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FEATURED ARTICLE

Cerebrospinal fluid biomarker panel of synaptic dysfunction in Alzheimer's disease and other neurodegenerative disorders

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Abstract

Introduction: Synaptic degeneration is a key part of the pathophysiology of neurodegenerative diseases, and biomarkers reflecting the pathological alterations are greatly needed.

Method: Seventeen synaptic proteins were quantified in a pathology-confirmed cerebrospinal fluid cohort of patients with Alzheimer's disease (AD; $n = 63$), frontotemporal lobar degeneration (FTLD; $n = 53$), and Lewy body spectrum of disorders (LBD; $n = 21$), as well as healthy controls (HC; $n = 48$).

Results: Comparisons revealed four distinct patterns: markers decreased across all neurodegenerative conditions compared to HC (the neuronal pentraxins), markers increased across all neurodegenerative conditions (14-3-3 zeta/delta), markers selectively increased in AD compared to other neurodegenerative conditions (neurogranin and beta-synuclein), and markers selectively decreased in LBD and FTLD compared to HC and AD (AP2B1 and syntaxin-1B).

Discussion: Several of the synaptic proteins may serve as biomarkers for synaptic dysfunction in AD, LBD, and FTLD. Additionally, differential patterns of synaptic protein alterations seem to be present across neurodegenerative diseases.

KEYWORDS
Alzheimer's disease, biomarkers, frontotemporal lobar degeneration, Lewy body spectrum of disorders, synaptic pathology, mass spectrometry

Highlights

- A panel of synaptic proteins were quantified in the cerebrospinal fluid using mass spectrometry.

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- We compared Alzheimer's disease, frontotemporal degeneration, and Lewy body spectrum of disorders.
- Pathology was confirmed by autopsy or familial mutations.
- We discovered synaptic biomarkers for synaptic degeneration and cognitive decline.
- We found differential patterns of synaptic proteins across neurodegenerative diseases.

1 | INTRODUCTION

Synaptic degeneration is a central pathophysiologic event of Alzheimer's disease (AD),[1] with a stronger association with the degree of cognitive decline in AD than amyloid plaque pathology.[2] This correlation makes a convincing argument for the development and implementation of synaptic in vivo biomarkers not only in the routine clinical assessment of AD to facilitate diagnosis, disease staging, and progression but also to monitor the efficacy and endpoints of treatments in drug trials. Other neurodegenerative diseases, including frontotemporal lobar degeneration (FTLD) and Lewy body spectrum of disorders (LBD), are also marked by synaptic dysfunction and degeneration.[3,4] It is thus of importance to study synaptic dysfunction across the range of neurodegenerative disorders.

Since the 1990s, when the first studies detecting synaptic proteins in the cerebrospinal fluid (CSF) emerged,[5] various synaptic proteins have been studied as potential biomarkers, and several methods for their quantification have been developed.[1] To identify candidate synaptic biomarkers and to identify a multi-biomarker profile of synaptic dysfunction in AD, we successfully developed a mass spectrometry–based assay quantifying a panel of 17 potential synaptic proteins,[6] selected based on a previous exploratory proteomics study.[7] The panel included syntaxins, vesicle-associated membrane protein 2 (VAMP-2), adaptor related protein complex 2 subunit beta 1 (AP2B1), complexin-2, synucleins, rab GDP dissociation inhibitor alpha (GDI1), neuronal pentraxins, phosphatidylethanolamine-binding protein 1 (PEBP-1), and members of the 14-3-3 protein family. The proteins all have a variety of synaptic functions and locations at the synapse, and several have been implicated in AD and other neurodegenerative diseases.[1,8–18]

When we studied the panel proteins in a small clinical sample, we found increased CSF levels of beta-synuclein, gamma-synuclein, neurogranin, PEBP-1, 14-3-3 proteins, and decreased CSF levels of the neuronal pentraxins in AD compared to healthy controls (HC), while the protein levels of complexin-2, the syntaxins, GDI1, and AP2B1 remained unchanged.[6] Thus, we concluded that several of the panel proteins could be potential synaptic pathology biomarkers for AD. However, it remained unclear if these protein alterations are specific to AD, or if any of these may also be markers of synaptic dysfunction in other neurodegenerative conditions. In this study, we aimed to validate these synaptic proteins in a larger independent sample of HC and AD, and we expand our study to other neurodegenerative diseases—LBD and FTLD.

2 | METHOD

2.1 | Patients and AD biomarker analysis

Participants had autopsy-confirmed AD ($n = 63$) and LBD ($n = 21$), or had FTLD ($n = 53$) pathologically confirmed by autopsy ($n = 42$) or with a familial form ($n = 11$, no autopsy data) determined by associated mutations (6 *C9orf72*, 2 *GRN*, 2 *MAPT*, 1 *TARDBP*).[19] At autopsy, primary AD pathology was determined by board-certified neurologists (EBL, JQT) according to established criteria for high or intermediate AD neuropathologic change (ADNC);[20] LBD pathology was determined by the accumulation of alpha-synuclein positive Lewy bodies,[21] and FTLD pathology was determined by misfolded tau or transactive response DNA-binding protein of 43 kDa.[22] One FTLD patient and 9 LBD patients had concomitant AD (high/intermediate ADNC).

Patients had been assayed for CSF amyloid beta 1-42 ($A\beta_{1\text{-}42}$), phosphorylated tau at Thr181 ($p\text{-}tau_{181}$), and total tau (t-tau), as previously described.[23] In addition, we included 48 HC subjects who were cognitively unimpaired, with a Mini-Mental State Examination (MMSE) score ≥28,[24] and who were biomarker-negative for AD with CSF $A\beta_{1\text{-}42}$ > 192 pg/mL (no autopsy data). In all cases, we excluded other potential causes of cognitive decline such as hydrocephalus, closed head injury, a history of central nervous system surgery, stroke, infection, metabolic factors such as hypothyroidism, and primary psychiatric disorders. The demographics of the sample are shown group-wise in Table 1. Consent was obtained according to the Declaration of Helsinki and approved by the Penn Institutional Review Board.

2.2 | Liquid chromatography tandem mass spectrometry analysis

An internal standard consisting of a mixture of stable-isotope-labeled peptides was added (25 μL, 0.032 pmol/μL) to 100 μL of CSF. Reduction, alkylation, and tryptic digestion were performed, followed by solid-phase extraction for purification purposes (for detailed sample preparation, refer to Nilsson et al.[6]). A micro-high-performance liquid chromatography mass spectrometry system (6495 Triple Quadrupole LC/MS system, Agilent Technologies) equipped with a Hypersil Gold reversed-phase C18 column (dim. = 100 × 2.1 mm, particle size = 1.9 μm, Thermo Fisher Scientific) was used for quantitation;

RESEARCH IN CONTEXT

1. **Systematic Review**: In the available scientific literature, numerous synaptic proteins have been identified as candidate biomarkers of synaptic degeneration. However, the validation of synaptic proteins across neurodegenerative diseases is needed to improve our understanding of both synaptic pathology and their potential as biomarkers of cognitive decline. We perform comparisons of a panel of synaptic proteins in rare pathology-confirmed cases of Alzheimer's disease (AD), Lewy body spectrum of disorders (LBD), and frontotemporal lobar degeneration (FTLD).
2. **Interpretation**: Our findings identify several synaptic dysfunction biomarkers for AD, LBD, and FTLD that could be used as possible prognostic and diagnostic biomarkers. We show differential patterns of synaptic protein alterations, both general to neurodegenerative disease and specific to AD. These findings have implications of overlapping and distinct pathological features of the synapse in AD, LBD, and FTLD.
3. **Future Directions**: Further studies in larger cohorts will be needed to validate the specificity of the biomarkers and the differential patterns presented herein.

for detailed settings see Table S1 in supporting information. Injections at regular intervals of two different quality control samples, consisting of pooled CSF samples, were used to monitor the performance of the assay over time.

2.3 | Data processing and statistical analysis

For data analysis, including peak inspection and adjustment of the chromatographic spectra's (Figure S1 in supporting information) Skyline 20.1 (MacCoss Lab Software) was used, and the relative peptide concentration was calculated using Suppl. Formula 1. R software (version 4.0.3) was used for statistical analysis and data visualization. A heatmap of the panel proteins was displayed by using the *heatmap2* R package; synaptic proteins were grouped according to hierarchical clustering with Spearman's correlation coefficient as distance. Demographic characteristics were evaluated by Kruskal–Wallis test and chi-square goodness of fit test for continuous and categorical variables, respectively (Table 1). Group-wise comparisons (HC, AD, FTLD, LBD) were assessed using rank-based analyses of covariance, including age and sex as covariates. Post hoc analyses in autopsy-confirmed patients tested how two analytes of interest—14-3-3 zeta/delta and beta-synuclein—differed by metrics of AD pathological severity (ADNC, Consortium to Establish a Registry for Alzheimer's Disease [CERAD] score, Braak stage); models included CSF to death interval and sex as covariates. All group comparisons were adjusted for multiple group comparisons with the false discovery rate approach. Associations between continuous variables were explored with Spearman rank correlation analysis. Receiver operating characteristic (ROC) curve contrasted groups and provided the area under the curve (AUC) to evaluate the discriminatory ability of the biomarkers.

3 | RESULTS

The analytical performance of the different proteins had a high precision within and between runs with a few exceptions (Table S2 in supporting information). For the proteins for which more than one peptide was analyzed, the peptide with the best analytical performance, in terms of repeatability and intermediate precision (lowest coefficient of variation), was chosen for the statistical analysis. Analysis of 14-3-3 eta did not meet quality control standards and was excluded completely.

To investigate associations between the synaptic proteins, cluster analysis (Figure 1A) was performed, and it emerged that some of the measured proteins correlated strongly with each other (Spearman's correlation coefficient [rho] > 0.75, $P \leq .0001$). Additionally, we found that the levels of several of these proteins were altered in neurodegenerative patients compared to HC and each other, and four differential patterns emerged (Figure 1B and Table S3 in supporting information).

First, increased neurogranin levels were found in AD compared to LBD ($P = .0074$) and FTLD ($P = .00054$) as well as increased beta-synuclein levels in AD compared to LBD ($P = .045$). Taken together, these proteins seem to exhibit a pattern of AD-specific increase compared to other neurodegenerative diseases. In the cluster analysis, beta-synuclein and neurogranin were closely associated with each other as well as with gamma-synuclein and GDI1 (rho = 0.77—0.89, $P \leq .0001$). However, despite a similar pattern, no changes were observed for GDI1 and gamma-synuclein.

Second, AP2B1 was found to have significantly decreased levels in FTLD compared to AD ($P = .039$) and both LBD ($P = .0054$) and FTLD ($P = .0017$) compared to HC, but not AD. Similarly, syntaxin-1B was found to have decreased levels in FTLD compared to HC ($P = .014$). Thus, AP2B1 and syntaxin-1B exhibited a pattern of non–AD-specific decrease compared to both HC and AD. AP2B1 and the syntaxin-1B correlated moderately to strongly with each other and with syntaxin-7, complexin-2, PEBP-1, and VAMP-2 (rho = 0.68—0.92, $P \leq .0001$). However, syntaxin-7, complexin-2, PEBP-1, and VAMP-2 showed no group differences.

Third, neuronal pentraxin-2 and the receptor had decreased levels in AD ($P \leq .0001$), FTLD ($P \leq .0001$), and LBD (NPTX2; $P = .0012$, NPTXR; $P = .0029$) compared to HC. In the cluster analysis, neuronal pentraxin-2 and the receptor were found to be closely associated, and both also correlated well with neuronal pentraxin-1 (rho = 0.71—0.92, $P \leq .0001$). Neuronal pentraxin-1 displayed decreased levels in AD ($P = .045$) and FTLD ($P = .0051$) compared to HC, but not in LBD. Together, the neuronal pentraxins exhibit a pattern of decreased levels of the same magnitude regardless of neurodegenerative disease.

TABLE 1 Demographic characteristics for autopsy patients and healthy controls

Demographic characteristics	HC	AD	LBD	FTLD	*P*-value
n	48	63	21	53	
Age at onset (years)	–	68.0 [59.0, 73.2]	64.0 [58.0, 66.0]	63.0 [55.0, 66.5]	.004
Age at CSF (years)	66.5 [63.0, 72.0]	72.0 [64.5, 78.5]	71.0 [65.0, 78.0]	66.0 [59.0, 71.0]	.001
Age at death (years)		79.0 [70.0, 85.0]	77.0 [71.0, 81.0]	67.5 [61.0, 73.8]	<.001
CSF to death (years)		6.0 [4.0, 8.0]	5.0 [2.0, 8.0]	3.0 [2.0, 5.0]	<.001
CSF A$\beta_{1\text{-}42}$ (pg/mL)	299.8 [248.5, 374.2]	132.0 [101.3, 156.3]	196.5 [156.1, 215.0]	258.5 [212.0, 304.5]	<.001
CSF p-tau$_{181}$ (pg/mL)	17.5 [13.4, 22.5]	28.7 [17.5, 44.1]	18.3 [11.8, 24.5]	12.0 [9.8, 16.0]	<.001
CSF t-tau (pg/mL)	45.0 [38.0, 61.0]	100.0 [65.4, 152.6]	37.0 [32.4, 59.5]	56.0 [43.8, 78.2]	<.001
MMSE (max = 30)	29.0 [29.0, 30.0]	23.0 [15.0, 26.0]	27.0 [25.0, 28.0]	23.0 [18.0, 27.2]	<.001
Education (years)	16.0 [13.5, 18.0]	16.0 [13.0, 18.0]	16.0 [13.0, 18.0]	16.0 [14.0, 18.0]	.860
Sex = male	14 (29.2%)	35 (55.6%)	19 (90.5%)	31 (58.5%)	<.001
ADNC					
Not		0 (0.0%)	3 (14.3%)	18 (45.0%)	
Low		0 (0.0%)	9 (42.9%)	21 (52.5%)	
Intermediate		4 (6.5%)	8 (38.1%)	0 (0.0%)	
High		58 (93.5%)	1 (4.8%)	1 (2.5%)	

Notes: Median (interquartile range) displayed for each demographic and pathologic variable. Kruskal–Wallis comparisons for continuous variables and chi-square goodness of fit test for the categorical variable performed across all groups: *P*-values are reported. In autopsy patients ADNC indicates severity of AD. Patients with intermediate or high levels of ADNC are considered positive for AD. MMSE is a measure of global cognition with higher scores indicating better cognitive function.

Abbreviations: Aβ, amyloid beta; AD, Alzheimer's disease; ADNC, Alzheimer's disease neuropathologic change; CSF, cerebrospinal fluid; FTLD, frontotemporal lobar degeneration; HC, health control; LBD, Lewy body disorder; MMSE, Mini-Mental State Examination; p-tau, phosphorylated tau; t-tau, total tau.

Last, increased 14-3-3 zeta/delta levels were found in AD ($P \leq .0001$) and FTLD ($P = .0027$) compared to HC, thus exhibiting a pattern of increased levels in neurodegenerative disease. Moreover, 14-3-3 zeta/delta was particularly elevated in AD, which also showed significantly increased levels compared to FTLD ($P = .0093$) and LBD ($P = .013$). 14-3-3 zeta/delta moderately correlated (rho = 0.61—0.65, $P \leq .0001$) with 14-3-3 epsilon and theta, although neither showed significant differences between groups.

When splitting the LBD group based on the presence of concomitant AD, no difference was found for any of the synaptic proteins between the patients with or without concomitant AD (Figure S2 in supporting information). Of note, none of the peptide levels differed between LBD and FTLD; thus, they are combined into a "non-AD" group in subsequent analyses.

Post hoc analyses examined the associations of the synaptic biomarkers with AD pathology (Figure 2); 14-3-3 zeta/delta and beta-synuclein were chosen as representatives of the protein groupings, which showed elevated levels in AD (previously explored for neurogranin[25]). 14-3-3 zeta/delta and beta-synuclein were significantly increased for high ADNC compared to not and low ($P < .05$), Braak stage 3 (widespread tau) compared to Braak stage 1 and 2 ($P < .05$), and CERAD score 3 (high plaque burden) compared to Braak stage 0 and 2 ($P < .05$).

Next, ROC analyses tested how proteins discriminated AD and non-AD (Table S4 in supporting information). 14-3-3 zeta/delta had the highest AUC (Figure 3A, AUC = 0.83, 95% confidence interval [CI] = 0.75–0.90) for AD versus HC, closely followed by neuronal pentraxin-2 (AUC = 0.78, 95% CI = 0.69–0.87). To discriminate non-AD from HC, neuronal pentraxin-2 had the highest AUC (AUC = 0.82, 95% CI = 0.74–0.89), followed by neuronal pentraxin receptor (AUC = 0.79; 95% CI = 0.71–0.87). Performance by analytes was generally less robust when discriminating AD from non-AD, with neurogranin having the highest AUC (AUC = 0.73, 95% CI = 0.65–0.82).

Because synaptic dysfunction has been linked to cognitive decline, we tested associations of synaptic proteins with MMSE. In AD, neuronal pentraxin-2 had the strongest correlation with MMSE (rho = 0.51, $P \leq .0001$, Figure 3B and Table S5 in supporting information). Neuronal pentraxin-2 also had the strongest correlation with MMSE in the non-AD group (rho = 0.28, $P = .016$). Furthermore, we tested associations between synaptic proteins and core AD biomarkers A$\beta_{1\text{-}42}$, t-tau, and p-tau$_{181}$ (Table S5). Within AD, all synaptic proteins correlated weakly to strongly with t-tau (rho = 0.34–0.81, $P \leq .01$) and weakly to moderately with p-tau$_{181}$ (rho = 0.25–0.51, $P \leq .05$, except 14-3-3 theta), but not with A$\beta_{1\text{-}42}$.

In the non-AD group, all synaptic proteins correlated weakly with p-tau$_{181}$ (rho = 0.24–0.48, $P \leq .05$, except 14-3-3 epsilon), as well as weakly to moderately with t-tau (rho = 0.30–0.71, $P \leq .05$, except 14-3-3 theta). Weak correlations with A$\beta_{1\text{-}42}$ (rho = 0.24–0.28, $P \leq .05$) were found for beta-synuclein, complexin-2, syntaxin-1B, AP2B1, and neuronal pentraxin receptor in the non-AD group.

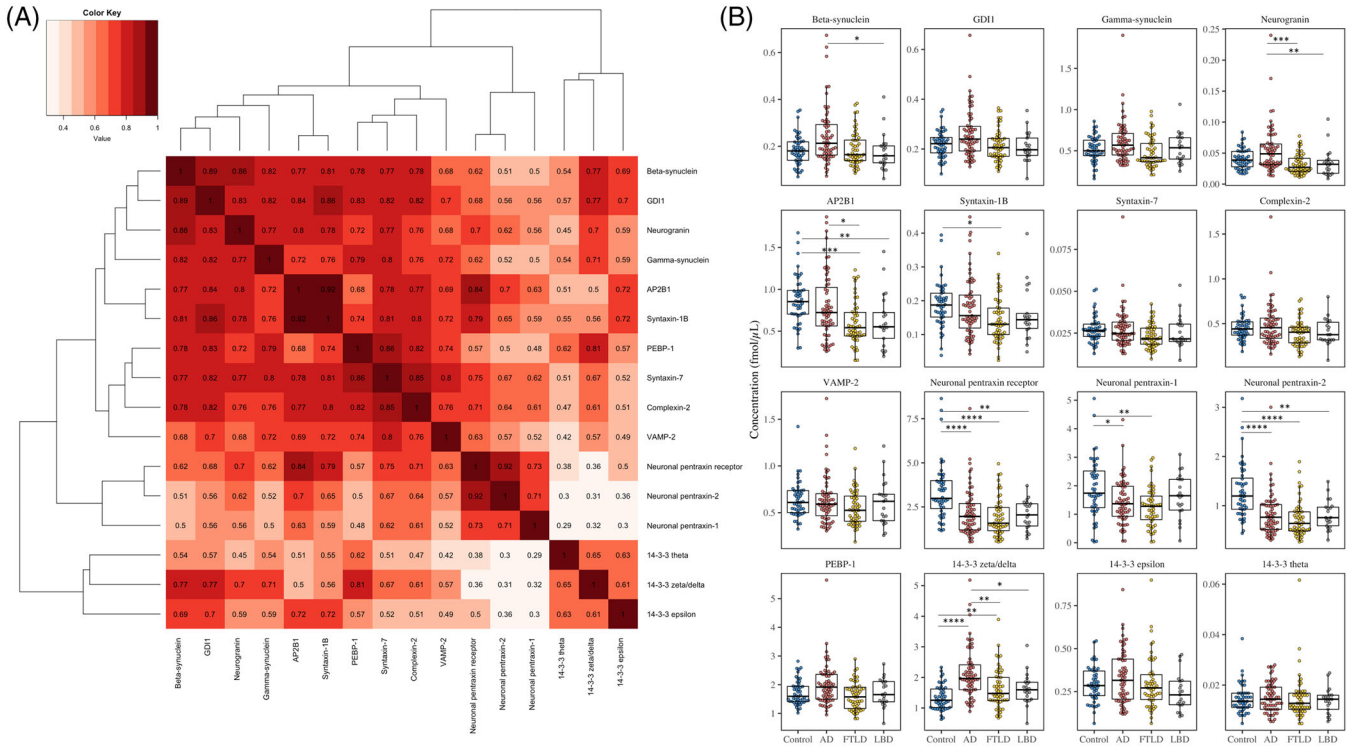

FIGURE 1 A, Hierarchical cluster analysis using Spearman rank correlation coefficient as distance. From the cluster analysis it emerged that some of the measured proteins correlated strongly with each other. B, Multiple reaction monitoring analysis of the synaptic panel proteins (one representative peptide for each protein) in the clinical sample consisting of healthy controls (HC, $n = 48$), and pathology-confirmed cases of Alzheimer's disease (AD, $n = 63$), Lewy body spectrum of disorders (LBD, $n = 21$), and frontotemporal lobar degeneration (FTLD, $n = 53$). Statistical comparison was performed with rank-based analyses of covariance, including age and sex as covariates, with *P*-value adjustment for multiple group comparisons. *P*-values: $^{*}P \leq .05$, $^{**}P \leq .01$, $^{***}P \leq .001$, and $^{****}P \leq .0001$

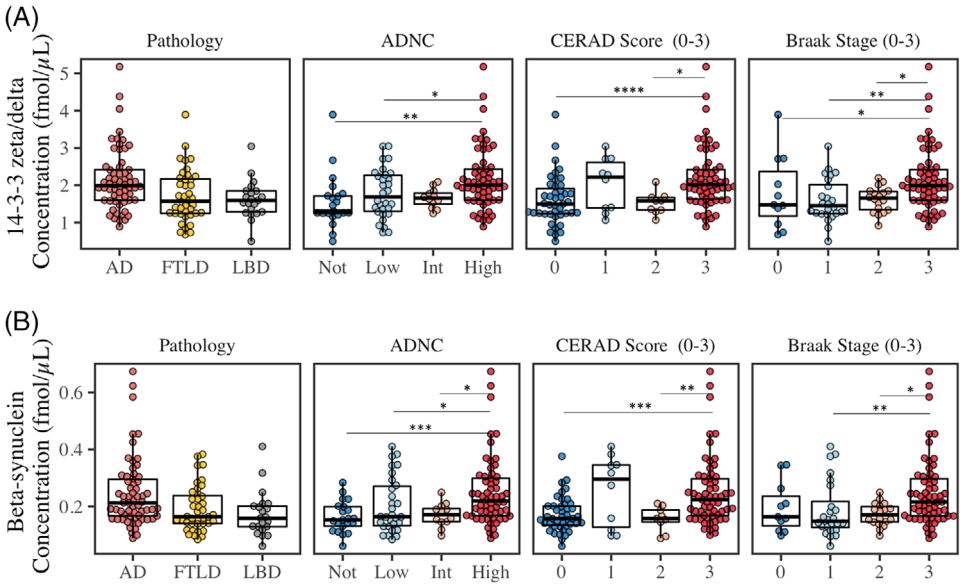

FIGURE 2 Cerebrospinal fluid (CSF) concentrations of 14-3-3 zeta/delta (A), and beta-synuclein (B) in the pathology-confirmed cases of Alzheimer's disease (AD, $n = 63$), Lewy body spectrum of disorders (LBD, $n = 21$), and frontotemporal lobar degeneration (FTLD, $n = 53$). From left to right; the groups are based on primary pathology group, Alzheimer's disease neuropathologic change (ADNC; not, low, intermediate [Int], high), Consortium to Establish a Registry for Alzheimer's Disease (CERAD) score, and Braak stage. Statistical comparison was performed with rank-based analyses of covariance adjusted for interval from CSF to death and sex. *P*-values: $^{*}P \leq .05$, $^{**}P \leq .01$, $^{***}P \leq .001$, and $^{****}P \leq .0001$

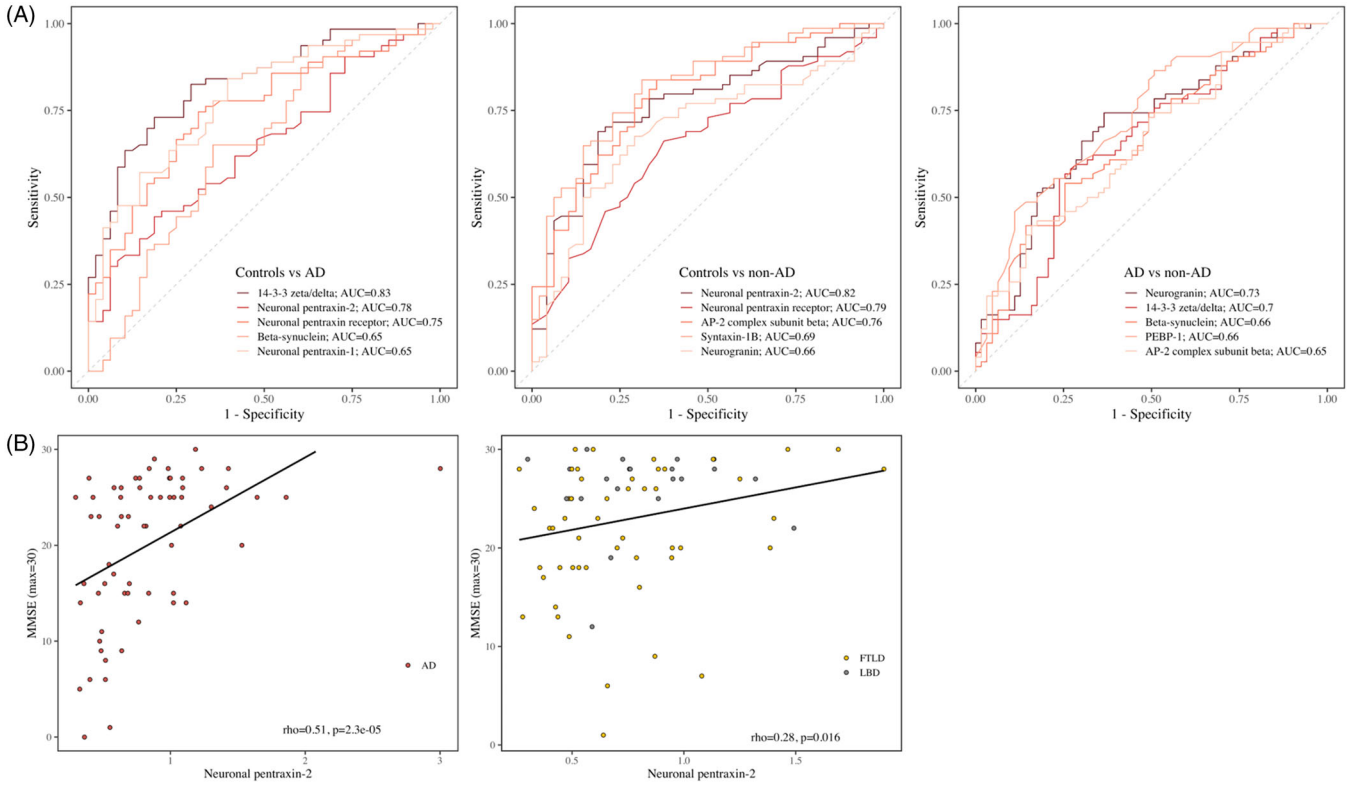

FIGURE 3 A, Receiver operating characteristic curves calculated for Alzheimer's disease (AD) versus healthy controls, healthy controls versus non-AD, and AD versus non-AD for the five synaptic proteins with the highest area under the curve (AUC) vales. B, Association between neuronal pentraxin-2 and Mini-Mental State Examination for AD and non-AD with Spearman rank correlation coefficient and *P*-value

4 | DISCUSSION

There is a need in clinical practice and trials to implement biomarkers that can reflect synaptic pathology, not only as early indicators of AD, but also to predict cognitive decline, monitor synaptic health, and facilitate differential diagnosis. In our previous study of the synaptic panel, we found that levels of beta-synuclein, gamma-synuclein, neurogranin, PEBP-1, and 14-3-3 proteins were increased while levels of the neuronal pentraxins were decreased in AD compared to HC[6] and identified these proteins as potential synaptic pathology biomarkers for AD. In the current study, we found four distinct biomarker patterns while validating this panel of synaptic proteins in an independent autopsy-confirmed sample of AD and expanding on our previous work to include two other neurodegenerative diseases: LBD and FTLD.

The first pattern was specifically increased CSF levels in AD compared to other neurodegenerative diseases, represented by beta-synuclein and neurogranin. Beta-synuclein, together with gamma-synuclein, is part of the presynaptic synuclein family also containing alpha-synuclein. Together, the synucleins have all been widely associated with neurodegenerative diseases,[26] especially alpha-synuclein, which is well-researched due to it being a major component of Lewy bodies as well as amyloid plaques.[27,28] The synucleins have been found to have increased CSF concentrations in AD compared to controls,[29] and more recently, this pattern has also been observed for serum beta-synuclein.[30] In corroboration of our findings of AD-specific changes of beta-synuclein, previous work also finds no difference in CSF in Parkinson's disease and in amyotrophic lateral sclerosis compared to non-neurodegenerative controls.[31] In general, the measured panel of proteins seems to have a better ability to discriminate HC from non-AD or AD patients than to discriminate non-AD from AD. One of the exceptions seems to be neurogranin, which is a postsynaptic protein involved in the regulation of calmodulin and, consequently calcium-mediated signaling pathways.[15] Both enzyme-linked immunosorbent assays and mass spectrometric methods have repeatedly found increased levels of neurogranin in AD compared to controls,[15,32] and also several meta-analyses have confirmed this.[32,33] We detected no significant difference comparing AD to HC; however, we did observe significantly increased neurogranin CSF levels in AD compared to other neurodegenerative diseases (non-AD). In fact, supporting our results, neurogranin has previously been reported to be specifically increased in AD not only compared to HC but also to other neurodegenerative diseases.[25] To summarize, we have found novel increased levels of beta-synuclein and neurogranin in AD compared to non-AD. However, we were not able to validate our earlier findings of increased levels in AD compared to HC for neurogranin and the synucleins,[6] possibly due to rigorous correction for multiple comparisons across multiple disease groups. We additionally observed novel findings of unchanged levels in non-AD neurodegenerative diseases for these proteins compared to HC. Neurogranin and beta-synuclein should therefore be studied further to discern their potential as possible diagnostic biomarkers of

AD and how they are specifically associated with AD, to cast light on possible AD-specific mechanisms.

The second pattern is represented by AP2B1 and syntaxin-1B, which have significantly decreased levels in non-AD compared to HC, but no change in AD. Syntaxin-1B is involved in vesicle exocytosis at the synapse and, consequently, neurotransmitter release as a SNARE protein.[34] AP2B1 belongs to a family of adaptor proteins involved in mediating endocytosis by linking clathrin to the plasma membrane.[35] This novel pattern is particularly interesting because these proteins do not show a significant difference between AD and HC, as in past findings,[6] but show significantly decreased levels in FTLD and LBD (only AP2B1) compared to HC. For AP2B1, this is also corroborated by Sjödin et al., finding no difference in AD but decreased levels in PD.[36] Additionally, decreased levels in LBD compared to controls have also been previously reported.[37] Endocytic impairment has been implicated to be a feature of many neurodegenerative diseases, not the least in AD.[38] However, we show that endocytic impairment, reflected by AP2B1 CSF levels, seems to be a more prominent feature of FTLD and LBD pathology than of AD. The similar findings for the CSF levels of syntaxin-1B might hence also be indicative of specific impairment of synaptic transport processes in FTLD pathology. The reason for the specific change in non-AD but not in AD compared to HC is certainly interesting but remains elusive. Further studies of AP2B1 and syntaxin-1B should investigate this in depth and also include a wider range of neurodegenerative diseases to discern the specificity of the changes.

The third pattern is decreased CSF levels in all explored diseases (AD, FTLD, and LBD), represented by the neuronal pentraxins. The neuronal pentraxins are proposed to be involved in the modulation of synaptic plasticity by AMPA-type glutamate receptors recruitment during exocytosis.[39,40] The interest in the pentraxin family in the context of neurodegenerative diseases has increased recently as several studies have reported reduced CSF levels of the neuronal pentraxins in AD,[8–12,41] DLB,[42] and FTLD[43] compared to controls. Furthermore, the levels have been found to constantly decrease from cognitively normal controls to mild cognitive impairment and last to AD.[8] Interestingly, in the present study, neuronal pentraxin-2 is the best correlate with MMSE in AD, corroborated by several studies,[6,8] and provides one of the best separations (AUC = 0.78) between AD and HC of all panel proteins. The pentraxin levels also correlate with MMSE in non-AD patients (LBD and FTLD) and are all decreased in FTLD and LBD pathologies compared to HC. In fact, neuronal pentraxin-2 of all panel proteins shows the best separation (AUC = 0.82) between non-AD and HC. Thus, the neuronal pentraxins may be potential monitoring biomarkers of general cognitive decline across neurodegenerative diseases, which should be further explored.

The last distinct pattern with higher CSF protein levels across all the investigated patient groups compared to HC was found for 14-3-3 protein zeta/delta, which belongs to a synapse enriched seven protein family.[13] The protein family has been associated with wide modulation abilities and a high number of binding partners and is consequently implicated in a number of neuronal functions. At the synapse, they regulate transmission and plasticity; however, their functions are still largely unknown in detail. The protein family are established biomarkers of Creutzfeldt-Jakob disease but are also associated with other neurodegenerative diseases.[13] In relation to AD, 14-3-3 proteins have been discovered to both colocalize in neurofibrillary tangles and interact with the key AD pathology protein tau.[44,45] They have similarly been found to be present in Lewy bodies.[46] To our knowledge, this is the first targeted CSF method to be used in the study of zeta/delta in neurological disease, even though several exploratory proteomics studies have suggested not only zeta/delta but also the other proteins in the family as potential biomarkers.[47,48] In the current study, 14-3-3 protein zeta/delta concentration was higher in the CSF of AD patients compared to HC, with the best separation of all the biomarkers (AUC = 0.83), while the rest of the 14-3-3 protein family members did not show any notable differences between any of the groups. 14-3-3 zeta/delta having the highest diagnostic potential of all synaptic proteins is corroborated by earlier exploratory data,[7] and it has been implicated to have the strongest connection with tau and its phosphorylation of all the 14-3-3s.[49] Increased 14-3-3 zeta/delta levels were also observed in FTLD patients compared to HC. However, 14-3-3 zeta/delta seems to have a stronger association with AD pathology, with higher levels in AD than in FTLD and LBD. Thus, 14-3-3 zeta/delta separated AD from non-AD (AUC = 0.70) almost as well as neurogranin (AUC = 0.73). We hence validate our previous findings that 14-3-3 zeta/delta is particularly increased in AD[6] and observe novel findings that the protein is also increased in non-AD, albeit not to the same degree. This indicates that 14-3-3 zeta/delta may be a general indicator of neurodegeneration and or cognitive decline across neuropathology, particularly affected by AD pathology.

Last, VAMP-2, PEBP-1, GDI1, syntaxin-7, and complexin-2 showed no significant differential patterns, which replicates our earlier findings,[6] with the exception of PEBP-1. These proteins seem accordingly to not be potential biomarkers for AD, FTLD, or LBD but should be confirmed in additional studies and possibly explored in other neurodegenerative diseases.

Two major strengths of this study were the use of gold-standard autopsy-confirmed pathology and the use of multiplexed mass spectrometry. The method allows for quantification in a small sample volume with high specificity of a range of biomarkers with diverse functions and localizations and has the ability to possibly discover, distinguish, and differentiate among general and specific pathological patterns. When the aim is to differentiate a range of neurodegenerative diseases and track disease progression, this is especially important due to the high complexity and pathological heterogeneity. Thus, unbiased mass spectrometry studies are an important step to biomarker discovery. However, these kinds of multiplex assays also carry the limitation of an increased analytical challenge due to the nonspecific sample preparation, which leads to a relatively broad concentration range and substantial general protein background. Several steps are needed to translate our findings here from bench to bedside.[50,51] Foremost, a challenge of future work is the "cross-technology translation gap"[50] and the need to bridge mass spectrometry findings to immunoassay to be implemented in a clinical context. Further analytical validation in clinical cohorts will be necessary, and histopathological

studies may be needed to understand the mechanism underlying the differences in synaptic protein patterns we observed between AD and FTLD/LBD. Another caveat to consider when interpreting our findings is that mixed pathology in all neurodegenerative diseases is common. Indeed, one FTLD patient and nine LBD patients had co-occurring AD pathology. While we did not see significant differences in analyte levels across LBD with and without AD, small sample sizes may preclude sufficient power to detect differences. Future work should further explore how co-pathologies might interact with synaptic CSF levels that we observed here. Additionally, while a strength of this study is the assessment of biomarkers across multiple neurodegenerative diseases, in this clinically and pathologically heterogeneous sample, we lacked a global measure of disease stage to be applied in the primary analyses. However, analyses within autopsy-confirmed patients included adjustment for CSF-to-death interval to help account for differences in disease severity at CSF collection and the lag to pathological assessment. Finally, while we studied CSF analytes in rare patients with known pathology, our groups were small, and we performed rigorous correction for multiple comparisons. Comparative studies are incredibly important for differential diagnosis, and we were able to identify a pattern of selective change in some analytes; however, additional work is needed to confirm our observations in larger sample sets as well as in longitudinal studies to explore the biomarker changes over time.

5 | CONCLUSION

The present study validated our previously published results that several of the synaptic proteins of our in-house mass spectrometric panel have the potential to be synaptic degeneration biomarkers in AD. We also find evidence that several of the proteins were also altered in the other neurodegenerative diseases: FTLD and LBD. Together, our results indicate differential patterns of synaptic protein alterations across neurodegenerative diseases. Thus, the synaptic panel proteins not only show promise as possible complements to other CSF and imaging markers to guide diagnostics, as prognostics, stage biomarkers, or to track cognitive decline, but may also give invaluable mechanistic input into the complex overlapping neuropathologies in neurodegenerative diseases and their differential impacts on synaptic function.

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CONFLICTS OF INTEREST

H.Z. has served on scientific advisory boards and/or as a consultant for Abbvie, Alector, Annexon, Artery Therapeutics, AZTherapies, CogRx, Denali, Eisai, Nervgen, Pinteon Therapeutics, Red Abbey Labs, Passage Bio, Roche, Samumed, Siemens Healthineers, Triplet Therapeutics, and Wave; has given lectures in symposia sponsored by Cellectricon, Fujirebio, Alzecure, Biogen, and Roche; and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program. K.B. has served as a consultant, on advisory boards, or on data monitoring committees for Abcam, Axon, Biogen, JOMDD/Shimadzu, Julius Clinical, Lilly, MagQu, Novartis, Roche Diagnostics, and Siemens Healthineers, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program. L.M.S. has served on scientific advisory boards and/or as a consultant for Biogen, Roche Diagnostics, Fujirebio, Siemens, and Diadem and has given lectures for Biogen, Roche, and Fujirebio. The rest of the authors have no disclosures to report. Author disclosures are available in the supporting information.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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