The Topography of Brain Damage at Different Stages of Parkinson's Disease

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Abstract: This study investigated gray matter (GM) and white matter (WM) damage in 89 patients at different clinical stages of Parkinson's disease (PD) (17 early, 46 mild, 14 moderate, and 12 severe) to differentiate the trajectories of tissue injury in this condition. PD patients had a very little GM atrophy even at the more advanced stages of the disease. Microstructural damage to the WM occurs with increasing PD severity and involves the brainstem, thalamocortical pathways, olfactory tracts, as well as the major interhemispheric, limbic, and extramotor association tracts. The most marked WM damage was found in moderate vs. mild cases. WM damage correlated with the degree of global cognitive deficits. WM abnormalities beyond the nigrostriatal system accumulate with increasing PD severity. WM damage is likely to contribute to the more severe motor and nonmotor dysfunctions occurring in patients at the later stages. *Hum Brain Mapp* 34:2798–2807, 2013. © 2012 Wiley Periodicals, Inc.

Key words: Parkinson's disease; white matter damage; DT MRI; brain atrophy; VBM

INTRODUCTION

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The main pathological substrates associated with motor symptoms in Parkinson's disease (PD) are the death of dopamine-producing neurons in the substantia nigra (SN) pars compacta, coupled with the presence of intracytoplasmic α -synuclein-positive inclusions in the cell body and processes [Braak and Braak, 2000]. However, other neurotransmitter systems do also degenerate in this condition, as suggested by the pathologic abnormalities in PD extending well beyond the nigrostriatal pathways and involving other projection neurons of the brainstem and cortex [Braak and Braak, 2000]. The nondopaminergic neural degeneration is likely to contribute to the nonmotor symptoms and signs of PD [Lim and Lang, 2010].

The in vivo topography of brain tissue loss in PD has been assessed by several structural magnetic resonance

TABLE I. Demographic, clinical, and cognitive data from healthy controls and PD patients stratified according to the Hoehn and Yahr stage scale

	Healthy controls	Early PD (HY 1/1.5)	Mild PD (HY 2/2.5)	Moderate PD (HY 3)	Severe PD (HY 4)	<i>P</i>
Number	42	17	46	14	12	—
Women/men	17/25	7/10	15/31	7/7	5/7	0.79
Age at MRI (years)	64 ± 7 (48–82)	64 ± 6 (54–73)	62 ± 6 (50–78)	65 ± 8 (52–78)	65 ± 7 (54–82)	0.55
Education (years)	13 ± 3 (4–18)	12 ± 3 (6–16)	12 ± 4 (4–25)	11 ± 4 (3–16)	11 ± 4 (4–16)	0.13
Age at onset (years)	—	62 ± 6 (50–72)	56 ± 8 (43–75)*	54 ± 9 (36–71)	51 ± 8 (36–65)	0.002
Disease duration (years)	—	2 ± 2 (0.5–7)	7 ± 5 (1–21)*	11 ± 5 (5–21)##	14 ± 6 (6–30)	<0.001
Side initially affected (right/left)	—	11/6	24/22	7/7	9/3	0.45
UPDRS III	—	15 ± 5 (9–26)	25 ± 9 (4–44)**	34 ± 12 (15–57)##	49 ± 8 (35–62)§§	<0.001
MMSE	29 ± 1 (26–30)	29 ± 1 (25–30)	28 ± 1 (25–30)	27 ± 2 (25–30)#	27 ± 2 (25–29)	0.002
WMH load (ml)	0.6 ± 1.1 (0–4.9)	0.4 ± 0.5 (0–1.8)	0.6 ± 1.1 (0–5.0)	0.7 ± 1.2 (0–4.6)	1.8 ± 2.2 (0–6.0)	0.35

Values are mean ± standard deviation (range) or number of patients. *P* indicates differences at the Kruskal-Wallis or Fisher's exact test when comparing all groups. Between-group comparisons using the Mann-Whitney *U*-test or Fisher's exact test: **P* < 0.05 and ***P* < 0.001 vs. early PD; #*P* < 0.05 and ##*P* < 0.001 vs. mild PD; and §§*P* < 0.001 vs. moderate PD.

imaging (MRI) studies. On the one hand, these studies showed no [Borghammer et al., 2010; Hattori et al., 2012; Nagano-Saito et al., 2005] or only slight frontal [Burton et al., 2004; Nishio et al., 2010] and temporal [Nishio et al., 2010] gray matter (GM) loss in early to mild nondemented PD patients, while on the other hand, there is a marked GM atrophy of the limbic, temporal, frontal, and parietal regions in demented PD patients [Burton et al., 2004]. In addition, white matter (WM) loss has been detected in early to moderate stages of the disease, in the pons, as well as in temporal and frontal regions [Jubault et al., 2009; Kostic et al., 2010].

Diffusion tensor (DT) MRI allows the interrogation in vivo of the microstructure of brain tissues by measuring directional changes in water diffusivity [Basser and Pierpaoli, 1996]. In PD patients, DT MRI is highly sensitive to microstructural changes occurring in the SN and basal ganglia [Peran et al., 2010]. However, only limited evidence is available regarding the overall pattern of WM damage in this condition, and the results of voxel-based DT MRI studies of nondemented PD patients are conflicting [Karagulle Kendi et al., 2008; Zhang et al., 2011]. Along with the different methodologies used to measure diffusion, the small samples studied and the clinical heterogeneity of the patients enrolled might have contributed to such inconsistent findings.

The aim of this study was to assess the topography of GM loss and WM tract damage associated with the different clinical stages of PD to differentiate the possibly different trajectories of tissue damage in this condition. Although we recognize that this is not a long-term longitudinal study—which is unlikely ever to be performed in this condition—we believe that our study would allow us to improve our understanding of PD pathobiology and will contribute to the identification of new markers for monitoring its evolution in vivo. We approached this issue

by using regionally unbiased, voxel-based MRI analyses in a large sample of PD patients stratified according to the Hoehn and Yahr (HY) stage score [Hoehn and Yahr, 1967]. We also explored the clinical relevance of GM and WM damage by evaluating the correlation with the motor and global cognitive status of PD patients.

METHODS

Subjects

One hundred thirteen PD patients [Hughes et al., 1992] were enrolled consecutively from the outpatient population attending the Centre for Neurodegenerative Diseases, University of Belgrade, Serbia. Clinical assessments were performed by experienced neurologists blinded to MRI results. At study entry, disease severity was assessed using the HY scale [Hoehn and Yahr, 1967] during OFF time. Patients were also administered the Unified Parkinson's Disease Rating Scale III (UPDRS III) [Fahn et al., 1987] and Mini-Mental State Examination (MMSE) [Folstein et al., 1975]. Patients were excluded if they had: (a) parkin, leucine-rich repeat kinase 2 (LRRK2), and glucocerebrosidase (GBA) gene mutations; (b) cerebrovascular disorders, traumatic brain injury history, or intracranial mass; (c) other major neurological and medical diseases; and (d) dementia [Dubois et al., 2007]. Eighty-nine patients fulfilled the inclusion/exclusion criteria and entered the study (Table I). Seventeen patients had early PD (HY 1/1.5), 46 mild PD (HY 2/2.5), 14 moderate PD (HY 3), and 12 severe PD (HY 4). Forty-two healthy subjects with no history of neurologic and psychiatric disease were recruited from spouses of patients and by word of mouth (Table I). Healthy controls underwent a multidimensional assessment, including neurological and global cognitive evaluation, and were included in the study only when all

items were normal. Approval was received from the local ethical standards committee on human experimentation, and written informed consent was obtained from all subjects participating in the study.

MRI Acquisition

MRI scans were obtained on a 1.5-T Avanto system (Siemens, Erlangen, Germany). The following sequences were acquired: (i) dual-echo (DE) turbo spin-echo (SE) [repetition time (TR) = 2,650 ms, echo time (TE) = 28/113 ms, echo train length = 5, 50 axial slices, thickness = 2.5 mm with no gap, matrix size = 256×256 , and field of view (FOV) = 250 mm^2]; (ii) three-dimensional T_1 -weighted magnetization-prepared rapid acquisition gradient echo [frequency direction = inferior–superior; TR = 2,000 ms, TE = 4.72 ms, flip angle = 12° , matrix size = $256 \times 224 \times 208$ (inferior–superior, anterior–posterior, and left–right), FOV = $236 \text{ mm} \times 236 \text{ mm} \times 236 \text{ mm}$]; and (iii) pulsed gradient SE single-shot echo-planar (TR = 8,100, TE = 95, flip angle = 90° , matrix size = 128×128 , FOV = 240 mm^2 ; 50 axial slices, and thickness = 2.5 mm with no gap), with diffusion-encoding gradients applied in 12 noncollinear directions, selected as the default in the scanner (b factor = 1,000 s/mm^2 and eight averages). The maximum amplitude of the diffusion gradients was 33 mT/m, and a multiple-channel head coil was used for signal reception.

MRI Analysis

MRI analysis was performed by an experienced observer, blinded to patients' identity. WM hyperintensities (WMHs), if any, were identified on DE scans. WMH load was measured using the Jim software package (Version 5.0, Xinapse Systems, Northants, UK, <http://www.xinapse.com>).

WM damage: Tract-based spatial statistics

DT MRI analysis was carried out using in-house software. DT MR images were first corrected for distortion induced by eddy currents [Studholme et al., 1997], then the DT was estimated by linear regression [Basser et al., 1994], and mean diffusivity (MD) and fractional anisotropy (FA) maps were computed [Basser and Pierpaoli, 1996]. Maps of axial diffusivity (axD), which is equivalent to the magnitude of the largest eigenvalue of the tensor, and radial diffusivity (radD), which is the average of the two smallest eigenvalues of the tensor, were also calculated [Basser and Pierpaoli, 1996]. Tract-based spatial statistics (TBSS) version 1.2 (<http://www.fmrib.ox.ac.uk/fsl/tbss/index.html>) was used to perform the multisubject DT MRI analysis [Smith et al., 2006]. FA volumes were aligned to a target image using the following procedure: (i) a target image was selected automatically as the most representative FA image by the FMRIB's Nonlinear Image Registra-

tion Tool (FNIRT), (ii) the nonlinear transformation that mapped each subject's FA to the target image was computed using FNIRT, (iii) the target image was transformed affinely to the Montreal Neurological Institute (MNI) 152 standard space, and (iv) the same transformation was used to align each subject's FA map to the standard space. A mean FA image was then created by averaging the aligned individual FA images and thinned to create a FA skeleton representing WM tracts common to all subjects [Smith et al., 2006]. The FA skeleton was thresholded at a value of 0.2 to exclude voxels with low FA values, which are likely to include GM or cerebrospinal fluid (CSF). Individual MD, FA, axD, and radD data were projected onto this common skeleton.

Brain atrophy: Voxel-based morphometry

Voxel-based morphometry (VBM) was performed using SPM8 and the Diffeomorphic Anatomical Registration Exponentiated Lie Algebra (DARTEL) registration method [Ashburner, 2007], as previously described [Canu et al., 2012]. Briefly, (i) T_1 -weighted images were segmented [Ashburner and Friston, 2005] to produce GM, WM, and CSF tissue probability maps in the MNI space; (ii) original T_1 -weighted images were imported in the common DARTEL space, rigidly aligned (using the rigid-body component of the normalization parameters from step i), segmented into GM, WM, and CSF (using the segmentation parameters from step i), and resampled to 1.5-mm isotropic voxels; (iii) the DARTEL template was created (using the resampled images); and (iv) the flow fields were obtained (from step iii), applied to the rigidly aligned segments to warp them to the common DARTEL space, and modulated using the Jacobian determinants. Because the DARTEL process warps to a common space that is smaller than the MNI space, we performed an additional transformation as follows: (v) the modulated images from DARTEL were normalized to the MNI template using an affine transformation estimated from the DARTEL GM template and the a priori GM probability map without resampling (<http://brainmap.wisc.edu/normalizeDARTELtoMNI>). Before the statistical computations, the images were smoothed with an 8-mm full width at half maximum (FWHM) Gaussian filter.

Statistical Analysis

Demographic and clinical variables were compared between groups using the Fisher's exact test for categorical variables and the Mann–Whitney U -test or the Kruskal–Wallis test for continuous variables (P value < 0.05 ; SAS Release 9.1, SAS Institute, Cary, NC).

DT MRI voxelwise statistics were performed using a permutation-based inference tool for nonparametric statistical thresholding ("randomize," part of FSL [Nichols and Holmes, 2002]). The number of permutations was set at 5,000 [Nichols and Holmes, 2002]. Two analyses were performed on TBSS data. First, a whole-brain analysis was

Figure 1.

WM damage: whole-brain analysis. Tract-based spatial statistics results in moderate + severe PD patients vs. healthy controls (A) and early + mild PD patients (B). Voxelwise group differences are shown in blue (MD), red (FA), green (radD), and yellow

(axD). Results are overlaid on the sagittal, coronal, and axial sections of the Montreal Neurological Institute standard brain in neurological convention (right is right) and displayed at $P < 0.05$, FWE corrected for multiple comparisons.

run to investigate the following contrasts: early + mild PD patients vs. healthy controls, moderate + severe PD patients vs. healthy controls, and moderate + severe vs. early + mild PD patients. MD, FA, axD, and radD values within the skeleton were tested between groups using permutation-based two-sample t -tests. The resulting statistical maps were thresholded at $P < 0.05$ corrected for multiple comparisons [family-wise error (FWE)] at a cluster level using the threshold-free cluster enhancement (TFCE) option in the "randomize" permutation-testing tool [Smith and Nichols, 2009]. Second, we perform a region of interest (ROI) analysis to investigate the differences between the single patient groups along the trajectory "early-mild-moderate-severe PD." To this end, an ROI was created including the MD and FA group differences obtained in the main contrast "moderate + severe PD patients vs. healthy controls" at $P < 0.10$ FWE corrected (Supporting Information Fig. 1). MD and FA results were combined and binarized to obtain a mask. Then, the following contrasts were tested within this mask: early PD vs. healthy

controls, mild vs. early PD, moderate vs. mild PD, and severe vs. moderate PD. The results were tested at $P < 0.05$ FWE corrected (TFCE).

Using SPM8, analyses of covariance were performed to assess GM differences between groups, adjusted for total intracranial volume (TICV). The VBM statistical analyses replicated those performed for TBSS results: a whole-brain analysis investigating the main contrasts (i.e., early + mild PD patients vs. healthy controls, moderate + severe PD patients vs. healthy controls, and moderate + severe vs. early + mild PD patients) and an ROI analysis investigating the differences between the single patient groups (within a mask of the GM differences obtained in the main contrast "moderate + severe PD patients vs. healthy controls" at $P < 0.10$ FWE corrected). The VBM results were tested at a statistical threshold of $P < 0.05$, FWE corrected.

To assess whether the UPDRS III and MMSE scores were associated with WM damage and GM atrophy, regression models in FSL and SPM8 (adjusted for TICV) were run within the WM and GM masks mentioned

Figure 2.

WM damage: ROI analysis. Tract-based spatial statistics results in moderate vs. mild PD patients. Voxelwise group differences are shown in blue (MD), red (FA), and green (radD). Results are overlaid on the sagittal, coronal, and axial sections of the Montreal Neurological Institute standard brain in neurological convention (right is right) and displayed at $P < 0.05$, FWE corrected for multiple comparisons.

above. To minimize the number of comparisons, axD and radD were not included in the correlation analysis. Results were assessed at $P < 0.05$ FWE corrected.

RESULTS

Demographic, Clinical, and Cognitive Features

Patient groups were similar in terms of age, gender, education, side initially affected, and WM hyperintensity load. Mild vs. early PD had lower age at onset ($P = 0.003$), longer disease duration ($P = 0.0005$), and higher UPDRS III ($P < 0.001$). Moderate vs. mild PD had longer disease duration ($P < 0.001$), higher UPDRS III ($P < 0.001$), and lower MMSE ($P = 0.03$). Severe vs. moderate PD had a higher UPDRS III score ($P < 0.001$).

WM Damage: Whole-Brain Analysis

Early + mild PD patients vs. healthy controls

No DT MRI differences were found between early + mild PD patients and controls ($P < 0.05$ FWE corrected).

Moderate + severe PD patients vs. healthy controls

Compared with controls, moderate + severe PD patients showed an increased MD in the midbrain, cerebellar WM, thalamic radiations, olfactory tracts, genu and anterior body of the corpus callosum, internal and external capsule, fornix, and temporal, parietal, occipital, and orbitofrontal WM bilaterally ($P < 0.05$ FWE corrected) (Fig. 1A). In moderate + severe PD patients relative to controls, the pattern of radD

increase resembled that of increased MD with additional regions of difference in the mid and posterior body of the corpus callosum, cingulum, superior longitudinal fasciculus, and dorsolateral and ventral superior frontal WM bilaterally ($P < 0.05$ FWE corrected) (Fig. 1A). There were no axD and FA differences between moderate + severe PD patients and controls ($P < 0.05$ FWE corrected).

Moderate + severe vs. early + mild PD patients

In moderate + severe compared with early + mild PD patients, regions of increased MD and radD were seen bilaterally and involved most of the WM, including brainstem, cerebellar WM, thalamic radiations, olfactory tracts, whole corpus callosum, internal and external capsule, and frontal, temporal, parietal, and occipital WM ($P < 0.05$ FWE corrected) (Fig. 1B). AxD was increased in the midbrain, thalamic radiations, external and internal capsule, superior longitudinal fasciculus, temporal, parietal, and occipital WM, bilaterally, and right olfactory tract ($P < 0.05$ FWE corrected) (Fig. 1B). Decreased FA was found in the genu and anterior body of the corpus callosum bilaterally, superior longitudinal fasciculus bilaterally (right > left), right external capsule, right olfactory tract, and right orbitofrontal WM ($P < 0.05$ FWE corrected) (Fig. 1B).

WM Damage: ROI Analysis

Early PD patients vs. healthy controls

No DT MRI differences were found between groups ($P < 0.05$ FWE corrected).

Figure 3.

GM atrophy. **(A)** Whole-brain analysis: areas of GM tissue loss in moderate + severe PD patients vs. healthy controls. **(B)** ROI analysis: areas of GM tissue loss in early PD patients vs. healthy controls and in moderate vs. mild PD patients. Results are superimposed on the sagittal, coronal, and axial sections of the Montreal Neurological Institute standard brain in neurological convention (right is right) and displayed at $P < 0.05$, FWE corrected for multiple comparisons.

Mild vs. early PD patients

No DT MRI differences were found between groups ($P < 0.05$ FWE corrected).

Moderate vs. mild PD patients

In moderate compared with mild PD patients, regions of increased MD were found in the anterior body of the corpus callosum bilaterally, right external and internal capsules, right olfactory tract, and left temporal WM ($P < 0.05$ FWE corrected) (Fig. 2). Increased radD was found in the whole corpus callosum bilaterally, right external capsule, internal capsules bilaterally, right superior longitudinal fasciculus, orbitofrontal (right > left) and parietal WM bilaterally, and olfactory tracts bilaterally ($P < 0.05$ FWE corrected) (Fig. 2). Compared with mild PD, moderate PD patients had regions of decreased FA in the anterior body of the corpus callosum, right external capsule, orbitofrontal (right > left) and parietal WM bilaterally, and right olfactory tract ($P < 0.05$ FWE corrected) (Fig.

2). There were no axD differences between groups ($P < 0.05$ FWE corrected).

Severe vs. moderate PD patients

No DT MRI differences were found between groups ($P < 0.05$ FWE corrected).

GM Atrophy: Whole-Brain Analysis

Early + mild PD patients vs. healthy controls

No GM difference was found between early + mild PD patients and controls ($P < 0.05$ FWE corrected).

Moderate + severe PD patients vs. healthy controls

Compared with controls, moderate + severe PD patients showed GM loss ($P < 0.05$ FWE corrected) in the right thalamus ($x = 9, y = -6, z = -2$, and t -value = 6.24), left middle frontal gyrus ($x = -37, y = 13, z = 47$, and t -value = 5.20), left superior medial frontal gyrus ($x = -11, y = 39, z = 43$, and t -value = 4.91), and right middle temporal gyrus ($x = 66, y = -17, z = -7$, and t -value = 4.79) (Fig. 3A).

Moderate + severe vs. early + mild PD patients

No GM difference was found between groups ($P < 0.05$ FWE corrected).

GM Atrophy: ROI Analysis

Early PD patients vs. healthy controls

Compared with controls, early PD patients showed GM loss ($P < 0.05$ FWE corrected) in the left middle frontal gyrus ($x = -43, y = 13, z = 38$, and t -value = 3.24) and right middle temporal gyrus ($x = 66, y = -19, z = -7$, and t -value = 3.80) (Fig. 3B).

Mild vs. early PD patients

No GM difference was found between groups ($P < 0.05$ FWE corrected).

Moderate vs. mild PD patients

Compared with mild PD, moderate PD patients showed GM loss in the right thalamus ($x = 7, y = -6, z = -2$, and t -value = 5.21; $P < 0.05$ FWE corrected) (Fig. 3B).

Severe vs. moderate PD patients

No GM difference was found between groups ($P < 0.05$ FWE corrected).

Figure 4.

WM damage vs. MMSE. Areas of WM tract damage associated with the MMSE scores in all PD patients. Voxelwise correlations are shown in red (FA). Results are overlaid on the sagittal, coronal, and axial sections of the Montreal Neurological Institute standard brain in neurological convention (right is right) and displayed at $P < 0.05$, FWE corrected for multiple comparisons.

Correlation Analysis

In PD patients, the UPDRS III score was not associated with DT MRI values ($P < 0.05$ FWE corrected). The MMSE score was correlated with the FA values (positive correlation) of the bilateral anterior body and right genu of the corpus callosum, fornix, right thalamic radiations, and right olfactory tract ($P < 0.05$ FWE corrected; Fig. 4). In PD patients, UPDRS III and MMSE scores were not correlated with GM atrophy.

DISCUSSION

This is the first study that used structural and DT MRI techniques to map in vivo the spatial distribution of GM loss and microstructural WM damage in a large sample of PD patients stratified according to disease status. We found that: (a) PD patients had a very little GM atrophy even at the more advanced stages of the disease; (b) microstructural damage to the WM occurs with increasing PD severity (starting at HY 3) and involves the brainstem, cerebellum, thalamocortical pathways, as well as the major interhemispheric, limbic, and extramotor association tracts; (c) the most striking difference in the pattern of WM damage was the one seen when patients with moderate PD (i.e., HY 3) were contrasted with mild PD cases (i.e., HY 2); and (d) the severity of WM damage correlates with the severity of the global cognitive status.

According to the well-established staging of brain pathology by Braak et al. [Braak and Braak, 2000; Braak et al., 2003, 2006], in the earliest pathological stages of PD, neural degeneration occurs in brainstem motor nuclei, olfactory bulbs, and limbic areas (Braak stages from I to III), propagates to the temporal and paralimbic cortex and thalamic nuclei (Braak stage IV), reaches associative areas such as the prefrontal lobes (Braak stage V), and finally involves the first-order motor and sensory areas (Braak stage VI). At Braak stage III, patients become symptomatic, expressing the typical features of PD as described in HY stage 1, i.e., tremor, rigidity, and bradykinesia, typically on one side of the body [Hawkes et al., 2010]. As the

patients enter the Braak stage IV, clinically the disease becomes bilateral (HY 2) and then symptoms are dominated by impairment of balance, frequent falls, and loss of physical independence. Furthermore, once the pathology has reached end stages, there is an increasing likelihood of cognitive disorders [Hawkes et al., 2010]. Considering that patients that participated in our study were all into the symptomatic stages of PD, we discuss our findings in the framework of the stagewise neuropathological ascension pattern [Braak and Braak, 2000; Braak et al., 2003, 2006], addressing both the elements of the results that are in accordance with the neuropathological staging and those that showed discrepancies.

We found that moderate and severe PD patients had a very little cortical atrophy compared with healthy controls, and this atrophy involved the thalamus and the frontotemporal cortex. In line with pathological studies [Braak and Braak, 2000; Braak et al., 2003, 2006], the ROI analysis showed that such a GM loss is already likely to be present in patients with early PD. Our findings also suggest that GM loss did not significantly worsen in the more advanced stages of the disease. In fact, the only region showing additional atrophy in moderate vs. mild PD cases was the thalamus. Although these findings disagree with the pattern of progression of cortical neuropathology as described by the Braak staging scheme [Braak and Braak, 2000; Braak et al., 2003, 2006], they are in line with a number of previous VBM studies in nondemented PD patients, which have generally found very little brain tissue loss [Whitwell and Josephs, 2007]. Indeed, some studies have failed to find any regions showing tissue loss [Borghammer et al., 2010; Hattori et al., 2012; Nagano-Saito et al., 2005] and in those that did, the results are scanty and rarely survive correction for multiple comparisons [Burton et al., 2004; Focke et al., 2011; Kostic et al., 2010; Nishio et al., 2010; Pereira et al., in press]. On the other hand, a seminal study using cortical thickness measurements in PD patients showed thinning of the inferior parietal, frontal, occipital, and temporal cortices compared with healthy controls at a corrected significant level [Pereira et al., in press].

Conversely, patients at the HY stages 3 (moderate PD) and 4 (severe PD), relative to healthy controls, showed

microstructural WM changes that were widespread and involved the brainstem, cerebellum, thalamocortical pathways, olfactory tracts, as well as the interhemispheric, limbic, and the majority of corticocortical association tracts. Although previous DT MRI findings in PD are somewhat conflicting, DT MRI abnormalities of the olfactory tracts and frontotemporal regions have been reported [Karagulle Kendi et al., 2008; Zhang et al., 2011]. ROI analysis showed that the most striking difference in the extent and distribution of WM damage was that found in moderate vs. mild PD patients. It is worth noting that the HY 3 is an important milestone of PD. Once stage 3 is reached, UPDRS score increases despite medication adjustments [Goetz et al., 2000]. Reaching HY 3 is also associated with a higher risk of developing dementia and a reduced patient survival [Roos et al., 1996]. The degeneration of the nigrostriatal pathway may play a role in the impaired motor performance of these patients. The accumulation of alterations in the cerebellum along the end stages of PD could also contribute to the steady increase of motor disability. Furthermore, the extension of neuronal degeneration to the corpus callosum and association WM tracts observed in moderate and severe PD cases is in line with the neuropathological spreading of cortical damage from the transentorhinal and hippocampal to the higher order cortical association regions, such as the temporal, prefrontal, and anterior cingulate areas, and finally with the progression to the entire brain [Braak and Braak, 2000; Braak et al., 2003, 2006]. The widespread WM damage in moderate and severe PD patients may be associated with the onset and accrual of nonmotor manifestations typically seen in the later stages of the disease [Braak et al., 2005; Hawkes et al., 2010]. In line with this hypothesis, we found that WM damage to the anterior corpus callosum, fornix, and thalamic radiations correlated with increased global cognitive deficits in PD patients. Our findings are also in agreement with two recent studies of nondemented PD patients, which showed that a reduced FA of the parietal WM was correlated with executive impairment [Matsui et al., 2007] and orbitofrontal WM loss with depression [Kostic et al., 2010]. Furthermore, in PD patients with mild cognitive impairment, FA values were found to be significantly decreased in the corpus callosum, SLF, inferior longitudinal fasciculus, uncinate fasciculus, and cingulum and correlated with the MMSE scores [Hattori et al., 2012]. A similar distributed pattern of WM damage has been observed in PD patients with dementia [Matsui et al., 2007].

WM alterations in moderate and severe PD could be due to axonal degeneration associated with injury/death of neuronal cell bodies [Braak and Braak, 2000; Braak et al., 2003, 2006]. Although this is a cross-sectional study and, therefore, it cannot fully address the temporal sequence or causal relations between GM and WM abnormalities in PD, it is tempting to speculate that WM damage beyond the nigrostriatal system is related to GM loss that we observed from the early stage of the disease. Nevertheless, DT MRI abnormalities were more widespread than GM atrophy and were

observed in WM regions not directly linked to atrophied GM areas. A primary WM pathology in Lewy body diseases is not well elucidated. Pathological studies have shown that ubiquitin and α -synuclein-positive inclusions can be located in portions of the axons [Braak et al., 1999]. In addition, there is evidence for an accumulation of axonally transported substances in cortical neurons of patients with Lewy body diseases [Katsuse et al., 2003], suggesting that impaired axonal transport may be associated with PD. Finally, Alzheimer-related lesions [Strobel, 2009] and cerebrovascular pathology [Bohnen and Albin, 2011] contribute to the progression of the neurodegenerative process in PD. This may be a third factor associated with the WM alterations observed in this study.

Some limitations of our study need to be addressed. First, the diagnosis of PD was not based on histopathological confirmation. However, this is not easy to obtain, especially in early patients, where normally many years would pass between MRI and histopathology, inevitably making the interpretation of MRI abnormalities challenging. Second, a comprehensive neuropsychological/behavioral assessment was not available for all subjects and, therefore, longitudinal studies are warranted to clarify whether structural and DT MRI can explain the role of GM and WM damage in determining specific nonmotor manifestations in PD. Third, in designing this study, we opted to assess patients with a similar age, but different degrees of functional worsening, as indicated by the HY stage scale. This approach allowed us to avoid the issue of comparing groups with a different aging-related pathology but also raised other problems. For example, the age at onset of the disease was different between groups. Although we excluded patients with parkin, LRRK2, or GBA gene mutations, we cannot completely rule out different genetic backgrounds, which may have had an impact on the reported findings. Finally, the proportion of women was higher in the moderate (50%) than in the mild (32%) PD groups; however, a reanalysis of the comparison between the two groups adjusting for gender yielded similar findings (data not shown).

In conclusion, our findings provide critical pieces of information that might contribute to the improvement of our understanding of the pathological mechanisms associated with the progression of PD. This study also suggests that structural and DT MRI techniques have the potential to provide novel in vivo markers of PD severity and that these markers are sensitive to the different phases of the disease.

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