic criteria [12, 13]. AD biomarkers were not taken into consideration as inclusion/exclusion criteria for DLB but were used to stratify DLB patients according to AD comorbidity according to our established cut-off for the CSF p-tau: $A\beta_{42}$ ratio (≥ 0.068 pg/mL for AD comorbidity in DLB) [9]. 16 DLB participants showed no signs of AD pathology and were classified as pure DLB. The remaining 28 were classified as DLB + AD. Inclusion criteria for controls required the absence of a cognitive or neurological disorder and normal CSF AD biomarkers.

CSF collection and biomarker assessment

CSF samples were collected following international consensus recommendations as previously described [8]. Samples were stored at − 80 °C and were not thawed prior to analysis. Commercially available immunoassays were used to determine levels of CSF $A\beta_{42}$, $A\beta_{40}$, t-tau, and p-tau 181 (Lumipulse® G assays β-Amyloid 1–40 and 1–42, total tau, p-tau 181 from Fujirebio, Ghent, Belgium). The *APOE* genotype was determined through Sanger sequencing of exon 4. VAMP-2 and SNAP-25 CSF levels were determined using ADx homebrew Simoa assays. Details about the VAMP-2 and SNAP-25 Simoa assay can be found in Galasko et al. and Goossens et al. [6, 14]. The samples were run in duplicate across 5 plates that included 3 run validation samples with concentrations of 35, 53 and 73 pg/ml for VAMP-2 (intra-run %CV 0.1–3.9%) and 3.8; 7.1 and 10.7 pg/ml for SNAP-25 (intra-run %CV 2.0 to 2.5%). The inter-run variation ranged from 10.6 to 18.2% CV. All of the controls and a subset of AD (prodromal and dementia stages) from [6] are included in this study and were measured in parallel with the DLB samples. All samples were blinded for clinical diagnosis and randomized before analysis.

Statistical analysis

Statistical analyses were performed in R (Version 4.3.1; R Core Team, 2024) developed by the R Foundation for Statistical Analysis, Vienna, Austria. Group differences of demographic variables were compared using χ2 test for categorical variables, and Kruskal-Wallis or Wilcoxon-test for linear variables. We used Spearman's method to test the correlation between the synaptic markers across groups. We used linear regression to compare CSF levels of the SNARE proteins across groups (including age as a covariate and controlling the false discovery rate for each group comparison using the Benjamini-Hochberg method), sex, *APOE* ε4 status and to determine the association of the SNARE proteins with age and AD biomarkers. As regression residuals deviated from a Gaussian distribution (Shapiro-Wilk $p < 0.05$), tests were performed on log2 transformed values, which did not differ from a Gaussian distribution (Shapiro-Wilk $p > 0.05$). Receiver-operating-characteristic curves were used to determine the diagnostic accuracy via the area-under-the-curve (AUC) and compared ROC curves using the DeLong test. We used standardized W-scores of the cognitive tests to control for the effects of age, sex, and years of education, based on the scores of cognitively unimpaired controls from the SPIN cohort [15] and tested for association with SNARE proteins using linear regression, controlling the false discovery rate using the Benjamini-Hochberg test. Regression residuals did not deviate from a Gaussian distribution (Shapiro-Wilk $p < 0.05$).

Results

Demographics of the study participants

Demographic and clinical data of the participants included in the study are shown in the Table 1. Age at analysis was significantly higher in the pure DLB ($p < 0.001$), DLB + AD ($p < 0.001$), and AD ($p < 0.001$) groups compared to controls. The percentage of females was comparable across groups ($p = 0.12$). The percentage of *APOE* ε4 carriers was lower in controls compared to the AD group ($p < 0.001$) but comparable in pure DLB ($p = 0.8$) and DLB + AD ($p = 0.09$). The mean number of years of education was significantly lower in the pure DLB ($p = 0.01$), DLB + AD ($p < 0.001$), and AD ($p < 0.001$) groups compared to controls. There were no differences in sex ($p = 0.35$), age ($p = 0.91$), percentage of *APOE* ε4 carriers ($p = 0.63$), global cognition ($p = 0.65$), or any of the other 17 cognitive tests included in this study (all $p > 0.09$) or global deterioration scale ($p = 0.86$), between pure DLB and DLB + AD. The DLB + AD group had significantly lower years of education compared to the pure DLB ($p = 0.03$). Results in the pure DLB group should be treated as exploratory due to the small number of samples following stratification by sex and *APOE* ε4 status.

Association of SNARE proteins in CSF with age, sex, and *APOE ε4* allele

As represented in Fig S1A, CSF VAMP-2 (r^2=0.09, $p = 0.01$) and SNAP-25 (Fig S1B) (r^2=0.05, $p = 0.04$) showed mild linear associations with age-at-analysis in controls. CSF VAMP-2 (r^2=0.04, $p = 0.02$) but not SNAP-25 (r^2=-0.002, $p = 0.39$) was significantly associated with age in AD. Neither SNARE protein was associated with age in pure DLB (VAMP-2 r^2=-0.05, $p = 0.60$; SNAP-25 r^2=0.03, $p = 0.20$) or DLB + AD (VAMP-2 r^2=-0.02, $p = 0.5$; SNAP-25 r^2=-0.03, $p = 0.6$).

CSF levels of VAMP-2 (Fig S2A) were comparable between males and females in controls ($p = 0.06$), pure

Table 1 Demographic and clinical data of the participants included in the study. Median values (IQR, range) are given for each variable across clinical and biomarker groups. Cut-off for AD comorbidity in DLB: CSF p-tau: Aβ42 ratio ≥ 0.068 pg/mL. MCI; mild cognitive impairment

Characteristic	Control, *N* = 62	Pure DLB, *N* = 16	DLB + AD, *N* = 28	AD, *N* = 113	*p*-value[1]
Disease stage	None	44%MCI, 56% dementia	43%MCI, 57% dementia	65%MCI, 35% dementia	
Age-at-analysis, years	61 (9, 53–74)	78 (9, 68–82)	77 (7, 64–86)	72 (8, 54–85)	< 0.001
Education, years	15.5 (9.0, 6.0–20.0)	9.0 (8.3, 2.0–20.0)	8.0 (2.5, 1.0–20.0)	10.0 (5.0, 1.0–20.0)	< 0.001
APOE ε4 allele carriers (%)	11 (18%)	4 (25%)	10 (37%)	69 (61%)	< 0.001
Female (%)	43 (69%)	6 (38%)	16 (57%)	70 (62%)	0.12
MMSE	29.0 (1.0, 26.0–30.0)	24.0 (3.8, 17.0–28.0)	23.0 (5.3, 8.0–30.0)	25.0 (4.5, 9.0–30.0)	< 0.001
Global deterioration scale	1 (0,1–2)	4 (1, 3–5)	4 (1, 3–5)	3 (1, 3–6)	< 0.001
Amyloid + (%)	0	0	93	100	
CSF Aβ42:40 ratio (A)	0.108 (0.009, 0.074–0.123)	0.101 (0.022, 0.064–0.116)	0.050 (0.010, 0.030–0.073)	0.043 (0.010, 0.026–0.060)	< 0.001
Tau + (%)	0	12.5	75	100	
CSF p-tau181 pg/ml (T)	39 (15, 21–58)	34 (14, 19–67)	98 (55, 27–303)	106 (55, 47–384)	< 0.001
CSF t-tau pg/ml (T)	267 (81, 138–438)	251 (102, 148–457)	546 (272, 161-1,902)	677 (309, 378-2,000)	< 0.001
Alzheimer morbidity (%)	0	0	100	100	
CSF p-tau181: Aβ42 ratio (T/A)	0.03 (0.01, 0.02–0.04)	0.04 (0.01, 0.03–0.07)	0.16 (0.09, 0.07–0.54)	0.20 (0.10, 0.09–0.76)	< 0.001
CSF NfL pg/ml	470 (229, 260-1,153)	795 (525, 519-3,004)	1,104 (518, 579-2,364)	923 (502, 409-2,952)	< 0.001
CSF VAMP-2 pg/ml	329 (134, 163–535)	243 (145, 49–415)	466 (318, 127-1,057)	384 (136, 173-1,043)	< 0.001
CSF SNAP-25 pg/ml	50 (19, 31–99)	42 (19, 23–76)	68 (31, 27–123)	81 (30, 31–208)	< 0.001

[1] *Kruskal-Wallis rank sum test; Pearson's Chi-squared test*

DLB ($p = 0.82$), and AD ($p = 0.60$) but female participants had higher levels of VAMP-2 in the DLB + AD ($p = 0.04$) group. We did not observe any sex-based differences in CSF SNAP-25 (Fig S2B) in controls ($p = 0.19$), pure DLB ($p = 0.20$), DLB + AD ($p = 0.14$), or AD ($p = 0.45$).

We observed no difference in CSF VAMP-2 (Fig S3A) or SNAP-25 (Fig S3B) levels between *APOE* ε4 carriers and non-carriers in controls (VAMP-2 $p = 0.32$, SNAP-25 $p = 0.33$) or AD (VAMP-2 $p = 0.82$, SNAP-25 $p = 0.45$). In the pure DLB group, carriers of the *APOE* ε4 allele had higher levels of CSF VAMP-2 ($p = 0.05$) and SNAP-25 ($p = 0.03$) than non-carriers. We observed similar associations in the DLB + AD group (VAMP-2 $p < 0.001$, SNAP-25 $p = 0.02$).

CSF profile of SNARE proteins in DLB and AD patients

As shown in Fig. 1A, there was a strong correlation between CSF VAMP-2 and SNAP-25 levels in controls ($r = 0.87$, $p < 0.001$), pure DLB ($r = 0.71$, $p = 0.002$), DLB + AD ($r = 0.9$, $p < 0.001$) and AD ($r = 0.75$, $p < 0.001$). Compared to controls, CSF VAMP-2 (Fig. 1B) levels were lower in the pure DLB group ($p < 0.001$) and elevated in the DLB + AD group ($p = 0.02$) but not in the AD group ($p = 0.2$). CSF SNAP-25 showed a similar profile (Fig. 1C) with lower levels in the pure DLB group ($p = 0.01$) and elevated levels in both the DLB + AD ($p = 0.02$) and AD ($p < 0.001$) groups compared to controls. CSF levels of VAMP-2 and SNAP-25 were significantly lower in pure DLB compared to DLB + AD ($p < 0.001$) and AD ($p < 0.001$). There were differences in SNAP-25 ($p = 0.01$) in DLB + AD compared to AD but not in VAMP-2 ($p = 0.05$). CSF VAMP-2 and SNAP-25 were able to distinguish between the pure DLB and DLB + AD groups with similar accuracy (DeLong $p = 0.63$), with AUC values of 0.85 (95% CI: 0.74–0.97) and 0.84 (95% CI: 0.72–0.96), respectively and between controls and pure DLB (VAMP-2 (AUC:0.73, 95% CI: 0.58–0.89); SNAP-25 (AUC:0.63, 95% CI: 0.46–0.80))(Fig. 1D).

Association of SNARE proteins with core AD and neurodegeneration biomarkers in CSF

Figure 2 shows the association between CSF VAMP-2 and SNAP-25 with CSF tau markers (p-tau181, t-tau), $A\beta_{42:40}$ ratio and CSF NfL. The strongest associations were observed between the SNARE proteins and tau markers; both p-tau181 (Fig. 2A-B; $r^2 = 0.51–0.86$, $p < 0.001$) and t-tau (Fig. 2C-D; $r^2 = 0.50–0.88$, $p < 0.001$), in all groups. VAMP-2 and SNAP-25 were also associated with the $A\beta_{42:40}$ ratio (Fig. 2E-F) in groups with pathological/low $A\beta_{42:40}$ ratios, i.e., the DLB + AD ($r^2 = 0.29–0.36$, $p < 0.002$) and AD ($r^2 = 0.12–0.23$, $p < 0.001$) but not in controls (r^2 = -0.01, $p > 0.60$) or pure DLB (r^2 = -0.01-0.06, $p > 0.19$). The SNARE proteins were associated with CSF NfL (Fig. 2G-H) in controls (r^2=0.10–0.11, $p < 0.01$), DLB + AD (r^2=0.10–0.15, $p < 0.05$) and AD (r^2=0.08–0.12, $p < 0.01$) but not in pure DLB ($p > 0.50$).

Association of the SNARE proteins with cognitive performance

We evaluated the association of the SNARE proteins with cognitive performance using standardized w-scores of 18 individual cognitive tests (Table S1) in DLB patients. CSF levels of VAMP-2 and SNAP-25 (Fig. 3A-B) showed a positive linear association with phonemic fluency, a

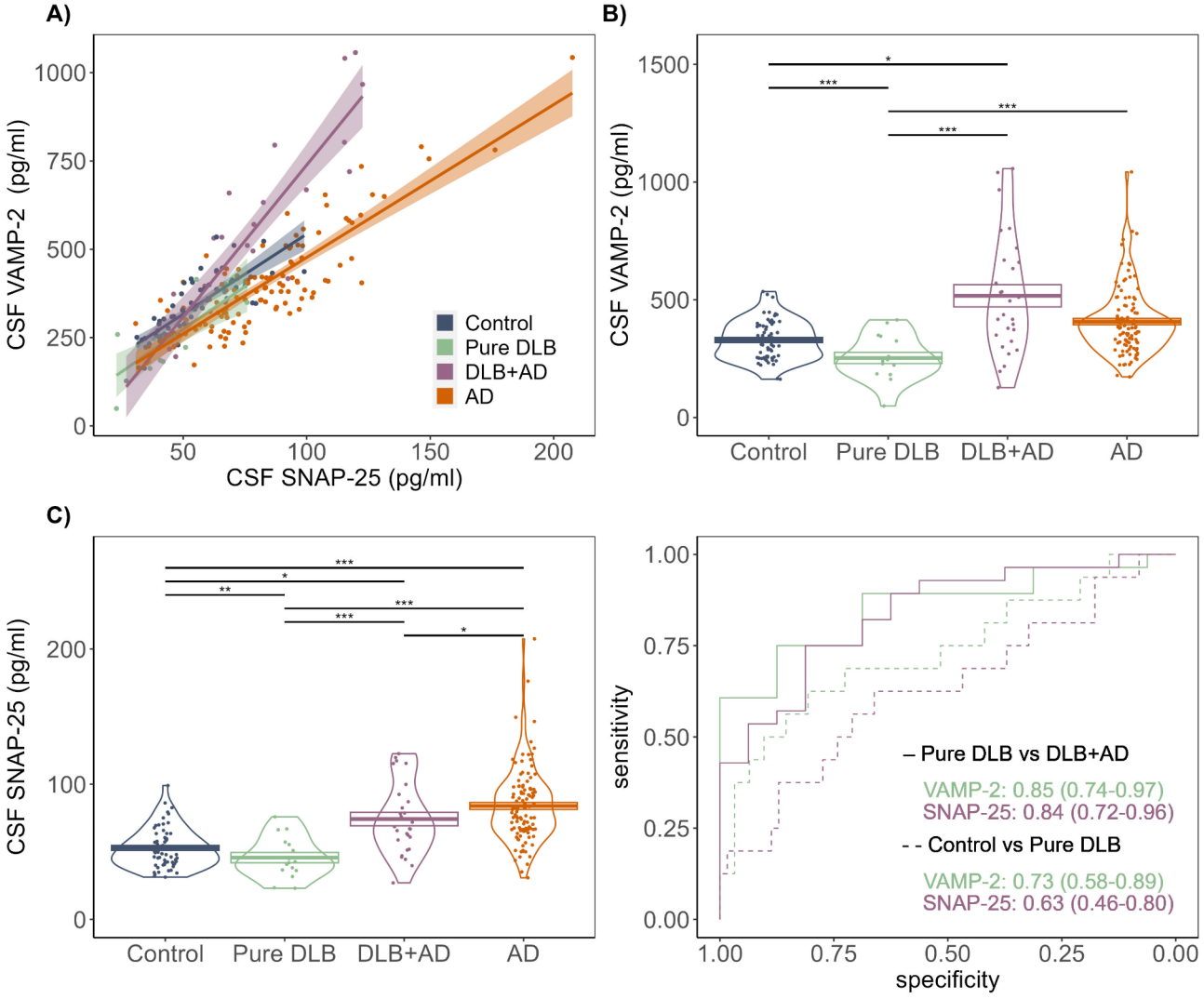

Fig. 1 CSF profile of synaptic markers in DLB and AD. (**A**) Pairwise correlation of VAMP-2 and SNAP-25 in controls, pure DLB, DLB + AD, and AD (see legend). Linear regression lines are shown for each group. Shaded areas represent standard error of the regression lines. (**B-C**) Distribution (violin), mean (horizontal line) and standard error (box) of CSF levels of VAMP-2 (**B**) and SNAP-25 (**C**) in cognitively healthy controls, pure DLB, DLB + AD, and AD subjects. *P*-values for linear regression using log2 and squared root transformed levels compared to controls are shown: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ (**D**) Receiver operating characteristic (ROC) curves of VAMP-2 and SNAP-25 to discriminate pure DLB from DLB + AD (solid lines) and controls from pure DLB (dotted lines) (Area under the curve, 95% Confidence Interval)

measure of executive function, in pure DLB (VAMP-2 adj.r^2=0.39, $p = 0.008$, SNAP-25 adj.r^2=0.28, $p = 0.025$). Thus, in pure DLB, where CSF VAMP-2 and SNAP-25 levels were lower than controls, lower VAMP-2 and SNAP-25 levels were associated with worse phonemic fluency. In DLB + AD where CSF VAMP-2 and SNAP-25 levels were elevated compared to controls, despite a mild negative linear association with phonemic fluency, neither association reached significance (VAMP-2 r^2=-0.007, $p = 0.373$, SNAP-25 r^2=0.051, $p = 0.144$). We found significant associations of CSF SNAP-25 (Fig. 3D) with the Clock drawing test, also part of the executive domain, in DLB + AD (r^2=0.152, $p = 0.031$) and the DLB (r^2=0.121, $p = 0.016$) group combined. We observed similar results when looking at global cognition, measured by the MMSE, where higher levels of CSF SNAP-25 (Fig. 3F) were associated with worse perfomance at the MMSE both in DLB + AD (r^2=0.137, $p = 0.030$) and DLB (r^2=0.079, $p = 0.036$) groups. These associations did not reach significance in the case of CSF VAMP-2 (Fig. 3C and E). The SNARE proteins were not associated with other cognitive tests included in the battery (Table S2). We next grouped the individual tests by cognitive domain and calculated the W-scores based on the mean of each domain (Table S3). SNAP-25 showed a mild association with executive function in the full DLB cohort only (adj. r^2=0.02, $p = 0.04$). This association was not replicated in

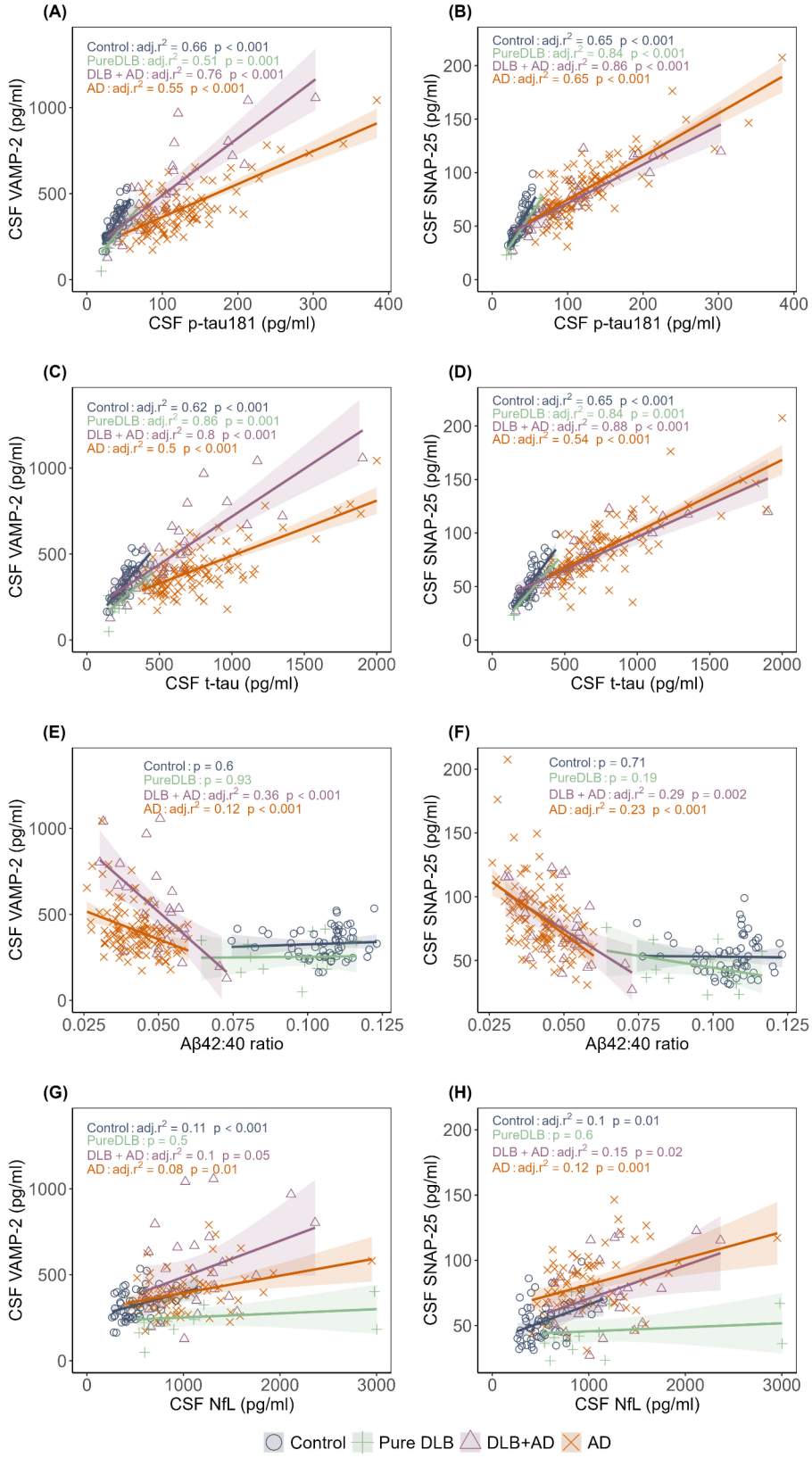

Fig. 2 Association of synaptic markers with core AD and neurodegeneration biomarkers in CSF. CSF VAMP-2 and SNAP-25 are plotted against core CSF AD biomarkers (p-tau181 (**A-B**), total tau (t-tau) (**C-D**), Aβ42:40 ratio (**E-F**) and Neurofilament-light chain (NfL) (**G-H**) in cognitively healthy controls, pure DLB, DLB + AD and AD subjects. Linear regression lines are shown for each group. Shaded areas represent standard error of the regression lines. Log-transformed values were used for statistical analyses

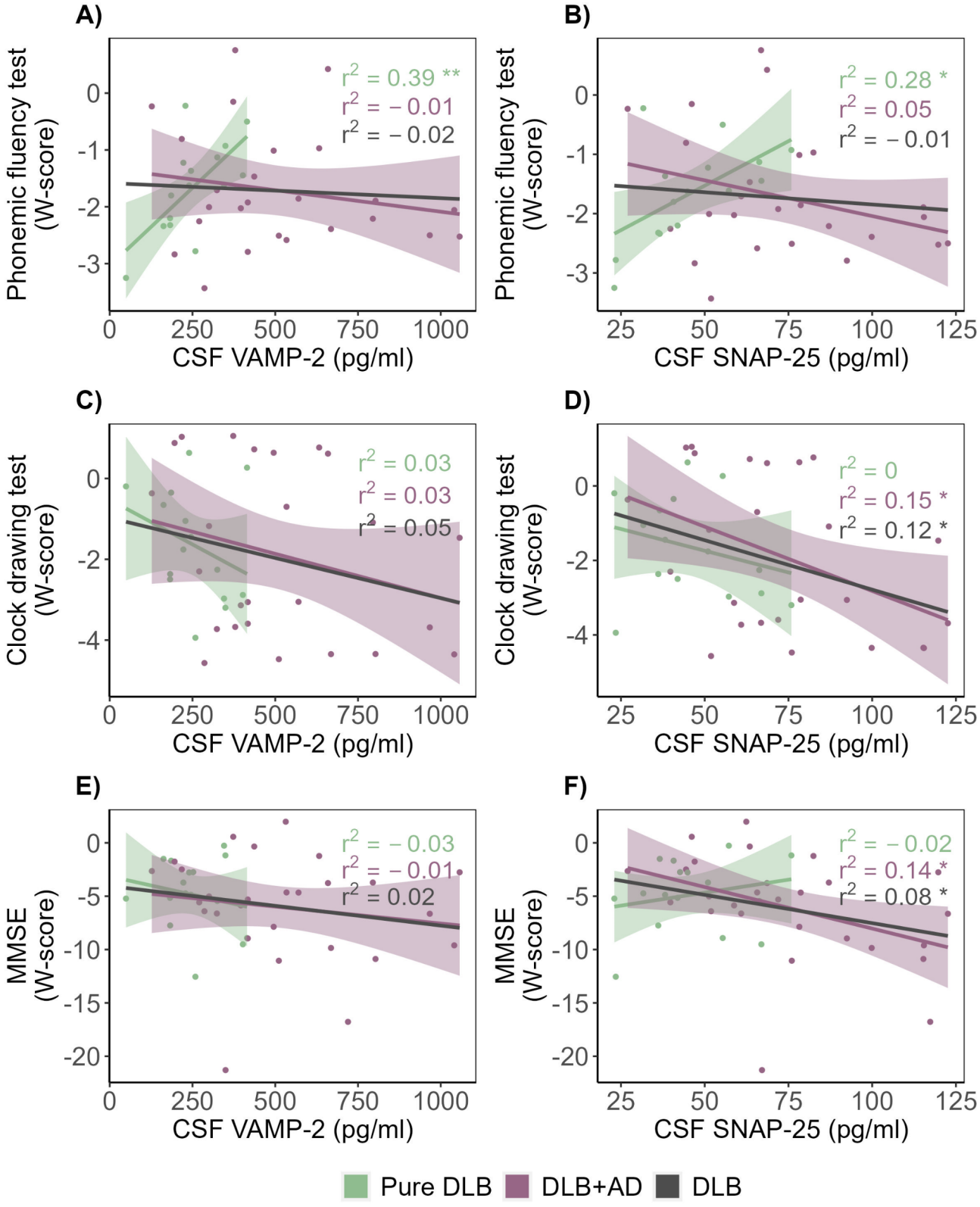

Fig. 3 Association of synaptic markers with cognitive performance. CSF VAMP-2 and SNAP-25 are plotted against the Phonemic fluency (**A-B**), Clock drawing test (**C-D**) and MMSE (**E-F**) w-scores in the pure DLB and DLB + AD groups (see legend). Linear regression lines are shown for each group (black line indicates the combined DLB group). Shaded areas represent the standard error of the regression lines

VAMP-2 ($p = 0.15$). None of the reported associations survived adjustment for multiple testing.

Discussion

Here we report low CSF levels of two SNARE proteins, VAMP-2 and SNAP-25, in the CSF of DLB patients compared to cognitively unimpaired controls. Moreover, we report that CSF levels of the two SNARE proteins were elevated in DLB patients in correlation with AD pathological load, such that both synaptic proteins showed good diagnostic accuracy to differentiate DLB patients with and without AD comorbidity. The distinct CSF profile of SNARE proteins related to AD pathophysiological markers in DLB that we report here suggests that different pathophysiological mechanisms may be at play at the synapse in pure DLB compared to DLB with AD comorbidity.

One potential explanation for low CSF levels of VAMP-2 and SNAP-25 in pure DLB could be the intracellular sequestration of SNARE proteins as a result of α-synuclein pathology. This is supported by studies reporting that (i) VAMP-2 colocalizes with α-synuclein in Lewy bodies purified from DLB subjects [16], (ii) oligomeric α-synuclein inhibits SNARE-dependent vesicle docking at the cytoplasmic membrane through its interaction with VAMP-2 [17], (iii) different proteoforms of α-synuclein can alter the distribution of SNARE proteins present at the synapse in in vitro models [18], and (iv) unbiased protemic study reported specific presynaptic signatures in confirmed Lewy body dementia brains compared to those of AD [19]. This hypothesis could also be extended to explain the low levels of SNARE proteins in CSF from participants at AD preclinical stage 1 (cognitively unimpaired amyloid-positive, tau-negative participants) that we previously reported [20] since Aβ40 and Aβ42 can also directly disrupt SNARE complex formation by affecting the interactions between VAMP-2 and SNAP-25 in vitro [21].

We propose that the elevated levels of SNARE proteins in the CSF in DLB patients with AD comorbidity (and that we and others have previously reported in the clinical stages of AD [6, 20, 22]) may reflect the impact of AD pathology at the synapse in DLB either directly or via further seeding of synuclein pathology. AD pathology in synucleinopathies is associated with increased α-synuclein pathology and worse prognosis [23, 24] and severity of Aβ pathology correlates with amount of α-synuclein pathology in DLB [4]. Future studies are needed to determine whether pathological Aβ and tau drive an increase in SNARE protein release from the membrane or active secretion of SNARE proteins. Either way, we propose a scenario whereby the arrival of SNARE proteins to the CSF may be diminished in individuals with single pathologies (e.g., Aβ or α-synuclein alone) but enhanced in participants with multiple pathologies (e.g., amyloid and tau in the presence or absence of synuclein).

The confounding effect of AD pathophysiological markers on CSF SNARE protein levels may be driven by either tau or Aβ, or perhaps both are required, as reported in AD [6, 20, 25]. That said, we found that CSF tau markers were better predictors of CSF VAMP-2 and SNAP-25 than CSF Aβ or neurodegeneration markers. CSF VAMP-2 and SNAP-25 were only associated with the amyloid marker in participants with AD morbidity (AD and DLB + AD). Furthermore, Aβ alone was not associated with elevated levels of synaptic markers in AD [6, 20]. The comparatively weak association of the SNARE proteins with the axonal degeneration marker, NfL, across all groups suggests that elevated CSF levels are unlikely a result of neurodegeneration alone.

AD comorbidity is prevalent in our DLB cohort, representing approximately 64% of the DLB cases, similar to what has been reported in other DLB cohorts [26, 27]. Despite this prevalence and the availability of AD pathophysiological biomarkers, AD comorbidity is often neglected in CSF biomarker studies in DLB. Whether these findings are generalizable to other synucleinopathies is unclear. In serum extracellular vesicles, VAMP-2 was decreased in Parkinson's disease (PD) patients while SNAP-25 levels remained comparable compared to controls [28]. Another study reported elevated levels of CSF SNAP-25 in PD subjects compared to healthy controls [29]. These studies reported no correlation with cognition, measured by the Montreal Cognitive Assessment, and stratification for AD comorbidity was not reported. While low levels of other synaptic proteins have been reported in DLB [30, 31], whether they also show different levels in CSF of pure DLB and DLB with AD comorbidity as the SNARE proteins have yet to be determined. The current study highlights the importance of segregating DLB patients by AD pathophysiological biomarkers to tease out associations that otherwise would have been missed. That said, the small sample size in the pure DLB group is a limitation of this study and validation in larger independent cohorts is required. Another limitation of this study is the absence of α-synuclein seeding amplification assay (SAA) data, which would have provided confirmation of Lewy body pathology in individuals clinically diagnosed with DLB. We report an inverse relationship between SNARE proteins in CSF and phonemic fluency in the pure DLB, in which SNARE proteins were lower than in controls. Phonemic fluency alterations are associated with higher Lewy body and tangle burden in frontal, temporal, and limbic regions of PD patients [32]. This finding thus provides further support that CSF SNARE proteins reflect α-synuclein-mediated synapse dysfunction in pure DLB. Notably, we observed a trend for association of SNARE proteins with phonemic fluency in

the opposing direction in the DLB + AD group, in which elevated levels of the SNARE proteins compared to controls were detected. Thus, these biomarkers are differentially associated with phonemic fluency in DLB patients with and without AD comorbidity, suggesting that the relationship between SNARE protein levels in the CSF and Lewy body burden may be affected by AD pathology. This is supported by our previous study that showed that VAMP-2 and SNAP-25 were associated with executive function in AD [6]. We also report an association of SNAP-25 in CSF with the MMSE test and the clock drawing test in the DLB + AD group. The clock drawing test is a cognitive screening measure for mild dementia, particularly sensitive to AD and DLB [33] and is associated with damage to the occipital and parietal cortex [34]. A digital version of the test has been shown to be a correlate of Aβ and tau burden [35] and studies have reported that patients with clinically diagnosed DLB and a low score on the clock drawing test had a shorter survival than patients with DLB and a high score [36].

Finally, we highlight other synaptic markers from the vesicle-mediated transport/secretory pathway, such as VGF, SCG2, and PDYN, all of which have been shown to be reduced in other DLB CSF cohorts compared to controls [30, 37]. CSF concentrations of all three proteins correlated with measures of global cognition, albeit that those findings were performed in the total dataset including controls, rather than in the DLB patients independently as reported here. Other synaptic proteins such as the pentraxins were reduced in multiple DLB cohorts and were associated with cognitive performance in the DLB dataset [30, 31]. Neurogranin and beta-synuclein on the other hand have been shown to be unaltered in most DLB studies [38–43]. Whether these other synaptic biomarkers differentiate AD comorbidity in DLB patients like the SNARE proteins reported here has yet to be reported.

In conclusion, CSF VAMP-2 and SNAP-25 are promising biomarkers of synapse degeneration in DLB that are associated with AD markers. The association with phonemic fluency supports the biological basis of the findings in DLB, due to the fact that reduced fluency is a typical cognitive profile in DLB [44]. However, care should be taken when interpreting CSF levels of these, and potentially other synaptic markers, in DLB in light of the confounding effect of AD pathophysiological markers. This study opens the door to future studies to better understand the complex interplay of pathophysiological mechanisms of synapse degeneration related to synuclein and AD pathologies.

Abbreviations

Aβ	Amyloid β
Aβ42	40 Amyloid β 42 over amyloid β 40 ratio
AD	Alzheimer's disease
APOE ε4	Apoliprotein E ε4 allele
CSF	Cerebrospinal fluid
DLB	Dementia with lewy bodies
NfL	Neurofilament-light chain
PD	Parkinson's disease
PET	Positron emission tomography
p-tau	Phosphorylated tau
ROC	Receiver operating characteristic
SNAP-25	Synaptosomal-associated protein-25 kDa
SNARE	Soluble N-ethylmaleimide-sensitive factor attachment protein receptors
SPIN	Sant pau initiative on neurodegeneration
t-tau	Total tau
VAMP-2	Vesicle-associated membrane protein 2

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13195-025-01685-y.

Supplementary Material 1: **Figure S1**: **Association of synaptic markers with age**. CSF VAMP-2 (**A**) and SNAP-25 (**B**) are plotted against age (years) across groups (see legend). Linear regression lines are shown for each group. Shaded areas represent the standard error of the regression lines. Log-transformed values were used for statistical analyses.

Supplementary Material 2: **Figure S2**: **Association of synaptic markers with sex**. CSF levels of synaptic markers VAMP-2 (**A**) and SNAP-25 (**B**) are plotted stratified by sex (M: male, F: female) across different groups. *P*-values from the t-test are indicated as follows: **p* < 0.05, ***p* < 0.01, ****p* < 0.001

Supplementary Material 3: **Figure S3**: **Association of synaptic markers with the APOE ε4 allele**. CSF levels of synaptic markers VAMP-2 (**A**) and SNAP-25 (**B**) are presented according to APOE ε4 allele status: ε4+ carriers (individuals with at least one ε4 allele) and ε4– non-carriers (individuals without the ε4 allele) across different groups. P-values from the t-test are indicated as follows: **p* < 0.05, ***p* < 0.01, ****p* < 0.001.

Supplementary Material 4: **Table S1**: **Cognitive w-scores in Pure DLB and DLB+AD**. Median values (IQR, range) are given for each cognitive test across DLB groups. *P*-values comparing cognitive scores using the Wilcoxon Rank Test across the two groups are shown.

Supplementary Material 5: **Table S2**: **Association Between Cognitive W-Scores and Synaptic Markers**. This table presents the results of regression models examining the associations between CSF VAMP-2 (left panel) and SNAP-25 (right panel) levels with the w-scores of 18 cognitive tests across three groups: pure DLB, DLB+AD, and combined DLB. The "adj.p" columns represent *p*-values adjusted for multiple testing using the Benjamini-Hochberg method.

Supplementary Material 6: **Table S3: Association Between Cognitive domains (W-Scores) and Synaptic Markers**. This table presents the results of regression models examining the associations between CSF VAMP-2 (left panel) and SNAP-25 (right panel) levels with cognitive domains (w-scores) across three groups: pure DLB, DLB+AD, and combined DLB. The "adj.p" columns represent *p*-values adjusted for multiple testing using the Benjamini-Hochberg method.

Acknowledgements

The authors thank all participants who agreed to take part in this study and all the members of the clinical teams involved in the selection and assessment. We would also like to thank Raúl Núñez Llaves, Soraya Torres, Shreyasee Das and Charlotte De Rocker for the scientific support.

Author contributions

A.C.G, J.G, O.B, A.L, E.V: Conceptualization. A.C.G, J.G, E.C, I.S, M.B.S, Í.R.B, L.L, D.P, D.A: Methodology, acquisition of data. A.C.G, J.G, O.B: Validation, Investigation, Formal analysis, Visualization, Writing– original draft. O.B, A.L, E.V, A.H: Funding acquisition. O.B, D.A, E.V.C, I.S, M.B.S, Í.R.B: Collection of patient samples and

clinical data. E.V, A.L, A.B, J.F: Supervision. All authors have reviewed and approved the final manuscript.

Funding
This work was supported by research grants from Institute of Health Carlos III (ISCIII), Spain (PI18/00327, PI21/00327, PI22/00611, DTS-22/00111) and the Joint Programming in Research to O.B. and Centro de Investigación Biomédica en Red en Enfermedades Neurodegenerativas (CIBERNED) Program 1, Alzheimer Disease to A.L. This is an EU Joint Programme - Neurodegenerative Disease Research (JPND) project. The project is supported through the following funding organisations under the aegis of JPND - www.jpnd.eu (Research Council of Finland, Spain ISCIII). This work has also been supported by the Department of Health Generalitat de Catalunya AGAUR program (AGAUR 2019 PROD 00088 to OB) and partly funded by Fondo Europeo de Desarrollo Regional (FEDER), Unión Europea, "Una manera de hacer Europa". The funders of this study played no role in the design of the study, collection, analysis or interpretation of data or writing the manuscript.

Data availability
The dataset(s) supporting the conclusions of this article is(are) included within the article (and its additional file(s).

Declarations

Ethics approval and consent to participate
This study was reviewed and approved by the IRB (Institut de Recerca de l'Hospital de la Santa Creu i Sant Pau– IIB Sant Pau) and was conducted in accordance with the Declarations of Helsinki. All participants gave their written informed consent to participate in the study.

Consent for publication
Not applicable.

Competing interests
E.V. is co-founder of ADx NeuroSciences NV, Zwijnaarde (Ghent), Belgium, J. G. is employee at ADx NeuroSciences NV. J.F. has served as a consultant for Novartis and Lundbeck, has received honoraria for lectures from Roche, NovoNordisk, Esteve and Biogen and served at advisory boards for AC Immune, Zambon and Lundbeck. D.A. participated in advisory boards from Fujirebio-Europe and Roche Diagnostics and received speaker honoraria from Fujirebio-Europe, Roche Diagnostics, Nutricia, Krka Farmacéutica S.L., Zambon S.A.U., Lilly and Esteve Pharmaceuticals. A.L. participated in advisory boards from Biogen, Eisai, Fujirebio-Europe, Novartis, NovoNordisk, Nutricia, Otsuka Pharmaceutical, and Zambón, and received speaker honoraria from Lilly, Biogen, KRKA and Zambon. D.A, J.F., A.L. and O.B. declare a filed patent application (WO2019175379 A1 Markers of synaptopathy in neurodegenerative disease) related to biomarkers included in this manuscript.

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Received: 19 November 2024 / Accepted: 26 January 2025
Published online: 24 February 2025

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