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Plasma extracellular vesicle tau, β-amyloid, and α-synuclein and the progression of Parkinson's disease: a follow-up study

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Abstract

Background: Plasma extracellular vesicle (EV) contents are promising biomarkers of Parkinson's disease (PD). The pathognomonic proteins of PD, including α-synuclein, tau, and β-amyloid, are altered in people with PD (PwP) and are associated with clinical presentation in previous cross-sectional studies. However, the dynamic changes in these plasma EV proteins in PwP and their correlation with clinical progression remain unclear.

Objective: We investigated the dynamic changes in plasma EV α-synuclein, tau, and β-amyloid and their correlation with/prediction of clinical progression in PwP.

Design: A cohort study.

Methods: In total, 103 PwP and 37 healthy controls (HCs) completed baseline assessment and 1-year follow-up. Clinical assessments included Unified Parkinson's Disease Rating Scale (UPDRS) parts II and III, Mini-Mental State Examination (MMSE), and Montreal Cognitive Assessment (MoCA). Plasma EVs were isolated, and immunomagnetic reduction-based immunoassay was used to assess α-synuclein, tau, and β-amyloid 1-42 (Aβ1-42) levels within the EVs.

Results: Compared with HCs, significant differences were noted in the annual changes in all three EV pathognomonic proteins in PwP. Although the absolute changes in plasma EV pathognomonic proteins did not significantly correlate with clinical changes, PwP with elevated baseline plasma EV tau (upper-half) levels demonstrated significantly greater decline in motor and cognition, and increased plasma EV α-synuclein levels were associated with postural instability and the gait disturbance motor subtype. For PwP with elevated levels of all three biomarkers, clinical deterioration was significant, as indicated by UPDRS-II scores, postural instability and gait disturbance subscores of UPDRS-III, and MMSE score.

Conclusion: The combination of plasma EV α-synuclein, tau, and Aβ1-42 may identify PwP with a high risk of deterioration. Our findings can elucidate the interaction between these pathognomonic proteins, and they may serve as treatment response markers and can be applied in treatment approaches for disease modification.

Keywords: biomarker, extracellular vesicle, Parkinson's disease, tau, α-synuclein, β-amyloid

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Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disease.[1] Its pathological hallmark is the loss of dopaminergic neurons in the substantia nigra, which is responsible for the motor symptoms of PD, including tremor, akinetic rigidity (AR), and postural instability and gait disturbance (PIGD).[2] The progression of nonmotor symptoms (NMSs), especially cognitive decline and dementia, is a major threat to people with PD (PwP); these symptoms markedly impair their functional capability and quality of life.[3] Cognitive decline in PD not only results from the loss of dopaminergic neurons, but also

involves serotonergic, glutaminergic, and cholinergic neurons in the subcortex and cortex.[4] Overall, considerable interindividual variation has been found in the speed of PD progression;[5–7] people with nontremor-dominant PD, depression, and baseline cerebrovascular disease exhibit a high risk of rapid deterioration.[8–10] Accordingly, better prediction models for identifying the high-risk groups are urgently required, as they are best candidates for therapeutic clinical trials.

The main pathognomonic protein of PD is α-synuclein; however, other proteins, such as tau and β-amyloid are also detected.[11] Numerous studies have investigated the use of these three proteins as PD biomarkers.[12] However, despite the understanding of the pathological role of these proteins in PD, their application as biomarkers remains challenging. The cerebrospinal fluid (CSF) is the best body fluid for identifying PD biomarkers, but its accessibility and application are low.[13] By contrast, although blood is more accessible and acceptable, inconsistent results have been obtained when using the blood concentrations of α-synuclein, tau, and β-amyloid as PD biomarkers for differentiating PwP from healthy controls (HCs).[12]

Plasma extracellular vesicles (EVs), especially exosomes, are novel targets of biomarker development for several diseases.[14–16] EVs cross the blood–brain barrier without loss of their integrity due to the lipid membrane structure,[17] and they carry the molecular information of neurons in terms of the inner protein and nucleotide profile to the periphery. Plasma EV α-synuclein is the most studied target for PD,[18–22] whereas, tau and β-amyloid,[23] neurofilament light chain,[24] cytokines,[25] trophic factors,[26] insulin receptor substrate,[27,28] and nucleotides[29] are also good candidates. However, most studies have been cross-sectional, precluding the analysis of whether dynamic changes in plasma EV α-synuclein, tau, and β-amyloid correlate with the clinical progression of PD. Besides, it is expected that the baseline biomarker could enable the identification of PwP with a high risk of deterioration.

In our previous work, we assessed the plasma EVs biomarkers in PwP.[18,23] We followed these patients for another 1 year to investigate the longitudinal change and the association of plasma EV biomarkers with clinical progression as well as the possibility that these baseline plasma EV biomarkers can be used to identify PwP with a high risk of progression.

Materials and methods

Study population

The participant recruitment approach has been described elsewhere.[23] We enrolled 138 PwP and 67 HCs at baseline, and 103 PwP and 37 HCs were followed at 1 year between 2018 and 2020 at the Department of Neurology, Shuang Ho Hospital. Figure 1 illustrated the reasons and number of the drop-out in PwP and HCs. PD was diagnosed based on the UK Parkinson's Disease Society Brain Bank Diagnostic Criteria.[30] Only patients with mild to moderate PD, defined as Hoehn and Yahr (H&Y) stages I–III, were included. HCs did not have any neurodegenerative, psychiatric, or major systemic diseases, such as malignant neoplasm and chronic kidney disease, and were regularly followed up in outpatient clinics for chronic conditions (hypertension, diabetes, or hyperlipidemia). This study was approved by the Joint Institutional Review Board of Taipei Medical University (approval numbers: N201609017 and N201801043).

Clinical assessment

All participants were interviewed to obtain their baseline demographic data. Their cognitive function was investigated by trained nurses using the Taiwanese versions of the Mini-Mental State Examination (MMSE) and Montreal Cognitive Assessment (MoCA). During an outpatient visit, all patients with PD were evaluated using Parts I–III of the Unified Parkinson's Disease Rating Scale (UPDRS). The time between the most recent dose of anti-PD medication and assessment conducted using UPDRS Part III was not recorded; PwP were assumed to be in their 'on' time. Tremor, AR, and PIGD scores were obtained from the subitems in UPDRS Part III and were calculated as described in a previous study,[31] with modifications. In brief, tremor score was from the subitems 20 and 21 of UPDRS-III; PIGD from the subitem 27–30 of UPDRS-III; AR from subitem of 18, 22, 23, 24, 25, 26, and 31 of UPDRS-III.

Plasma EV isolation and validation

The details of plasma EV isolation and validation have been published previously.[23,24] In brief,

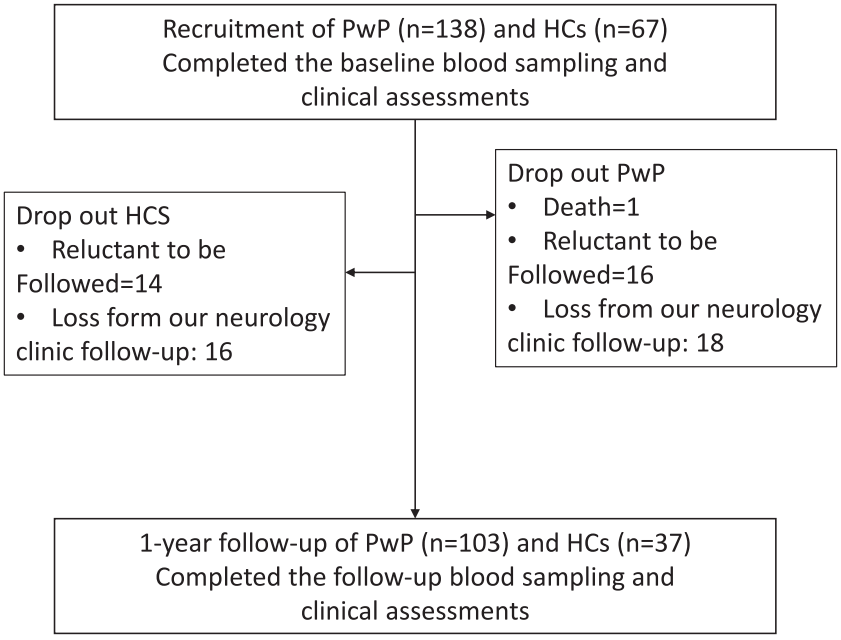

Figure 1. The flow chart of case selection, follow-up, and reasons of drop-out.

plasma EV was isolated using the exoEasy Maxi kit according to the manufacturer's instructions. The isolated EVs were validated based on the presence of surface markers, such as CD63, CD9, and CD81; their morphology was determined using transmission electron microscopy; and particle size analysis was conducted through nanoparticle tracking.

Immunomagnetic reduction assay for quantifying α-synuclein, tau, and β-amyloid

The details of the immunomagnetic reduction assay for quantifying plasma EV α-synuclein, tau, and β-amyloid have been described previously;[18,23] these analyses were conducted by MagQu Co. (New Taipei, Taiwan). According to their instructions, the assay limit of detection was 1.39, 26, and 77 fg/ml for α-synuclein, tau, and Aβ1-42, respectively.

Statistical analysis

All statistical analyses were performed using IBM SPSS, version 26 (IBMin, Armonk, NY, USA). The nonparametric Mann–Whitney *U* test was used to compare the changes in plasma EV α-synuclein, tau, and Aβ1-42 between PwP and HCs. Multivariable logistic regression was employed to investigate the association between the changes in plasma EV α-synuclein, tau, and Aβ1-42 levels, and the changes in motor or cognitive function scores in PwP after adjustment for age, sex, disease duration, and baseline motor or cognitive score. Repeated-measures analysis of variance (ANOVA) was used to compare the changes in clinical parameters of HCs and PwP between baseline and follow-up, and repeated-measures analysis of co-variance (ANCOVA) with estimated marginal means was used to assess the effect of the high baseline plasma EV α-synuclein, tau, and Aβ1-42 levels on the follow-up motor or cognitive function with the adjustment of age, sex, disease duration, and H&Y stage (for cognition only). $p < 0.05$ was considered statistically significant.

Results

Demographic data

Participants baseline information is summarized in Table 1. In brief, 37 HCs and 103 PwP completed baseline and 1-year follow-up examinations. No significant between-group differences in age and sex distributions were observed. HCs had significantly better baseline cognition, as assessed through MMSE and MoCA. Among PwP, at baseline, the mean disease duration was 2.69 years, with the mean UPDRS-II/III scores of 8.50 and 22.74, respectively. At follow-up, HCs had significant improvement in MoCA, but not

Table 1. Baseline demographic data of study participants (with completed baseline and follow-up).

	HCs, *n* = 37	PwP, *n* = 103	*p*-value (HCs *versus* PwP)
Age (years)	66.61 ± 10.77	68.17 ± 10.02	0.43
Women, *n* (%)	12 (32.4)	48 (46.6)	0.18
Baseline			
MMSE	27.00 ± 2.93	25.20 ± 4.18	0.006
MoCA	22.77 ± 3.77	20.73 ± 5.74	0.019
Disease duration	–	2.69 ± 2.23	–
UPDRS-II	–	8.50 ± 8.59	–
UPDRS-III	–	22.74 ± 9.26	–
1-year follow-up			
MMSE	27.14 ± 3.21	24.89 ± 5.59	0.004
MoCA	24.33 ± 4.86	20.85 ± 6.50	0.001
UPDRS-II	–	11.29 ± 6.50*	
UPDRS-III	–	21.43 ± 9.41	

HC, healthy control; MMSE, Mini-Mental State Examination; MoCA, Montreal Cognitive Assessment; PwP, people with Parkinson's disease; UPDRS, Unified Parkinson's Disease Rating Scale.
*$p<0.05$ between baseline and 1-year follow-up.

MMSE, scores. Among PwP, no significant changes in UPDRS-III, MMSE, and MoCA scores were noted between baseline and follow-up; however, UPDRS-II scores significantly increased, which indicated the deterioration of life activities.

Changes in plasma EV α-synuclein, tau, and Aβ1-42 and cognition in PwP and HCs

Compared with baseline plasma EV α-synuclein, tau, and Aβ1-42, the difference of these three plasma EV target proteins was significantly higher in PwP than in HCs (Figure 2(a)–(c)). In general, for PwP, plasma EV tau, Aβ1-42, and α-synuclein levels tended to increase at follow-up (median change in plasma EV tau: 1.71 pg/ml, Aβ1-42: 0.21 pg/ml, α-synuclein: 0.016 pg/ml), whereas for HCs, these markers tended to remain unchanged or decrease (median change in plasma EV tau: –0.27 pg/ml, Aβ1-42: –0.01 pg/ml, α-synuclein: –0.027 pg/ml). Significant differences were found in the change in plasma EV α-synuclein ($p=0.008$), tau ($p<0.001$), and Aβ1-42 ($p=0.016$) between PwP with HCs. There was no significant difference in the change of plasma EV tau and Aβ1-42 between PwP at H&Y stage III with stage I/II. However, there was a trend of greater increase of plasma EV α-synuclein in PwP at stage III (stage I/II: 0.0056 ± 0.0482, stage III: 0.0466 ± 0.0580 pg/ml, $p=0.07$) (Supplementary Table 1).

Correlation of changes in plasma EV α-synuclein, tau, and Aβ1-42 with changes in clinical motor and cognition

The changes in plasma EV α-synuclein, tau, and Aβ1-42 did not significantly correlate with the changes in clinical scores, including UPDRS-II and III, MMSE, and MoCA scores after adjustment for age, sex, disease duration, and baseline assessment scores (Table 2). Considering the huge heterogeneity of clinical progression in PwP, PwP were grouped based on the disease progression speed, as determined in clinical assessments.

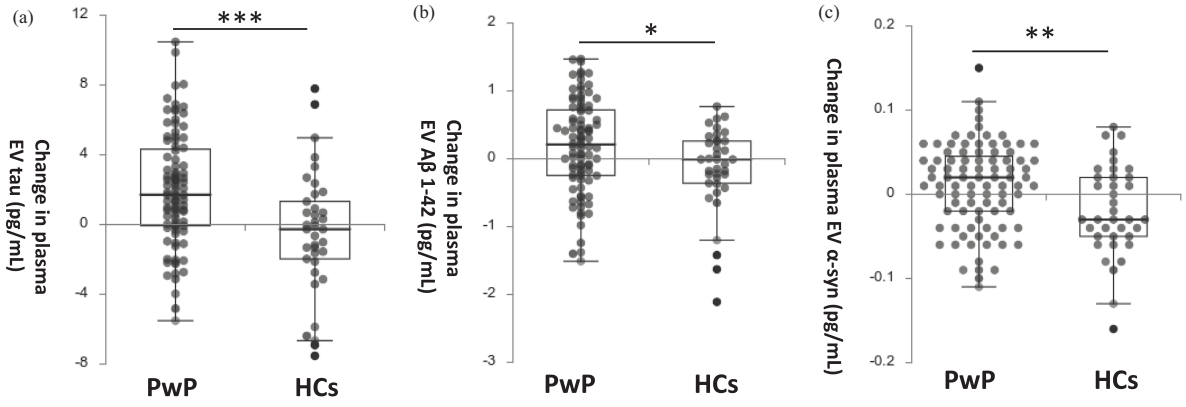

Figure 2. Changes in plasma extracellular vesicle tau (a), β-amyloid 1-42 (Aβ1-42) (b), and α-synuclein (α-syn) (c) between baseline and follow-up in people with Parkinson's disease (PwP) and healthy controls (HCs).
Data are presented as median (interquartile range).
*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

It was found that for PwP with greater annual progression in the PIGD subscore (fourth quartile, increase equal or more than 0.25 point of PIGD subscore), increases in plasma EV α-synuclein were significant between baseline and follow-up after adjusting age and disease duration ($p = 0.025$). There was no significant change of plasma EV α-synuclein in PwP without rapid PIGD subscore increase (Figure 3).

Elevated baseline plasma EV α-synuclein, tau, or β-amyloid and PD progression

To investigate the prediction of worse disease condition using any one of the baseline plasma EV proteins of α-synuclein, tau, and Aβ1-42, PwP were grouped with the median baseline plasma EV α-synuclein, tau, and Aβ1-42 levels as the cut-off. Only high baseline plasma EV tau levels significantly predicted worse disease condition. Repeated-measures of ANCOVA was conducted to obtain the estimated marginal means (EMM) of each clinical parameters at baseline and follow-up of PwP with high or low baseline plasma EV tau, which was calculated with a constant age, sex, disease duration, and H&Y stage (for cognition) in each individual. PwP with high baseline plasma EV tau exhibited significantly worse of EMM in PIGD subscore and MMSE at follow-up (Figure 4(a) and (b)). Regarding MMSE decline, applying the identical cut-off value of plasma EV tau (similar to the median levels applied for grouping PwP), HCs with high baseline plasma EV tau levels did not exhibit significantly greater MMSE decline (Supplementary Figure 1). Finally, PwP with high baseline plasma EV tau levels had significantly worse EMM of executive/visuospatial function at follow-up with the adjustment of age, sex, and disease duration, which was assessed by MoCA Part I (Figure 4(c)).

Elevations in baseline levels of all three plasma EV pathognomonic proteins predict worse PD conditions at follow-up

Finally, 24 of 103 PwP had increased levels of all three plasma EV biomarkers (above the median) at baseline were identified. Among all PwP, PwP with increased levels of all three EV biomarkers had worse clinical presentation in terms of the EMM of UPDRS-II scores at follow-up, whereas EMM of PIGD subscore and MMSE scores at both baseline and follow-up. Moreover, the differences of EMM in UPDRS-II scores, PIGD subscore, and MMSE scores between PwP with increased levels of all three EV biomarkers and the other PwP increased during follow-up with the adjustment of age, sex, disease duration, and H&Y stage (MMSE only), indicating faster clinical deterioration (Figure 5(a)–(c)).

Discussion

We demonstrated that the annual changes in plasma EV α-synuclein, tau, and Aβ1-42 levels were significantly different between PwP and HCs, and that the increase in these proteins among PwP was not age-related. Although the changes in the absolute values of plasma EV

Table 2. Association between the changes in plasma extracellular vesicle (EV) pathognomonic proteins with the changes in clinical assessments in people with Parkinson's disease after adjustment for disease duration and baseline corresponding clinical assessment.

	Changes in plasma extracellular vesicle		
	tau	**Aβ 1-42**	**α-synuclein**
Changes in			
UPDRS-II	−0.002 (0.981)	0.018 (0.863)	0.042 (0.684)
UPDRS-III	0.113 (0.269)	0.105 (0.308)	0.134 (0.208)
MMSE	0.083 (0.425)	0.036 (0.732)	−0.111 (0.283)
MoCA	0.050 (0.649)	0.039 (0.362)	0.106 (0.334)

MMSE, Mini-Mental State Examination; MoCA, Montreal Cognitive Assessment; UPDRS, Unified Parkinson's Disease Rating Scale. Data are presented as standardized beta value (*p*-value).

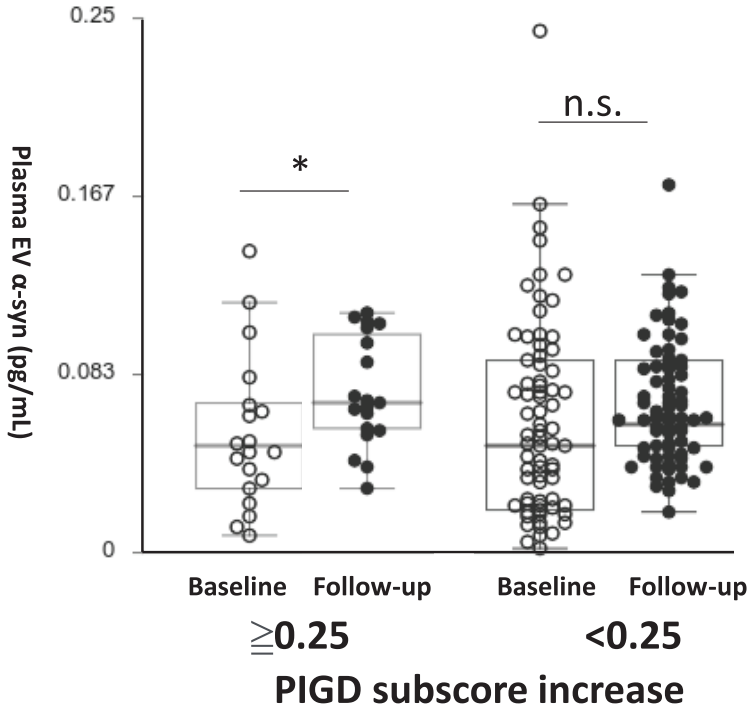

Figure 3. Changes in plasma extracellular vesicle α-synuclein (α-syn) in subgroup PwP [postural instability and gait disturbance (PIGD) subscore increase ≧0.25].
Data are presented as median (interquartile range). n.s., non-significant.
*$p < 0.05$.

α-synuclein, tau, or Aβ1-42 did not correlate with the changes in UPDRS-II/III, MMSE, and MoCA scores, elevated baseline plasma EV tau levels (above the median) could identify PwP at a high risk of disease progression. Furthermore, the elevated baseline value of all three plasma EV biomarkers was a good indicator of rapid deterioration in life activity and cognition. To the best of our knowledge, this study is the first to report the dynamic changes in plasma EV α-synuclein, tau, and Aβ1-42 in PwP and to use these biomarkers to predict disease progression.

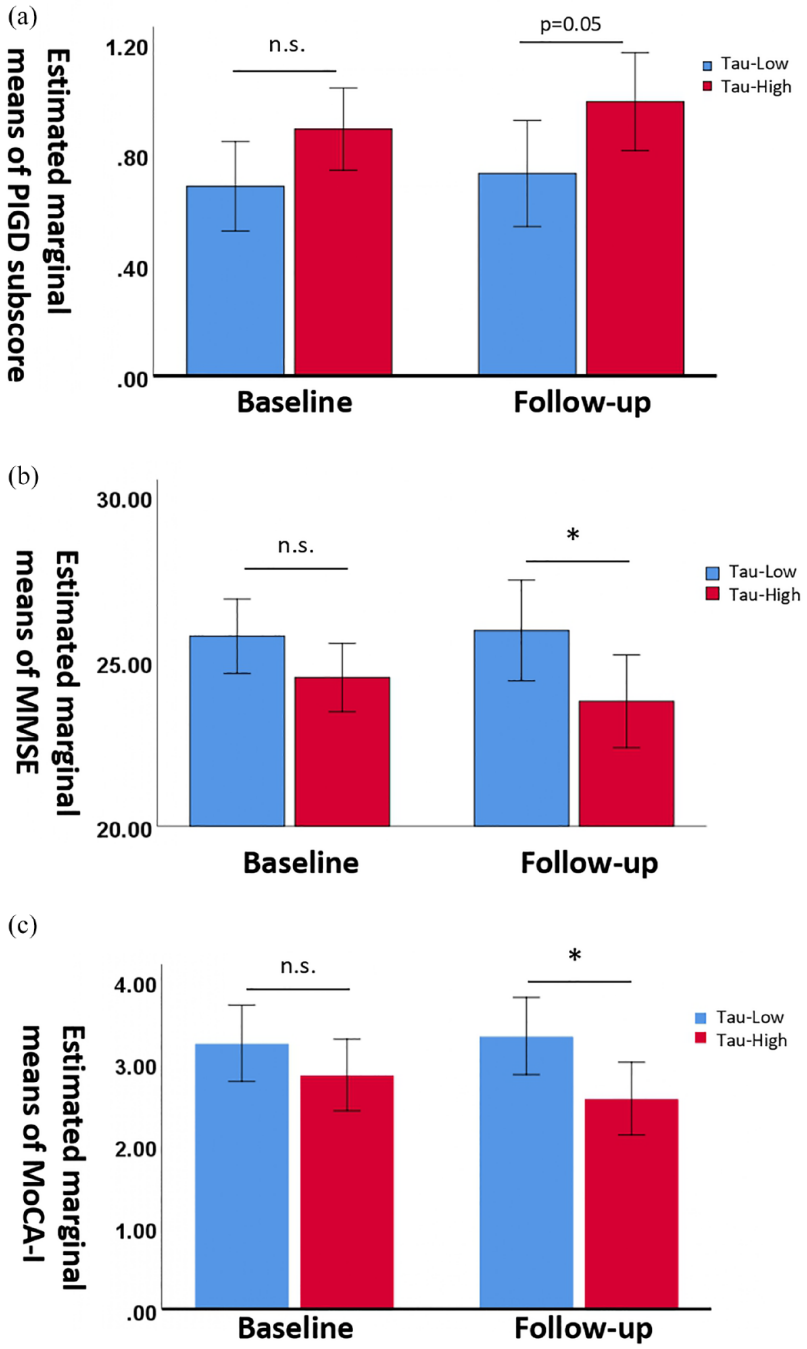

Figure 4. Changes in estimated marginal means obtained from constant age, sex, and disease duration of postural instability and gait disturbance (PIGD) subscore (a), Mini-Mental Status Examination (MMSE) (b), and Montreal Cognitive Assessment (MoCA) Part I (c) in people with Parkinson's disease with/without elevated baseline plasma extracellular vesicle (EV) tau (above the median).
Data were presented as mean with 95% confidence interval. n.s., non-significant.
*$p < 0.05$, **$p < 0.01$.

PD is a neurodegenerative disease with considerable interindividual variation in progression. According to the ongoing Parkinson Progression Markers Initiative cohort study, there were about one-third of untreated PwP progressed rapidly during the first 2 years of follow-up, distinct from

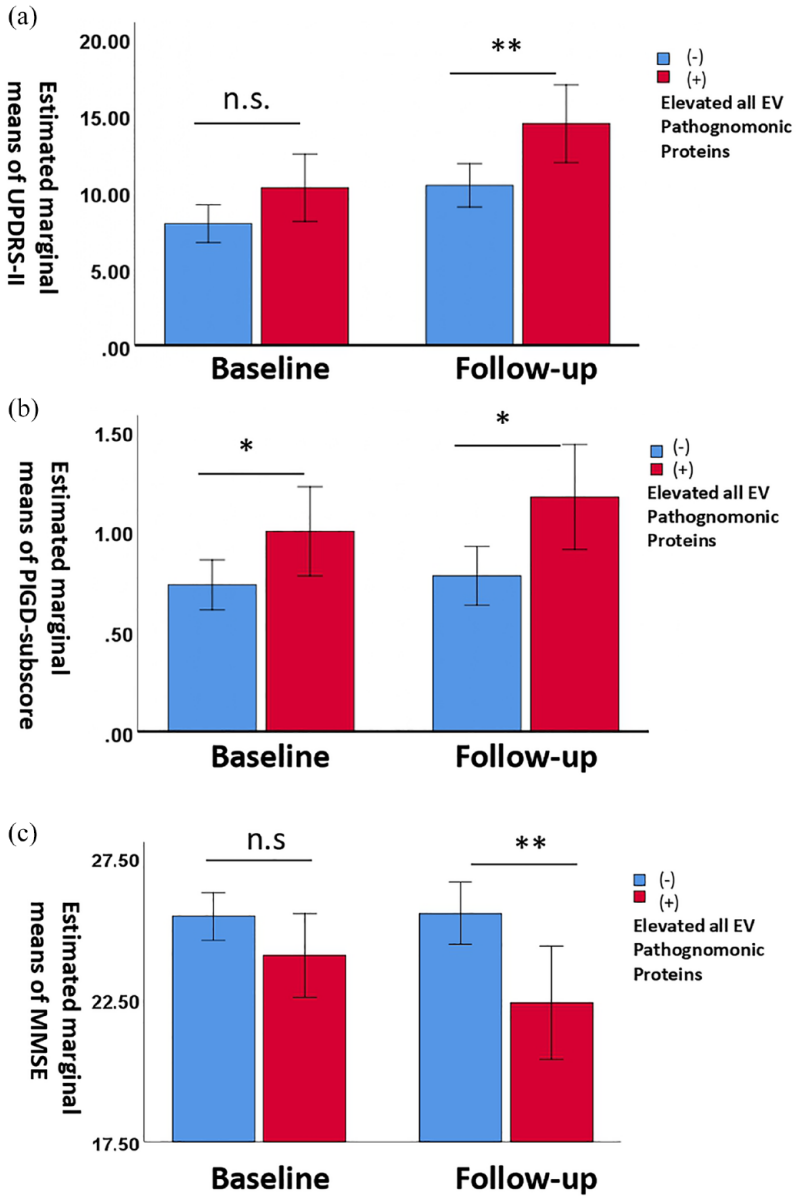

Figure 5. Changes in estimated marginal means obtained from constant age, sex, and disease duration of Unified Parkinson's Disease Rating Scale (UPDRS)-II (a), postural instability and gait disturbance (PIGD) subscore (b), and Mini-Mental Status Examination (MMSE) (c) in PwP with/without elevated baseline all three plasma EV pathognomonic proteins (above the median).
Data were presented as mean with 95% confidence interval. n.s., non-significant.
*$p < 0.05$, **$p < 0.01$.

the slowly progressive course in the rest of PwP.[32] Several factors are associated with rapid progression, but evidence on the use of blood biomarkers of rapid disease progression is limited. However, the clinical presentations of PD are heterogeneous; for example, the severity of different motor symptoms[33] and variable NMSs[6] do not progress in parallel and the impact also varies. For instance, among the motor symptoms, the progression of PIGD and AR, rather than tremors, causes substantial impairment in functional capability and quality of life.[34–36] Thus, a separate subscale should be developed in UPDRS-III (the most popular assessment of the motor symptoms of PD) to assess tremors, AR, and PIGD. UPDRS-II is also a crucial assessment despite the low

interest from researchers compared with their use of UPDRS-III. It reflects motor function in daily living, which correlates with disability and activities of daily living without the interference of temporary drug effects, as in UPDRS-III.[37,38] Regarding NMSs, cognitive impairment is one of the most devastating problems for PwP; it severely undermines the functional capability and quality of life of both PwP and their caregivers.[39–42] We focused on the progression of motor symptoms and cognition in PwP and analyzed UPDRS-I/II/III, MMSE, and MoCA scores. The tremor, AR, and PIGD scores in UPDRS-III and the seven subitems of MoCA provide more information on disease progression.

Tauopathy is widely found in postmortem PwP brains,[43] and tau-related neurofibrillary tangles are prevalent in PwP with dementia.[44] A meta-analysis reported higher CSF tau levels in PwP with dementia than in those without cognitive impairment.[45] Our previous study demonstrated that although plasma EV tau levels were not different between PwP with HCs, they were significantly higher in PwP with cognitive impairment or dementia than in PwP with normal cognition. Moreover, compared with other factors, such as plasma EV α-synuclein and Aβ1-42, age, and sex, plasma EV tau is the most-weighted factor for determining cognitive impairment in PwP.[23] In the present study, plasma EV tau increased in PwP at follow-up, but not in HCs, indicating its role in brain pathology. However, among PwP, higher baseline plasma EV tau is also associated with greater disease progression in terms of not only cognition (MMSE and executive/visuospatial function in MoCA) but also motor performance (PIGD and UPDRS-II). The rapid progression in PIGD for PwP with high baseline plasma EV tau may be secondary to cognitive decline or nondopaminergic degeneration. Gait and postural problems are complex issues in PD, with the suspected involvement of motor, cognition, and mood aspects.[46] Cholinesterase inhibitors, which delay cognitive decline in PwP, also improve gait.[47] Both gait disturbance and dementia are crucial factors affecting functional disability in PwP.[39,40] However, tauopathy may directly influence motor symptoms; for example, phosphorylated tau in the brain facilitates α-synuclein accumulation – resulting in further motor dysfunction.[48]

Plasma EV α-synuclein has been studied more widely in PwP than in any other protein targets because α-synuclein plays a critical role in PD. It can be used to differentiate PwP from HCs and people with atypical parkinsonisms.[22] In addition, it has been found that the plasma neuronal exosome α-synuclein was associated with the UPDRS I and the summation of I/II/III cross-sectionally, which may indicate the reflection of α-synuclein burden. Besides, the increase of neuronal exosomal α-synuclein is significantly associated with progression of motor symptoms in PwP, whereas high or low baseline neuronal exosomal α-synuclein cannot distinguish between PwP with/without progression of motor phenotype.[19] Nevertheless, this longitudinal study was obtained from small group of PwP ($n = 18$ in total), and further study is warranted to validate this result. On the other hand, the results of a comparison between PwP and HCs using other body fluid EV α-synuclein are inconsistent.[49,50] The present study demonstrated that the annual changes in plasma EV α-synuclein were significantly higher in PwP than in HCs. In addition, PwP with the baseline PIGD subtype had significant increases in plasma EV α-synuclein, and PwP with greater deterioration in the PIGD subscore exhibited higher increases in plasma EV α-synuclein. These findings indicate that plasma EV α-synuclein is a potential PIGD progression biomarker. As the most devastating motor subtype of PD, PIGD is associated with falls,[51] dementia,[52] and mortality.[53] Plasma EV α-synuclein as a biomarker of PIGD progression may help identify PwP for optimal treatment. Finally, Aβ1-42 has been traditionally thought to be related more to Alzheimer's disease (AD) than to PD. However, because PD dementia may interact with AD pathology, the role of Aβ1-42 in PD, especially in cognition, has been studied.[43] Our previous study revealed that plasma EV Aβ1-42 levels were significantly different among PwP with normal cognition, mild cognitive impairment, or dementia. It is also the second weighted factor that is used to identify the PD cognitive impairment.[23] The present study revealed a significant difference in the changes in plasma EV Aβ1-42 in PwP compared with HCs, and PwP with greater MMSE decline had significantly higher baseline and follow-up plasma EV Aβ1-42 levels, and the difference between with PwP and HCs increased at the 1-year follow-up.

Our data indicated that each of the three plasma EV pathognomonic proteins can predict PD progression. However, considering cerebral

multimorbidity and the heterogeneous nature of PD,[54] a combination of several biomarkers achieves better prediction. This panel is more likely to identify PwP with a higher risk of rapid progression. Here, we grouped approximately one-fourth of PwP who had elevated levels of all three plasma EV pathognomonic proteins at baseline. They tended to have worse motor and cognition function at baseline compared with the remaining PwP. At the 1-year follow-up, their progression was faster, and the difference in UPDRS-II, MMSE, and PIGD scores widened. These PwP are at a high risk of rapid progression and may therefore be suitable candidates for intensive treatments, such as cognitive rehabilitation, gait training, and inclusion in clinical trials of neuroprotective therapy.

The major strength of the present study was the demonstration of temporary changes in plasma EV α-synuclein, tau, and Aβ 1-42 in PwP, and their association with clinical progression. Previous cross-sectional studies have provided evidence supporting the use of single plasma EV pathognomonic proteins or a panel of these proteins as diagnostic biomarkers of PD or segregating biomarkers of PD cognitive impairment. However, because of the development of multiple diagnostic tools for PD and the high accuracy of clinical diagnosis by experienced neurologists or movement disorder specialists,[55] the prediction of disease progression is currently the most urgent issue for both PwP and clinicians. Temporal information on plasma EV biomarkers from clinical assessments may facilitate the identification of PwP with a high risk of progression. We also investigated the three major protein targets and the progression of not only motor symptoms but also cognition. Considering the role of AD pathology in PD and the significance of cognitive decline in PD progression, examining two cardinal AD pathognomonic proteins, namely, tau and Aβ1-42, and the changes in cognition in this cohort enriched the application of plasma EV biomarkers for PD. Cognitive decline is a crucial marker of disease progression,[56–59] and disease modification trials have investigated the efficacy of treatments for halting cognitive decline.[60–62] Our results may guide the selection of study candidates in future clinical trials and monitoring the treatment response.

This study has some limitations. The present PD cohort was mono-centric and small scale, and lack of the information, such as olfactory test, neuroimaging, motor fluctuation, dyskinesia, and full spectrum of NMSs evaluation. The results form our PD cohort required further validation. Regarding the HCs, despite the exclusion of people with dementia, some of them may be at mild cognitive impairment (MCI) status. Besides, there was no disease control, such as people with AD or atypical Parkinsonism. The 1-year follow-up is short and may not demonstrate the full scale of progression. In addition, MMSE and MoCA may lack sensitivity to detect trivial changes in cognition, especially in PwP with PD-MCI. The International Movement Disorder Society recommends several cognitive assessment batteries for the diagnosis of PD-MCI,[63] which are more sensitive for detecting the progression of cognitive impairment. However, the completion of the assessments is time consuming, and the utilization of MMSE and MoCA is an acceptable compromise. The diagnostic accuracy of MoCA for PD-MCI has been confirmed, and the whole test is less time consuming.[64] Moreover, MMSE and MoCA can be conducted by trained medical staff, whereas the comprehensive neuropsychological tests are to be conducted by board-certified neuropsychologists. Another limitation is the assessment of motor symptoms; when administering UPDRS-III during the 'on' period of time, dopaminergic medications may mask the severity of symptoms; thus, UPDRS-III may fail to represent disease progression. In addition, there was no restriction of the adjustment of dopaminergic medications, which may also affect the evaluation of motor performance. In fact, in the present study, for overall PwP, no significant changes were noted in total UPDRS-III scores. However, an average 3-point increase was noted in the UPDRS-II score, implying the progression of motor symptom-related functional disability. UPDRS-II is less affected than UPDRS-III by temporal factors, such as on or off. In the present study, PwP with an elevation in baseline plasma EV tau alone or in all three pathognomonic proteins had significant deterioration in UPDRS-II, thereby indicating the application of these pathognomonic proteins as biomarkers for predicting motor progression.

Conclusion

The present study used a follow-up PD cohort to demonstrate that the changes in plasma EV α-synuclein, tau, and Aβ 1-42 were significantly

different between PwP and HCs. An elevation of tau alone or all three plasma EV proteins could predict progression of motor and cognition dysfunction at the 1-year follow-up. These results indicate the association of plasma EV α-synuclein, tau, and Aβ 1-42 with the brain pathology in PwP. Future studies should include longer follow-up durations to establish the prediction efficacy.

Declarations

Ethics approval and consent to participate

This study was approved by the Joint Institutional Review Board of Taipei Medical University (TMU-JIRB approval nos. 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 authorship.

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Availability of data and materials

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

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Supplemental material

Supplemental material for this article is available online.

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