

Motor progression and nigrostriatal neurodegeneration in Parkinson's disease

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Abbreviations: CR=contrast ratio, NM-MRI=neuromelanin-sensitive magnetic
resonance imaging, PD=Parkinson's disease, SN=substantia nigra, SPECT=single
photon emission computed tomography, SUR=striatal uptake ratio.

Abstract

Objective: The motor severity in Parkinson's disease (PD) is believed to parallel with dopaminergic terminal degeneration in the striatum although the terminal was reported to be virtually absent by 4 years post-diagnosis. Meanwhile, neuromelanin-laden dopamine neuron loss in the substantia nigra (SN) elucidated a variability at early stages and gradual loss with less variability 10 years post-diagnosis. Here, we aimed to clarify the correlation between motor impairments and striatal dopaminergic terminal degeneration and nigral neuromelanin-laden dopamine neuron loss at early to advanced stages of PD.

Methods: Ninety-three PD patients were divided into early and advanced subgroups based on motor symptom duration and whether motor fluctuation was present. Striatal dopaminergic terminal degeneration was evaluated using a presynaptic dopamine transporter tracer, ^{123}I -ioflupane SPECT. Nigral neuromelanin-laden dopamine neuron density was assessed by neuromelanin-sensitive MRI (NM-MRI).

Results: In patients with early-stage (motor symptoms for ≤ 8 or 10 years), motor dysfunction during the drug-off state was paralleled by a decline in ^{123}I -ioflupane uptake in the striatum despite the absence of a correlation with reductions in NM-MRI signals in SN. Meanwhile, in patients with advanced-stage (motor symptoms for > 8 or 10 years and with fluctuation), the degree of motor deficits during the drug-off state was not correlated with ^{123}I -ioflupane uptake in the striatum, despite its significant negative correlation with NM-MRI signals in SN.

Interpretation: We propose striatal dopaminergic terminal loss measured using ^{123}I -ioflupane SPECT and nigral dopamine neuron loss assessed with NM-MRI as early-stage and advanced-stage motor impairments biomarkers, respectively.

Introduction

Series of studies demonstrated that the severity of motor symptoms in patients with Parkinson's disease (PD) during the drug-off state parallels with the level of dopaminergic terminal degeneration in the striatum using single photon emission computed tomography (SPECT) and positron emission tomography (PET).¹⁻⁸ Meanwhile, in vivo imaging data indicate that dopaminergic terminal degeneration decay cannot be fixed and is faster at early stages than at late stages.⁹⁻¹⁵ Pathological observations also showed that dopaminergic terminals in the post-commissural striatum degenerate rapidly at early stages and are virtually absent by 4 years post-diagnosis, yet motor impairments still continue to progress beyond that period.¹⁶

Multiple studies showed that the level of motor deficits in early stages of PD, approximately <8 years post-diagnosis, is not correlated with the degree of global loss of neuromelanin-sensitive MRI (NM-MRI) signals in the substantia nigra (SN).¹⁷⁻¹⁹ In contrast, several reports covering various disease durations from several months to 15 years demonstrated that the severity of motor manifestations is correlated to decreases in NM-MRI signals in SN.^{20,21} Post-mortem data elucidated a considerable variability in NM-laden dopamine neuron loss in SN at early disease stages, with several patients displaying a profound loss of approximately 80% and some demonstrating preserved loss, which is almost the lower limit in healthy controls; gradual loss showed significantly less variability 10 years following diagnosis.¹⁶

To further elucidate the etiology of PD and determine novel therapies, it is critical to obtain information on the correlation between clinical parameters and

neurodegeneration. Accordingly, this study aimed to investigate the correlation between motor impairments and dopaminergic terminal degeneration in the striatum and NM-laden dopamine neuron loss in SN in early to advanced stages of PD. Striatal dopaminergic terminal degeneration was evaluated using a presynaptic dopamine transporter tracer, ^{123}I -ioflupane SPECT. Nigral NM dopamine neuron density was also assessed by NM-MRI. As evidence from postmortem brains and genetic animal models suggests that dopaminergic axons degenerate at early stages, whereas cell body loss occurs later,^{16,22} we hypothesized that dopaminergic terminals in the striatum and NM-laden cell bodies in SN degenerate at different paces and may be candidate biomarkers of motor disturbance in patients with different stages of PD. We divided PD patients into early and advanced subgroups based on motor symptom duration and whether motor fluctuation was present. Although there is no single definition of advanced-stage, according to physicians' judgment in an international study of 2615 patients, the average disease duration was 11.0 years for patients with advanced PD and 4.3 years for those with non-advanced PD,²³ while an Australian study of 100 patients reported a median time since diagnosis of 12.8 and 5.3 years among patients with advanced and non-advanced PD, respectively.²⁴ The midpoint was 7.7 years in the larger sample and 9.1 years in the smaller sample, therefore, we selected 8 years as the cut-off for categorization of patients into early or advanced stages. To confirm the findings, we also subdivided patients with cut-off values of 10 years and conducted the same analyses.

Methods

Participants

Ninety-three patients with various durations from symptom onset (mean $\pm$ standard deviation [SD], range; age, 63.7 ± 9.3 , 41-85 years; disease duration, 8.2 ± 4.4 , 0.5-20.2 years; 50 female) participated at the Kyoto University Hospital between September 2016 and September 2019. Patients were diagnosed according to the Movement Disorder Society (MDS) Clinical Diagnostic Criteria for PD,²⁵ and no patients had a history of any neurological or psychiatric disorders other than PD. All patients provided written informed consent in accordance with the dictates of the Ethics Committee, Kyoto University Graduate School and Faculty of Medicine.

Patients were divided into the early and advanced subgroups according to two different perspectives. Patients with PD-related motor symptoms for ≤ 8 years were considered to have early PD, whereas those with PD-related motor symptoms for > 8 years were considered to have advanced PD (Table 1). We also subdivided patients into those diagnosed with PD for ≤ 10 years or > 10 years and conducted the same analysis since no consensus exists on disease duration to classify early and advanced PD (Table 2). Second, we classified patients with PD based on whether motor fluctuation was present; patients were considered to have motor fluctuation if MDS Unified Parkinson Disease Rating Scale (UPDRS)²⁶ Part IV 4.1 or 4.3 score was ≥ 1 , whereas patients were considered to not have motor fluctuation if MDS-UPDRS Part IV 4.1 and 4.3 score were 0 (Table 3).

Behavioral measures

Motor symptom severity was evaluated using the Hoehn and Yahr stage and MDS-UPDRS Part III score in a practically defined drug-off state after withdrawing all anti-Parkinsonian medications for at least 12 h²⁷⁻²⁹ and also in a drug-on state in which optimal dopaminergic medication was administered. We calculated the levodopa daily equivalent dose (LEDD).³⁰

¹²³I-Ioflupane SPECT data acquisition

SPECT scans were conducted using the Infinia GE Healthcare system (Waukesha, Wisconsin, USA); 65 slice images were accordingly acquired. Three hours after the intravenous injection of 167 MBq ¹²³I-Ioflupane, projection data were obtained using extended low-energy general purpose collimators with the following parameters: energy window, 159 keV $\pm$ 10 %; scan mode, continuous; step angle, 3°; 120 steps of 7 s; 2 rotations; total acquisition time, 28 min; zoom, 1.33; and 128 $\times$ 128 matrix. Data were reconstructed by filtered back projection (iteration 2, subset 10). The voxel size of the reconstructed image was 3.3 $\times$ 3.3 $\times$ 3.3 mm.

MRI data acquisition

MRI scans were performed using the 3 tesla Magnetom Skyra system with a 32-channel head coil (Siemens, Erlangen, Germany). To obtain NM-MRI images, we utilized a single-slab 3D gradient echo sequence with the following parameters: repetition time, 35.0 ms; echo time, 2.55 ms; flip angle, 20°; field of view, 178 × 178 mm; slices, 60; and voxel size, 0.7 × 0.7 × 0.7 mm. An off-resonance magnetization transfer pulse (offset frequency, 1200 Hz) was added. The three-dimensional slab of the NM-MRI scan was placed on a mid-sagittal slice parallel to the cerebral aqueduct. The center slice was located in a manner confirming that SN was located at the center of the three-dimensional scan slab.

Whole brain T1-weighted anatomical images were acquired using a magnetization-prepared rapid gradient echo sequence (MPRAGE) using the following parameters: repetition time, 1900 ms; echo time, 2.58 ms; inversion time, 900 ms; flip angle, 9°; field of view, 230 × 230 mm; slices, 192; and voxel size, 0.9 × 0.9 × 0.9 mm. Conventional T2-weighted and T2*-weighted images were acquired to confirm the absence of structural MRI abnormalities associated with other causes of parkinsonism.

¹²³I-Ioflupane SPECT data analysis

Image processing was performed using DaTQUANT software for Xeleris version 3.1 (GE Healthcare, Waukesha, Wisconsin, USA). This software spatially normalizes single SPECT images to the template generated from healthy subjects to set striatal volumes of

interest (VOI) and occipital lobe VOI as reference backgrounds. To evaluate ^{123}I -ioflupane uptake, we calculated the striatal uptake ratio (SUR) as follows: $\text{SUR} = (\text{mean counts in striatal VOI} - \text{mean counts in occipital lobe VOI}) / (\text{mean counts in occipital lobe VOI})$. Subsequently, SUR was averaged across the left and right sides for statistical analysis.

MRI mask definition

To quantitatively evaluate NM-MRI images, study-specific masks were created in NM-positive regions in SN and reference regions in the pons using the NM-MRI and MPAGE images of 48 healthy controls (HCs) (mean $\pm$ SD, range; age, 60.1 ± 10.4 , 41-81; 30 females) using the aforementioned acquisition protocols. Imaging data were processed using tools from the FMRIB Software Library version 6.0.0.³¹ After brain extraction, NM-MRI data were registered to individual MPAGE images by rigid body transformation and spatially normalized to the Montreal Neurological Institute (MNI) 1-mm template using nonlinear registration.³² We created an NM-MRI template by averaging the normalized NM-MRI images of the 48 HCs (Fig. 1). We specified the NM-positive region in SN by manually outlining the hyperintense area on eight contiguous axial template slices.²¹ The average SN volume of one side was 317.9 ± 63.6 mm³ in HCs. Furthermore, the reference region was set in the rostral pontine area known to have minimal NM content on three contiguous axial template slices.³³

MRI data analysis

Masks in NM-positive and reference regions in the MNI 1-mm space were transformed into individual MPRAGE images and subsequently NM-MRI images using nonlinear and rigid body transformation.³⁴ Individual NM-MRI images were smoothed with a Gaussian 1.4-mm FWHM kernel. Accordingly, the signal intensity was normalized by dividing by the average intensity of the rostral pontine reference region.³³ The corresponding images were submitted for further analysis.

To evaluate NM-positive regions, we defined the contrast ratio (CR) of SN as the mean signal intensity of voxels having above the 50th percentile value in the projected NM map in SN to avoid noise-related errors. We set the threshold as 50th percentile following the judgment of three board-certified neurologists (K.F., A.S., and N.S.) who agreed that the hyperintense area in the NM-MRI data of each HC corresponded to the SN (Fig. 1).

Moreover, we computed the volume of the NM-positive region with suprathreshold intensity in the registered NM map in SN. We searched an optimum threshold between 1.01 and 1.20 according to a previous study.¹⁸ We subsequently set the threshold at 1.05 based on agreement among the three board-certified neurologists that the suprathreshold area in HC data corresponded to the SN (Fig. 1). In each patient, the ratio of the volume of the NM-positive region relative to the average volume of HCs was calculated according to a previous report.³⁵ Both the CR and volume of the NM-positive region in SN were averaged across left and right sides for statistical analysis.

Statistical analysis

We compared the demographic data of patients between the early and advanced PD groups and between the PD without and with motor fluctuation groups using an independent t-test and Pearson's chi-square test. The SUR of the striatum on ^{123}I -Ioflupane SPECT and the CR and volume of SN in NM-MRI were also compared between the aforementioned groups.

The linear relationship between the MDS-UPDRS Part III score during the drug-off state and imaging biomarkers was analyzed in early and advanced PD groups and without and with motor fluctuation groups. The correlation was evaluated with SUR of the striatum, CR as well as the volume of SN. Since age and biological sex influence the dopaminergic terminal density and neuromelanin accumulates, we adjusted for these effects by a linear regression, and then estimated the linear relationship between the variables. Given that the 3 groupings of early- and advanced-stage (disease duration with cut-off values of 8, 10 years and without or with motor fluctuation) highly correlated with each other and were not independent observations, for each of the 6 groups, 3 hypothesis testing during the drug-off state (18 comparisons) were controlled for using false discovery rate (FDR) with Benjamini-Hochberg procedure.

We also performed group comparisons of the slopes for regression lines between motor symptoms during the drug-off state and imaging biomarkers, even when

the linear relationship between motor symptoms and individual imaging biomarkers was not statistically significant in each group.

For additional reference, the correlation was also investigated during the drug-on state using the FDR method (18 comparisons), although interpretation of the correlation is not straightforward, given the individualized dose and type of medication.

The correlation was analyzed in all patients with PD combined as well. Additionally, the correlation between the SUR and the CR and volume of the NM-positive region was analyzed. Statistical analyses were performed using JMP® version 15 (SAS Institute Inc., Cary, NC, USA). A P -value <0.05 was considered statistically significant.

Results

Clinical characteristics

The advanced PD and PD with motor fluctuation groups demonstrated a significantly longer disease duration, higher LEDD, and higher Hoehn and Yahr stage and MDS-UPDRS Part III score than the early PD and PD without motor fluctuation groups during the drug-off state (Tables 1, 2 and 3). During the drug-on state, the advanced PD and PD with motor fluctuation groups had higher Hoehn and Yahr stage than the early PD and PD without motor fluctuation group; however, the MDS-UPDRS Part III score was not different between the groups. The group differences of Hoehn and Yahr stage

and MDS-UPDRS Part III score were always larger during the drug-off state than during the drug-on state.

Many patients in the early PD (disease duration ≤ 8 years: 28/45, 62.2%; disease duration ≤ 10 years: 29/66, 43.9%) group did not have motor fluctuation, yet most patients with advanced PD (disease duration > 8 years: 45/48, 93.8%; disease duration > 10 years: 25/27, 92.6%) had motor fluctuation.

Motor symptoms, dopaminergic terminal, and NM density between stages during the drug-off state

In the early PD group (disease duration ≤ 8 years), during the drug-off state, the MDS-UPDRS Part III score was significantly negatively correlated with the striatum SUR ($r = -0.39$; $P_{\text{FDR}} < 0.05$), whereas the motor score was not correlated with the CR or volume of the NM-positive region in SN (CR: $r = -0.16$; $P_{\text{FDR}} = 0.55$; volume: $r = -0.15$; $P_{\text{FDR}} = 0.49$) (Fig. 2). In contrast, in the advanced PD group (disease duration > 8 years), during the drug-off state, the motor score was not significantly correlated with the striatum SUR ($r = -0.13$; $P_{\text{FDR}} = 0.44$), yet it was significantly correlated with the CR and volume of the NM-positive region in SN (CR: $r = -0.41$; $P_{\text{FDR}} < 0.05$; volume: $r = -0.36$; $P_{\text{FDR}} < 0.05$).

We conducted the same statistical analysis with a cut-off value of 10 years and obtained similar results. In the early PD group (disease duration ≤ 10 years), during the drug-off state, the MDS-UPDRS Part III score was significantly negatively correlated with the striatum SUR ($r = -0.40$; $P_{\text{FDR}} < 0.05$), whereas the motor score was

not significantly correlated with the CR or volume of the NM-positive region in SN (CR: $r=-0.12$; $P_{\text{FDR}}=0.52$; volume: $r=-0.12$; $P_{\text{FDR}}=0.48$). In contrast, in the advanced PD group (disease duration >10 years), during the drug-off state, the motor score was not significantly correlated to striatum SUR ($r=-0.09$; $P_{\text{FDR}}=0.96$), yet it was correlated with the CR and tended to be correlated with the volume of NM-positive regions in SN (CR: $r=-0.45$; $P_{\text{FDR}} < 0.05$; volume: $r=-0.42$; $P_{\text{FDR}}=0.06$).

The slopes for the regression lines between motor symptoms during the drug-off state and striatum SUR were not different between the early and advanced PD groups (cut-off value of 8 years: $P=0.76$; cut-off value of 10 years: $P=0.23$). The group comparison of the differences in the slopes for the motor symptoms and the CR of the NM-positive region in SN did not attain statistical significance (cut-off value of 8 years: $P=0.09$; cut-off value of 10 years: $P=0.05$). The groups were not significantly different in the slopes for the motor symptoms and the volume of the SN (cut-off value of 8 years: $P=0.19$; cut-off value of 10 years: $P=0.07$).

When all PD patients were analyzed together, the MDS-UPDRS Part III score during the drug-off state was weakly negatively correlated with the striatum SUR ($r=-0.33$; $P < 0.01$). In all patients with PD, the motor score during the drug-off state was also weakly negatively correlated with both the CR and volume of the NM-positive region in SN (CR: $r=-0.24$; $P < 0.05$; volume: $r=-0.24$; $P < 0.05$).

Motor symptoms, dopaminergic terminal, and NM density without and with fluctuation groups during the drug-off state

In the PD without motor fluctuation group, during the drug-off state, a correlation between the motor score and the striatum SUR did not reach statistical significance ($r=-0.35$; $P_{\text{FDR}}=0.10$), and a correlation between the motor score and the CR or volume of the NM-positive region in SN was not significant (CR: $r=-0.02$; $P_{\text{FDR}}=0.98$; volume: $r=-0.04$; $P_{\text{FDR}}=0.92$) (Fig 3). Nevertheless, in the PD with motor fluctuation group, during the drug-off state, the motor score was not correlated with the striatum SUR ($r=-0.12$; $P_{\text{FDR}}=0.46$), whereas the motor score was significantly negatively correlated with the CR and volume of the NM-positive region in SN (CR: $r=-0.38$; $P_{\text{FDR}} < 0.05$; volume: $r=-0.34$; $P_{\text{FDR}} < 0.05$).

The slopes for the regression lines between motor symptoms and striatum SUR were not different between the PD without and with motor fluctuation groups ($P=0.63$). If the issue of multiple comparisons was not addressed, the slopes for the motor symptoms and the CR of the NM-positive region in SN were significantly different between the groups ($P < 0.05$). The slopes for the motor symptoms and the volume of the SN were not different between the groups ($P=0.17$).

Motor symptoms, dopaminergic terminal, and NM density between stages during the drug-on state

During the drug-on state, the motor score was not correlated with striatum SUR, CR, or volume of the NM-positive region in SN in the early PD (disease duration ≤ 8 years: SUR: $r=-0.07$; $P_{\text{FDR}}=0.74$; CR: $r=-0.12$; $P_{\text{FDR}}=0.72$; volume: $r=-0.10$; $P_{\text{FDR}}=0.71$) and

advanced PD (disease duration >8 years: SUR: $r=-0.14$; $P_{\text{FDR}}=0.72$; CR: $r=-0.23$; $P_{\text{FDR}}\geq 1$; volume: $r=-0.13$; $P_{\text{FDR}}=0.69$) groups.

Similar findings were observed when the cut-off value of 10 years was used.

The motor score was not correlated with striatum SUR, CR, or volume of the NM-positive region in SN in the early PD (disease duration ≤ 10 years: SUR: $r=-0.09$; $P_{\text{FDR}}=0.69$; CR: $r=-0.17$; $P_{\text{FDR}}=0.75$; volume: $r=-0.13$; $P_{\text{FDR}}\geq 1$) and advanced PD (disease duration >10 years: SUR: $r=-0.12$; $P_{\text{FDR}}=0.72$; CR: $r=-0.20$; $P_{\text{FDR}}=0.83$; volume: $r=-0.12$; $P_{\text{FDR}}=0.68$) groups.

When all patients with PD were analyzed together, during the drug-on state, the MDS-UPDRS Part III score was not significantly correlated with striatum SUR ($r=-0.08$; $P=0.44$). In all patients with PD, the motor score during the drug-on state was also not correlated with the CR or volume of the NM-positive region in SN (CR: $r=-0.18$; $P=0.09$; volume: $r=-0.12$; $P=0.27$).

Motor symptoms, dopaminergic terminal, and NM density without and with fluctuation groups during the drug-on state

During the drug-on state, the motor score was not correlated with the striatum SUR, CR or volume of the NM-positive region in SN in the PD without motor fluctuation group (SUR: $r=-0.19$; $P_{\text{FDR}}=0.94$; CR: $r=0.04$; $P_{\text{FDR}}=0.86$; volume: $r=0.02$; $P_{\text{FDR}}=0.89$) and with motor fluctuation group (SUR: $r=-0.12$; $P_{\text{FDR}}=0.80$; CR: $r=-0.32$; $P_{\text{FDR}}=0.20$; volume: $r=-0.19$; $P_{\text{FDR}}=0.85$).

Dopaminergic terminal and NM density in PD subgroups

The SUR of the striatum on ^{123}I -Ioflupane SPECT was significantly higher in the early PD group than in the advanced PD group (cut-off value of 8 years: early PD, 0.87 ± 0.32 ; advanced PD, 0.71 ± 0.19 ; $P < 0.005$; cut-off value of 10 years: early PD, 0.83 ± 0.29 ; advanced PD, 0.68 ± 0.19 ; $P < 0.05$). In contrast, the CR and volume of the SN on NM-MRI were not different between the early and advanced PD groups (cut-off value of 8 years: CR: early PD, 1.09 ± 0.03 ; advanced PD, 1.10 ± 0.02 ; $P = 0.29$; volume: early PD, 0.90 ± 0.24 ; advanced PD, 0.92 ± 0.25 ; $P = 0.71$; cut-off value of 10 years: CR: early PD, 1.10 ± 0.03 ; advanced PD, 1.10 ± 0.03 ; $P = 0.71$; volume: early PD: 0.93 ± 0.24 ; advanced PD, 0.87 ± 0.24 ; $P = 0.26$).

The striatum on ^{123}I -Ioflupane SPECT had a higher SUR in the PD without motor fluctuation group than in the PD with motor fluctuation group (without fluctuation, 0.95 ± 0.33 ; with fluctuation, 0.71 ± 0.20 ; $P < 0.0001$). Nevertheless, the CR and volume of SN on NM-MRI were at a similar level in the PD without and with motor fluctuation groups (CR: without fluctuation, 1.10 ± 0.03 ; with fluctuation, 1.10 ± 0.02 ; $P = 0.98$; volume: without fluctuation, 0.91 ± 0.23 ; with fluctuation, 0.91 ± 0.25 ; $P = 1.00$).

In the early PD group (disease duration ≤ 8 years), the striatum SUR was positively correlated with the CR and was not correlated with the volume of the NM-positive region in SN (CR: $r = 0.33$; $P < 0.05$; volume: $r = 0.25$; $P = 0.10$) (Fig. 4). In the advanced PD group (disease duration > 8 years), the striatum SUR was also

correlated with the CR and tended to be correlated with the volume of the NM-positive region in SN (CR: $r=0.43$; $P<0.01$; volume: $r=0.27$; $P=0.06$).

Similar relationships were observed when a cut-off value of 10 years was used. In the early PD group (disease duration ≤ 10 years), the striatum SUR had a positive linear relationship with CR and was almost significantly correlated with the volume of the NM-positive region in SN (CR: $r=0.30$; $P<0.05$; volume: $r=0.22$; $P=0.07$). In the advanced PD group (disease duration >10 years), the striatum SUR was almost significantly correlated with the CR, but not with the volume of the NM-positive region in SN (CR: $r=0.38$; $P=0.05$; volume: $r=0.14$; $P=0.46$).

In the PD without motor fluctuation group, the striatum SUR was positively correlated with the CR and volume of the NM-positive region in SN (CR: $r=0.45$; $P<0.05$; volume: $r=0.44$; $P<0.05$) (Fig. 5). In the PD with motor fluctuation group, the striatum SUR was positively correlated with the CR of the NM-positive region, but not its volume (CR: $r=0.30$; $P<0.05$; volume: $r=0.16$; $P=0.21$).

Discussion

The findings of this study demonstrated that motor dysfunction severity in patients with early-stage PD during the drug-off state was paralleled by a decline in ^{123}I -ioflupane uptake in the striatum, even though it is not correlated with decreases in NM-MRI signals in SN. Nevertheless, the degree of motor deficits in patients with advanced-stage PD and those with motor fluctuation during the drug-off state was not correlated with ^{123}I -ioflupane uptake in the striatum, even though it is significantly negatively

correlated with NM-MRI signals in SN. The findings of this study revealed that the level of dopaminergic terminal degeneration in the striatum measured with dopamine transporter imaging was significantly correlated with the degree of motor symptoms in patients with early-stage PD who had motor symptoms for ≤ 8 or 10 years, while the degree of NM-laden dopamine neuron loss in SN assessed with MRI was significantly correlated with the severity of motor symptoms in patients with advanced-stage PD and those with motor fluctuation. We suggest monitoring the progression of motor diseases for future disease-modifying therapies using dopaminergic terminal degeneration in the striatum as an early-stage biomarker and NM-laden dopamine neuron loss in SN as an advanced-stage marker.

The findings of this study demonstrating the correlation between motor manifestations in patients with early-stage during the drug-off state and dopaminergic terminal loss in the striatum are in line with the idea that motor deficits, especially bradykinesia and rigidity, result from dopamine depletion in the striatum and the consequent dysfunction of the cortico-basal ganglia circuit that controls movements.³⁶ This idea was also in line with the present observation that both motor symptoms during the drug-off state and dopaminergic terminal loss in the striatum became significantly worse in patients with advanced-stage disease and those with motor fluctuation than in patients at early stages and those without motor fluctuation.

In contrast, we did not find a correlation between motor symptom severity and striatal dopaminergic terminal damage in patients with advanced-stage PD and those with motor fluctuation. These findings are consistent with the results of

1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated monkey investigation, human pathological and imaging studies suggesting rapid decreases in striatal dopamine function at early stages and slow reductions and a possible plateau at advanced stages^{12-14,16,37-38} Motor disabilities were prominent during the drug-off state, despite the relatively preserved motor function during the drug-on state in patients with advanced-stage PD and those with motor fluctuation when compared with that in patients with early-stage PD and those without motor fluctuation. We found that, during the drug-on state, the Hoehn-Yahr stage was significantly different between groups, but the MDS-UPDRS Part III score was not significantly different. This discrepancy may be due to the fact that the Hoehn-Yahr stage is more heavily weighted towards postural instability and mobility problems than the MDS-UPDRS Part III score, which comprehensively assesses major PD symptoms. In any case, the Hoehn and Yahr stage and MDS-UPDRS Part III scores were more different between groups during the drug-off state than during the drug-on state. The findings of this study implicate that deterioration in patients with advanced-stage PD and PD with motor fluctuation may be partly attributable to dopaminergic transmission at striatal sites with no or low

¹²³I-ioflupane binding affinity such as the serotonergic system, or at extra-striatal sites.³⁹

Severe dopaminergic terminal loss, as shown in patients with advanced-stage PD, has been considered essential to develop motor fluctuation, though the relationship between the degree of presynaptic denervation and motor fluctuation risk remains to be clarified. When dopaminergic presynaptic terminals in the striatum are profoundly injured and dopamine content was reduced to <20% of control values in a rodent PD

model, extracellular dopamine concentration upon treatment with levodopa became markedly higher at peak levels.⁴⁰ This larger swing observed in the striatum and SN is likely to contribute to motor fluctuation development.⁴¹ In contrast, a direct dopamine agonist, apomorphine, induced a shorter response duration in PD patients with motor fluctuation than in de novo patients, suggesting that postsynaptic mechanisms also play a role.⁴² Accordingly, it is plausible that a loss of nigrostriatal dopamine input entails a profound adaptation of both pre- and postsynaptic mechanisms, causing motor fluctuations in patients with PD.

This study demonstrated a correlation between motor symptoms during the drug-off state and NM density in SN in patients with advanced-stage PD and those with motor fluctuation. Meanwhile, in early-stage patients, the density of NM in SN varied greatly, with several patients displaying preserved levels while others demonstrated profound loss. This large variability among patients with early-stage PD seemingly induces an insignificant difference in the density of NM between early- and advanced-stage patients, as well as between patients with and without motor fluctuation. Though the functional significance of the observed correlation between motor disability and NM density remains unclear, animal experiments proposed that dopaminergic transmission in SN pars reticulata may have a functional role in PD. Dopamine is synthesized, stored, and released from the SN pars compacta neurons that arborize in the SN pars reticulata,⁴³ and restoring dopaminergic tone in the SN alleviated motor symptoms in a PD animal model.⁴⁴ NM-laden dopamine neuron degeneration in the SN, therefore, may be related to motor deterioration at advanced stages. Midbrain dopamine

neurons seem difficult to measure accurately with ^{123}I -ioflupane SPECT but can be scrutinized using a high uptake tracer with high spatial resolution, such as dihydrotetrabenazine (DTBZ) PET.⁴⁵ Future studies should clarify the possible differences in the pathophysiology underlying motor disturbance between patients with early- and advanced-stage PD.

The findings of this study are in line with the hypothesis that neuronal degeneration occurs earlier in dopaminergic terminals and progresses to the soma since the terminal in the striatum rapidly decreases at early stages, while the NM in SN has a considerably variable density at early stages but gradual loss with much less variability at advanced stages. This hypothesis is in line with postmortem data showing that, at the time of motor symptom onset, dopaminergic terminal capacity in the striatum decreased to <50% of baseline values, whereas NM in SN was reduced by approximately 70% of control values.⁴⁶ Additionally, the experimental data from a mouse transgenic model suggest that primary insult in PD pathology occurs at dopaminergic terminals.⁴⁷ Distal axon vulnerability in the nigrostriatal system is proposed based on the facts that mitochondrial dysfunction is implicated in both sporadic and familial PD⁴⁸ and that subsequent energy failure predisposes hyper-branched axons, such as dopamine neurons, to degeneration, especially at the terminals.⁴⁹

Midbrain dopamine cells projecting to the striatum are spatially divided into partially overlapped motor, limbic, and cognitive regions.⁵⁰ Voxel wise analysis of clinical deficits and NM-MRI signals found a correlation between motor deficits related to the posterolateral SN, mood/behavioral symptoms related to the anteromedial SN,

and cognitive impairments related to the superior and anterior SN in patients with early-stage PD involving average disease duration of <5 years.³⁵ The findings of that study imply that the NM-laden cell bodies in SN have spatial variability, whereas our findings propose that this degeneration has temporal variability. The lack of correlation between motor deficits and NM-MRI signals in patients with early-stage PD in this study may be due to the global signals of SN we measured and the variability in motor, limbic, and cognitive disabilities among the patients in this study. Alternatively, three-dimensional acquisition using 0.7-mm cubic data in the present study, rather than two-dimensional acquisition using 3-mm thickness and 0.4-mm in-plane resolution, may affect the evaluation of SN with sizes of approximately 10 mm in the rostral-caudal and ventral-dorsal axes of the brainstem.^{51,52}

The slopes for the regression lines between motor symptoms during the drug-off state and imaging biomarkers were largely not different between the early and advanced PD groups and between the PD without and with motor fluctuation groups. This lack of group difference may be attributable to low statistical power caused by the small sample size and high variability associated with *in vivo* imaging techniques in the present study. Alternatively, the lack of difference may reflect genuine pathological characteristics. The origin should be clarified in future studies using methodologies such as higher signal-to-noise ratio 7 tesla MRI or longitudinal large cohorts.

Since this is not a clinicopathologic or clinicogenetic study, one may argue that the larger variability in NM-laden neuron loss in patients with early-stage PD is due to the higher ratio of misdiagnosed early-stage patients with other causes of

parkinsonism and related disorders. However, this is unlikely because, in addition to applying the MDS clinical diagnostic criteria, with high sensitivity and specificity of around 90%,²⁵ the diagnostic accuracy of PD was enhanced in all patients by confirming the absence of structural MRI abnormalities associated with other causes of parkinsonism, decreased striatal uptake of ¹²³I-ioflupane, and presence of levodopa responses during the drug-on state when compared with the drug-off state.

In conclusion, this cross-sectional study found that motor deficits in patients with early-stage PD were firmly associated with dopaminergic terminal loss in the striatum. In patients with advanced-stage PD, striatal presynaptic dopaminergic functional imaging was a suboptimal marker of motor disease progression, and motor disabilities were associated with the severity of NM-laden neuron loss in SN, as assessed using NM-MRI. A multimodal neuroimaging approach accelerates research related to etiology as well as therapy development and provides biomarkers to define and measure disease progression for neuroprotective or neuromodulatory therapy in PD.

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Author Contributions

KF, AS, TO, RT and NS contributed to study concept and design. KF, AS, DK, AN, IW, HS, KY, YT, YS, MM, MS, EN, YT, HY, YF, YN, and NS contributed to data acquisition and analysis. KF, AS, and NS contributed to drafting the manuscript and the figures.

Potential Conflicts of Interest

R.T. received research grant from Nihon Medi-Physics Co., Ltd., a manufacture of ^{123}I -Ioflupane. The remaining authors have nothing to report.

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Figure Legends

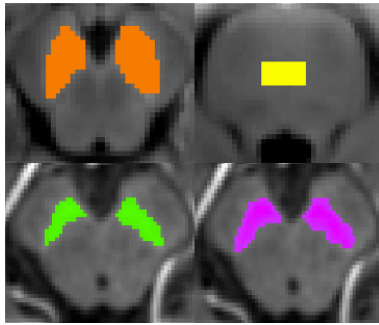


Figure 1 Definition of the neuromelanin (NM)-positive region in the substantia nigra (SN) and reference region in the rostral pons. *Top left:* A group-averaged NM map was delineated in the SN by manually outlining the hyperintense area on the NM-magnetic resonance imaging (MRI) template generated from healthy control participants (orange). NM accumulates in dopamine neurons which are rich in the SN pars compacta, although boundaries between the SN pars compacta and SN pars reticulata are difficult to identify with MRI. *Top right:* The reference region was set in the rostral pontine area on the NM-MRI template (yellow). The reference region on the template was transformed on an individual NM-MRI to normalize the signal intensity by removing inter-participant variability. *Bottom left:* The group-averaged NM map on the template was projected on the individual NM-MRI acquired perpendicular to the cerebral aqueduct, and hyperintense voxels having above 50th percentile value in the projected NM map were selected. For display purposes, representative healthy control participant data are represented (green). The mean signal intensity of the selected voxels was computed as the contrast ratio (CR) of SN. *Bottom right:* Hyperintense voxels having suprathreshold intensity, that is, 1.05, in the registered NM map were identified,

and total volumes were calculated as the volume of NM-positive regions.

Representative healthy control participant data are displayed (magenta).

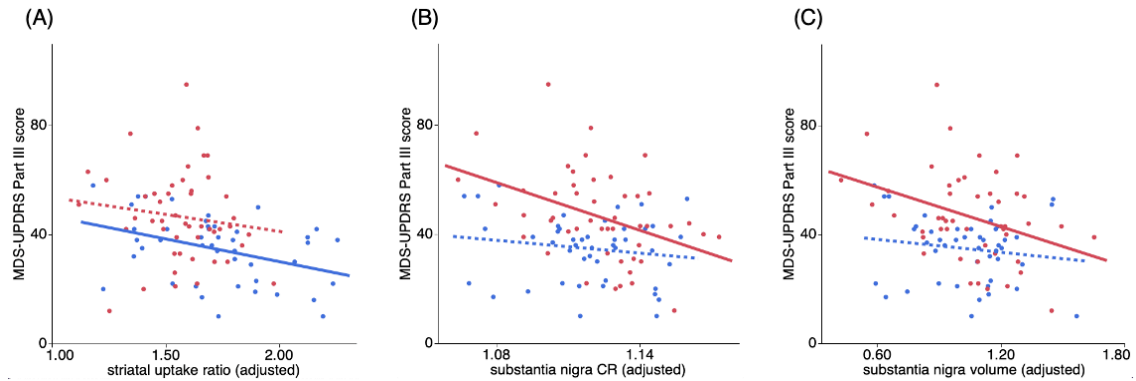


Figure 2 Correlation between MDS-UPDRS Part III score and the striatal uptake ratio (A) and the contrast ratio (CR) (B) and volume (C) of the neuromelanin (NM)-positive region in the substantia nigra (SN) at early- (disease duration ≤ 8 years: blue) and advanced-stage (disease duration > 8 years: red) of Parkinson's disease. The striatal uptake ratio, substantia nigra CR and volume were adjusted for the effects of age and sex by linear regression. Regression lines are presented for each stage (solid line: statistically significant; dotted line: nonsignificant). The MDS-UPDRS Part III score was evaluated during the drug-off state.

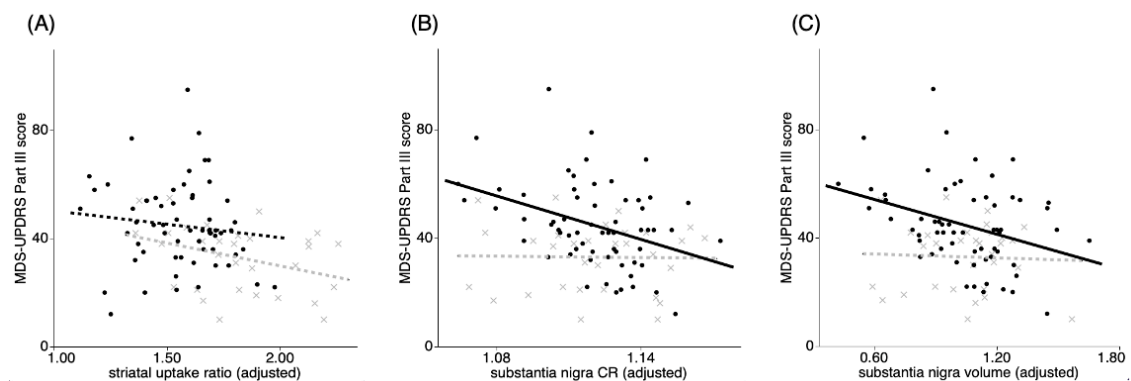


Figure 3 Correlation between MDS-UPDRS Part III score and the striatal uptake ratio (A) and the contrast ratio (CR) (B) and volume (C) of the neuromelanin

(NM)-positive region in the substantia nigra (SN) in Parkinson's disease (PD)

patients without (gray cross) and with motor fluctuation (black dot). The striatal uptake ratio, substantia nigra CR and volume were adjusted for the effects of age and sex by linear regression. Regression lines are presented for each group (PD without motor fluctuation: gray; PD with motor fluctuation: black; solid line: statistically significant; dotted line: nonsignificant). The MDS-UPDRS Part III score was evaluated during the drug-off state.

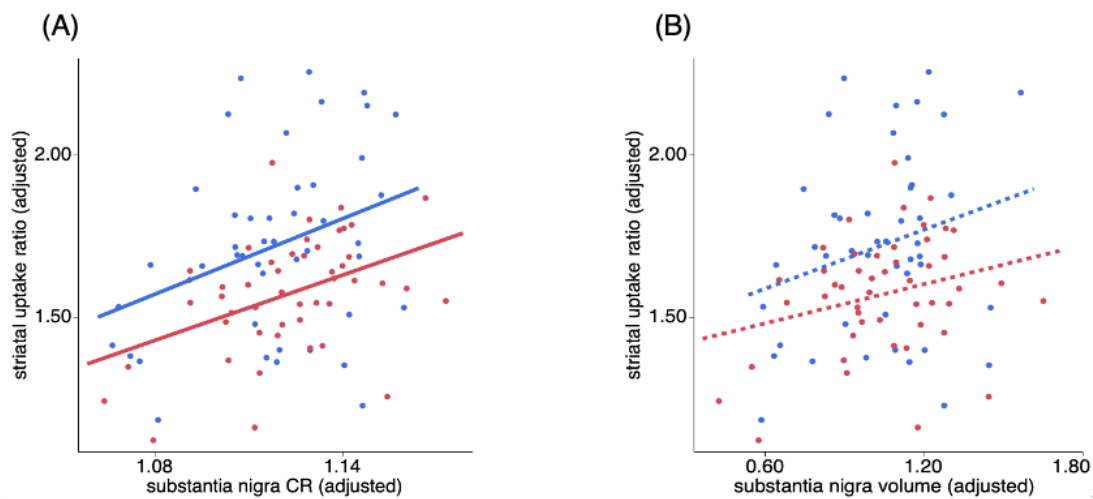


Figure 4 Correlation between the striatal uptake ratio and the contrast ratio (CR)

(A) and volume (B) of the neuromelanin (NM)-positive region in the substantia nigra (SN) at early- (disease duration ≤ 8 years: blue) and advanced-stage (disease duration > 8 years: red) of patients with Parkinson's disease. Regression lines are presented for each stage (solid line: statistically significant; dotted line: nonsignificant).

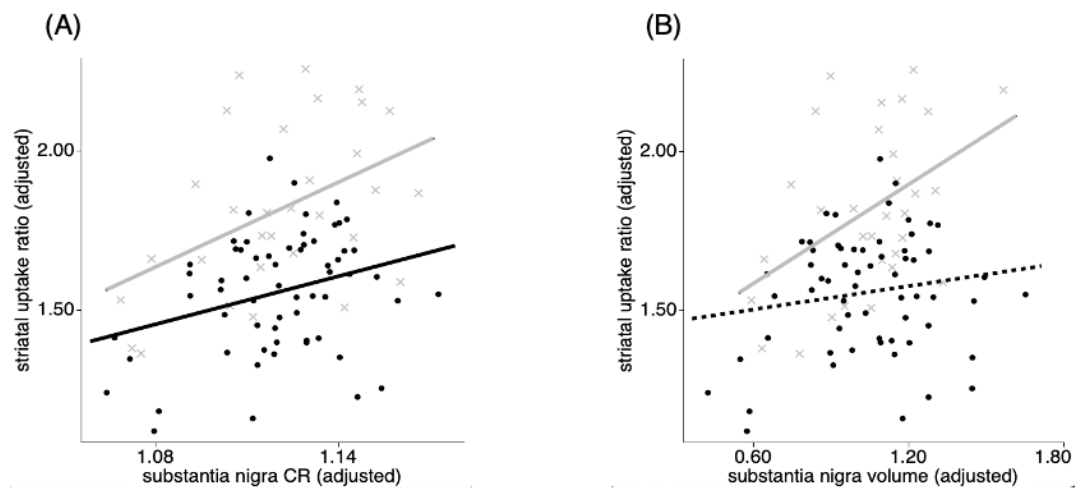


Figure 5 Correlation between the striatal uptake ratio and the contrast ratio (CR)

(A) and volume (B) of the neuromelanin (NM)-positive region in the substantia

nigra (SN) in Parkinson's disease (PD) patients without (gray cross) and with

motor fluctuation (black dot). Regression lines are presented for each group (PD

without motor fluctuation: gray; PD with motor fluctuation: black; solid line:

statistically significant; dotted line: nonsignificant).

Table 1. Clinical characteristics of patients with early (disease duration ≤ 8 years) and advanced Parkinson's disease (> 8 years)

	≤ 8 years (n=45)	> 8 years (n=48)	<i>P</i> value
Age	63.3 $\pm$ 10.9	64.1 $\pm$ 7.6	0.71 ^a
Female:Male	22:23	28:20	0.36 ^b
Disease duration	4.7 $\pm$ 2.3	11.5 $\pm$ 3.2	$<0.0001^a$
LEDD	407.8 $\pm$ 362.7	807.2 $\pm$ 376.1	$<0.0001^a$
Hoehn and Yahr (off)	2.3 $\pm$ 0.9	3.3 $\pm$ 1.2	$<0.0001^a$
Hoehn and Yahr (on)	1.9 $\pm$ 0.5	2.1 $\pm$ 0.5	$<0.01^a$
MDS-UPDRS Part III score (off)	34.8 $\pm$ 12.1	46.5 $\pm$ 16.6	$<0.001^a$
MDS-UPDRS Part III score (on)	21.3 $\pm$ 10.2	20.8 $\pm$ 10.8	0.81 ^a

^aIndependent t-test and ^bPearson's chi-square test used for both groups. On = drug-on state; off = drug-off state. LEDD = levodopa daily equivalent dose

Table 2. Clinical characteristics of patients with early (disease duration ≤ 10 years) and advanced Parkinson's disease (> 10 years)

	≤ 10 years (n=66)	> 10 years (n=27)	<i>P</i> value
Age	63.6 $\pm$ 9.9	63.9 $\pm$ 7.8	0.89 ^a
Female: Male	34:32	16:11	0.50 ^b
Disease duration	6.0 $\pm$ 2.7	13.7 $\pm$ 2.7	$<0.0001^a$
LEDD	482.5 $\pm$ 361.9	935.2 $\pm$ 377.2	$<0.0001^a$
Hoehn and Yahr (off)	2.4 $\pm$ 0.9	3.9 $\pm$ 1.1	$<0.0001^a$
Hoehn and Yahr (on)	1.9 $\pm$ 0.5	2.2 $\pm$ 0.6	$< 0.05^a$
MDS-UPDRS Part III score (off)	38.1 $\pm$ 13.6	47.4 $\pm$ 18.4	$< 0.01^a$
MDS-UPDRS Part III score (on)	21.3 $\pm$ 10.5	20.3 $\pm$ 10.7	0.65 ^a

^aIndependent t-test and ^bPearson's chi-square test for the two groups. On=drug-on state; off=drug-off state. LEDD = levodopa daily equivalent dose.

Table 3. Clinical characteristics of Parkinson's disease patients without and with motor fluctuation

	Without motor fluctuation (n=31)	With motor fluctuation (n = 62)	<i>P</i> value
Age	64.9 ± 12.0	63.1 ± 7.6	0.37 ^a
Female:Male	17:14	33:29	0.88 ^b
Disease duration	4.3 ± 3.1	10.2 ± 3.7	< 0.0001 ^a
LEDD	237.4 ± 233.3	802.2 ± 360.3	< 0.0001 ^a
Hoehn and Yahr (off)	2.0 ± 0.7	3.2 ± 1.1	< 0.0001 ^a
Hoehn and Yahr (on)	1.8 ± 0.5	2.1 ± 0.5	< 0.01 ^a
MDS-UPDRS Part III score (off)	33.0 ± 12.4	44.7 ± 15.7	< 0.001 ^a
MDS-UPDRS Part III score (on)	22.4 ± 11.3	20.3 ± 10.1	0.37 ^a

^aIndependent t-test and ^bPearson's chi-square test for the two groups. On = drug-on state; off = drug-off state. LEDD = levodopa daily equivalent dose.