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RESEARCH ARTICLE

Enzymatic properties and clinical associations of serum alpha-galactosidase A in Parkinson's disease

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Abstract

Objective: Recent studies have revealed an association between Parkinson's disease (PD) and Fabry disease, a lysosomal storage disorder; however, the underlying mechanisms remain to be elucidated. This study aimed to investigate the enzymatic properties of serum alpha-galactosidase A (GLA) and compared them with the clinical parameters of PD. **Methods**: The study participants consisted of 66 sporadic PD patients and 52 controls. We measured serum GLA activity and calculated the apparent Michaelis constant (K_m) and maximal velocity (V_{max}) by Lineweaver–Burk plot analysis. Serum GLA protein concentration was measured by enzyme-linked immunosorbent assay. We examined the potential correlations between serum GLA activity and GLA protein concentration and clinical features and the plasma neurofilament light chain (NfL) level. **Results**: Compared to controls, PD patients showed significantly lower serum GLA activity ($P < 0.0001$) and apparent V_{max} ($P = 0.0131$), but no change in the apparent K_m value. Serum GLA protein concentration was lower in the PD group ($P = 0.0168$) and was positively associated with GLA activity. Serum GLA activity and GLA protein concentration in the PD group showed a negative correlation with age. Additionally, serum GLA activity was negatively correlated with the motor severity score and the level of plasma NfL, and was positively correlated with the score of frontal assessment battery. **Interpretation**: This study highlights that the lower serum GLA activity in PD is the result of a quantitative decrement of GLA protein in the serum and that it may serve as a biomarker of disease severity.

Introduction

Parkinson's disease (PD) is a neurodegenerative disorder characterized by the degeneration of dopaminergic neurons in the substantia nigra with intraneuronal alpha-synuclein aggregates.[1] Recent genetic epidemiological studies have demonstrated an association between PD and mutations in the *GBA1* gene, which encodes the lysosomal enzyme glucocerebrosidase.[2] A reduction in glucocerebrosidase activity has been revealed in biological samples, such as brain and cerebrospinal fluid, in PD patients associated with the accumulation of alpha-synuclein.[3–5] Moreover, other lysosomal storage disorder gene variants have also been linked to an increased risk of PD,[6] suggesting a critical role of lysosomal dysfunction in its pathogenesis.

Fabry disease, which is an X-linked lysosomal storage disease caused by a deficiency of alpha-galactosidase A (GLA), has also been implicated in the development of PD.[7,8] Some studies have shown lower GLA activity in biological samples of PD patients,[9,10] but other studies have not observed this reduction.[11,12] This discrepancy

highlights the need to further investigate the relationship between lysosomal dysfunction and clinical manifestations and disease severity of PD.

In this study, we aimed to characterize the serum GLA in PD patients with olfactory dysfunction or decreased cardiac ^{123}I-metaiodobenzylguanidine (MIBG) uptake through enzyme kinetic assays and enzyme-linked immunosorbent assays (ELISAs). We also examined the association of these GLA enzyme characteristics with clinical features, including age, sex, disease duration, motor and non-motor symptoms, and plasma neurofilament light chain (NfL),[13] to gain insight into the link between serum GLA and the clinical presentation and biomarkers in PD.

Methods

Subjects

We recruited 72 consecutive PD patients admitted to Fujita Health University Hospital between May 2020 and September 2021. All PD cases fulfilled the MDS clinical diagnostic criteria for PD.[14] Among them, we enrolled 66 clinically established PD patients who had olfactory dysfunction (Odor Stick Identification Test for Japanese (OSIT-J) score ≤6)[15] or decreased cardiac ^{123}I-MIBG uptake (heart/mediastinum ratio <2.2),[16–18] which provides a specificity >80% for the differential diagnosis of PD from other parkinsonian conditions.[14] We also enrolled 52 age- and sex-matched controls from our ongoing aging cohort study at Fujita Health University, Japan. Controls were included based on the following criteria: (1) cognitively normal with Mini-Mental State Examination (MMSE) scores >24[19] without a history of neurological or psychiatric disorders and (2) no observable anatomical abnormality in the brain detected by magnetic resonance imaging.

This study was approved by the ethics committees of Fujita Health University Hospital, and all subjects provided written informed consent before participation and opt-out consent.

Clinical evaluation

We evaluated the PD patients during the "on" condition. Motor and non-motor symptoms related to PD were assessed using the Japanese version of the Movement Disorder Society's Unified PD Rating Scale (MDS-UPDRS). We calculated motor severity using Hoehn and Yahr scale and MDS-UPDRS III score. Cognitive performance was evaluated using the Frontal Assessment Battery (FAB), the Japanese version of the Addenbrooke's Cognitive Examination-Revised (ACE-R), Mini-Mental State Examination (MMSE), and the Japanese version of the Montreal Cognitive Assessment (MoCA-J). We also assessed subjects with the Parkinson's Disease Questionnaire-39 Summary Index (PDQ-39 SI), the Geriatric Depression Scale-15 (GDS-15), the OSIT-J score, the Japanese version of the REM Sleep Behavior Disorder Screening Questionnaire (RBDSQ-J), the Scales for Outcomes in Parkinson's Disease-Autonomic (SCOPA-AUT), the Epworth Sleepiness Scale (ESS), and the Japanese version of the Questionnaire for Impulsive-Compulsive Disorders in Parkinson's Disease (J-QUIP). The levodopa equivalent daily dose (LEDD) was calculated according to established formulae[20] with additional consideration of opicapone and safinamide intake.[21]

Blood sample preparation

To obtain serum and plasma from all recruited subjects, we collected blood samples after more than 6 h of fasting. The samples were centrifuged for 10 min at 1500 ***g***, and 500 μL aliquots of serum and plasma were immediately frozen at −80°C until assay. We divided each aliquot to avoid repeated freezing and thawing. We used serum for the analysis of GLA activity and GLA protein concentration and plasma for plasma NfL measurement.

GLA activity measurement

GLA activity in serum was assayed using the 4-methylumbelliferyl (4-MU) derivative of the substrate (Calbiochem, La Jolla, CA, USA), as described previously.[22] Briefly, N-acetyl-D-galactosamine (Sigma-Aldrich, St. Louis, MO, USA) was used as an inhibitor of α-N-acetylgalactosaminidase (NAGA, MIM104170, EC3.2.1.49), formerly called α-galactosidase B. Next, 20 μL of sera was incubated with 40 μL of 5 mmol/L substrate solution for 4 h at 37°C, and the reaction was stopped by adding ice cold 0.2 mol/L glycine-NaOH buffer, pH 10.4, to a final volume of 260 μL.

Substrate concentration in the measurement for GLA activity was adopted as 5 mmol/L to exceed the apparent Michaelis constant (K_m) value of serum GLA in the present study. Fluorescence of the liberated 4-methylumbelliferone was then measured (excitation, 355 nm; emission, 460 nm). One unit of enzyme activity was defined as the amount of enzyme that hydrolyzes 1 nmol of substrate/h at 37°C (nmol/h/mL).

The kinetic parameters of GLA were determined by performing the same measurements as above at 4 substrate concentrations (0.417, 0.625, 1.25, and 5 mmol/L). The values of apparent K_m and maximal velocity (V_{max}) were calculated from Lineweaver–Burk plot analysis.

Measurement of serum GLA protein concentration

Serum GLA protein concentration was quantified by ELISA using the BIO-RAD Benchmark microplate reader and Microplate Manager version 5.2.1 software. The measurement of GLA protein concentration in serum was performed with ELISA kits (Human Alpha-Galactosidase A/GLA ELISA Kit; RayBiotech, Norcross, GA, USA) according to the manufacturer's instructions. The samples and standards were measured in duplicate, and the means of duplicates were used for statistical analyses.

Plasma NfL measurement

NfL is a structural component of the neuronal cytoskeleton. Recent studies show that plasma NfL values are significantly related with motor and cognitive decline,[23–25] suggesting broad cortical involvement in PD.[26] In this study, we evaluated plasma NfL as a disease severity marker in PD. Plasma NfL levels were determined by single molecule array (Simoa) using the Simoa Human Neurology 4-Plex E kit (Quanterix, Billerica, MA, USA), according to the manufacturer's protocol. Plasma samples were tested in duplicate. In the analysis of plasma NfL, we excluded three patients with PD who had a recent traumatic episode. To ensure consistency with previously reported plasma NfL values in controls, 18 out of the 52 control subjects in this study were consecutively selected as a reference in reverse chronological order based on the timing of measurement using Simoa. These 18 control subjects were age-and sex-matches with the PD group.

Statistical analysis

JMP software, version 16 (SAS Institute, Cary, NC, USA) was used for statistical analyses. Significant differences were defined at $P < 0.05$. Fisher's exact test was used to compare the sex distribution among the two groups. We tested the normality of the variables and homoscedasticity with the Shapiro–Wilk test and Levene's test, respectively. The Wilcoxon rank sum test was used to compare continuous variables between the two groups because assumptions of normality or homogeneity of variance were violated. Correlations between continuous valuables, including GLA activity, GLA protein concentration, and clinical indices, were assessed with Spearman's rank correlation test. To assess the association of GLA with disease severity, multiple regression analysis, with age at examination, gender, LEDD, disease duration, and serum GLA activity added, was applied to uncover the influence of each explanatory variable on Hoehn and Yahr scale. Similarly, to assess the association of GLA with cognitive function, multiple regression analysis, with age at examination, gender, disease duration, and serum GLA activity added, was applied to clarify the influence of each explanatory variable on FAB score. Then, we conducted likelihood tests in the comparison between full regression model and the reduced model with the zero-coefficient for serum GLA activity to prove the significant association of serum GLA activity to Hoehn and Yahr scale and FAB score after excluding the influences of confounding factors. Continuous variables were expressed as mean ± standard deviation.

Because some serum samples showed extreme values of GLA protein concentration, we performed the analyses of GLA protein concentration both excluding and including these outliers to exclude the possibility that the associations were driven by extreme values. Outliers were defined as data points that were greater than the third quartile by 1.5-fold the interquartile range.[27] A total of 15 subjects, 6 in the PD group and 9 in the control group, were judged as outliers.

Results

Demographic and clinical characteristics of the participants

Table 1 summarizes the demographic and clinical characteristics of the participants in the PD and control group. PD patients showed significantly higher depression scores (GDS-15: $P < 0.0001$) and lower scores on global cognitive scales (MMSE: $P = 0.0002$, ACE-R and MoCA-J: $P < 0.0001$) than controls but did not differ in age at examination and sex. In the PD group, 22 patients (33.3%) were diagnosed with PD with dementia (PDD), according to the algorithm for diagnosing PDD at Level I proposed by the MDS Task Force.[28]

GLA activity and kinetic parameters

The serum GLA activity was significantly lower in the PD group than in the control group (PD group: 1.75 ± 0.34 nmol/h/mL; control group: 2.25 ± 0.60 nmol/h/mL; $P < 0.0001$) as shown in Figure 1A. The kinetic parameters calculated from Lineweaver–Burk plot analysis showed significantly lower apparent V_{max} in the PD group than in the control group (PD group: 2.59 ± 0.70 nmol/h/mL; control group: 3.09 ± 1.03 nmol/h/mL; $P = 0.0131$) as depicted in Figure 1B. There was no significant difference in the apparent K_m values between the two groups (PD group: 3.30 ± 1.09 mmol/L; control group: 2.99 ± 0.83 mmmol/L; $P = 0.1441$) as indicated in Figure 1C.

Table 1. The clinical characteristics of the subjects in the PD and control group.

Characteristics	Control (N = 52) mean ± SD	PD (N = 66) mean ± SD	P-value
Age at examination (years)	69.8 ± 5.2	71.1 ± 8.2	0.0893
Male/female	23/29	38/28	0.1943
Age at onset (years)		63.5 ± 9.2	
Disease duration (months)		91.7 ± 51.2	
LEDD (mg)		618.5 ± 326.3	
Hoehn and Yahr scale		3.1 ± 1.1	
MDS-UPDRS I		11.1 ± 5.9	
MDS-UPDRS II		14.5 ± 8.2	
MDS-UPDRS III		36.4 ± 17.0	
MDS-UPDRS IV		5.4 ± 4.7	
PDQ-39 SI		28.9 ± 14.9	
SCOPA-AUTO		15.5 ± 8.9	
GDS-15	3.0 ± 2.3	6.8 ± 3.7	**<0.0001**
J-QUIP		0.6 ± 0.9	
RBDSQ-J		5.5 ± 3.1	
ESS		9.0 ± 6.0	
OSIT-J score		3.0 ± 2.1	
MMSE	28.3 ± 1.5	25.7 ± 4.8	**0.0002**
ACE-R	93.9 ± 4.1	80.8 ± 16.2	**<0.0001**
MoCA-J	24.7 ± 2.8	20.5 ± 5.5	**<0.0001**
FAB		12.6 ± 2.7	

Bold letters indicate a statistically significant difference. Significance was tested using the Wilcoxon rank sum test. PD, Parkinson's disease; NC, normal control; LEDD, Levodopa equivalent daily dose; MDS-UPDRS, Movement Disorder Society's Unified Parkinson's Disease Rating Scale; PDQ-39 SI, Parkinson's Disease Questionnaire-39 Summary Index; SCOPA-AUT, scales for outcomes in Parkinson's disease-autonomic; GDS-15, Geriatric Depression Scale-15; J-QUIP, Japanese version of the Questionnaire for Impulsive-Compulsive Disorders in Parkinson's Disease; RBDSQ-J, Japanese version of the REM Sleep Behavior Disorder Screening Questionnaire; ESS, Epworth Sleepiness Scale; OSIT-J, Odor Stick Identification Test for Japanese; MMSE, Mini-Mental State Examination; ACE-R, Addenbrooke's Cognitive Examination-Revised; MoCA-J, Japanese version of the Montreal Cognitive Assessment; FAB, Frontal Assessment Battery.

GLA protein concentration and its correlation with GLA activity

The 15 outliers (6 subjects in the PD group and 9 subjects in the control group) in the GLA protein concentration data were not associated with specific clinical findings. In the comparison without those outliers, the serum GLA protein concentration was significantly lower in the PD group than in the control group (PD group: 0.49 ± 0.13 ng/mL; control group: 0.57 ± 0.15 ng/mL; P = 0.0168) as presented in Figure 1D. The analysis including 15 outliers also showed significantly lower serum GLA protein concentration in the PD group compared to the control group (P = 0.0071).

In the PD group, GLA protein concentration showed a significant positive correlation with GLA activity (rs = 0.4430, P = 0.0004) as demonstrated in Figure 2A. Conversely, in the control group, GLA protein concentration showed no significant correlation with GLA activity (rs = 0.1948, P = 0.2106) as depicted in Figure 2B.

Sub-analysis of serum GLA enzymatic properties in excluding the PDD cases from the PD group

As one-third of PD patients were diagnosed with PDD, separate analyses were needed to determine whether the observed results could be attributed to dementia rather than PD itself. To this end, we excluded PDD cases from the PD group and found that the comparison of serum GLA activity, its related kinetic parameters, and protein concentration between the PD and control groups yielded similar results (Figure S1A–D).

Relationships between GLA activity and protein concentration with clinical parameters

Table 2 summarized the relationships between serum GLA activity and protein concentration with clinical indices in the PD group. The age at examination and age at onset were significantly negatively correlated with serum GLA activity (Fig. 3A and B). Age at examination also showed a significant negative correlation with GLA protein concentration (Fig. 3C), whereas age at onset did not (Fig. 3D). Additionally, serum GLA activity showed a significant negative correlation with the Hoehn and Yahr scale (rs = −0.3753, P = 0.0048) as presented in Figure 3E and a positive correlation with the score of FAB (rs = 0.2559, P = 0.0396) as shown in Figure 3F, whereas GLA protein concentration did not. Additionally, in multiple regression analyses for serum GLA activity and potential confounding clinical factors affecting Hoehn and Yahr scale and FAB score in the PD group, the likelihood tests in the comparison between full regression model and the reduced model with the zero-coefficient for serum GLA activity demonstrated the significant association of serum GLA activity with Hoehn and Yahr scale and FAB score in the PD group (Hoehn and Yahr scale: P = 0.0044, FAB score: P = 0.0475) as presented in Table S1. Scores in MMSE, ACE-R, and MoCA-J also showed a tendency

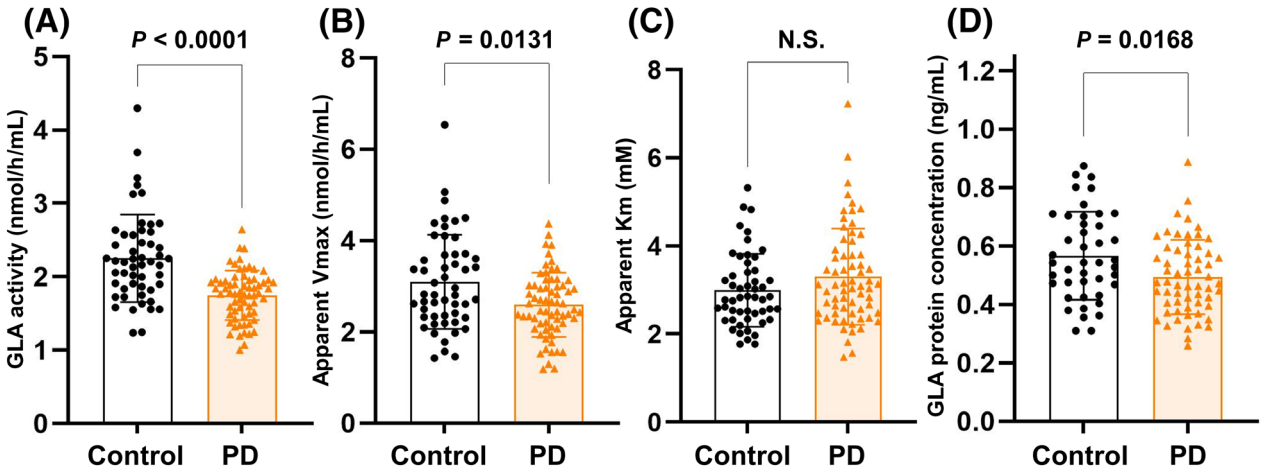


Figure 1. Comparison of serum GLA enzymatic properties between the PD and control groups. (A) Comparison of serum GLA activity at 5 mmol/L substrate concentration. (B) Comparison of apparent V_{max} values. (C) Comparison of apparent K_m values. (D) Comparison of serum GLA protein concentration without outliers. Data are presented as mean ± SD. Significance was tested using the Wilcoxon rank sum test. GLA, α-galactosidase A; PD, Parkinson's disease; V_{max}, maximal velocity; K_m, Michaelis constant; N.S., not significant.

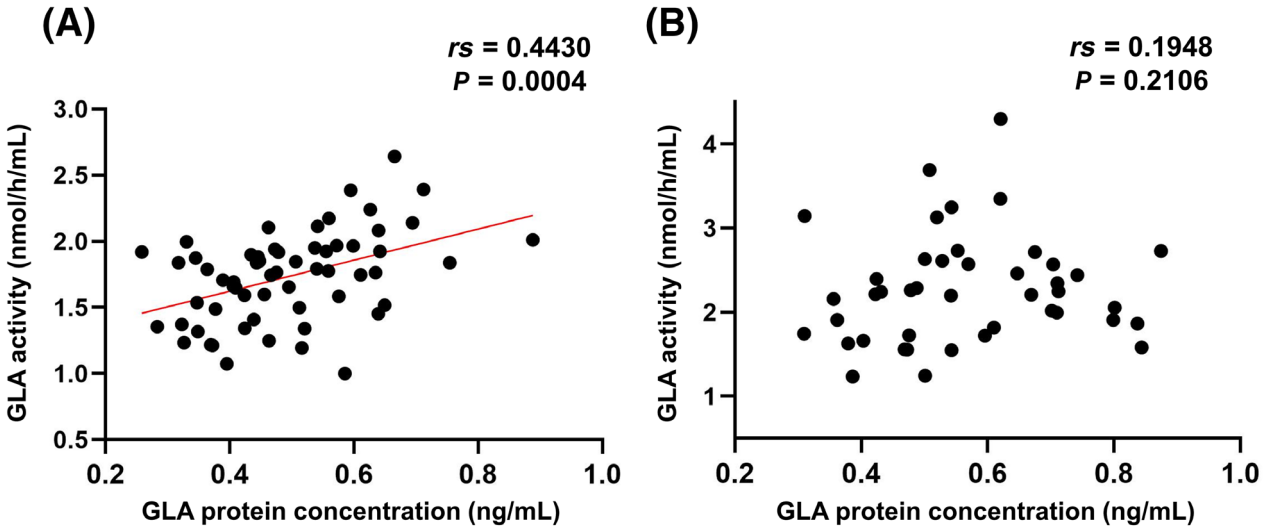


Figure 2. The relationship between GLA protein concentration and GLA activity in sera in each group. GLA activity in serum was measured at a substrate concentration of 5 mmol/L. Analysis of GLA protein concentration was performed excluding outliers. (A) Correlation between GLA protein concentration and GLA activity in sera in the PD group. (B) Correlation between GLA protein concentration and GLA activity in sera in the control group. Spearman's rank correlation test was used to determine significant correlations. GLA, α-galactosidase A; PD, Parkinson's disease.

for a positive, but not significant, correlation with GLA activity. By contrast, in the control group, serum GLA activity and protein concentration showed no significant correlation with age at examination and other clinical parameters (MMSE, ACE-R, MoCA-J, and GDS-15), except that the male controls exhibited significantly higher serum GLA activity than the female controls ($P = 0.0357$). (Table S2).

Plasma NfL levels and GLA activity in the PD group

Plasma NfL levels observed in the control group were consistent with these previous reports.[29,30] Plasma NfL was significantly higher in the PD group than in the control group ($P < 0.0001$) as depicted in Figure 4A. In the PD group, plasma NfL was negatively correlated with

Table 2. Correlations between GLA activity or GLA protein concentration and clinical indices in the PD group.

	GLA activity		GLA protein level	
	rs	*P*-value	*rs*	*P*-value
Age at examination	**−0.3025**	**0.0136**	**−0.2969**	**0.0213**
Gender		0.1631		0.1119
Age at onset	**−0.3030**	**0.0134**	−0.1957	0.1339
Disease duration	0.1456	0.2472	−0.0164	0.9017
MDS-UPDRS I	−0.0899	0.4730	0.0319	0.8089
MDS-UPDRS II	−0.0489	0.6968	−0.0946	0.4723
MDS-UPDRS III	−0.0039	0.9760	0.0743	0.5862
MDS-UPDRS IV	0.2169	0.0961	0.0726	0.6019
Hoehn-Yahr scale	**−0.3753**	**0.0048**	−0.0955	0.5139
PDQ-39 SI	−0.1129	0.3669	−0.1230	0.3491
SCOPA-AUTO	−0.0630	0.6151	0.0372	0.7776
GDS-15	−0.1922	0.1250	−0.1394	0.2925
J-QUIP	0.0516	0.6830	0.0645	0.6274
RBDSQ-J	−0.1316	0.2962	−0.0693	0.6021
ESS	0.1192	0.3443	0.1309	0.3230
OSIT-J score	0.1000	0.4245	0.0178	0.8928
MMSE	0.2138	0.0847	0.0761	0.5634
ACE-R	0.2285	0.0671	0.1540	0.2443
MoCA-J	0.2296	0.0658	0.1113	0.4014
FAB	**0.2559**	**0.0396**	0.2528	0.0534

Bold letters indicate a correlation with a statistically significant difference. GLA activity in serum was measured at a substrate concentration of 5 mmol/L. The analysis regarding GLA protein concentration was performed excluding outliers. Spearman's rank correlation test was used to determine significant correlations. GLA, α-galactosidase A; PD, Parkinson's disease; MDS-UPDRS, Movement Disorder Society's Unified Parkinson's disease Rating Scale; PDQ-39 SI, Parkinson's Disease Questionnaire-39 Summary Index; SCOPA-AUT, Scales for Outcomes in Parkinson's Disease-Autonomic; GDS-15, Geriatric Depression Scale-15; J-QUIP, Japanese version of the Questionnaire for Impulsive-Compulsive Disorders in Parkinson's Disease; RBDSQ-J, Japanese version of the REM Sleep Behavior Disorder Screening Questionnaire; ESS, Epworth Sleepiness Scale; OSIT-J, Odor Stick Identification Test for Japanese; MMSE, Mini-Mental State Examination; ACE-R, Addenbrooke's Cognitive Examination-Revised; MoCA-J, Japanese version of the Montreal Cognitive Assessment; FAB, Frontal Assessment Battery.

clinical indices centered on cognitive rating scales (Data not shown). Furthermore, unlike GLA protein concentration, GLA activity demonstrated a significant negative correlation with plasma NfL ($rs = -0.4185$, $P = 0.0006$) as shown in Figure 4B and C.

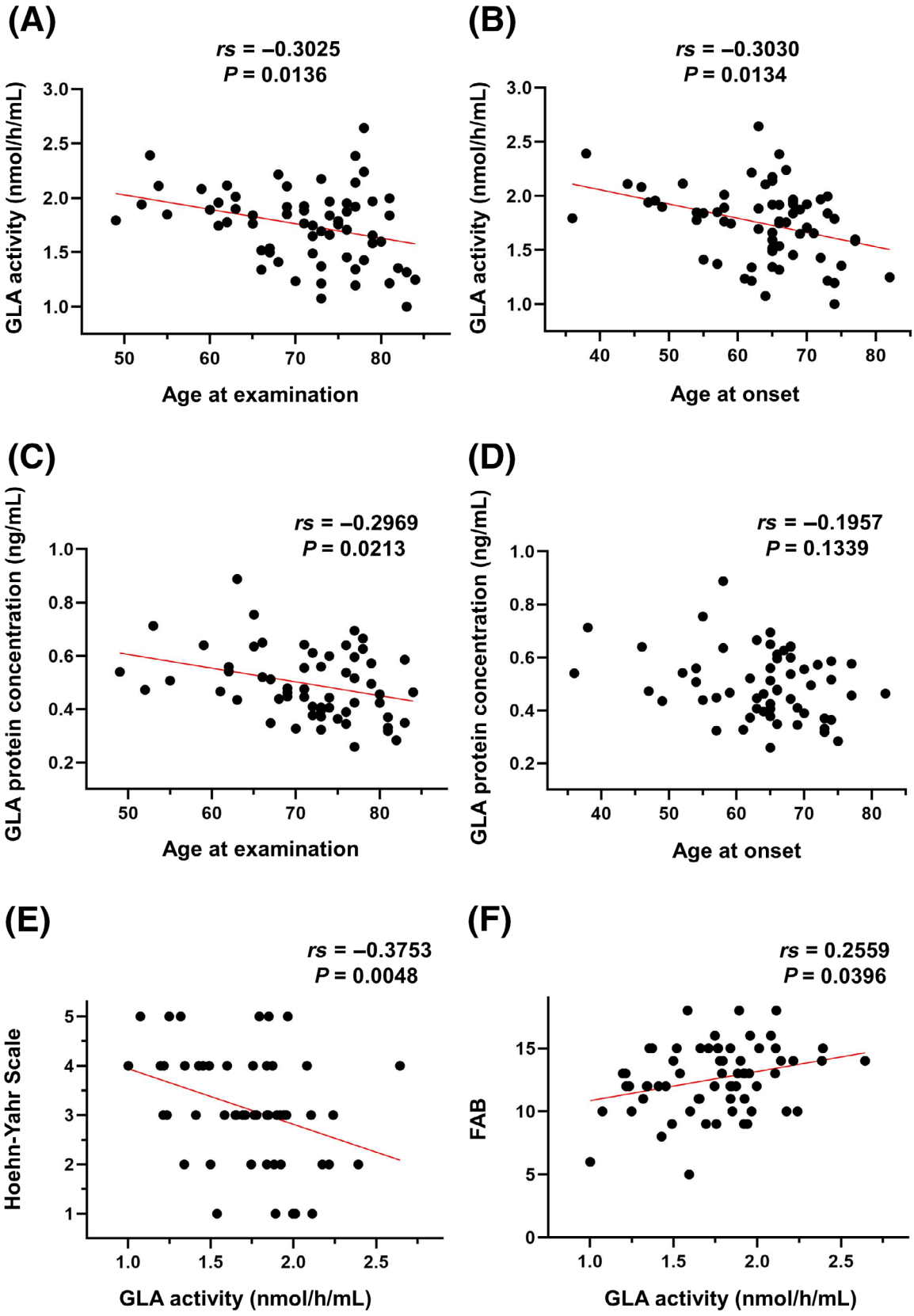
Discussion

In this study, we found lower serum GLA activity and protein levels in patients with PD compared to unaffected controls. Similar results were obtained even in a separate analysis in which PDD cases were excluded from the PD group. The GLA activity was associated with age, disease severity, cognitive decline, and plasma NfL level. A negative correlation was found between GLA activity or protein level and age in the PD group, but not in the control group, indicating that age-related reductions in GLA protein could be a disease-related pathological process in PD.

Regarding the association between Fabry disease with PD pathogenesis, studies on Fabry mouse models and Gaucher mouse models have demonstrated α-synuclein and tau aggregates in the central nervous system.[31] Furthermore, an autopsy study conducted on a male patient with Fabry disease, exhibiting predominant hypokinesia, revealed severe neuronal loss in the substantia nigra pars compacta and Lewy pathology corresponding to neuropathological stage 4 of PD.[32] Collectively, these studies suggest the potential for PD pathology to manifest within the context of Fabry disease. In addition, regarding the relationship between GLA and PD, previous studies have shown lower GLA activity in various tissues such as brain tissue,[5,9] dried blood spots,[10] and leukocytes[33] of patients with PD, yet the underlying mechanisms remain unclear. Our findings showed significantly lower serum GLA activity and protein level, but not substrate affinity, in patients with PD compared with controls. A positive correlation was also observed between GLA activity and protein level in the PD group. These results indicate abnormalities in GLA-related trafficking in peripheral tissues in PD. Nelson et al. also showed a significant positive correlation between GLA activity and GLA protein level in central tissue such as brain of patients with PD.[9] These results imply that the lower serum GLA activity in PD could be a result of a reduction in protein level rather than by a change in substrate affinity.

In PD patients, GLA activity was negatively related to the Hoehn and Yahr scale and positively related to the FAB score. General cognitive tests such as MMSE, ACE-R, and MoCA-J scores also showed a tendency for a positive correlation with GLA activity. Moreover, GLA activity

Figure 3. Correlation between serum GLA activity or GLA protein concentration and clinical parameters in the PD group. GLA activity in serum was measured at a substrate concentration of 5 mmol/L. The analysis of GLA protein concentration was performed excluding outliers. (A, B) Correlation between serum GLA activity and age at examination (A) and between serum GLA activity and age at onset (B) in the PD group. (C, D) Correlation between serum GLA protein concentration and age at examination (C) and between serum GLA protein concentration and age at onset (D) in the PD group. (E, F) Correlation between serum GLA activity and the Hoehn and Yahr scale (E) and between serum GLA activity and the score of FAB (F) in the PD group. Spearman's rank correlation test was used to determine significant correlations. GLA, α-galactosidase A; PD, Parkinson's disease; FAB, frontal assessment battery.



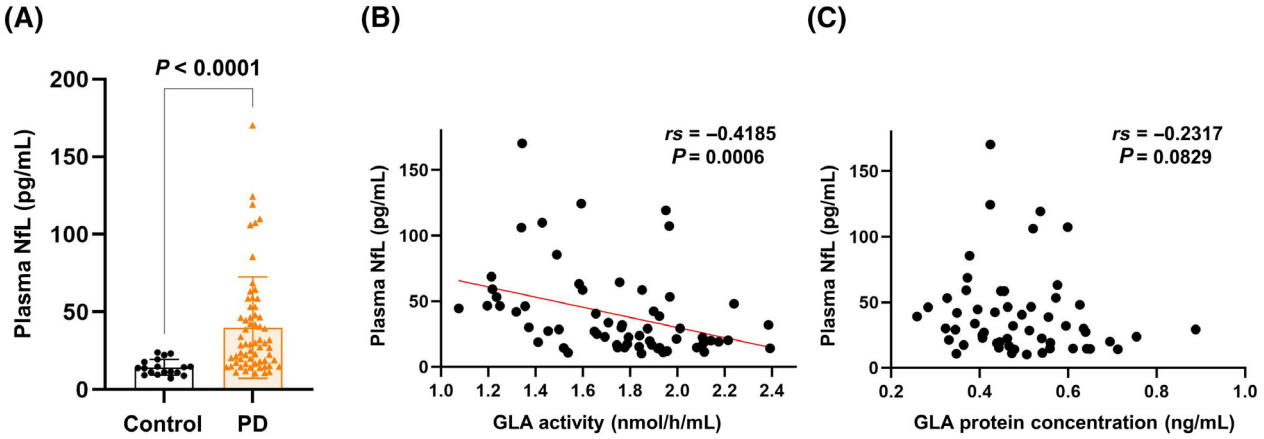


Figure 4. Intergroup comparison of plasma NfL and association with serum GLA in the PD group. (A) Comparison of plasma NfL levels between the PD and control groups. Data are presented as mean ± SD. Significance was tested using the Wilcoxon rank sum test. (B, C) Correlation between serum GLA activity and plasma NfL level (B) and between serum GLA protein concentration and plasma NfL level (C) in the PD group. GLA activity in serum was measured at a substrate concentration of 5 mmol/L. Analysis of GLA protein concentration was performed excluding outliers. Spearman's rank correlation test was used to determine significant correlations. NfL, neurofilament light chain; PD, Parkinson's disease; GLA, α-galactosidase A.

was negatively correlated with plasma NfL, a biomarker that has been linked to motor impairment and cognitive decline in PD[12,13] and considered to reflect cortical neurodegeneration.[34] A clinicopathological study of post-mortem PD brain samples showed significantly lower GLA activity in the temporal cortex correlated with the accumulation of phosphorylated alpha-synuclein.[9] Although the relationship between GLA activities in brain and blood remains unclear, alterations in serum GLA activity may be indicative of widespread brain involvement, similar to the relationship between sphingolipid metabolic indices in peripheral blood and pathological changes in the brain.[35]

In patients with PD, the activity and protein concentration of GLA were found to be negatively correlated with age at examination. The origin of lysosomal acid hydrolase, including GLA, in serum remains unclear; however, a study revealed a correlation between serum lysosomal acid hydrolase activity and the activity of vascular endothelial cells.[36] Additionally, human endothelial cells displayed age-related dysfunction of the autophagy–lysosomal pathway and impaired lysosomal acidification resulting from ATP shortage related to mitochondrial dysfunction.[37] Conversely, the control group did not show a significant reduction in GLA activity or protein concentration with age. The relationship between GLA activity and aging in normal subjects is yet to be fully understood, with some previous studies failing to establish a significant correlation.[5,33] Mitochondrial dysfunction plays a key role in the pathogenesis of PD.[38] In addition, the study using fibroblasts with mutations in the PD linked gene revealed decreased secretion of lysosomal hydrolases due to impaired lysosomal exocytosis.[39] Further study is necessary, but it is posited that age-related impairment of the autophagy–lysosomal pathway and mitochondrial function might accelerate the reduction in peripheral GLA activity and protein concentration in PD patients who are predisposed to lysosomal dysfunction due to their genetic background.[40,41]

In this study, 33% patients diagnosed with PD were classified as PDD. A recent systematic review and meta-analysis indicated that the global pooled dementia frequency was 26.3%.[42] The relatively high prevalence of PDD in this study's population may be due to inclusion criteria, which were designed to exclude patients with other types of parkinsonism and dementia with Lewy bodies by enrolling individuals with PD who had olfactory impairment or reduced ^{123}I-MIBG cardiac accumulation. As a result, higher proportion of advanced-stage patients with PD was included in the analysis. However, these inclusion criteria were considered rational, as the objective of the study was to investigate GLA alterations and their correlation with clinical presentation in patients with PD pathology.

The present study has some limitations that should be acknowledged. Firstly, this was a single-center study, and the number of participants was relatively limited. Secondly, outliers were observed in the GLA protein concentration data and the biological significance of these extreme values is unclear. To address this, we performed the analyses with and without these outliers, yielding similar results for the comparison of GLA protein

concentration between the groups and the association between GLA protein concentration and age at examination. Thus, it is believed that these outliers do not significantly impact the results of this study. Thirdly, for statistical age matching with the PD group, subjects under the age of 61 were not recruited to the control group. This may explain the absence of significant negative correlations between GLA activity and protein concentration and age at examination in the control group. However, even after adding control subjects under the age of 61 to the control group, no significant correlations were found between GLA activity or GLA protein concentration and age at examination (data not shown). Finally, we did not perform genotyping of GLA in this study. As reported previously, lower GLA activity and protein concentration are observed in males with Fabry disease and female carriers of GLA variants.[43] Nevertheless, in a previous genetic study consisting of 126 male and female patients with PD, only four female GLA mutant carriers were present (3.2%).[8]

In conclusion, this study indicated that lower serum GLA activity is due to the decrement of enzyme protein in sera but not due to a change of substrate affinity in the PD group when compared with the control group. Negative correlation of GLA activity or GLA protein level and age was observed in PD but not in control, suggesting that age-related reduction in GLA protein can be a disease-related pathological process in PD. In addition, disease severity, cognitive decline, and plasma NfL level were associated with lower GLA activity. Taken together, altered serum GLA activity in PD may reflect widespread pathological involvement, including the cerebral cortex and periphery, as well as age-related reduction in GLA protein in serum.

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Author Contributions

YM designed the study, performed the measurements, and analyzed the data, and drafted the manuscript. KN contributed to the measurements of GLA enzyme activity and protein concentration. RO, KM, SS, AU, and MI performed the clinical evaluations of subjects. HT and TT contributed to the measurement of plasma neurofilament light chain. JY provided intellectual advice on statistical analysis methods and contributed to the execution of analyses. TM contributed to the general conception and supervised the execution of the study on biochemical aspects and revised the manuscript. HW supervised the study, advised on the study design and statistical analyses, and revised the manuscript.

Conflict of Interest

Nothing to report.

Data Availability Statement

The data generated during the current study are available from the corresponding author on reasonable request.

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Supporting Information

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

Figure S1. Comparison of serum GLA enzymatic properties between the PD and control groups in excluding PDD patients from the PD group. (A) Comparison of serum GLA activity at 5 mmol/L substrate concentration. (B) Comparison of apparent V_{max} values. (C) Comparison of apparent K_m values. (D) Comparison of serum GLA protein concentration without outliers. Data are presented as mean ± SD. Significance was tested using the Wilcoxon rank sum test. GLA, α-galactosidase A; PD, Parkinson's disease; PDD, PD with dementia; V_{max}, maximal velocity; K_m, Michaelis constant; N.S., not significant.

Table S1. Multiple regression analyses for serum GLA activity and potential confounding factors affecting Hoehn and Yahr scale and FAB score in the PD group. GLA, α-galactosidase A; PD, Parkinson's disease; FAB, Frontal Assessment Battery; LEDD, Levodopa equivalent daily dose.

Table S2. Correlations between GLA activity or GLA protein concentration and clinical indices in the control group. GLA activity in serum was measured at a substrate concentration of 5 mmol/L. The analysis regarding GLA protein concentration was performed excluding outliers. Spearman's rank correlation test was used to determine significant correlations. GLA, α-galactosidase A; GDS-15, Geriatric Depression Scale-15; MMSE, Mini-Mental State Examination; ACE-R, Addenbrooke's Cognitive Examination-Revised; MoCA-J, Japanese version of the Montreal Cognitive Assessment. *Bold letters indicate a statistically significant difference.

