

RESEARCH ARTICLE

Cerebrospinal Fluid Biomarkers of Synaptic Dysfunction are Altered in Parkinson's Disease and Related Disorders

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ABSTRACT: Background: Synaptic dysfunction and degeneration are central contributors to the pathogenesis and progression of parkinsonian disorders. Therefore, identification and validation of biomarkers reflecting pathological synaptic alterations are greatly needed and could be used in prognostic assessment and to monitor treatment effects.

Objective: To explore candidate biomarkers of synaptic dysfunction in Parkinson's disease (PD) and related disorders.

Methods: Mass spectrometry was used to quantify 15 synaptic proteins in two clinical cerebrospinal fluid (CSF) cohorts, including PD ($n_1 = 51$, $n_2 = 101$), corticobasal

degeneration (CBD) ($n_1 = 11$, $n_2 = 3$), progressive supranuclear palsy (PSP) ($n_1 = 22$, $n_2 = 21$), multiple system atrophy (MSA) ($n_1 = 31$, $n_2 = 26$), and healthy control (HC) ($n_1 = 48$, $n_2 = 30$) participants, as well as Alzheimer's disease (AD) ($n_2 = 23$) patients in the second cohort.

Results: Across both cohorts, lower levels of the neuronal pentraxins (NPTX; 1, 2, and receptor) were found in PD, MSA, and PSP, compared with HC. In MSA and PSP, lower neurogranin, AP2B1, and complexin-2 levels compared with HC were observed. In AD, levels of 14-3-3 zeta/delta, beta- and gamma-synuclein were higher compared with the parkinsonian disorders. Lower pentraxin

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levels in PD correlated with Mini-Mental State Exam scores and specific cognitive deficits (NPTX2; $\rho = 0.25$ – 0.32 , $P < 0.05$) and reduced dopaminergic pre-synaptic integrity as measured by DaTSCAN (NPTX2; $\rho = 0.29$, $P = 0.023$). Additionally, lower levels were associated with the progression of postural imbalance and gait difficulty symptoms (All NPTX; β -estimate = -0.025 to -0.038 , $P < 0.05$) and cognitive decline (NPTX2; β -estimate = 0.32 , $P = 0.021$).

Conclusions: These novel findings show different alterations of synaptic proteins in parkinsonian disorders

compared with AD and HC. The neuronal pentraxins may serve as prognostic CSF biomarkers for both cognitive and motor symptom progression in PD. © 2022 The Authors. *Movement Disorders* published by Wiley Periodicals LLC on behalf of International Parkinson and Movement Disorder Society.

Key Words: Parkinson's disease; multiple system atrophy; progressive supranuclear palsy; biomarkers; synaptic dysfunction

Introduction

Parkinson's disease (PD) affects millions of people as the second most common neurodegenerative disease.¹ PD belongs to a group of parkinsonian disorders clinically characterized by bradykinesia, rigidity, tremor, and non-motor symptoms, including cognitive decline and autonomic dysfunction. Atypical forms encompass corticobasal degeneration (CBD), progressive supranuclear palsy (PSP), and multiple system atrophy (MSA). As synucleinopathies, PD and MSA have abnormal intracellular aggregation and accumulation of alpha-synuclein as a central pathogenic feature in Lewy bodies and glial cytoplasmic inclusions, respectively. PSP and CBD often display overlapping clinical presentations with PD, but differ regarding their pathologic characteristics, being characterized by tau inclusions. However, parkinsonian disorders are commonly characterized by a loss of dopaminergic neurons in the substantia nigra pars compacta.² Furthermore, it is believed that synaptic dysfunction, another major hallmark of neurodegenerative diseases, precedes neuronal loss through mechanisms that are largely unexplored.³ In monogenetic PD, more than 50% of the numerous identified causative genes appear to function at the synapse, and similar mechanisms are believed to be important in idiopathic PD.³ For example, in the healthy brain, alpha-synuclein is a pre-synaptic protein that has been suggested to have critical functions in the clustering of synaptic vesicles and synaptic vesicle exocytosis.⁴ Therefore, synaptic dysfunction in parkinsonian disorders is of great interest to elucidate pathological mechanisms in both genetic and idiopathic disease. The study of synaptic proteins is also of importance to find synaptic dysfunction biomarkers to improve diagnostic and prognostic assessment, especially in the early disease phase, and for monitoring treatment responses in future drug trials.

Although synaptic proteins in the cerebrospinal fluid (CSF) have been extensively studied as potential biomarkers in Alzheimer's disease (AD),^{5–7} they remain under-explored in parkinsonian disorders. A few studies mainly focused on PD found no differences compared with controls or inconsistent results for synaptic

proteins, including neurogranin,^{8–10} GAP-43,^{11,12} and SNAP-25.^{12,13} However, a proteome study in postmortem human brain found that synaptic proteins were able to differentiate PD with dementia from controls and AD and that they associated with cognitive decline.¹⁴

We have previously identified 15 synaptic proteins in the CSF as potential synaptic biomarkers and studied them in AD.¹⁵ The panel encompasses AP-2 subunit complex beta (AP2B1), complexin-2, rab GDI alpha (GDI-1), phosphatidylethanolamine-binding protein-1 (PEBP-1), and several members of the protein families of the 14-3-3 s, syntaxins, synucleins, and neuronal pentraxins (NPTX1, NPTX2, and NPTXR). This study aimed to investigate these synaptic proteins in two well-characterized clinical parkinsonian disorder cohorts, including PD, CBD, PSP, and MSA. The proteins were investigated concerning clinical and imaging parameters of disease severity to characterize their diagnostic and prognostic properties.

Method

Study Populations

The discovery cohort ($n = 154$) included patients from Sahlgrenska University Hospital (Gothenburg, Sweden) diagnosed between 1999 and 2016 according to the Movement Disorder Society (MDS) clinical diagnostic criteria¹⁶ for PD ($n = 51$), Gilman's criteria¹⁷ for MSA ($n = 31$), MDS criteria¹⁸ for PSP ($n = 22$), and Armstrong's criteria¹⁹ for CBD ($n = 11$). Healthy controls (HC, $n = 48$) were recruited among orthopedic patients undergoing lower limb surgery between 2018 and 2020. Additional cohort descriptions can be found in the Supporting Data.

The second cohort ($n = 143$) included patients from Umeå University Hospital (Umeå, Sweden), part of the New Parkinsonism in Umeå (NYPUM, $n = 94$, 2004–2009), a population-based incidence study of unselected cases of new-onset idiopathic parkinsonism. Additional cases of idiopathic parkinsonism were included from the consecutive follow-up study in the same institution (PARKNY, $n = 49$, 2009–present), using the same inclusion criteria. Patients were diagnosed according to

the United Kingdom (UK) PD Society Brain Bank criteria²⁰ for PD patients (n = 95), Gilman's criteria¹⁷ for MSA (n = 26), National Institute of Neurological Disorders and Stroke and Society for PSP (NINDS-SPSP) criteria²¹ for PSP (n = 22), and Armstrong's criteria¹⁹ for CBD (n = 3). Detailed inclusion criteria and cohort characterization are described elsewhere.²² In short, pre-synaptic dopamine transporter (DAT) imaging by ¹²³I-FP-CIT single-photon emission computed tomography (DaTSCAN; GE Healthcare BV, Eindhoven, Netherlands) was performed in most patients (n = 125), all showing pathological uptake, and patients with dementia at baseline (clinical symptoms and Mini-Mental State Exam [MMSE] < 24) or secondary parkinsonism were excluded. Patients were included in the early motor phase while naïve to dopaminergic medications. Motor function was assessed at baseline and then yearly with Unified Parkinson's Disease Rating Scale (UPDRS), with subscores divided into tremor (sum of items 20 and 21) and postural imbalance and gait difficulty (PIGD) scores (sum of items 13–15, 29, and 30).²³ Motor assessments were made in the “off” (drug-naïve) state at baseline and in the “on” state at follow-up visits. HCs (n = 30) were recruited by advertisements and among relatives. Inclusion criteria for the HC group included no neurological diseases, a normal neurological exam, and normal DaTSCAN brain imaging. Additionally, an AD (n = 23) group fulfilling the Diagnostic and Statistical Manual of Mental Disorders (DSM)-5 criteria for AD dementia²⁴ from BioFINDER-2 was added to evaluate whether the CSF synaptic profiles in the parkinsonian disorders differed from those in AD. The demographics and clinical characteristics of the cohorts are shown in Table 1.

All participants gave written informed consent. The study was approved by the Regional Ethics Review Board in Gothenburg, Sweden (DNR 460-13, T217-15/ad/460-13, T1083-18/ad/460-13, 2019-00756/1226-18), the Regional Ethical Committee in Lund (DNR 2016-1053), Sweden and the Regional Medical Ethics Board in Umeå, Sweden (DNR 03-387, 2014-163-31 M).

CSF Analysis, SPECT Imaging, and Neuropsychological Testing

All participants underwent lumbar puncture CSF collection according to standard procedures, and samples were stored at –80°C until analysis. In the second cohort, participants underwent CSF analysis of alpha-synuclein, total tau, phosphorylated tau at position 181, and amyloid beta peptide 1–42/1–40 ratio as well as DaTSCAN imaging at baseline, according to previously described methods.^{25,26} The HCs were used to derive normal reference values,^{25,27} and reduction of DAT uptake in the putamen and caudate was quantified in standard deviations (SDs) of the HC group values. Complete

neuropsychological testing was performed at baseline, and follow-ups in the NYPUM study cohort, with further details, described elsewhere.²⁶ Briefly, evaluations were made based on MDS level 2 criteria, and performance in specific domains were investigated by merging results into five domains (working memory and attention, visuospatial function, language, episodic memory, and executive function) following the partition suggested by the MDS.¹⁶

Liquid Chromatography with Tandem Mass Spectrometry Analysis of Synaptic Panel

Sample preparation included heavy standard addition to 100 µL CSF, followed by sequential reduction, alkylation, and digestion. For the detailed procedure, refer to previously published work.¹⁵ The liquid chromatography with tandem mass spectrometry (LC–MS/MS) analysis (for details, refer to Table 1) was carried out on a micro-high-performance liquid chromatography-mass-spectrometry system (6495 Triple Quadrupole LC/MS System, Agilent Technologies, CA, USA) equipped with a Hypersil Gold reversed-phase column (dim. = 100 × 2.1 mm, p.s = 1.9 µm, Thermo Fisher Scientific, MA, USA). Injections of quality controls were used to monitor the performance over time.

Data Processing and Statistical Analysis

Skyline 20.1 (MacCoss Lab Software) was used for peak inspection, area calculation, and adjustment if required. Standardization of relative peptide levels was performed by z-scoring biomarker values relative to the HC groups. ANOVA and χ^2 goodness of fit test were used to compare demographic continuous and categorical variables between groups, respectively. The group-wise comparisons were assessed using linear models, controlling for the effect of age and sex, with adjustment for multiple comparisons using the false discovery rate (FDR) approach.²⁸ Heatmap with hierarchical clustering (Spearman's correlation coefficient as distance) for the panel proteins was displayed by using the *heatmap* R package. The receiver operating characteristics (ROC) curves contrasting groups provided the area under the curve (AUC), and the DeLong test was used to compare the AUC values head-to-head.²⁹ Correlation coefficients were calculated using partial Spearman rank tests, adjusted for age. Linear mixed models adjusted for baseline, age, and sex, including random slopes and intercepts, were used. We included interaction terms between predictors (biomarkers and covariates) with time to enable the assessment of the association between biomarkers and longitudinal symptom trajectories. Statistical analysis was performed with R software (version 4.0.3), and a two-sided alpha = 0.05 determined statistical significance.

TABLE 1 Cohort demographics and baseline characteristics

Cohort	Characteristics	HC	AD	PD	MSA	PSP	CBD	P-value
Cohort 1 - discovery	Total, n	48		51	31	22	11	
	Sex, n (Female, %)	29 (60.4%)		13 (25.5%)	17 (54.8%)	13 (59.1%)	7 (63.6%)	0.003**
	Age, y	70.4 (11.9)		62.6 (11.2)	65.4 (8.5)	69.9 (7.5)	69.5 (8.8)	0.003**
	Aβ _{42/40} ratio	0.97 (0.09)		0.92 (0.11)	0.97 (0.12)	0.94 (0.11)	0.97 (0.12)	0.173
	P-tau ₁₈₁ , ng/L	30.9 (8.5)		28.7 (10.6)	25.7 (9.4)	30.1 (8.0)	30.3 (10.7)	0.189
	T-tau, ng/L	253.7 (67.4)		253.8 (123.0)	271.6 (104.0)	284.6 (101.7)	274.7 (103.9)	0.704
	Total, n	30	23	95	26	22	3	
Cohort 2 - Validation	Sex, n (female, %)	14 (46.7%)	14 (60.9%)	40 (41.2%)	13 (50.0%)	11 (50.0%)	1 (33.3%)	0.706
	Age, y	69.0 (5.8)	71.7 (8.0)	68.4 (8.8)	66.6 (10.1)	72.6 (5.8)	60.0 (3.2)	0.025*
	MMSE score	29.1 (0.8)	21.8 (4.7)	28.6 (1.4)	28.4 (2.1)	26.8 (3.8)	25.0 (7.1)	<0.001***
	P-tau ₁₈₁ /Aβ ₄₂ ratio	0.070 (0.055)		0.077 (0.053)	0.088 (0.061)	0.077 (0.040)		0.727
	T-tau, ng/L	304.2 (139.5)		301.1 (182.5)	311.1 (126.5)	325.4 (182.7)		0.948
	alpha-synuclein (total), ng/L	0.82 (0.30)		0.82 (0.38)	0.87 (0.44)	0.61 (0.28)		0.275
	BMI	25.2 (4.2)		25.7 (3.6)	25.0 (3.0)	29.3 (3.5)		0.041*
	Time to CSF sampling, y	0.10 (0.55)		0.33 (0.32)	0.65 (1.01)	0.25 (0.70)		0.026*
	Disease duration, y			1.6 (1.3)	1.8 (0.9)	2.0 (2.1)	3.0 (1.4)	0.363
	Hoehn and Yahr stage			2.1 (0.6)	2.7 (1.1)	2.4 (0.6)	1.5 (0.7)	<0.001***
	Total UPDRS score			33.8 (12.7)	37.7 (17.4)	36.6 (11.4)		0.337
	MADRS	0.3 (0.5)		4.9 (4.0)	8.9 (7.7)	7.9 (10.1)		<0.001***
	Systolic BP drop, mm Hg			6.7 (16.5)	27.4 (18.1)	3.7 (11.5)		0.003**
Median LEDD, mg			0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)		NA	
TUG, s			8.5 (2.8)	8.8 (1.7)	17.0 (10.5)		<0.001***	

Note: Data presented as mean (standard deviation) unless otherwise specified as median (IQR) or counts (%). Disease duration is the time from first motor symptom onset, as reported by the patient, to baseline. For the validation cohort, Aβ₄₀ was not available and P-tau181/Aβ₄₂ ratio is instead shown as according to Hansson et al.²⁵ Analysis of variance (ANOVA) and χ^2 goodness of fit test were used to compare demographic continuous and categorical variables between groups.

Abbreviations: AD, Alzheimer's disease; Aβ₄₂, amyloid beta peptide 1-42; Aβ₄₀, amyloid beta peptide 1-40; BMI, body mass index; BP, blood pressure; CBD, corticobasal degeneration; CSF, cerebrospinal fluid; HC, healthy controls; LEDD, levodopa equivalent daily dose; MADRS, Montgomery-Åsberg Depression Rating Scale; MMSE, Mini-Mental State Exam; MSA, multiple system atrophy; PD, Parkinson's disease; PSP, progressive supranuclear palsy; P-tau181, phosphorylated tau at position 181; T-tau, total tau; TUG, timed up and go; UPDRS, Unified Parkinson's Disease Rating Scale.

*P ≤ 0.05
 **P ≤ 0.01
 ***P ≤ 0.001

Availability of Data and Materials

Derived data supporting the findings of this study are available from the corresponding author on request, providing data transfer agrees with the participating center's national legislation and institutional review center.

Results

The analytical performance of the different markers presented high within- and between-run precision, described in detail in Supplementary Table S2. Three proteins (beta-synuclein, 14-3-3 epsilon, and 14-3-3 theta) were excluded from the statistical analysis in the discovery CSF cohort because of poor analytical performance.

Synaptic Biomarker Levels Across Groups and Diagnostic Accuracy

In the discovery cohort (Fig. 1, Supplementary Table S3), neurogranin and neuronal pentraxins showed lower concentrations in MSA and PSP in comparison with HC. NPTX2 was the only biomarker found to have lower levels in PD and CBD in comparison with HC.

We aimed to validate these findings in the second cohort, and an AD group was included (Fig. 2A, Supplementary Table S4). Again, we found lower levels of NPTX2 and NPTXR in MSA, PD, and PSP in comparison with HC. In addition, lower concentrations of NPTX2 and NPTXR were also found in the AD group compared to HC. For NPTX1, lower levels were only found in PSP compared with HC. Unlike the discovery

cohort, lower levels of AP2B1 were found in MSA and PSP compared to HC. Similarly, complexin-2 demonstrated lower levels in PSP compared with HC and AD. No differences were found for neurogranin in comparison with HC. However, higher CSF concentrations of neurogranin were found in AD in comparisons with PD, PSP, and MSA, with the HC group, found in-between. A similar pattern was also observed for beta- and gamma-synuclein. Finally, 14-3-3 zeta/delta had higher levels in AD compared to both HC and parkinsonian disorders. Few patients had started dopaminergic medications at the time of CSF collection (11 PD, 1 PSP, and 1 MSA with available levodopa equivalent daily dose), and there was no correlation between these medications and synaptic protein levels. A cluster analysis (Supplementary Fig. S1) was performed to investigate associations between the synaptic proteins and showed that some of the measured proteins correlated strongly with each other. Specifically, closely associated were first the pentraxins ($\rho > 0.88$, P -value ≤ 0.0001), second, 14-3-3 zeta/delta with 14-3-3 epsilon ($\rho = 0.62$, P -value ≤ 0.0001), and last the rest of the synaptic proteins ($\rho > 0.73$, P -value ≤ 0.0001), with the exception of 14-3-3 theta. A second cluster analysis (Supplementary Fig. S2) was also performed of the synaptic proteins with the addition of other CSF biomarkers. All synaptic proteins correlated moderately-to-strongly ($\rho = 0.48$ – 0.91 , P -value ≤ 0.0001) with total tau, tau phosphorylated at Thr181, and alpha-synuclein whereas for amyloid-beta peptide 1-42 only weak correlations ($\rho = 0.18$ – 0.39) were found for the pentraxins, syntaxin-7 and 14-3-3 theta. When analyzing median fold-changes against HC in the total combined sample (Supplementary Fig. S3,

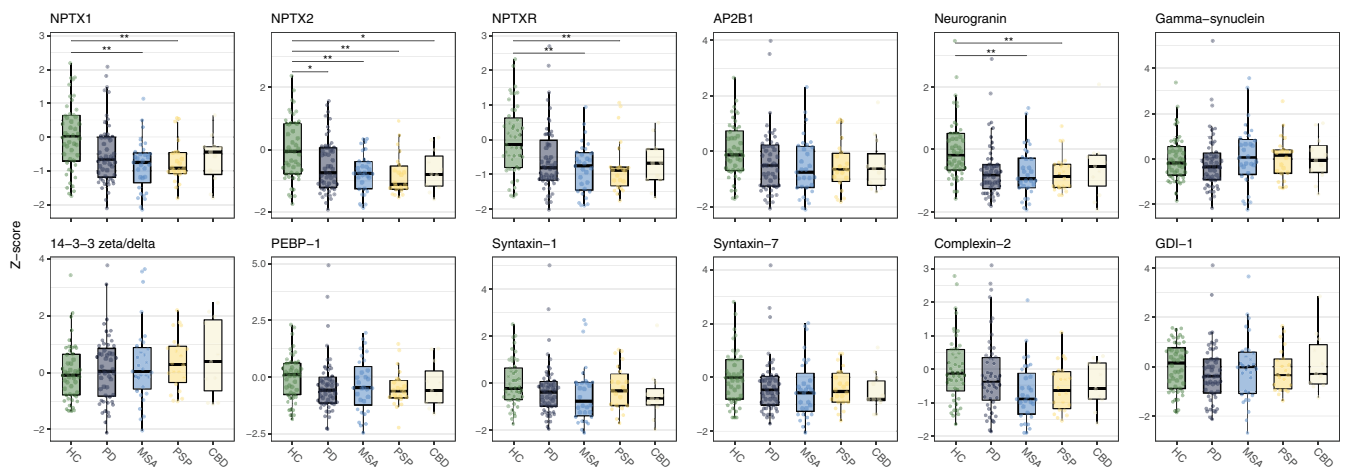


FIG. 1. Multiple reaction monitoring (MRM) analysis of the synaptic panel proteins (one representative peptide for each protein) in the discovery cohort consisting of healthy controls (HC, $n = 48$), Parkinson's disease (PD, $n = 51$), corticobasal degeneration (CBD, $n = 11$), progressive supranuclear palsy (PSP, $n = 22$), and multiple system atrophy (MSA, $n = 31$). Statistical comparison was performed with linear models, adjusted for age and sex, corrected for multiple group comparisons with the false discovery rate approach. P -values: * $P \leq 0.05$, and ** $P \leq 0.01$. Results are presented as standardized peptide levels (z-scores relative to control group) where the lower and upper hinges of the box plot denote the 25th and 75th percentiles, respectively, whereas the medians are represented by vertical lines, and the whiskers extend to the most extreme points within $1.5 \times$ interquartile range of the 25th and 75th percentiles.

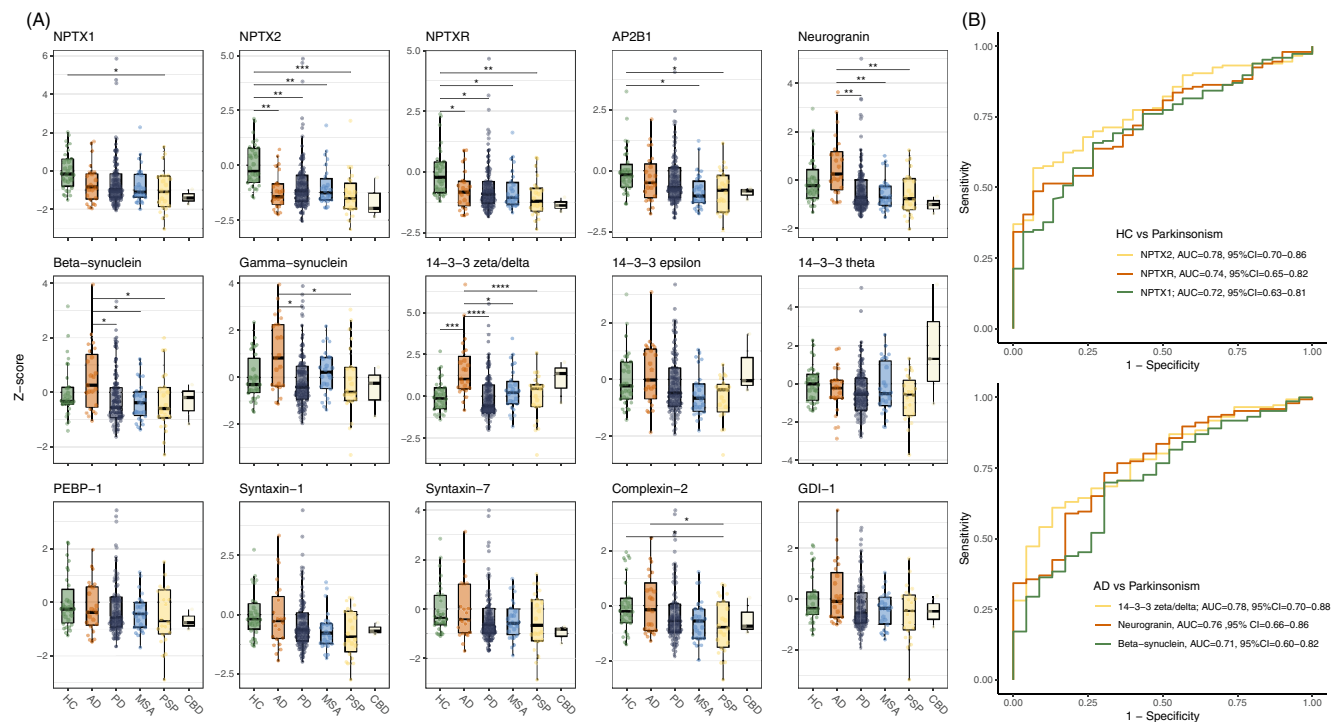


FIG. 2. (A) Multiple reaction monitoring (MRM) analysis of the synaptic panel proteins (one representative peptide for each protein) in the validation cohort consisting of healthy controls (HC, n = 30), Parkinson’s disease (PD, n = 101), corticobasal degeneration (CBD, n = 3, excluded from statistics), progressive supranuclear palsy (PSP, n = 21), and multiple system atrophy (MSA, n = 26). Statistical comparison was performed with linear models, adjusted for age and sex, corrected for multiple group comparisons with the false discovery rate approach. *P*-values: **P* ≤ 0.05, ***P* ≤ 0.01, ****P* ≤ 0.001, and *****P* ≤ 0.0001. Results are presented as standardized peptide levels (z-scores relative to control group) where the lower and upper hinges of the box plot denote the 25th and 75th percentiles, respectively, whereas the medians are represented by vertical lines, and the whiskers extend to the most extreme points within 1.5 × interquartile range of the 25th and 75th percentiles. (B) Receiver operating curves calculated for healthy controls versus parkinsonism patients, and Alzheimer’s disease versus parkinsonism patients for the three synaptic proteins with the highest area under the curve values.

TABLE 2 Partial Spearman correlation, adjusted for age, for the neuronal pentraxins against cognitive scores at the baseline evaluation

Variable	Neuronal pentraxin receptor		Neuronal pentraxin-1		Neuronal pentraxin-2	
	ρ	<i>P</i> -value	ρ	<i>P</i> -value	ρ	<i>P</i> -value
Domain – visuospatial function	0.085	0.52	0.088	0.50	0.28	0.028*
Domain – language	0.12	0.34	0.022	0.86	0.27	0.034*
Domain – episodic memory	0.14	0.26	0.094	0.46	0.24	0.059
Domain – executive function	0.065	0.61	0.0030	0.98	0.32	0.0096**
Domain – working memory and attention	0.12	0.33	0.055	0.67	0.29	0.019*
DatScan; most affected – caudate	0.19	0.14	0.19	0.14	0.29	0.023*
DatScan; most affected – putamen	0.062	0.61	0.072	0.55	0.10	0.41
MMSE score	0.18	0.10	0.20	0.069	0.25	0.023*
PIGD score	−0.21	0.093	−0.22	0.078	−0.20	0.094
Tremor score	0.39	0.00092***	0.37	0.0019**	0.35	0.0033**
Total UPDRS score	−0.14	0.22	−0.11	0.32	−0.16	0.16

Note: Data are shown as spearman correlation coefficient, ρ with corresponding *P*-values.
 Abbreviations: MMSE, Mini-Mental State Exam; PIGD, postural imbalance and gait difficulty; UPDRS, Unified Parkinson’s Disease Rating Scale.
 **P* ≤ 0.05
 ***P* ≤ 0.01
 ****P* ≤ 0.001

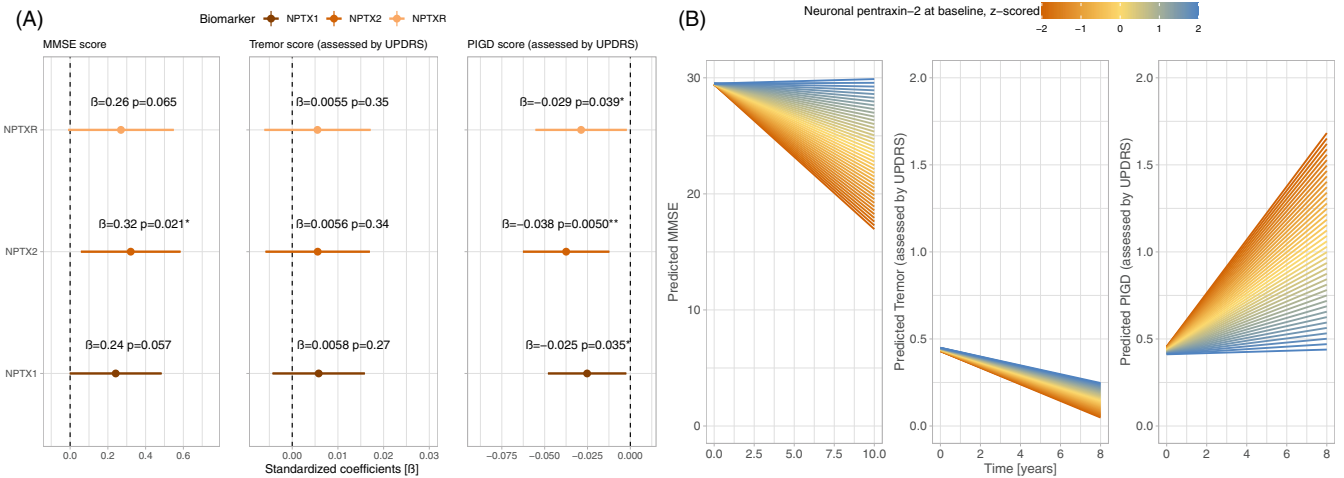


FIG. 3. Associations with the longitudinal performance of mini-mental state exam (MMSE), tremor, and postural imbalance and gait difficulty (PIGD) score for the neuronal pentraxins using linear mixed models adjusted for age, sex, and baseline values. (A) Forest plot of the pentraxins with respective standardized coefficients [β] and P -value for the interaction of the standardized biomarker levels with time. (B) Plots for neuronal pentraxin-2 with lines reflecting estimated marginal means from mixed effect models analyses.

Supplementary Table S5), similar biomarker patterns to those of the separate cohorts were observed, which strengthened the observations.

ROC analyses (Fig. 2B, Supplementary Table S6) were performed in the second cohort to test how the synaptic proteins differentiated patients with parkinsonism from HC and AD. NPTX2 had the highest AUC (AUC = 0.78, DeLong $P < 0.05$), higher than all other proteins for patients with parkinsonism versus HC, closely followed by the other pentraxins (NPTX1; AUC = 0.72, NPTXR; AUC = 0.74). To discriminate patients with parkinsonism from AD, however, the pentraxins fared not as well (AUC > 0.55). The highest discrimination was instead found for 14-3-3 ζ/δ (AUC = 0.78, DeLong $P < 0.05$), higher than all other proteins, except for neurogranin (AUC = 0.76).

Biomarker Associations With Baseline Clinical Features

Because synaptic dysfunction has been linked with cognitive decline, we investigated associations of the neuronal pentraxins in PD with clinical parameters of disease severity at baseline (Table 2, Supplementary Fig. S3). Lower CSF NPTX2 concentrations correlated with lower scores in visuospatial function, language, executive function, and working memory and attention domains ($\rho = 0.27-0.32$, $P < 0.05$). Lower NPTX2 levels were also associated with cognitive dysfunction at baseline, measured by MMSE ($\rho = 0.25$, $P = 0.023$), and with DaTSCAN measures of the striatal binding ratio of caudate ($\rho = 0.29$, $P = 0.023$), but not with putamen. Interestingly, higher levels of the pentraxins were found to be associated ($\rho = 0.35-0.39$, $P < 0.01$) with higher tremor scores.

Biomarker Associations With Longitudinal Clinical Features

We next examined the relationship of the pentraxins with longitudinal measurements of disease progression in PD—as measured by performance in MMSE, tremor, and PIGD scores (Fig. 3). Lower baseline levels of all pentraxins were found to be associated (β -estimate = -0.025 to -0.038 , $P < 0.05$) with faster progression of PIGD, but not with tremor scores. For longitudinal performance in MMSE, lower NPTX2 showed an association (β -estimate = 0.32, $P = 0.021$). Therefore, lower levels of NPTX2 were associated with faster progression in both motor- and cognitive aspects of PD.

Discussion

There is a need for synaptic dysfunction biomarkers in neurodegenerative diseases, which can detect or monitor synaptic degeneration in routine clinical diagnostics or drug monitoring and predict early cognitive decline. The synaptic proteins included in the panel have been implicated in neurodegenerative diseases, including parkinsonian disorders, and we have previously shown several of them to be altered in AD.¹⁵ Furthermore, studies in AD have found synapse loss as the pathological mechanism that best correlates with cognitive decline.³⁰ Across two cohorts, we now demonstrate lower CSF levels of the neuronal pentraxins in PD, MSA, and PSP and that lower levels are related to more severe PD symptoms in both motor and cognitive domains of the disease. Indications of lower levels of other synaptic proteins, including AP2B1, neurogranin, and complexin-2, were also observed. However, primarily, with the exceptions of the aforementioned

proteins, no difference was observed for the other synaptic proteins quantified, indicating specific alterations in particular synaptic proteins and not a general process of synaptic loss in parkinsonian disorders.

The neuronal pentraxins encompass the two secreted glycoproteins, NPTX1 and NPTX2, and their receptor, NPTXR, which is anchored to the plasmatic membrane. They are synaptic proteins that associate and form heteromultimers functionally implicated in the clustering of glutamate receptors and therefore, play vital roles in synaptic function and plasticity.³¹ The pentraxins have recently gained attention as potential synaptic pathology biomarkers in AD, for which their concentrations have repeatedly been found to be lower³²⁻³⁷ compared to controls. Still, few studies have been performed on other neurodegenerative diseases such as PD and atypical parkinsonism. Interestingly, NPTX2 has explicitly been implicated in PD. Not only has it been found in Lewy bodies in the substantia nigra and colocalize with alpha-synuclein, but also its messenger RNA (mRNA) expression was substantially upregulated in both the substantia nigra and frontal cortex of PD patients.³⁸ In the same study, NPTX1 was reported to be upregulated, and NPTXR downregulated. Here, we confirmed lower CSF levels of all three pentraxins across PD, PSP, and MSA in two clinical cohorts compared to HC. Lower CSF levels of the pentraxins also seem to be found in CBD, although this needs to be validated because of the low number of CBD patients in the present cohorts. Corroborating our results, studies using explorative proteomics have found lower levels of pentraxins in PD and atypical parkinsonism (PSP, MSA, and CBS).^{39,40} Lower levels have been reported, using targeted approaches, of NPTXR in PSP and MSA, NPTX1 in PSP and DLB, and NPTX2 in DLB compared to HC, as well as correlations with alpha-synuclein and cognitive function, and unchanged levels for NPTX2 in PD.⁴⁰⁻⁴² This study is the first to use a targeted approach to quantify all three pentraxins concurrently in PD and atypical parkinsonism. In the current study, we find that of the three pentraxins, NPTX2 shows better performance than the other pentraxins, which is also corroborated by our previous study,¹⁵ although they correlate strongly with each other. The reason remains elusive, but can be attributed to analytical variation or pathological mechanistic causes. We and others have previously shown that the NPTX2 seems to have stronger associations with cognitive measurements in AD than other synaptic biomarkers.^{15,32} Presently, we show that this is also true for PD, with lower CSF levels of NPTX2 associated with dysfunction in specific cognitive domains that are often affected in PD, as well as with a faster cognitive decline over time. Lower CSF levels of NPTX2 were also correlated with the progression of PIGD symptoms and, in contrast, less severe tremor symptoms at

baseline. Therefore, lower pentraxin levels seem to be correlated with both motor and cognitive disease severity in PD, and specifically with non-tremor symptoms. Since motor symptoms were assessed in the “on” state at follow-ups, it is also possible that the lower pentraxin levels more strongly correlated with the progression of motor symptoms partly unresponsive to dopaminergic therapy (eg, PIGD as compared with tremor). Additionally, CSF NPTX2 levels correlated with early denervation in the caudate nucleus in PD, but not in the putamen. Notably, a higher risk of developing dementia is associated with impaired function of the caudate nucleus,^{43,44} and a tremor-dominant PD phenotype is associated with lower dementia risk and a more benign prognosis.⁴⁵

It has been speculated that the reduction found for NPTX2 in the CSF of AD patients is because of a downregulation of NPTX2 and that it has a central role in the alteration of synaptic function preceding the general release of other synaptic proteins.^{32,37,46} However, NPTX1, also present at lower concentrations in the CSF, seems to be increased or unchanged in post-mortem brain tissue of AD patients.^{46,47} Furthermore, the expression of the two pentraxins is oppositely regulated in response to a reduction of neuronal activity,^{48,49} where the expression of NPTX1 is induced while NPTX2 is reduced. As previously stated, NPTX1 and NPTX2 are found to have substantially increased mRNA expression in PD. Interestingly, increased expression of both has been connected with neurotoxicity. For instance, increased NPTX1 expression has been associated with mediation of amyloid- β neurotoxicity,⁵⁰ apoptotic neuronal death,⁴⁷ and synaptic pruning through activation of the complement cascade.⁵¹ Although the increased expression of NPTX2 may be synaptogenic,^{48,49} it has also been implicated in the meditation of highly selective non-apoptotic death of dopaminergic neurons.^{38,52} In a mouse model, overexpression of NPTX2 was found in the striatum after levodopa treatment and contributed to the development of levodopa-induced dyskinesia.⁵³ In light of our findings and these previous reports, future studies should therefore, focus on the pentraxins and their mechanistic pathways during pathological conditions to elucidate the potential drivers behind the lower NPTXs levels in PD and other neurodegenerative diseases.

Furthermore, the presence of NPTX2 in Lewy bodies might connect the lower levels in PD to inclusion pathology, and investigations should explore the other pentraxins in this context. Taken together, these findings raise the question if the pentraxins functionally contribute to the disease process (eg, to the formation of Lewy bodies and cell death in the substantia nigra). Hence, increased expression of NPTX1 and NPTX2, which are found in PD, seems connected to several pathways of neurotoxicity. Furthermore, because the

pentraxins have functions tied to the disease pathogenesis of PD, this might explain the association of the protein levels with PD pathology not found for the other synaptic proteins. However, because the pentraxins were found in lower concentrations in the CSF across several neurodegenerative diseases, not only in the spectrum of parkinsonian disorders, this could suggest the presence of a general junction of neuronal dysfunction (ie, possible overlapping mechanistic pathways). To conclude, the pentraxins do not seem to be useful as differential diagnostic biomarkers, as alterations were not found to be disease-specific. However, based on the associations between pentraxin levels and symptoms found in the present paper, they do seem to be potential monitoring biomarkers of disease severity, progression, and decline in parkinsonian disorders, in both motor and cognitive domains.

Of the other synaptic panel proteins, only AP2B1, complexin-2, and neurogranin had changed CSF levels in parkinsonian disorders compared to HC. The proteins vary in their results across the two cohorts, but in the total sample, all three proteins showed decreased levels in MSA and PSP compared with HC. The clathrin adaptor protein, AP2B1, mediates synaptic vesicle endocytosis, whereas complexin-2 is an accessory protein, which binds and modulates the soluble N-ethylmaleimide-sensitive factor attachment receptor (SNARE) complex, and is therefore, involved in the process of synaptic vesicle exocytosis. Nevertheless, CSF AP2B1 and complexin-2 are mostly unexplored in neurodegenerative diseases. Very few studies investigate complexin-2 in parkinsonian disorders, and, to our knowledge, this is the first study of the protein in PSP and MSA, finding lower CSF levels compared to HC. A growing body of evidence suggests a strong connection between dysregulation of the endocytic membrane-trafficking pathway and neurodegenerative diseases, particularly in PD.⁵⁴ AP2B1 has not only been genetically linked as a risk factor for PD,⁵⁴ but has also been found to have a functional interaction with the key influencing factor LRRK2.⁵⁵ However, in the current study, we only found lower CSF levels of AP2B1 in MSA and PSP in comparisons with HC and not in PD, which previously have been reported.^{42,56} Corroborated by our previous work, the CSF levels of AP2B1 and complexin-2 were unchanged in AD compared to HC,¹⁵ indicating specific alterations in parkinsonian disorders.

Moreover, the postsynaptic protein neurogranin, whose function is tied to calmodulin regulation, is one of the most well studied synaptic proteins, and higher levels have been confirmed in AD in comparison with controls.^{7,9} Neurogranin is less explored in parkinsonian disorders. In addition to the lower levels found in PSP and MSA compared to HC in the total sample, we found lower levels in PD, MSA, and PSP compared to

AD, with the HC group in between. Therefore, neurogranin seems to display oppositely changed levels with lower levels in parkinsonian diseases and higher levels in AD compared to controls. Unlike the pentraxins, neurogranin, AP2B1, and complexin-2 seem to be indicative of parkinsonism-related alterations, possibly especially affected in PSP and MSA. Therefore, they should be further studied to elucidate mechanistic pathways potentially differentially affected by AD and parkinsonian disorders.

Beta- and gamma-synuclein belong to the same pre-synaptic protein family as alpha-synuclein, which is involved in the regulation of synaptic plasticity; however, less is known regarding their exact mechanistic function.⁵⁷ Interestingly, beta-synuclein has been found to inhibit the aggregation of alpha-synuclein and its mRNA overexpressed in the caudate nucleus of PD patients.⁵⁸ 14-3-3 zeta/delta, on the other hand, belongs to a synapse-enriched protein family, which is involved in the regulation of transmission and plasticity.⁵⁹ 14-3-3 zeta/delta has been found to interact with key proteins associated with PD onset and progression, such as LRRK2, alpha-synuclein, and Parkin. It has also been found to colocalize with Lewy bodies⁶⁰ and neurofibrillary tangles.^{61,62} 14-3-3 zeta/delta, beta-synuclein, and gamma-synuclein showed unchanged levels comparing parkinsonian disorders with HC and higher levels in AD compared with parkinsonian disorders. Corroborating this finding, we and others, have previously found these proteins' concentrations to be higher in AD compared with controls.^{15,63} Furthermore, 14-3-3 ζ/δ was the protein in the panel to best discriminate patients with parkinsonism from AD (AUC = 0.78), together with neurogranin. Therefore, the CSF levels of 14-3-3 zeta/delta, beta-synuclein, and gamma-synuclein seem to be specifically associated with AD pathology and not with alpha-synuclein pathology or primary tau pathology. These proteins should, therefore, be studied to unveil possible AD-specific mechanistic pathways of synaptic dysfunction. Last, GDI-1, the syntaxins, PEBP-1, 14-3-3 epsilon, and theta showed no differential changes.

A major limitation of the study is the small number of patients in each subgroup, especially for CBD. Future studies replicating our findings are needed, especially for these smaller subgroups. However, the validation of the results across two extensively investigated and well-characterized independent clinical cohorts strengthens our findings. A significant strength of the study when exploring synaptic pathology is the use of multiplexed mass spectrometry, which allows for the quantification of multiple biomarkers with diverse functions and localizations in a single analysis run. Such methods require low sample volume, have high detection specificity, and sample preparation is simple and robust. Furthermore, although the results are promising, the use of CSF hampers the translation to clinical practice because of the invasive

nature of lumbar puncture. Future studies should explore testing in peripheral fluids (eg, blood). However, it does not come without challenges, such as peripheral protein expression and low abundance of brain-derived proteins compared to high total protein content.

Conclusion

The present study found novel evidence that several of the synaptic proteins quantified by our in-house mass spectrometric panel seem to be altered in parkinsonian disorders. These synaptic proteins should be further investigated to provide additional insights into the complex impacts different neuropathologies have on synaptic function. Although none of the synaptic proteins seemed to have potential as differential diagnostic biomarkers, we want to highlight the performance of the neuronal pentraxins, which show promise to depict disease severity and track both motor and cognitive symptoms of PD. ■

Data Availability Statement

Derived data supporting the findings of this study are available from the corresponding author on request, providing data transfer agrees with the participating centre's national legislation and institutional review centre.

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Supporting Data

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.