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Parkinson's Disease with Fatigue: Clinical Characteristics and Potential Mechanisms Relevant to α-Synuclein Oligomer

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Background and Purpose The aim of this study was to identify the clinical characteristics and potential mechanisms relevant to pathological proteins in Parkinson's disease (PD) patients who experience fatigue.

Methods PD patients (n=102) were evaluated using a fatigue severity scale and scales for motor and nonmotor symptoms. The levels of three pathological proteins—α-synuclein oligomer, β-amyloid $(A\beta)_{1-42}$, and tau—were measured in 102 cerebrospinal fluid (CSF) samples from these PD patients. Linear regression analyses were performed between fatigue score and the CSF levels of the above-listed pathological proteins in PD patients.

Results The frequency of fatigue in the PD patients was 62.75%. The fatigue group had worse motor symptoms and anxiety, depression, and autonomic dysfunction. The CSF level of α-synuclein oligomer was higher and that of $A\beta_{1-42}$ was lower in the fatigue group than in the non-fatigue group. In multiple linear regression analyses, fatigue severity was significantly and positively correlated with the α-synuclein oligomer level in the CSF of PD patients, after adjusting for confounders.

Conclusions PD patients experience a high frequency of fatigue. PD patients with fatigue have worse motor and part nonmotor symptoms. Fatigue in PD patients is associated with an increased α-synuclein oligomer level in the CSF.

Key Words Parkinson's disease, fatigue, motor symptoms, nonmotor symptoms, α-synuclein oligomer, cerebrospinal fluid.

INTRODUCTION

Parkinson's disease (PD) is a progressive neurodegenerative disorder with typical motor symptoms and various nonmotor symptoms, including neuropsychiatric symptoms, autonomic dysfunctions, abnormal sense, sleep disorders, and fatigue. According to the International Classification of Diseases (ICD)-10, the signs and symptoms of fatigue include asthenia, debility, general physical deterioration, lethargy, and tiredness.[1] Currently there is less attention paid to fatigue than to other nonmotor symptoms of PD. The PaRkinson And non Motor symptOms study found that fatigue was present in 58.1% of 1072 PD patients, ranging from 37.7% in the early stage to 81.6% in the advanced stage.[2] Importantly, about 50% of PD patients reportedly consider fatigue to be one of the most disabling nonmotor symptoms.[3] Fatigue dramatically compromises the activities of daily living and the quality of life for PD patients.[4,5]

While the epidemiology of PD with fatigue has been investigated, how fatigue is related to demographic information, motor symptoms, and other nonmotor symptoms of PD is still uncertain, with uncertain inclusion criteria having been applied. Exploring these issues may identify the most important clinical characteristics and underlying factors of fa-

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tigue, thereby yielding potential therapeutic targets for PD with fatigue.

While fatigue is very common in PD patients and impacts their quality of life, little is known about the underlying mechanisms. The presence of any correlations between fatigue and pathological proteins in the cerebrospinal fluid (CSF) is still unclear. α-synuclein oligomer is the main component of Lewy bodies—the pathological hallmark of PD—in both neuronal bodies and neurites. β-Amyloid $(A\beta)_{1-42}$ is the core component of neuroinflammatory plaques in the extracellular space, and phosphorylated tau (P-tau) is the main component of neurofibrillary tangles in the brains of Alzheimer's disease (AD) patients. Pathological changes associated with AD are also found in PD brains;[6] for example, PD patients without dementia have a large $A\beta_{1-42}$ burden in the brain.[7-9] Moreover, increasing attention is being paid to $A\beta_{1-42}$ and tau burdens in both early and late PD.[10,11] These data imply that PD may be a complex clinicopathological entity. Past studies have revealed relationships between the above-mentioned pathological proteins and motor symptoms of PD. For example, it has been reported that decreased CSF levels of α-synuclein and total tau (T-tau) are associated with an increased severity of motor symptoms in PD patients.[11] α-synuclein is also related to nonmotor symptoms such as olfactory dysfunction.[12] Our previous work has demonstrated that an increased CSF α-synuclein oligomer level is associated with rapid-eye-movement-sleep behavior disorder (RBD).[13] Moreover, tau and Aβ are all involved in PD with dementia.[14] However, the relationships between fatigue and the above-mentioned pathological proteins—α-synuclein oligomer, $A\beta_{1-42}$, T-tau, and P-tau—are uncertain.

In this study we investigated the clinical characteristics and potential mechanisms relevant to pathological proteins in PD patients with fatigue, with the aim of identifying clues for the early identification of and effective clinical interventions for PD with fatigue.

METHODS

Subjects

Patients with PD. In total, 102 PD patients were recruited from the neurodegenerative outpatient clinics in the Department of Geriatrics and Neurology, Beijing Tiantan Hospital, Capital Medical University. Demographic information including sex, age, and education level, and disease duration as well as levodopa equivalent daily doses was recorded (Table 1). Among the 102 PD patients, 51 cases (50.0%) were male and 51 (50.0%) were female with ages ranging from 30 to 85 years (60.4±10.4 years, mean±SD). The disease duration varied from 1 month to 22 years, with a median of 2.1 years [interquartile range (IQR): 4.3 years]. The demographic characteristics are listed in Table 1. Patients were diagnosed with PD according to criteria of Parkinson's UK Brain Bank.[15] PD patients with any type of dementia (according to criteria of the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition) and systemic diseases including hypertension, anemia, hepatosis, heart failure, pulmonary disorders, chronic liver/renal failure, severe hypothyroidism, and diabetes were excluded. PD patients with an Epworth Sleepiness Scale score of >6[16] or an Apathy Scale score of ≥14 were excluded.[17]

Control subjects. In total, 31 age-matched controls from Beijing Tiantan Hospital were selected based on the following criteria: 1) no essential tremor, PD, secondary parkinsonism, or Parkinson-plus syndrome; 2) no systemic diseases affecting sleep or fatigue, such as hypertension, anemia, hepatosis, heart failure, pulmonary disorders, chronic liver/renal failure, severe hypothyroidism, diabetes, or epilepsy history; 3) no dysarthria or mental illness that affect expression; 4) no obvious apathy, cognitive impairment, or psychiatric symptoms; and 5) no alcohol or drug abuse. Six control subjects whose CSF levels of α-synuclein oligomer were below 0.078 ng/mL were excluded.

The controls were also patients, but their diseases were not related to and did not influence the results of this investigation, such as peripheral neuropathy and headache caused by high intracranial pressure.

Assessment of fatigue

The Fatigue Severity Scale (FSS) satisfies the criteria of a "recommended" fatigue scale in PD (both for screening and severity rating) because it has been shown to have good psy-

Table 1. Demographic variables in the control, fatigue, and non-fatigue groups

Variable	Control group (*n*=25)	Non-fatigue group (*n*=38)	Fatigue group (*n*=64)	*p*
Male/total [cases/total (%)]	12/25 (48.0)	17/38 (44.7)	33/64 (51.5)	0.45
Age (years, mean±SD)	56.7±11.2	55.9±12.2	60.8±9.9	0.23
Education level [cases/total (%)]				0.12
Primary school and below	8/25 (32.0)	11/38 (28.9)	20/64 (31.2)	
Middle and high school	10/25 (40.0)	20/38 (52.6)	34/64 (53.1)	
Bachelor's degree and above	7/25 (28.0)	7/38 (18.4)	10/64 (15.6)	

chometric properties (including for discriminating between fatigued and non-fatigued patients) in PD patients and has also been used in previous studies.[18] It is a self-administered nine-item fatigue rating scale that encompasses several aspects of fatigue and their impact on the daily functioning of patients. Patients were asked to rate how each item described their fatigue level from 1 ("strongly disagree") to 7 ("strongly agree"). The total FSS score was obtained by dividing the sum of all item scores by 9. When it was used with a cutoff of 4 points, the sensitivity and specificity were 75.5% and 74.5%, respectively. Patients with total FSS scores of >4 points and ≤4 points were classified into the fatigue and non-fatigue groups, respectively.[19]

This study was approved by the review board of Beijing Tiantan Hospital. Written informed consent was obtained from all participants.

Clinical assessments of motor and nonmotor symptoms

The severity of PD was assessed based on the Hoehn and Yahr (H-Y) stage. Motor symptoms were evaluated by Unified Parkinson's Disease Rating Scale (UPDRS) III, in which items 20 and 21 were for tremor, item 22 was for rigidity, items 23−26 and 31 were for bradykinesia, and items 27−30 were for postural and gait abnormalities. The score for each motor symptom was calculated by summing up the score for the relevant items in UPDRS III. Nonmotor symptoms were evaluated using the following scales: Hamilton Depression Scale (HAMD) (24 items for depression), Hamilton Anxiety Scale (HAMA) (14 items for anxiety), Scale for Outcomes in Parkinson's Disease for Autonomic Symptoms (SCOPA-AUT) for autonomic dysfunctions, Mini Mental State Examination for cognitive decline, and Restless Legs Syndrome Rating Scale for restless legs symptoms.

Collection of CSF samples

Antiparkinsonian drugs were withheld for 12−14 hours prior to sampling the CSF. Three milliliters of CSF were obtained using a lumbar puncture between 7 a.m. and 10 a.m. under a fasting condition, and placed in a polypropylene tube. Approximate 0.5 mL volumes of CSF were aliquoted into separate Nunc cryotubes and kept frozen at -80°C until used in assays. The use of a separate aliquot for each measure avoided freeze-thawing and the associated potential degradation of proteins. We analyzed the validity of the measurements of the CSF levels of α-synuclein oligomer, which showed acceptable intrarun and interrun precisions, with coefficients of variation of 8.5% and 9.8%, respectively.

We took the following actions in order to avoid blood contamination resulting in misleading measurements of the CSF α-synuclein oligomer level: 1) we performed the lumbar puncture very carefully to avoid injury; 2) we examined the CSF sample of each PD patient or control subject, and excluded samples if they were red or turbid, or red blood cells were evident; and 3) all CSF samples were centrifuged at 3,000 rpm for 10 minutes to remove cells and debris. Since the limit of detection (analytical sensitivity) for α-synuclein oligomer was 0.078 ng/mL, six control subjects with CSF α-synuclein oligomer levels below 0.078 ng/mL were excluded. Eventually, totals of 102 PD patients and 25 control subjects were recruited into this study.

Detection of pathological proteins

The CSF levels of pathological proteins were determined using an enzyme-linked immunosorbent assay, including those of α-synuclein oligomer, $A\beta_{1\text{-}42}$, T-tau, and tau phosphorylated at the following positions: threonine 181 (P-tau_{181t}), threonine 231 (P-tau_{231t}), serine 396 (P-tau_{396s}), and serine 199 (P-tau_{199s}). CSB-E18033h, CSB-E10684h, and CSB-E12011h kits for measuring α-synuclein oligomer, $A\beta_{1\text{-}42}$, and T-tau, respectively, were obtained from CUSABIO (Wuhan, China), while KHB7031, KHB7041, KHB8051, and KHO0631 kits for measuring P-tau_{396s}, P-tau_{199s}, P-tau_{231t}, and P-tau_{181t}, respectively, were obtained from Invitrogen (Carlsbad, CA, USA). The levels of α-synuclein oligomer, $A\beta_{1\text{-}42}$, T-tau, P-tau_{181t}, P-tau_{231t}, P-tau_{396s}, and P-tau_{199s} were measured using a quantitative sandwich enzyme immunoassay.

Data analyses

Statistical analyses were performed with SPSS Statistics (version 20.0, SPSS Inc., Chicago, IL, USA). Demographic information was compared among the control, fatigue, and non-fatigue groups. Motor and nonmotor symptoms were compared between the fatigue and non-fatigue groups. The CSF levels of pathological proteins were detected and compared among the control, fatigue, and non-fatigue groups. Continuous variables that were normally distributed are reported as mean±SD values, with two-tailed *t*-tests used for two-group comparisons and ANOVAs used for three-group comparisons. Continuous variables that were not normally distributed are reported as median (IQR) values, with the Kruskal-Wallis test used for both two- and three-group comparisons.

Multiple linear regression analyses were used to investigate the associations between the scores for fatigue severity and the CSF levels of pathological proteins in three models: Model 1, unadjusted; Model 2, adjusted for age, sex, disease duration, and levodopa equivalent daily doses; and Model 3, additionally adjusted for UPDRS III, rigidity, bradykinesia, HAMA, HAMD, and SCOPA-AUTO scores. Probability values of $p<0.05$ were considered to indicate statistically sig-

nificant differences.

RESULTS

Frequency of fatigue, demographic information, motor symptoms, and nonmotor symptoms in the fatigue and non-fatigue groups

Fatigue was present in 64 (62.7%) of the 102 PD patients. The median fatigue severity scores in the fatigue and non-fatigue groups were 5.7 (IQR=1.8) and 2.2 (IQR=1.89) points, respectively (Table 2). Ten (15.6%) of the 64 PD patients with fatigue had experienced fatigue before the onset of motor symptoms. The fatigue group showed a more advanced H-Y stage, higher total UPDRS III scores, and higher scores for rigidity and bradykinesia according to UPDRS III relative to the non-fatigue group. The fatigue group also exhibited higher HAMA, HAMD, and SCOPA-AUTO scales than the non-fatigue group, suggesting that the fatigued individuals had worse anxiety, depression, and autonomic dysfunctions. Age, sex, education level, disease duration, and levodopa equivalent daily dose did not differ between the fatigue and non-fatigue groups (Table 2).

Comparisons of pathological protein levels in the CSF among the control, fatigue, and non-fatigue groups

Table 3 compares the CSF levels of α-synuclein oligomer, $A\beta_{1\text{-}42}$, T-tau, P-tau_{231t}, P-tau_{181t}, P-tau_{199s}, and P-tau_{396s} among the control, fatigue, and non-fatigue groups. The level of α-synuclein oligomer in the CSF of the fatigue group was strikingly elevated relative to the control and non-fatigue groups ($p<0.017$), while the CSF level of $A\beta_{1\text{-}42}$ in the fatigue group was remarkably decreased relative to the control group ($p<0.017$).

The levels of P-tau_{231t}, P-tau_{181t}, P-tau_{199s}, and P-tau_{396s} in the CSF were higher in both the fatigue and non-fatigue groups than in the control group, while the T-tau level was markedly higher in the fatigue group than in the control group ($p<0.017$). The CSF levels of P-tau_{231t}, P-tau_{181t}, P-tau_{199s}, and P-tau_{396s} did not differ significantly between the fatigue and non-fatigue groups (Table 3).

Correlations between fatigue severity and the CSF levels of pathological proteins in PD patients

Multiple linear regression models were constructed in which the FSS score was set as a dependent variable in order to investigate the associations between the score for the fatigue severity and the CSF levels of pathological proteins (Models 1, 2, and 3).

The multiple linear regression equation was statistically significant for Model 1 ($F=16.998$, $p<0.0001$), Model 2 ($F=11.576$, $p=0.002$), and Model 3 ($F=11.12$, $p=0.003$). Our data indicate that the α-synuclein oligomer level in the CSF was the only factor influencing the FSS score in the PD group [regression coefficient=0.78, $p=0.003$]. The association remained significant after adjusting for each group of confounders. Among the demographic factors and clinical vari-

Table 2. Clinical variables associated with fatigue in Parkinson's disease (PD)

Variable	Non-fatigue group (n=38)	Fatigue group (n=64)	p
Disease duration [years, median (IQR)]	2.1 (3.1)	3.2 (4.3)	0.05
Fatigue severity [score, median (IQR)]	2.2 (1.9)	5.7 (1.8)	<0.001*
Levodopa equivalent daily dose (mg, mean±SD)	302.8±109.4	313.2±113.5	0.347
Motor symptoms			
H-Y stage (mean±SD)	1.8±0.7	2.2±0.8	<0.001*
UPDRS III scores [median (IQR)]	21.0 (17.1)	26.5 (17.0)	<0.001*
Tremor	4.0 (4.0)	4.0 (4.0)	0.49
Rigidity	1.0 (3.0)	2.0 (3.0)	<0.001*
Bradykinesia	9.0 (8.0)	10.0 (8.0)	<0.001*
Postural and gait abnormalities	4.0 (3.0)	4.0 (3.0)	0.06
Nonmotor symptoms			
HAMD score (mean±SD)	38.0±9.2	56.0±14.6	<0.001*
HAMA score (mean±SD)	29.0±8.7	36.0±13.2	<0.001*
SCOPA-AUTO score (mean±SD)	32.6±8.9	38.7±8.1	<0.001*
MMSE score (mean±SD)	26.8±4.0	26.4±3.9	0.186
RLSRS score [median (IQR)]	3.0 (15.8)	5.0 (18.0)	0.289

*$p<0.01$.
HAMA: Hamilton Anxiety Scale, HAMD: Hamilton Depression Scale, H-Y: Hoehn and Yahr, IQR: interquartile range, MMSE: Mini Mental State Examination, RLSRS: Restless Legs Syndrome Rating Scale, SCOPA-AUTO: Scale for Outcomes in Parkinson's Disease for Autonomic Symptoms, UPDRS III: Unified Parkinson's Disease Rating Scale, Part III.

Table 3. Cerebrospinal fluid (CSF) levels of pathological proteins in the control, fatigue, and non-fatigue groups

Protein	Control group (*n*=25)	Non-fatigue group (*n*=38)	Fatigue group (*n*=64)	P	P1	P2	P3
α-synuclein oligomer [ng/mL, median (IQR)]	0.08 (0.02)	0.29 (0.19)	0.70 (0.32)	<0.001†	<0.001†	<0.001†	0.004†
$A\beta_{1-42}$ [ng/mL, median (IQR)]	83.3 (49.2)	41.9 (29.4)	21.6 (24.0)	<0.001†	0.021	<0.001†	0.030
T-tau [pg/mL, median (IQR)]	34.2 (117.5)	96.9 (112.8)	105.7 (119.9)	<0.001†	0.052	<0.001†	0.036
P-tau_{231t} (pg/mL, mean±SD)	78.1±37.8	116.6±59.8	138.2±60.4	0.018*	0.016*	0.011*	0.442
P-tau_{181t} (pg/mL, mean±SD)	31.4±15.8	66.3±6.3	78.9±28.8	0.022*	0.026*	<0.001†	0.224
P-tau_{199s} (pg/mL, mean±SD)	3.3±1.3	6.4±1.5	6.5±1.8	0.012*	0.019*	<0.001†	0.812
P-tau_{396s} (pg/mL, mean±SD)	31.4±15.3	72.0±27.3	78.0±32.4	0.013*	0.018*	<0.001†	0.654

*p<0.05, †p<0.01, ‡p<0.017.
P: Kruskal-Wallis test was used to compare CSF levels of α-synuclein oligomer, $A\beta_{1-42}$, and T-tau among control, fatigue, and non-fatigue groups; p<0.05, statistically significant. ANOVA was used to compare P-tau_{231t}, P-tau_{181t}, P-tau_{199s}, and P-tau_{396s} CSF levels among control, fatigue, and non-fatigue groups; p<0.05, statistically significant. P1: Control group vs. non-fatigue group; Kruskal-Wallis test was used to compare CSF levels of α-synuclein oligomer, $A\beta_{1-42}$, and T-tau between control and non-fatigue groups; p<0.017, statistically significant. Two-tailed t-test was used to compare P-tau_{231t}, P-tau_{181t}, P-tau_{199s}, and P-tau_{396s} CSF levels between the control and non-fatigue groups, p<0.05 was defined as statistically significant. P2: Control group vs. fatigue group; Kruskal-Wallis test was used to compare CSF levels of α-synuclein oligomer, $A\beta_{1-42}$, and T-tau between control and fatigue groups; p<0.017, statistically significant. Two-tailed t-test was used to compare CSF levels of P-tau_{231t}, P-tau_{181t}, P-tau_{199s}, and P-tau_{396s} between control and fatigue groups; p<0.05, statistically significant. P3: Fatigue group vs. non-fatigue group; Kruskal-Wallis test was used to compare CSF levels of α-synuclein oligomer, $A\beta_{1-42}$, and T-tau between fatigue and non-fatigue groups; p<0.017, statistically significant. Two-tailed t-test was used to compare CSF levels of P-tau_{231t}, P-tau_{181t}, P-tau_{199s}, and P-tau_{396s} between fatigue and non-fatigue groups; p<0.05, statistically significant. $A\beta_{1-42}$: β-$amyloid_{1-42}$.

Table 4. Linear regression analyses between CSF pathological proteins and fatigue severity (*n*=102)

	Model I	Model 2	Model 3
α-synuclein oligomer (ng/mL)	0.8 (0.4~1.2)*	0.7 (0.2~1.2)*	0.6 (0.1~1.3)*
$A\beta_{1-42}$ (ng/mL)	8.0 (-7.8~23.8)	7.7 (-10.3~25.0)	7.6 (-9.5~26.2)
T-tau (pg/mL)	-0.3 (-1.2~2.2)	-0.3 (-2.5~5.7)	-2.4 (-2.0~5.1)
P-tau_{231t} (ng/mL)	0.0 (-0.1~0.1)	0.0 (-0.1~0.1)	0.0 (-0.2~0.2)
P-tau_{181t} (ng/mL)	0.0 (-0.2~0.2)	0.0 (-0.2~0.2)	0.0 (-0.5~0.2)
P-tau_{199s} (ng/mL)	0.1 (-2.5~2.6)	-0.5 (-3.8~2.8)	-1.6 (-4.0~2.6)
P-tau_{396s} (ng/mL)	0.0 (-0.2~0.1)	0.0 (-0.2~0.2)	0.0 (-0.3~0.2)

Data β (95% confidence interval) values. Model 1: unadjusted. Model 2: adjusted for age, sex, disease duration, and levodopa equivalent daily doses. Model 3: additionally adjusted for UPDRS III, rigidity, bradykinesia, HAMA, HAMD, and SCOPA-AUTO scores.
*p<0.01.
CSF: cerebrospinal fluid, HAMA: Hamilton Anxiety Scale, HAMD: Hamilton Depression Scale, SCOPA-AUTO: Scale for Outcomes in Parkinson's Disease for Autonomic Symptoms, UPDRS III: Unified Parkinson's Disease Rating Scale, Part III.

ables, an increased CSF level of α-synuclein oligomer was associated with the fatigue severity as assessed by the FSS (Table 4).

DISCUSSION

Fatigue was firstly noted by James Parkinson in 1817 in his original description of PD, but it was not investigated further until very recently. The overall frequency of fatigue (defined as an FSS mean score of >4 points) in this study was 62.5%, indicating that fatigue is a very common nonmotor symptom of PD. This frequency of fatigue among our PD patients is higher than those found in a previous study,[2] which may be due to differences in disease durations.

According to ICD-10, fatigue includes asthenia, debility, general physical deterioration, lethargy, and tiredness.[1] While there is generally considered to be no relationship between fatigue and the severity of motor symptoms,[3] the PD patients with fatigue in the present study showed a more advanced H-Y stage and higher total UPDRS III score (Table 2), demonstrating that fatigue worsens with disease progression and severity.[20-22] Importantly, further analyses of each motor symptom in the PD patients revealed that the scores for rigidity and bradykinesia as evaluated by the corresponding items in UPDRS III were significantly higher in the fatigue group than in the non-fatigue group (Table 2), whereas the scores for tremor and postural and gait abnormalities did not differ between the fatigue and non-fatigue groups. Bradykinesia is caused by the defective preparation of voluntary movement and alteration in movement execution,[23] which may contribute to fatigue in PD patients.[24] No previous study has investigated the relationship between rigidity and fatigue,

and so the present study is the first to reveal that rigidity is related to PD with fatigue. Similar to bradykinesia, rigidity worsens at an annual rate and is an unremitting motor symptom, which together with rigidity[25-27], may contribute to fatigue in PD. There is no correlation between fatigue and tremor. Recent studies suggest that tremor is usually an episodic phenomenon and does not worsen at a fixed rate,[28] suggesting that the pathophysiology underlying tremor differs from those of bradykinesia and rigidity.[29,30] In contrast to a previous report, the scores for postural and gait abnormalities are higher in PD patients with fatigue than in those without fatigue.[31] The present study found no relationship between postural and gait abnormalities and fatigue, which may be due to the different fatigue scales and cutoff points adopted. Additionally, the early stage of PD is characterized by tremor, rigidity, and bradykinesia, with or without axial involvement, while postural and gait abnormalities usually occur at the late stage. Most of the PD patients included in the present study were in the early stage, which may explain the absence of a correlation between fatigue and postural and gait abnormalities. It may therefore be worthwhile to further investigate the relationship between fatigue and postural and gait abnormalities among patients in the late stage. Each individual motor symptom of PD might not be independently correlated with fatigue, but when multiple symptoms are present they may work together and amplify the effect of each other, significantly contributing to PD with fatigue. In this study, the control, fatigue, and non-fatigue groups showed no significant differences in demographic information (including age, sex, and education level), disease duration, and levodopa equivalent daily dose (Table 1), indicating that fatigue is not related to those factors.[32-34]

Previous studies have explored the relationship between fatigue and other nonmotor symptoms of PD, but no definitive conclusions were drawn. This study found that PD patients in the fatigue group had worse depression, anxiety, and autonomic dysfunctions than those in the non-fatigue group (Table 2). Pavese reported that fatigue in PD patients was related to the serotonin (5-HT) system by using ^{11}C-DASB [N,N-dimethyl-2-(2-amino-4-cyanophenylthio) benzylamine].[35] We directly investigated the relationship between fatigue severity and the level of 5-HT in the CSF of PD patients, and found that fatigue severity was negatively and significantly related to the CSF 5-HT level (r=-0.45, p=0.003; unpublished data) (Table 5). Several studies have suggested that serotonergic dysfunction is relevant to the development of fatigue as well as depression and anxiety in PD patients,[36,37] implying that these three symptoms may share the same neurobiological mechanisms.[37] For example, depression and anxiety may precipitate the occurrence of fatigue,[38,39] and depression and autonomic dysfunctions have been found to exacerbate the subjective perception of fatigue.[40] When Lewy bodies are deposited in the lower raphe nuclei, magnocellular portions of the reticular formation, and the locus coeruleus of PD patients, they may manifest as autonomic dysfunction and fatigue, both of which may share the same pathological mechanism.[41] Our previous study showed that the FSS score is positively correlated with the SCOPA-AUTO score (r=058, p=0.035, unpublished data) (Table 5). These data are indicative of the strong correlation of fatigue with depression, anxiety, and autonomic dysfunction. A few studies have explored the relationship between fatigue and cognitive impairment, as well as restless legs symptom. The present study found no significant difference in cognitive function and restless legs symptom between the fatigue and non-fatigue groups.

Table 5. Correlations of fatigue severity with CSF 5-HT level and autonomic dysfunction score in the PD group

Fatigue severity	*r*	*p*
5-HT (ng/μL)	-0.45	0.003†
SCOPA-AUTO score	0.58	0.035*

*p<0.05, †p<0.01.
CSF: cerebrospinal fluid, PD: Parkinson's disease, SCOPA-AUTO: Scale for Outcomes in Parkinson's Disease for Autonomic Symptoms.

We investigated the potential mechanisms of fatigue in PD by measuring the levels of pathological proteins in the CSF. The pathological hallmark of PD is the formation of α-synuclein-containing Lewy bodies. The oligomer is the most neurotoxic form of α-synuclein in Lewy bodies.[42] α-synuclein oligomer has been observed in the extracellular space,[43] and it may be released from degenerative or dead neurons. An excessive level of the α-synuclein oligomer in the CSF may reflect an elevated level of α-synuclein oligomer in the brain.[44]

In the current study, the CSF level of α-synuclein oligomer in the fatigue group was remarkably enhanced relative to those in the non-fatigue and control groups. In multiple linear regression the FSS score was associated with the elevated α-synuclein oligomer level in the CSF of PD patients. Moreover, even after adjusting for demographic factors and additional clinical variables, the increased CSF level of α-synuclein oligomer was still associated with the fatigue severity as assessed by the FSS. The levels of $A\beta_{1\text{-}42}$, T-tau, P-tau_{231t}, P-tau_{181t}, P-tau_{199s}, and P-tau_{396s}, and the UPDRS III, rigidity, bradykinesia, HAMA, HAMD, and SCOPA-AUTO scores did not enter in the regression equation. These results imply that the increased α-synuclein oligomer in the brain may be the pathogenesis of fatigue in PD patients. A previous study demonstrates that motor symptoms and some nonmotor symptoms, such as cognitive impairment and RBD, are both correlated with the CSF level of α-synuclein oligomer.[10,13,45] The present study is the first to explore the relationship be-

tween PD with fatigue and the level of pathological proteins in the CSF, and it finds that fatigue is correlated with an enhanced level of α-synuclein oligomer in the CSF. A previous study found that missense and multiplication mutations in the α-synuclein gene were linked to clinical and pathological phenotypes in PD,[46] highlighting a direct role of α-synuclein overexpression in the pathogenesis of PD. The CSF level of α-synuclein oligomer has previously been reported to be higher in PD patients than in normal brains.[44,47-49] However, some studies found the opposite result, which may be explained by cerebral accumulation of the α-synuclein oligomer.[50] The patients included in previous studies had a higher H-Y stage (higher than stage 2.5),[51] and an increased CSF α-synuclein level might be a marker of more intense synaptic degeneration in PD.[52] However, most of the PD patients in the present study were from geriatric clinics, and their H-Y stage was 2.05±0.80. Thus, the CSF α-synuclein oligomer level might vary during the course of PD progression: it might be elevated in the early stage of PD and then decreased in the later stage. Therefore, we postulate that degenerative or dead brain cells can release more α-synuclein oligomer into the extracellular spaces of fatigue-related areas, and thereby cause sustained brain cell death, and eventually induce the occurrence of fatigue in PD patients. The results obtained in this study suggest that the α-synuclein oligomer could serve as a useful biomarker indicating the severity of PD with fatigue.

It has been reported that the decreased $A\beta_{1-42}$ level in the CSF is related to memory impairment in PD patients.[8,11,53,54] A reduced $A\beta_{1-42}$ level in the CSF even tends to precede the onset of PD with mild cognitive impairment.[55] Studies have revealed common mechanisms in AD and PD. However, no previous study has investigated the relationship between fatigue and $A\beta_{1-42}$ level in the CSF. The present study is therefore the first to show that the CSF level of $A\beta_{1-42}$ in PD patients with fatigue is significantly decreased relative to control patients. The level of $A\beta_{1-42}$ in the CSF did not differ between the fatigue and non-fatigue groups. However, our analysis of multiple linear regression models revealed no association between fatigue severity and decreased $A\beta_{1-42}$ level. These data imply that $A\beta_{1-42}$ alone might not be associated with fatigue, but it could still combine with other mechanisms to contribute to PD with fatigue. Furthermore, most of patients in our study were in the early stage, and $A\beta_{1-42}$ aggregation might not play a key role in the pathological progression of PD with fatigue at that time.

It has been reported that tau is involved in the mechanisms underlying PD.[56] In the present study the CSF levels of P-tau_{231t}, P-tau_{181t}, P-tau_{199s}, and P-tau_{396s} were significantly higher in both the fatigue and non-fatigue groups than in the control group, but there were no differences between the fatigue and non-fatigue groups. The T-tau level in the CSF did not differ among the control, fatigue, and non-fatigue groups. These data indicate that T-tau, P-tau_{231t}, P-tau_{181t}, P-tau_{199s}, and P-tau_{396s} are more relevant to PD itself than the fatigue symptom that appears with PD.

In summary, the frequency of fatigue in the current PD patients was 62.7%. PD with fatigue was associated with advanced H-Y stage, worse motor symptoms (especially rigidity and bradykinesia), and several worse nonmotor symptoms (e.g., anxiety, depression, and autonomic dysfunctions). The increased level of α-synuclein oligomer in the CSF appears to be associated with fatigue severity in PD patients. This study has highlighted the clinical characteristics and potential mechanisms relevant to α-synuclein oligomer in PD patients with fatigue.

This study was subject to some limitations. Firstly, relatively few CSF samples were analyzed due to the difficulties of obtaining CSF from PD patients, which may have weakened the statistical power of the analyses, such as of the correlation between PD with fatigue and the $A\beta_{1-42}$ level in the CSF. Secondly, this study was designed to identify the clinical characteristics and potential mechanisms relevant to pathological proteins in PD patients who experience fatigue, it is a cross-sectional study, therefore, causal relationships between the levels of pathological proteins in the CSF of PD patients and fatigue could not be determined.

Conflicts of Interest

The authors have no financial conflicts of interest.

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