

Plasma extracellular vesicle synaptic proteins as biomarkers of clinical progression in patients with Parkinson's disease: A follow-up study

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Abstract

Synaptic dysfunction plays a key role in Parkinson's disease (PD), and plasma extracellular vesicle (EV) synaptic proteins are emerging as biomarkers for neurodegenerative diseases. This study assessed the efficacy of plasma EV synaptic proteins as biomarkers in PD and their association with disease progression. In total, 144 participants were enrolled, including 101 people with PD (PwP) and 43 healthy controls (HCs). The changes in plasma EV synaptic protein levels between baseline and 1-year follow-up did not differ significantly in both PwP and HCs. In PwP, the changes in plasma EV synaptic protein levels were significantly associated with the changes in unified PD rating scale (UPDRS) part II and III scores. Moreover, PwP with elevated levels (first quartile) of any one plasma EV synaptic proteins (synaptosome-associated protein 25, growth-associated protein 43 or synaptotagmin-1) had significantly greater disease progression in UPDRS part II score and the postural instability and gait disturbance subscore in UPDRS part III than did the other PwP after adjustment for age, sex, and disease duration. These results indicate the promising potential of plasma EV synaptic proteins as clinical biomarkers of disease progression in PD. However, a longer follow-up period is warranted to confirm their role as prognostic biomarkers.

eLife assessment

This **useful** study presents data regarding the presence of synaptic proteins in the extracellular vesicle present in the blood of Parkinson's patients and healthy people, trying to correlate changes in such levels with the progression of Parkinson's symptoms. The results are preliminary, suggesting that these biomarkers might be **useful** for this purpose. The evidence is **incomplete**, and more adequate methods to isolate the extracellular vesicles and quantify the proteins are recommended. Also, a better presentation of the results will help the reader to understand the significance of the report, and in addition, more focused Introduction and Discussion sections are recommended.

Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disease [1]. It is well known for its progression, which involves increased disability and burden [2]. Worsening is noted not only in motor symptoms but also in nonmotor ones, particularly cognition. The rate of disease progression varies among people with PD (PwP). In the ongoing Parkinson's Progression Markers Initiative cohort study, approximately one-third of untreated PwP exhibited rapid progression during the first 2 years of follow-up. By contrast, the remaining PwP had a slow progressive course [3]. Unfortunately, no disease modifying therapy for halting disease progression and no predictor for assessing disease progression are available.

Synapses are sites of neuronal communication, and synaptic degeneration is an early functional pathogenic event in neurodegenerative diseases such as Alzheimer's disease (AD) and PD [4, 5]. Assessment of synaptic proteins in the cerebrospinal fluid (CSF) can reflect synaptic loss in patients with neurological diseases and is a key area of research interest. Substantial research efforts have been focused on assessing the CSF synaptic pathology to improve the diagnosis of neurodegenerative diseases at an early stage, before neuronal loss, and to monitor clinical progression [6–8]. Several synaptic proteins are promising biomarkers of synaptic function. Synaptosome-associated protein 25 (SNAP-25) is a presynaptic protein that plays a crucial role in neuronal survival, vesicular exocytosis, and neurite outgrowth [9]. Increased CSF levels of SNAP-25 have been reported in PwP [8]. In addition, growth-associated protein 43 (GAP-43) is a presynaptic protein anchored to the cytoplasmic side of the presynaptic plasma membrane [10]. CSF levels of GAP-43 have been reported to be significantly higher in patients with AD than in HCs [11]. Synaptotagmin-1 is a calcium sensor vesicle protein that is vital for rapid synchronous neurotransmitter release in hippocampal neurons [12]. Significantly increased CSF levels of synaptotagmin-1 have been reported in patients with AD and mild cognitive impairment [13]. However, the CSF collection process is moderately invasive, is inevitable during clinical assessment, and can result in some side effects, such as postpuncture headaches. Although studies have assessed blood biomarkers for PD diagnosis and progression, the results are conflicting. A lack of correlation between the peripheral blood content and the brain because of the blood–brain barrier (BBB) is a major obstacle to the identification of blood biomarkers for neurodegenerative diseases [14]. Assessment of peripheral blood extracellular vesicle (EV) proteins can be an alternative approach. EVs are tiny vesicles covered with a lipid membrane. They contain proteins, lipids, and nucleic acid responsible for cell-to-cell signal transmission. The integrity of EVs can be maintained when crossing the BBB [15]. Plasma EV biomarkers being rapidly developed for PD [16]. EV-cargo α -synuclein has been the most studied target that has exhibited strong potential for distinguishing PwP from HCs and other patients with atypical parkinsonism [17–21]. EV-cargo tau, β -amyloid, neurofilament light chain, brain-derived neurotrophic factor, and insulin receptor substrate have also been assessed in PwP [22–26]. These results indicate the potential role of EV content as biomarkers in PD.

The levels of synaptic proteins in blood exosomes, a specific type of EV, decrease in patients with AD and frontotemporal dementia [27]. Moreover, blood exosomal SNAP-25, GAP-43, neurogranin, and synaptotagmin-1 levels are lower in patients with AD. A combination of exosomal synaptic protein biomarkers could predict cognitive impairment [28]. A cross-sectional study also had demonstrated that the synaptic proteins inside the blood neuron-derived exosomes are reduced in PwP compared with healthy controls (HCs), which can distinguish PwP with HCs with around 80% accuracy [29]. However, there is no information from the cohort study. Therefore, this study assessed the association between plasma EV synaptic proteins and PD progression and determined whether plasma EV synaptic proteins could be used as clinical biomarkers to predict the progression of PD.

Results

The participants' demographic data at baseline and 1-year follow-up are presented in **Table 1**. In total, 144 participants (101 PwP and 43 HCs) were followed up. No significant difference was noted in plasma EV SNAP-25, GAP-43, and synaptotagmin-1 levels at baseline and follow-up between PwP and HCs after adjustment for age and sex (**Figures 1A** [representative image] and B–D [dot plot]) (Supplementary Figure 1 for the original blot of the representative image).

We assessed the association between the changes in plasma EV synaptic proteins levels and the changes in clinical parameters in PwP through the generalized linear model (**Table 2**). The changes in the total score of unified Parkinson disease rating scale (UPDRS)-II was positively associated with the change of plasma EV synaptic proteins (SNAP-25, GAP-43, and synaptotagmin-1); the change of total score of UPDRS-III and akinetic rigidity (AR) subscore were significantly associated with the change of plasma EV GAP-43 and synaptotagmin-1. The changes in mini-mental status examination (MMSE) and Montreal cognitive assessment (MoCA) scores were non-significantly associated with the changes in plasma EV synaptic protein levels.

We further assessed the association between the severity of the clinical parameters of PD at follow-up and the baseline plasma EV SNAP-25, GAP-43, and synaptotagmin-1 levels. After adjustment for age, sex, and disease duration, the plasma EV SNAP-25, GAP-43, and synaptotagmin-1 levels were nonsignificantly associated with the UPDRS-II, UPDRS-III, MMSE, and MoCA scores at follow-up (**Figure 2**; for details, refer to Supplementary Table 1). However, after we categorized the UPDRS-III scores into tremor, AR, and postural instability and gait disturbance (PIGD) subscores, the baseline plasma EV SNAP-25 and GAP-43 levels exhibited a significant positive correlation with the PIGD subscores at follow-up; a similar trend was noted in the plasma EV synaptotagmin-1 level.

Finally, we grouped the PwP on the basis of their baseline plasma EV synaptic protein level, with a cutoff at the first quartile. Overall, PwP with elevated baseline levels of plasma EV synaptic proteins had poor total scores of UPDRS-II and UPDRS-III and poor PIGD subscores of UPDRS-III (**Table 3**). Moreover, PwP with elevated baseline levels of plasma EV synaptic proteins exhibited significantly greater deterioration, as assessed using the UPDRS-II scores and PIGD subscores in UPDRS-III. After adjustment for age, sex, and disease duration, repeated-measures analysis of covariance revealed that the estimated marginal mean UPDRS-II scores and PIGD subscores of PwP with elevated levels of any one plasma EV synaptic protein were significantly worse at follow-up (but not at baseline) than those of PwP without elevated plasma EV synaptic protein levels, indicating significantly faster deterioration in the former patient group (**Figure 3**).

Discussion

In this study, although no significant difference in plasma EV synaptic protein levels was noted between PwP and HCs, changes in plasma EV synaptic protein levels in PwP were associated with motor decline. In addition, baseline plasma EV synaptic protein levels were associated with clinical outcomes, as assessed using PIGD subscores at follow-up. PwP with elevated baseline levels of any one plasma EV synaptic protein (SNAP-25, GAP-43, or synaptotagmin-1) exhibited significant deterioration in activities of daily living (as assessed by UPDRS-II scores and PIGD subscores in UPDRS-III) between baseline and follow-up. These results indicate the promising potential of plasma EV synaptic proteins as biomarkers for PD, particularly its progression.

Synaptic dysfunction is a remarkable phenomenon in PD. Postmortem studies have revealed a substantial loss of dopamine terminals in the putamen and amygdala [30, 31]. In addition, the loss of glutamatergic corticostriatal synapses has been reported [32]. Mitochondrial

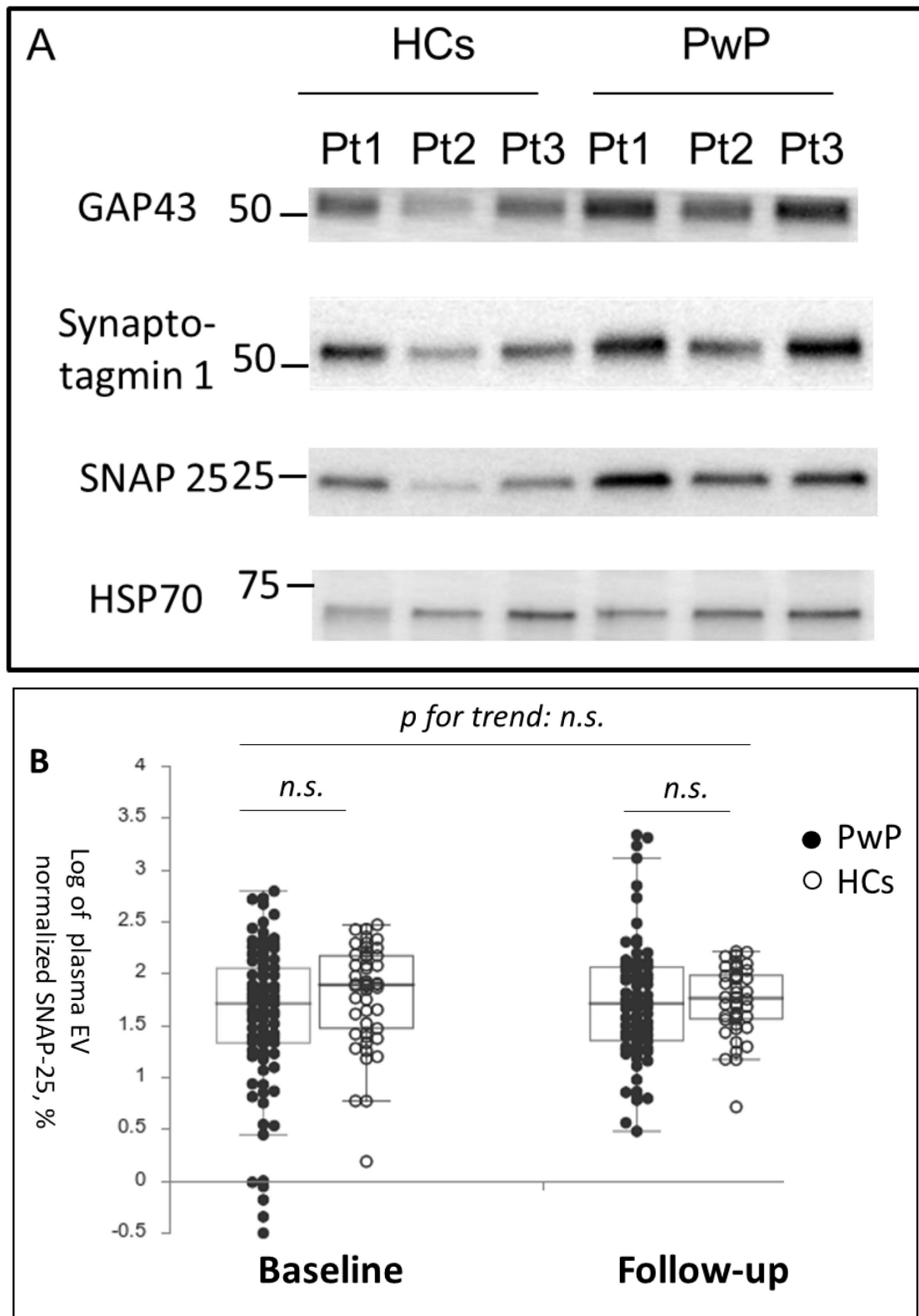


Figure 1.

Baseline and follow-up synaptic protein levels in plasma extracellular vesicles (EVs) between patients with Parkinson's disease (PwP) and healthy controls (HCs). (A) Representative protein blot images of different synaptic proteins, including SNAP-25, GAP-43, and synaptotagmin-1. Heat shock protein 70 (HSP-70) was the protein loading control. (B–D) Comparison of plasma SNAP-25, GAP-43, and synaptotagmin-1 levels between PwP and HCs at baseline and follow-up. Data are presented using a dot plot displaying the median and first and third quartile values. *n.s.*, non-significant.

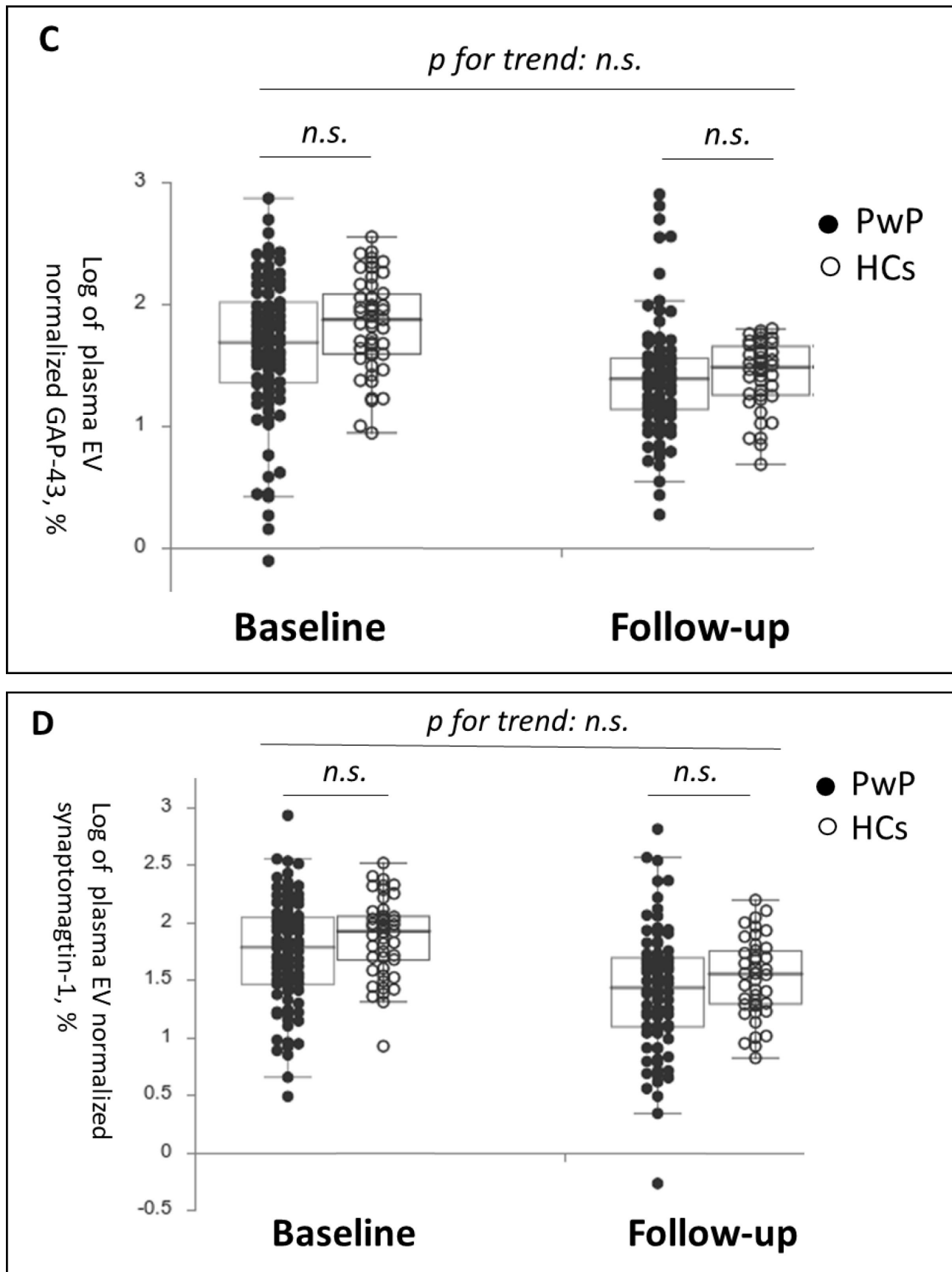


Figure 1. (continued)

	HCs, n = 43	PwP, n = 101
Age (years)	65 (10.24)	69 (7.76)
Women	15	48
Baseline		
MMSE	27 (3.92)	26 (4.15)
MoCA	23 (4.63)	21 (5.70)
Disease duration (years)	-	2 (2.24)
UPDRS-II	-	8 (5.58)
UPDRS-III	-	22 (9.30)
1-year follow-up		
MMSE	28 (4.12)	27 (5.61)
MoCA	24 (5.82)	23 (6.45)
UPDRS-II	-	11 (6.41)
UPDRS-III	-	19 (9.39)

Data was presented as median (standard deviation). HC, healthy control; PwP, people with Parkinson’s disease; MMSE, Mini-Mental State Examination; MoCA, Montreal cognitive assessment; UPDRS, unified Parkinson’s disease rating scale.

Table 1.
Demographic data of study participants

	UPDRSII	UPDRSIII				MMSE	MoCA
			Tremor	AR	PIGD		
SNAP-25*Follow	0.218 (0.049)	0.312 (0.076)	0.004 (0.432)	0.016 (0.066)	0.009 (0.440)	-0.007 (0.932)	0.048 (0.647)
GAP-43*Follow	0.984 (0.031)	1.711 (0.018)	0.001 (0.972)	0.089 (0.011)	0.073 (0.115)	-0.099 (0.767)	-0.054 (0.901)
Synaptomagtin-1 *Follow	1.543 (0.012)	2.205 (0.024)	0.007 (0.815)	0.107 (0.023)	0.109 (0.080)	-0.361 (0.421)	-0.260 (0.661)

UPDRS, unified Parkinson Disease rating scale; AR, akinetic rigidity; PIGD, postural instability and gait disturbance; MMSE, mini-mental status examination; MoCA, Montreal cognitive assessment.

Table 2.

Generalized linear model investigated the association between the change of plasma EV synaptic proteins (per 100%) with the change of clinical severity as dependent variable in people with Parkinson's disease, presented as coefficient with p value. n.s., non-significant

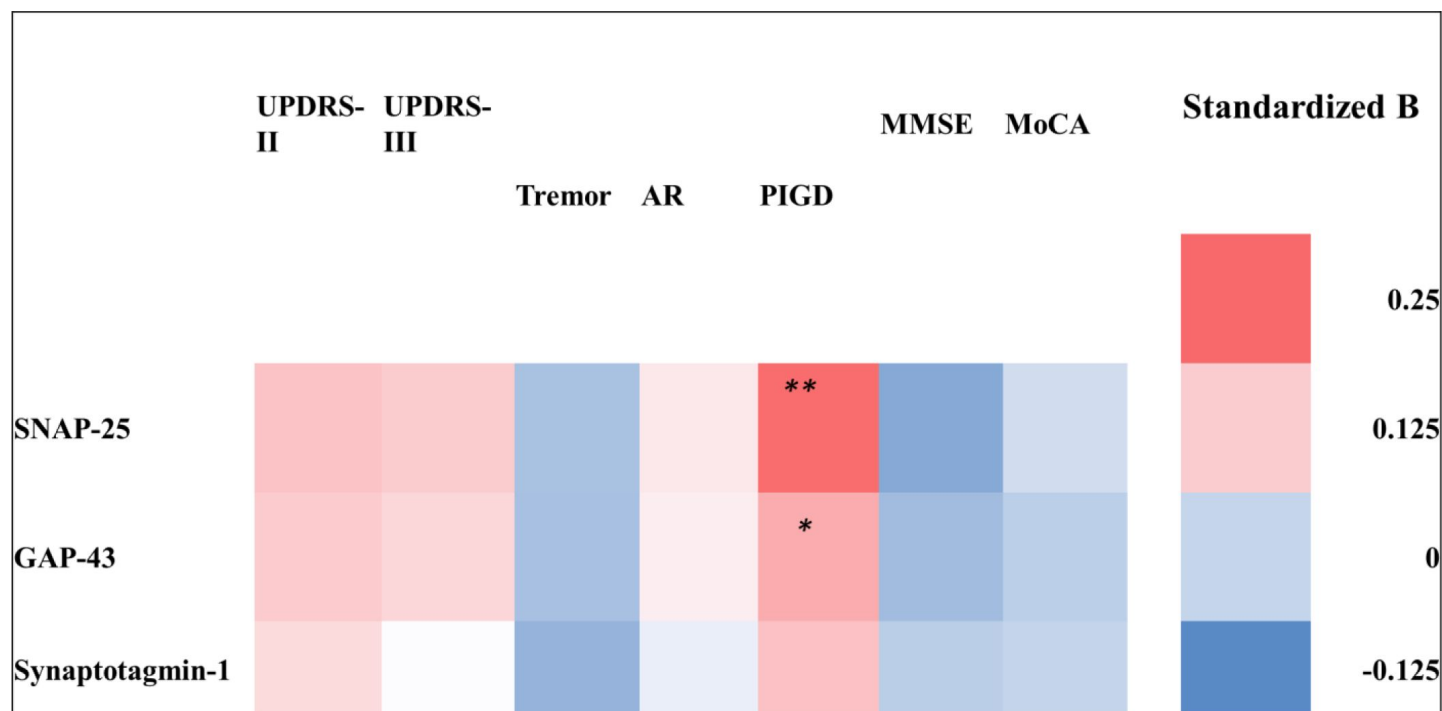


Figure 2.

Heatmap of the association between baseline plasma extracellular vesicle (EV) synaptic protein levels and clinical assessment parameters at follow-up in patients with Parkinson's disease (PwP). The logistic regression model was used to assess the baseline plasma EV SNAP-25, GAP-43, and synaptotagmin-1 levels. The motor symptoms were assessed based on the Unified Parkinson Disease Rating Scale (UPDRS)-II and UPDRS-III scores and tremor, akinetic rigidity (AR), and postural instability and gait disturbance (PIGD) subscores, and cognitive function was assessed using the Mini-Mental State Examination (MMSE) and Montreal Cognitive Assessment (MoCA) scores. The association is presented using standardized β values. Detailed results of the regression model are provided in Supplementary Table. *, $p < 0.05$; **, $p < 0.01$.

Plasma		SNAP-25				GAP-43				Synaptotagmin-1			
EV		L, n=74	H, n=28	p- value	p for trend	L, n=74	H, n=28	p- value	p for trend	L, n=74	H, n=28	p- value	p for trend
UPDRS-II	Baseline	8.26±	9.04±	0.530	<0.001	8.53±	8.32±	0.868	<0.001	8.46±	8.50±	0.974	<0.001
		5.80	4.90			5.67	5.33			5.70	5.24		
	Follow-up	10.38±	13.36±	0.035		10.65±	12.64±	0.161		10.57±	12.86±	0.107	
UPDRS-III	Baseline	6.11	6.72			6.30	6.53			6.25	6.58		
		22.31±	23.89±	0.445	0.259	22.22±	24.14±	0.352	0.244	21.97±	24.79±	0.174	0.145
	Follow-up	9.54	8.65			9.66	8.25			9.37	8.93		
Tremor	Baseline	20.01±	24.21±	0.042		19.96±	24.36±	0.033		20.04±	24.14±	0.048	
		9.33	8.87			9.25	8.99			9.39	8.70		
	Follow-up	0.34±	0.46±	0.127	0.037	0.34±	0.46±	0.105	0.034	0.33±	0.47±	0.072	0.014
AR	Baseline	0.46	0.38			0.34	0.37			0.34	0.38		
		0.27±	0.35±	0.181		0.27±	0.35±	0.181		0.28±	0.32±	0.595	
	Follow-up	0.26	0.32			0.26	0.32			0.29	0.22		
PIGD	Baseline	1.04±	1.09±	0.628	0.300	1.03±	1.11±	0.449	0.325	1.03±	1.13±	0.317	0.195
		0.46	0.44			0.47	0.42			0.46	0.45		
	Follow-up	0.96±	1.10±	0.164		0.95±	1.12±	0.076		0.96±	1.10±	0.155	
MMSE	Baseline	0.46	0.42			0.45	0.43			0.46	0.42		
		0.76±	0.81±	0.658	0.023	0.77±	0.77±	0.963	0.046	0.74±	0.86±	0.351	0.027
	Follow-up	0.61	0.44			0.60	0.45			0.56	0.58		
MoCA	Baseline	0.74±	1.07±	0.023		0.78±	0.98±	0.157		0.73±	1.11±	0.008	
		0.59	0.75			0.61	0.74			0.56	0.80		
	Follow-up	25.32±	25.29±	0.967	0.483	25.58±	24.61±	0.293	0.470	25.62±	24.50±	0.225	0.342
MoCA	Baseline	4.45	3.29			4.16	4.11			3.91	4.69		
		24.78±	25.14±	0.774		25.05±	24.43±	0.616		25.23±	23.96±	0.310	
	Follow-up	5.93	4.64			5.79	5.10			5.61	5.50		
MoCA	Baseline	20.68±	21.21±	0.680	0.834	21.14±	20.04±	0.390	0.899	21.12±	20.07±	0.411	0.926
		6.00	5.00			5.77	5.62			5.33	6.69		
	Follow-up	20.60±	21.46±	0.551		21.10±	20.18±	0.526		21.19±	19.93±	0.382	
		6.85	5.37			6.55	6.30			6.33	6.80		

L: 2nd to 4th quartile at baseline; H: 1st quartile at baseline; UPDRS, unified Parkinson Disease rating scale; AR, akinetic rigidity; PIGD, postural instability and gait disturbance; MMSE, mini-mental status examination; MoCA, Montreal cognitive assessment.

Table 3.

The clinical severity in people with Parkinson's disease with and without elevated (1st quartile) baseline plasma extracellular vesicle (EV) synaptosome-associated protein 25 (SNAP-25), growth-associated protein 43 (GAP-43) and synaptotagmin-1.

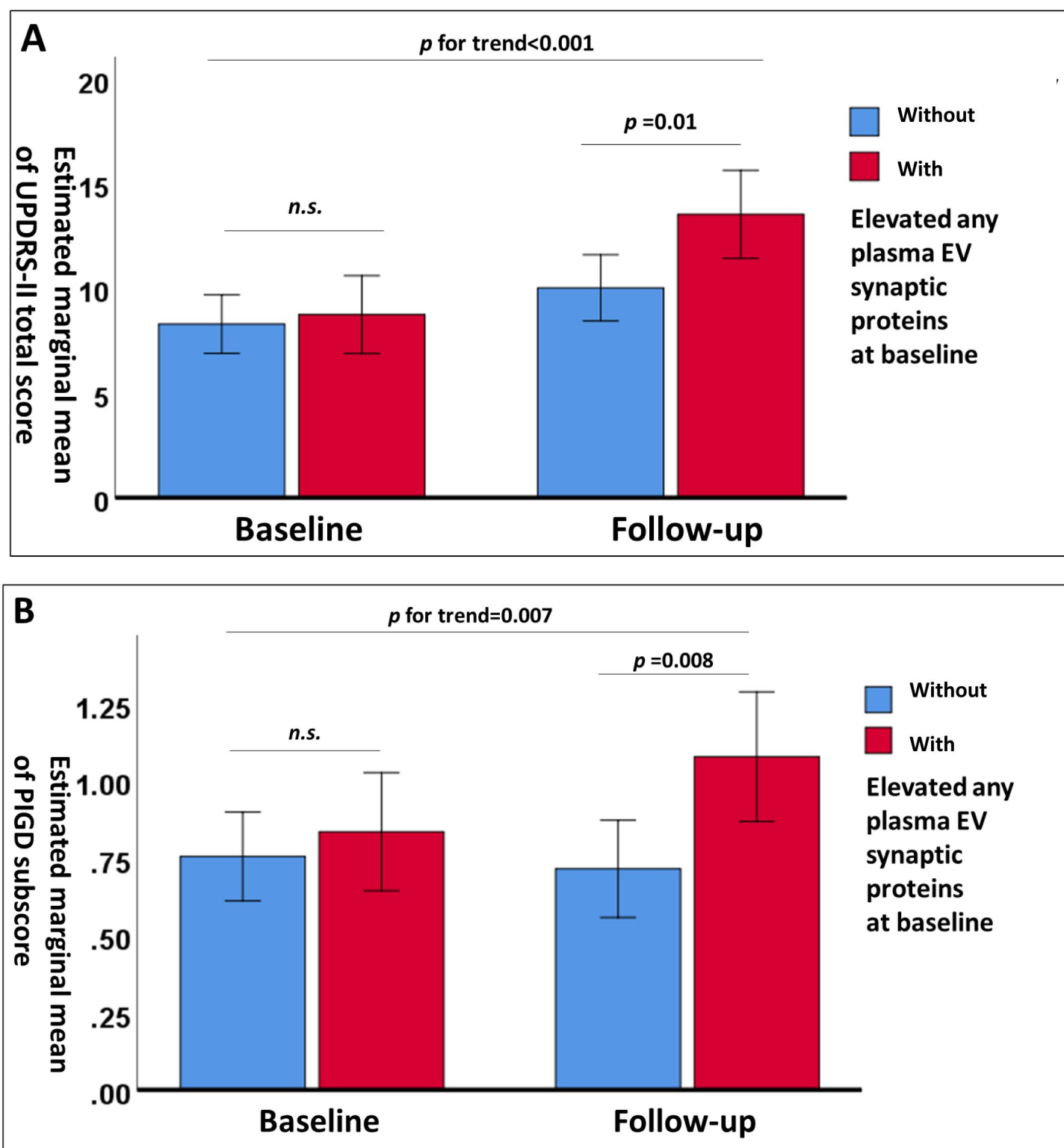


Figure 3.

Changes in estimated marginal means of Unified Parkinson Disease Rating Scale (UPDRS)-II total scores (A) and postural instability and gait disturbance (PIGD) subscores (B) after adjustment for age, sex, and disease duration in patients with Parkinson's disease (PwP) with and without elevated levels of any one plasma extracellular vesicle synaptic protein (first quartile) at baseline and follow-up. Data are presented as means with 95% confidence intervals. n.s., nonsignificant.

dysfunction-related metabolic burden accounts for part of the synaptic loss in PD, and the aggregation of α -synuclein, a synaptic protein that is the major pathognomonic protein in PD, contributes to the pathogenesis of PD [33]. Several synaptic proteins have been assessed as biomarkers for PD. One study reported an increase in the level of CSF SNAP-25 but not Ras-related protein 3A or neurogranin. Moreover, treated PwP exhibited higher CSF levels of SNAP-25 than did their drug-naïve counterparts [34]. However, synaptic proteins in plasma EVs have not been assessed. Plasma EVs remain stable up to 90 days [35]; this prevents the fluctuation of free-form synaptic proteins because of transient surge or degradation. Moreover, SNAP-25 is transported in the blood by EVs [36]. This study assessed the role of plasma EV synaptic proteins as biomarkers for PD. The results reveal that the changes in plasma EV synaptic protein levels were significantly associated with the changes in UPDRS-II, UPDRS-III and AR subscore, indicating the efficacy of plasma EV synaptic proteins as indicators of motor deterioration.

Significant worsening of UPDRS-II scores and PIGD subscores in UPDRS-III was noted in PwP with higher baseline levels of plasma EV synaptic proteins (first quartile). UPDRS-II is a crucial but underestimated parameter for assessing PwP; it can be used to assess the daily functional capability related to motor symptoms in PwP without temporary drug interruptions, as in the case of UPDRS-III [37, 38]. Assessment of the motor subtypes of PD revealed that the PIGD subtype is associated with an increased Lewy body and α -synuclein burden; rapid decline; and increased risks of cognitive impairment, falls, and mortality in comparison with the tremor-dominant (TD) subtype, which has a relatively benign course [39–42]. PwP may convert from the TD subtype to the PIGD subtype during disease progression [43]. Moreover, PIGD-related motor symptoms respond to dopaminergic medications to a lesser extent than do tremor-, akinesia-, and rigidity-related symptoms [44], which may more directly reflect the progression of the disease. We observed that PwP with elevated levels of any one plasma EV synaptic protein exhibited greater deterioration, as assessed using UPDRS-II scores and PIGD subscores. High EV synaptic protein levels conventionally indicate increased synaptogenesis; this contrasts the synaptic loss pattern noted in PD. Decreased blood exosomal synaptic protein levels have been reported in patients with AD and frontotemporal dementia [27]. However, in PwP who are at the early disease stage, compensatory synaptic sprouting and increased synaptic plasticity are noted in the striatum. This compensation, also known as motor reserve, may temporarily decrease the clinical disease burden. However, the patient would be more vulnerable to deterioration if the compensation is overshadowed by degeneration [45]. For instance, in one study, PwP who engaged in higher premorbid exercises exhibited milder motor symptoms despite having a similar gradient of striatal dopaminergic reduction at baseline; however, they exhibited more rapid deterioration [46]. Because our study mainly included PwP who had the early stage of disease (mean disease duration of less than 3 years) and a mild disease burden (baseline UPDRS-II score = 8.49), the elevated plasma EV synaptic protein levels may indicate the activation of the compensatory process. Such PwP are at an increased risk of rapid deterioration and disease progression and should be candidates for disease-modifying interventions, including pharmacological and nonpharmacological treatments.

The strength of this study is that it is the first study to assess the changes in plasma EV synaptic protein levels and determine the association between the changes in plasma EV synaptic protein levels and cognitive decline in PwP. Considering the well-established role of synaptic degeneration and plasticity in PD, the theoretical background of the use of these synaptic proteins as plasma EV biomarkers for PD is confirmed. Moreover, most synaptic proteins are neuron derived, thereby preventing contamination from nonneuronal tissues. The significant association between the changes in plasma EV synaptic proteins levels and the changes in UPDRS-II, UPDRS-III and AR subscore suggests the efficacy of these proteins in detecting motor decline in PwP, which can serve as a objective parameter for further disease-modification clinical trial. Elevated baseline plasma EV synaptic protein levels can also predict rapid deterioration in PwP, highlighting the importance of motor reserve and synaptic plasticity in the progression of PD for future research. Although any single synaptic protein cannot precisely predict the progression of PD, these protein targets may

be considered for use in the biomarker panel and analyzed using an artificial intelligence–assisted artificial neural network, which is widely used to predict the outcomes of several neurological diseases [47–50].

This study has some limitations. Semiquantitative assessment of plasma EV synaptic protein (SNAP-25, GAP-43, and synaptotagmin-1) levels was performed using western blot analysis. The lack of absolute values limits further clinical application. In addition, because numerous synaptic proteins are involved in the pathogenesis of PD, the three selected proteins may not reflect all features of the synaptic condition. The selection of HSP-70 as the control for the synaptic proteins quantification of plasma EV is not undisputable. Two types of EV proteins are used for this role: membrane proteins and intravesicular proteins. Membrane proteins include CD9, CD63, and CD81; intravesicular proteins include TSG101, annexins, and chaperone proteins, such as HSP-70. Since the expression of HSP-70 is usually steady, we used the HSP-70 as the internal control, but it is a limitation of the present study. Moreover, this study assessed the total plasma EVs instead of neuron-derived exosomes. Synaptic proteins are mainly derived from neurons. Even neuron-derived exosomes do not originate solely from the brain, resulting in contamination from the peripheral nervous system. It is also worth mentioning that the 1-year follow-up period to assess the progression of PD was relatively short and may have been insufficient to detect significant disease progression. In addition, synaptic dysfunction is a commonly shared phenomenon of several neurological diseases, which is not specific to PD. Therefore, the HCs in the present study may be contaminated with concurrent neurological diseases, which accounts for the non-significant difference between PD and HCs. However, this approach also delineated the role of synaptic dysfunction in the progression of PD, which helped to monitor the disease progression, especially for disease modification clinical trials. Choosing the first quartile as a cut-off value of plasma EV synaptic proteins is also one of the limitations of the study. While developing new biomarkers, there was no clear cut-off value as reference for the continuous variable, and percentile is considered for predicting the prognosis[51]. Further studies are required to validate this application. Lastly, the results form a mono-centric, small-scale and short-period PD cohort required further validation

In conclusion, this study revealed that changes in the levels of plasma EV synaptic proteins, namely SNAP-25, GAP-43, and synaptotagmin-1, are associated with motor decline in PwP. Elevated baseline plasma EV synaptic protein levels can predict increased deterioration of motor function, particularly PIGD symptoms, in PwP. Our results indicate that plasma EV synaptic proteins have the potential to be used as biomarkers of PD progression and detection. A longer longitudinal follow-up is warranted to clearly assess the prognostic efficacy of plasma EV synaptic proteins in PwP.

Methods

Study participants

This study included 101 PwP and 43 HCs. PD was diagnosed in accordance with the criteria used in another study [52]. Patients diagnosed as having early-to-mid-stage PD (Hoehn and Yahr stage I–III) were invited to participate in this study. HCs were excluded if they had comorbidities, such as neurodegenerative, psychiatric, or major systemic diseases (malignant neoplasm or chronic kidney disease). HCs were mainly recruited from neurological outpatient clinics; they had minor chronic health conditions, such as hypertension, diabetes, or hyperlipidemia. This study was approved by the Joint Institutional Review Board of Taipei Medical University (approval no. N201609017 and N201801043).

Clinical assessments

The participants' background data were obtained through a personal interview. Their cognitive function was assessed by trained nurses using the Taiwanese versions of the MMSE and MoCA. The severity of PD was assessed using parts I, II, and III of the UPDRS during clinic visits. PwP were assumed to be in their "on" time. Tremor, AR, and PIGD subscores were calculated from the subitems in UPDRS-III as described previously [53], with some modifications.

Plasma EV isolation and characterization

Venous blood was collected by from PwP and HCs after their outpatients clinic (non-fasting) by 21 gauge needle, and the plasma was isolated through centrifugation at $13,000 \times g$ for 20 min immediately. Plasma was storage in the -80°C freezer before EV isolation. Plasma EVs were isolated from 1 mL of plasma by exoEasy Maxi Kit in accordance with the manufacturer's instructions and stored in the -80°C freezer. The isolated plasma EVs were then eluted and stored. Usually, 400 μL of eluate is obtained per mL of plasma. The isolated plasma EVs were validated by 1.markers, including the presence of CD63, CD9, tumor susceptibility gene 101 protein and negative of cytochrome c 2. The nanoparticle tracking analysis, which demonstrated the majority of the size of EV are within 50-100nm 3. The morphology from the electron microscopy analysis. The validation had been described previously [24–26].

Quantification of plasma EV synaptic proteins

The isolated plasma EVs were directly lysed using protein sample buffer (RIPA Lysis Buffer, Millipore) and analyzed using protein sodium dodecyl sulfate–polyacrylamide gel electrophoresis. Antibodies against SNAP-25 (GeneTex, GTX113839, 1:1000), GAP-43 (GeneTex, GTX114124, 1:5000), and synaptotagmin-1 (GeneTex, GTX127934, 1:1000) were used for the analysis. The antibodies were prepared in Tris-buffered saline containing 0.1% Tween 20 and 5% bovine serum albumin. Secondary antibodies, including antimouse immunoglobulin G (IgG)-conjugated horseradish peroxidase (HRP; 115-035-003) and antirabbit IgG-conjugated HRP (111-035-003), were purchased from Jackson ImmunoResearch. Protein blot intensities were quantified using ImageJ software. The expression levels of plasma EV synaptic proteins were normalized to that of heat shock protein 70 (Proteintech, Cat.10995-1-AP, 1:2000). For each participant, equal volume of EV suspension (5 μL) was applied to the protein quantification. To ensure that the data could be compared between different gels, all the data were normalized to the average of the control group in the same gel.

Statistical analyses

All statistical analyses were performed using SPSS for Windows 10 (version 26; SPSS Inc., Chicago, IL, USA). A linear mixed model was used to assess whether the changes in plasma EV synaptic protein levels differed between PwP and HCs after adjustment for age and sex. A generalized linear model was used to determine the association between the changes in plasma EV synaptic protein levels and the changes in clinical parameters in PwP after adjustment for age, sex, and disease duration. Multivariate logistic regression was performed to assess the association between plasma EV synaptic proteins and clinical parameters at follow-up in PwP after adjustment for age, sex, and disease duration. Repeated-measures analysis of covariance with estimated marginal means was employed to compare the changes in clinical parameters between baseline and follow-up in PwP with elevated baseline levels (first quartile) of any one plasma EV synaptic protein. Finally, p values < 0.05 were considered statistically significant.

Declarations

Ethics approval and consent to participate

This study was approved by the Joint Institutional Review Board of Taipei Medical University (TMU-JIRB approval no. N201609017 and N201801043). Written informed consent was obtained from all participants for participation in the study.

Consent for publication

All authors have read and approved the final version of the manuscript. All authors agree to the present state of authorship and have signed a statement attesting to the authorship.

Availability of data and materials

Please contact the corresponding author (CT Hong). The availability of data and materials requires permission from the TMU-JIRB.

Competing interests

The authors declare that there are no competing interests.

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Authors' contributions

Study conception and design: CT Hong, L Chan, and CC Chung. Data acquisition and analysis: CT Hong, L Chan, and CC Chung. Data interpretation: CT Hong and RC Yu. Manuscript writing and revision: CT Hong, CC Chung, L Chan, and RC Yu. Provision of resources and administrative oversight: CT Hong. All authors have read and approved the final manuscript.

Data Availability

Please contact the corresponding author (CT Hong). The availability of data and materials requires permission from the TMU-JIRB.

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None

References

1. de Lau L. M., Breteler M. M. (2006) **Epidemiology of Parkinson's disease** *Lancet Neurol* **5**:525–35
2. Bloem B. R., Okun M. S., Klein C (2021) **Parkinson's disease** *The Lancet* **397**:2284–2303
3. Chen-Plotkin A. S., Albin R., Alcalay R., Babcock D., Bajaj V., Bowman D., Buko A., Cedarbaum J., Chelsky D., Cookson M. R., Dawson T. M., Dewey R., Foroud T., Frasier M., German D., Gwinn K., Huang X., Kopil C., Kremer T., Lasch S., Marek K., Marto J. A., Merchant K., Mollenhauer B., Naito A., Potashkin J., Reimer A., Rosenthal L. S., Saunders-Pullman R., Scherzer C. R., Sherer T., Singleton A., Sutherland M., Thiele I., van der Brug M., Van Keuren-Jensen K., Vaillancourt D., Walt D., West A., Zhang J (2018) **Finding useful biomarkers for Parkinson's disease** *Sci Transl Med* **10**
4. Picconi B., Piccoli G., Calabresi P (2012) **Synaptic dysfunction in Parkinson's disease** *Adv Exp Med Biol* **970**:553–72
5. Arendt T (2009) **Synaptic degeneration in Alzheimer's disease** *Acta Neuropathol* **118**:167–79
6. Lleó A., Núñez-Llaves R., Alcolea D., Chiva C., Balateu-Paños D., Colom-Cadena M., Gomez-Giro G., Muñoz L., Querol-Vilaseca M., Pegueroles J., Rami L., Lladó A., Molinuevo J. L., Tainta M., Clarimón J., Spires-Jones T., Blesa R., Fortea J., Martínez-Lage P., Sánchez-Valle R., Sabidó E., Bayés À., Belbin O (2019) **Changes in Synaptic Proteins Precede Neurodegeneration Markers in Preclinical Alzheimer's Disease Cerebrospinal Fluid*** *Molecular & Cellular Proteomics* **18**:546–560
7. Milà-Alomà M., Brinkmalm A., Ashton N. J., Kvartsberg H., Shekari M., Operto G., Salvadó G., Falcon C., Gispert J. D., Vilor-Tejedor N., Arenaza-Urquijo E. M., Grau-Rivera O., Sala-Vila A., Sanchez-Benavides G., González-de-Echávarri J. M., Minguillon C., Fauria K., Niñerola-Baizán A., Perissinotti A., Kollmorgen G., Suridjan I., Zetterberg H., Molinuevo J. L., Blennow K., Suárez-Calvet M, Study o. b. o. t. A (2021) **CSF Synaptic Biomarkers in the Preclinical Stage of Alzheimer Disease and Their Association With MRI and PET A Cross-sectional Study** *97*:e2065–e2078
8. Bereczki E., Bogstedt A., Höglund K., Tsitsi P., Brodin L., Ballard C., Svenningsson P., Aarsland D (2017) **Synaptic proteins in CSF relate to Parkinson's disease stage markers** *NPJ Parkinsons Dis* **3**:7–7
9. Antonucci F., Corradini I., Fossati G., Tomasoni R., Menna E., Matteoli M (2016) **SNAP-25, a Known Presynaptic Protein with Emerging Postsynaptic Functions** *Front Synaptic Neurosci* **8**
10. Benowitz L. I., Routtenberg A (1997) **GAP-43: an intrinsic determinant of neuronal development and plasticity** *Trends Neurosci* **20**:84–91
11. Sjögren M., Davidsson P., Gottfries J., Vanderstichele H., Edman A., Vanmechelen E., Wallin A., Blennow K (2001) **The cerebrospinal fluid levels of tau, growth-associated protein-43 and soluble amyloid precursor protein correlate in Alzheimer's disease, reflecting a common pathophysiological process** *Dement Geriatr Cogn Disord* **12**:257–64

12. Courtney N. A., Bao H., Briguglio J. S., Chapman E. R (2019) **Synaptotagmin 1 clamps synaptic vesicle fusion in mammalian neurons independent of complexin** *Nat Commun* **10**
13. Öhrfelt A., Brinkmalm A., Dumurgier J., Zetterberg H., Bouaziz-Amar E., Hugon J., Paquet C., Blennow K (2019) **A Novel ELISA for the Measurement of Cerebrospinal Fluid SNAP-25 in Patients with Alzheimer's Disease** *Neuroscience* **420**:136–144
14. Qin X. Y., Zhang S. P., Cao C., Loh Y. P., Cheng Y (2016) **Aberrations in Peripheral Inflammatory Cytokine Levels in Parkinson Disease: A Systematic Review and Meta-analysis** *JAMA Neurol* **73**:1316–1324
15. Jan A. T., Malik M. A., Rahman S., Yeo H. R., Lee E. J., Abdullah T. S., Choi I (2017) **Perspective Insights of Exosomes in Neurodegenerative Diseases: A Critical Appraisal** *Front Aging Neurosci* **9**:317–317
16. Leggio L., Paternò G., Vivarelli S., Falzone G. G., Giachino C., Marchetti B., Iraci N (2021) **Extracellular Vesicles as Novel Diagnostic and Prognostic Biomarkers for Parkinson's Disease** *Aging Dis* **12**:1494–1515
17. Chung C. C., Chan L., Chen J. H., Hung Y. C., Hong C. T (2021) **Plasma Extracellular Vesicle α -Synuclein Level in Patients with Parkinson's Disease** *Biomolecules* **11**
18. Niu M., Li Y., Li G., Zhou L., Luo N., Yao M., Kang W., Liu J (2020) **A longitudinal study on α -synuclein in plasma neuronal exosomes as a biomarker for Parkinson's disease development and progression** *Eur J Neurol* **27**:967–974
19. Zheng H., Xie Z., Zhang X., Mao J., Wang M., Wei S., Fu Y., Zheng H., He Y., Chen H., Xu Y (2021) **Investigation of α -Synuclein Species in Plasma Exosomes and the Oligomeric and Phosphorylated α -Synuclein as Potential Peripheral Biomarker of Parkinson's Disease** *Neuroscience* **469**:79–90
20. Si X., Tian J., Chen Y., Yan Y., Pu J., Zhang B (2019) **Central Nervous System-Derived Exosomal Alpha-Synuclein in Serum May Be a Biomarker in Parkinson's Disease** *Neuroscience* **413**:308–316
21. Stüendl A., Kraus T., Chatterjee M., Zapke B., Sadowski B., Moebius W., Hobert M. A., Deuschle C., Brockmann K., Maetzler W., Mollenhauer B., Schneider A (2021) **α -Synuclein in Plasma-Derived Extracellular Vesicles Is a Potential Biomarker of Parkinson's Disease** *Movement Disorders* **36**:2508–2518
22. Athauda D., Gulyani S., Karnati H. k., Li Y., Tweedie D., Mustapic M., Chawla S., Chowdhury K., Skene S. S., Greig N. H., Kapogiannis D., Foltynie T (2019) **Utility of Neuronal-Derived Exosomes to Examine Molecular Mechanisms That Affect Motor Function in Patients With Parkinson Disease: A Secondary Analysis of the Exenatide-PD Trial** *JAMA Neurology* **76**:420–429
23. Chou S.-Y., Chan L., Chung C.-C., Chiu J.-Y., Hsieh Y.-C., Hong C.-T (2020) **Altered Insulin Receptor Substrate 1 Phosphorylation in Blood Neuron-Derived Extracellular Vesicles From Patients With Parkinson's Disease** *Frontiers in Cell and Developmental Biology* **8**
24. Chung C. C., Huang P. H., Chan L., Chen J. H., Chien L. N., Hong C. T (2020) **Plasma Exosomal Brain-Derived Neurotrophic Factor Correlated with the Postural Instability and Gait Disturbance-Related Motor Symptoms in Patients with Parkinson's Disease** *Diagnostics (Basel)* **10**

25. Chung C. C., Chan L., Chen J. H., Bamodu O. A., Hong C. T (2020) **Neurofilament light chain level in plasma extracellular vesicles and Parkinson's disease** *Ther Adv Neurol Disord* **13**
26. Chung C. C., Chan L., Chen J. H., Bamodu O. A., Chiu H. W., Hong C. T (2021) **Plasma extracellular vesicles tau and β -amyloid as biomarkers of cognitive dysfunction of Parkinson's disease** *FASEB J* **35**
27. Goetzl E. J., Kapogiannis D., Schwartz J. B., Lobach I. V., Goetzl L., Abner E. L., Jicha G. A., Karydas A. M., Boxer A., Miller B. L (2016) **Decreased synaptic proteins in neuronal exosomes of frontotemporal dementia and Alzheimer's disease** *FASEB J* **30**:4141–4148
28. Jia L., Zhu M., Kong C., Pang Y., Zhang H., Qiu Q., Wei C., Tang Y., Wang Q., Li Y., Li T., Li F., Wang Q., Li Y., Wei Y., Jia J (2021) **Blood neuro-exosomal synaptic proteins predict Alzheimer's disease at the asymptomatic stage** *Alzheimers Dement* **17**:49–60
29. Agliardi C., Meloni M., Guerini F. R., Zanzottera M., Bolognesi E., Baglio F., Clerici M (2021) **Oligomeric α -Syn and SNARE complex proteins in peripheral extracellular vesicles of neural origin are biomarkers for Parkinson's disease** *Neurobiology of Disease* **148**
30. Iseki E., Kato M., Marui W., Ueda K., Kosaka K (2001) **A neuropathological study of the disturbance of the nigro-amygdaloid connections in brains from patients with dementia with Lewy bodies** *Journal of the Neurological Sciences* **185**:129–134
31. Kordower J. H., Olanow C. W., Dodiya H. B., Chu Y., Beach T. G., Adler C. H., Halliday G. M., Bartus R. T (2013) **Disease duration and the integrity of the nigrostriatal system in Parkinson's disease** *Brain* **136**:2419–2431
32. Stephens B., Mueller A. J., Shering A. F., Hood S. H., Taggart P., Arbuthnott G. W., Bell J. E., Kilford L., Kingsbury A. E., Daniel S. E., Ingham C. A (2005) **Evidence of a breakdown of corticostriatal connections in Parkinson's disease** *Neuroscience* **132**:741–754
33. Gcwenza N. Z., Russell D. L., Cowell R. M., Volpicelli-Daley L. A (2021) **Molecular Mechanisms Underlying Synaptic and Axon Degeneration in Parkinson's Disease** *Frontiers in Cellular Neuroscience* **15**
34. Berezcki E., Bogstedt A., Höglund K., Tsitsi P., Brodin L., Ballard C., Svenningsson P., Aarsland D (2017) **Synaptic proteins in CSF relate to Parkinson's disease stage markers** *NPJ Parkinsons Dis* **3**
35. Kalra H., Adda C. G., Liem M., Ang C. S., Mechler A., Simpson R. J., Hulett M. D., Mathivanan S (2013) **Comparative proteomics evaluation of plasma exosome isolation techniques and assessment of the stability of exosomes in normal human blood plasma** *Proteomics* **13**:3354–64
36. Agliardi C., Guerini F. R., Zanzottera M., Bianchi A., Nemni R., Clerici M (2019) **SNAP-25 in Serum Is Carried by Exosomes of Neuronal Origin and Is a Potential Biomarker of Alzheimer's Disease** *Molecular Neurobiology* **56**:5792–5798
37. Rodriguez-Blazquez C., Rojo-Abuin J. M., Alvarez-Sanchez M., Arakaki T., Bergareche-Yarza A., Chade A., Garretto N., Gershanik O., Kurtis M. M., Martinez-Castrillo J. C., Mendoza-Rodriguez A., Moore H. P., Rodriguez-Violante M., Singer C., Tilley B. C., Huang J., Stebbins G. T., Goetz C. G., Martinez-Martin P (2013) **The MDS-UPDRS Part II (motor experiences of daily living) resulted useful for assessment of disability in Parkinson's disease** *Parkinsonism Relat Disord* **19**:889–93

38. Sampaio C (2009) **Can focusing on UPDRS Part II make assessments of Parkinson disease progression more efficient?** *Nature Reviews Neurology* **5**:130–131
39. Johnson A. R., Bucks R. S., Kane R. T., Thomas M. G., Gasson N., Loftus A. M (2016) **Motor Subtype as a Predictor of Future Working Memory Performance in Idiopathic Parkinson's Disease** *PLOS ONE* **11**
40. Burn D. J., Rowan E. N., Allan L. M., Molloy S., O'Brien J. T., McKeith I. G (2006) **Motor subtype and cognitive decline in Parkinson's disease, Parkinson's disease with dementia, and dementia with Lewy bodies** *Journal of neurology, neurosurgery, and psychiatry* **77**:585–589
41. Ren J., Hua P., Li Y., Pan C., Yan L., Yu C., Zhang L., Xu P., Zhang M., Liu W (2020) **Comparison of Three Motor Subtype Classifications in de novo Parkinson's Disease Patients** *Frontiers in Neurology* **11**
42. Nutt J. G (2016) **Motor subtype in Parkinson's disease: Different disorders or different stages of disease?** *Movement Disorders* **31**:957–961
43. Lee J. W., Song Y. S., Kim H., Ku B. D., Lee W. W (2019) **Alteration of Tremor Dominant and Postural Instability Gait Difficulty Subtypes During the Progression of Parkinson's Disease: Analysis of the PPMI Cohort** *Frontiers in Neurology* **10**
44. Kempster P. A., Williams D. R., Selikhova M., Holton J., Revesz T., Lees A. J (2007) **Patterns of levodopa response in Parkinson's disease: a clinico-pathological study** *Brain* **130**:2123–8
45. Chung S. J., Lee J. J., Lee P. H., Sohn Y. H (2020) **Emerging Concepts of Motor Reserve in Parkinson's Disease** *J Mov Disord* **13**:171–184
46. Sunwoo M. K., Lee J. E., Hong J. Y., Ye B. S., Lee H. S., Oh J. S., Kim J. S., Lee P. H., Sohn Y. H (2017) **Premorbid exercise engagement and motor reserve in Parkinson's disease** *Parkinsonism & Related Disorders* **34**:49–53
47. Chung C. C., Hong C. T., Huang Y. H., Su E. C., Chan L., Hu C. J., Chiu H. W (2020) **Predicting major neurologic improvement and long-term outcome after thrombolysis using artificial neural networks** *J Neurol Sci* **410**
48. Chung C.-C., Chan L., Bamodu O. A., Hong C.-T., Chiu H.-W (2020) **Artificial neural network based prediction of postthrombolysis intracerebral hemorrhage and death** *Scientific Reports* **10**
49. James C., Ranson J. M., Everson R., Llewellyn D. J (2021) **Performance of Machine Learning Algorithms for Predicting Progression to Dementia in Memory Clinic Patients** *JAMA Network Open* **4**:e2136553–e2136553
50. Chou S.-Y., Bamodu O. A., Chiu W.-T., Hong C.-T., Chan L., Chung C.-C (2022) **Artificial neural network-boosted Cardiac Arrest Survival Post-Resuscitation In-hospital (CASPRI) score accurately predicts outcome in cardiac arrest patients treated with targeted temperature management** *Scientific Reports* **12**
51. Duarte P. S (2021) **Cut Point Identification of Continuous Biomarkers: A Challenge That Goes Beyond Statistical Aspects** *Journal of Nuclear Medicine* **62**:1833–1833

52. Hughes A. J., Daniel S. E., Kilford L., Lees A. J (1992) **Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases** *J Neurol Neurosurg Psychiatry* **55**:181–4
53. Lewis S. J., Foltynie T., Blackwell A. D., Robbins T. W., Owen A. M., Barker R. A (2005) **Heterogeneity of Parkinson's disease in the early clinical stages using a data driven approach** *J Neurol Neurosurg Psychiatry* **76**:343–8

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Reviewer #1 (Public Review):

The study isolated extracellular vesicles (EV) from healthy controls (HCs) and Parkinson patients (PwP), using plasma from the venous blood of non-fasting people. Such EVs were characterized and validated by the presence of markers, their size, and their morphology. The main aim of the manuscript is to correlate the presence of synaptic proteins, namely SNAP-25, GAP-43, and SYNAPTOTAGMIN-1, normalized with HSP70, with the clinical progression of PwP. Changes in synaptic proteins have been documented in the CSF of Alzheimer's and Parkinson's patients. The demographics of participants are adequately presented. One

important limiting, as well as puzzling aspect, is the fact that authors did not find differences between groups at the beginning of the study nor after one year, after age and sex adjustment. Tables in general are hard to follow. Specifically, Table 2 does not convey a clear message nor in the text of the Table itself, and the per 100% of change needs to be explained in the corresponding legend. It is only when PwP were classified as a first quartile that a significantly greater deterioration was found. However, in the case of tremor, the top 25% had values going from 0.46-0.47 to 0.32-0.35, whereas the lower three quarters went from 0.33-0.34 to 0.27-0.28 depending on the protein analyzed. This needs to be clarified in the text. Table 3 is hard to read and some of the values seem repetitive, especially for tremor, AR, and PIGD. It looks as if Figure 2 represents the same information as Table 3. The text and figure legends are not helpful in guiding the reader to understand the presented information.

Reviewer #2 (Public Review):

Hong and collaborators investigated variations in the amount of synaptic proteins in plasma extracellular vesicles (EV) in Parkinson's Disease (PD) patients on one-year follow-up. Their findings suggest that plasma EV synaptic proteins may be used as clinical biomarkers of PD progression.

It is a preliminary study using semi-quantitative analysis of synaptic proteins.

The authors have a cohort of PD patients with clinical examination and a know-how on EV purification. Regarding this latter part, they may improve their description of EV purification. EV may be broken into smaller size EV after freezing. Does it explain the relatively small size in their EV preparation? Do the authors refer to the MISEV guidelines for EV purity? Regarding synaptic protein quantification, the choice of western blotting may not be the best one. ELISA and other multiplex arrays are available. How the authors do justify their choice? Do the authors try to sort plasma EV by membrane-associated neuronal EV markers using either vesicle sorting or immunoprecipitation?

Many technical aspects may be improved. Such technical questions weakened the authors' conclusions.

The discussion is pretty long to justify the data. It may be shortened by adding some information in the introduction.