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Imaging nigral pathology and clinical progression in Parkinson's disease

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Abstract

Background—The pattern of dopamine cell loss in Parkinson's disease is known to be prominent in the ventrolateral and caudal substantia nigra, but less severe in the dorsal and rostral region. Both diffusion tensor imaging and R2* relaxometry of the substantia nigra have been

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reported as potential markers for Parkinson's disease, but their relative ability to mark disease progression and differences in pathophysiological bases remains unclear.

Methods—High resolution T2-weighted, R2*, and diffusion tensor imaging were obtained from 28 controls and 40 Parkinson's disease subjects [15 early-stage (disease duration 1 year), 14 mid-stage (duration 2–5 years), and 11 late-stage (duration >5 years)]. Fractional anisotropy and R2* values in both rostral and caudal substantia nigra were obtained for all subjects, and clinical measures (disease duration; levodopa-equivalent daily dosage; “off”-drug Unified Parkinson's Disease Rating Scale motor score) were obtained for Parkinson's subjects.

Results—There was no correlation between fractional anisotropy and clinical measures, whereas R2* was strongly associated with disease progression. Compared to controls, fractional anisotropy in caudal substantia nigra was significantly decreased in Parkinson's disease patients of all stages, whereas in rostral substantia nigra it was decreased significantly only in the late-stage group. R2* in both substantia nigra regions was significantly increased in the mid-stage and late-stage, but not early-stage, of Parkinson's disease subjects.

Conclusions—These findings suggest that fractional anisotropy changes may mark early pathological changes in caudal substantia nigra, whereas the changes in R2* may more closely track Parkinson's disease's clinical progression after symptom onset.

Keywords

Parkinson's disease (PD); substantia nigra; diffusion tensor imaging (DTI); transverse relaxation rate (R2*); magnetic resonance imaging (MRI)

INTRODUCTION

Parkinson's disease (PD) is marked by the loss of dopaminergic neurons in the substantia nigra (SN) of the basal ganglia. The neuronal loss is known to occur primarily in the ventrolateral and caudal region of the SN pars compacta, with less severe pathology in the dorsal and rostral region.¹ This pattern of cell loss was confirmed in another pathological study that used a different subdivision framework for the SN.² Both the understanding of the PD-associated cell loss and the evaluation of potential neuroprotective therapies have been hindered by the lack of reliable and objective *in vivo* markers for cell loss and progression in living human subjects.

Magnetic resonance imaging (MRI) is widely available, and has been explored extensively to study pathological processes in PD.^{3–7} For instance, several MRI studies have demonstrated that effective transverse relaxation rate ($R2^*=1/T2^*$) in the SN is correlated with iron concentration *in vivo*,^{4,6,8} and is increased in the SN of PD patients.^{4,6,9,10} Concomitantly, efforts have been made to apply diffusion tensor imaging (DTI) approaches to measure microstructure disorganization due to PD-related pathology in the SN.^{5,7,9,11} Correlation between dopaminergic neuron loss and DTI changes has been reported in mice.¹² Consistent with this, several human studies have demonstrated reduced FA values in the SN of PD patients.^{5,7,9,10}

Despite the potential of these two promising MRI measurements to mark PD-related changes in the SN,¹³ there is neither clear understanding of their relevance to dopamine neuron death in the SN, nor the clinical signs of PD progression. This is critical for elucidating the pathophysiological underpinnings of these potential markers, and for evaluating their utility in both clinical and research settings. Recent data from our laboratory has suggested that R2* and FA changes in the SN are not correlated, and may reflect different aspects of PD-related pathological processes.¹⁰ To address these issues, the current

study evaluated the regionally-specific changes of $R2^*$ and FA in rostral and caudal regions of the SN in PD subjects, and their relationship to clinical progression.

Methods

Subjects

Forty PD and 28 healthy control subjects were recruited from a tertiary movement disorders clinic (see Table 1 for detailed demographic information). Of these subjects, 16 with PD and 16 controls were data that was reported earlier.¹⁰ PD diagnosis was confirmed by a movement disorder specialist (XH) according to published criteria.^{14,15} Unified Parkinson's Disease Rating Scale-III motor scores (UPDRS-III) were obtained for each PD subject after withholding all PD medication overnight (~12 hr), this being an operational “off” stage.¹⁶ Levodopa-equivalent daily dose (LEDD) was estimated for PD subjects according to a published formula.¹⁷ All subjects were free of major acute medical issues such as liver, kidney, or thyroid abnormalities and/or deficiencies of B12 and folate, and all subjects had Mini Mental Status Exam (MMSE) scores >24. All subjects gave written informed consent that was reviewed and approved by the Penn State University Institutional Review Board, and was consistent with the Declaration of Helsinki.

It is believed that the rate of nigrostriatal dopamine neurons and terminals loss in PD is greatest shortly after diagnosis, but this absolute rate decreases or even plateaus with disease progression. This slow-down phase typically occurs within 5-10 years of diagnosis¹⁸⁻²⁰ and may be related to either fewer cells remaining or the presence of dopaminergic cells resistant to PD-related cell death.^{18,21} Thus, we categorized PD patients into three clinical stages: **PD_{ES}** - “early-stage,” newly diagnosed PD subjects within the first year of diagnosis; **PD_{MS}** - “mid-stage,” the rapidly progressing stage 2-5 years following diagnosis; and **PD_L** - “late-stage,” >5 years since initial diagnosis.

Data acquisition

All subjects were scanned with a 3.0 Tesla MRI system (Trio, Siemens Magnetom, Erlangen, Germany, 8-channel phased array head coil) with high-resolution T2-weighted, multi-gradient-echoes (T2* weighted), and diffusion tensor imaging (DTI) sequences. A fast-spin-echo sequence was used to obtain T2-weighted images with TR/TE=2500/316, FOV=256 mm × 256 mm, matrix=256 × 256, slice thickness=1 mm (with no gap), slice number=176. A multi-gradient-echo sequence was used to estimate the transverse relaxation rate, $R2^*$ ($R2^*=1/T2^*$). Six echoes with TE ranging from 7 to 47 ms and an interval of 8 ms were acquired with TR=54 ms, flip angle=20°, FOV=256 mm × 256 mm, matrix=256 × 256, slice thickness=1 mm (with no gap), slice number=64. The middle slice of the multiple gradient echoes images was placed on the line between the anterior and posterior commissures (AC and PC, respectively). For DTI, acquisition parameters were as follows: TR/TE=8300/82 ms, b value=1000 s/mm², diffusion gradient directions=42 and 7 b=0 scans, FOV=256 mm × 256 mm, matrix=128 × 128, slice thickness=2 mm (with no gap), slice number=65.

Image processing and analysis

Generation of $R2^*$ and DTI maps—The magnitude images of multi-echo SWI images were used to generate $R2^*$ maps by employing a voxel-wise non-linear Levenberg-Marquardt algorithm to fit a monoexponential function with free baseline using an in-house MATLAB (The MathWorks, Inc., Natick, MA) tool. DTI image quality control and tensor reconstruction was done by using DTIPrep (Neuro Image Research and Analysis Laboratory, University of North Carolina, Chapel Hill, NC) that first checks DWI images for appropriate quality by calculating the inter-slice and inter-image intra-class correlation,

and then corrects for the distortions induced by eddy currents and head motion.²² Then, diffusion tensor maps were estimated via weighted least squares.²³ FA, mean diffusivity (MD), axial diffusivity (AD), and radial diffusivity (RD) maps were finally generated for subsequent analysis.

Segmentation of substantia nigra—The SN was delineated manually using ITK-SNAP²⁴ by an investigator (GD) blinded to subject diagnosis. To obtain a comparable region-of-interest (ROI) definition with pathological studies,^{2,25} individual high resolution T2-weighted images were reformatted by positioning the AC-PC line to the center of the image and then rotating the AC-PC line 35° superior to the PC point and along the mid-sagittal plane, as shown in Figure 1A. The 35° rotation was used to minimize the inclusion of the subthalamic nucleus that lays superior-anterolateral to the SN.^{26,27} The SN was defined as a hypo-intensity band between the red nucleus and cerebral peduncle in axial sections on multi-planar reformatted T2-weighted images as illustrated in Figure 1. Segmentation of the SN was started at one slice lower than the level of the red nucleus showing the largest radius and is shown as the yellow band in Figure 1A. The relationship of the SN, red nucleus, and subthalamic nucleus is depicted in Figure 1B. A total of six slices (from the rostral to caudal part of the SN) were used as the SN ROI (Figure 1C). The first three slices were treated as the rostral SN segment and the remaining three slices were considered the caudal segment. Figures 1D 1-6 show the six slices segmented for the SN starting with the most rostral slice (Figure 1D 1) and gradually growing the SN ROI to include the more caudal region (Figure 1D 2-6). All ROIs then were visually assessed by a second trained neuroscientist blinded to subject diagnosis to validate and fix potential mislabeling of the SN definition.

After manual segmentation, the ROIs were mapped to R2* and FA maps, respectively, by co-registering the T2-weighted images to R2* maps and the B0 images of DTI data by an affine registration followed with a b-spline non-rigid registration implemented in 3D Slicer (www.slicer.org).²⁸ R2* and DTI (FA, MD, AD, and RD) values in both the rostral and caudal segments of the SN of each subject were calculated using trimmed means (from the 5%-95% percentile) to reduce variability introduced by manual segmentation and imperfect registration processes.

Statistical Analysis

PD and Control demographics were compared using two-sample t-tests and Fisher's exact tests as appropriate. The association between clinical (disease duration, LEDD, and “off” drug UPDRS-III scores) and Regional MRI measures (R2* and DTI) was quantified via partial Pearson correlation coefficient analysis with adjustment for age. R2* and DTI values were compared separately between PD and control subjects by analysis of covariance (ANCOVA) with adjustments for age and gender. PD subgroups and control subjects also were compared using ANCOVA with adjustments for age and gender. All statistical analyses were performed using SAS 9.2 (SAS Institute Inc., Cary, NC, USA).

Results

Demographics

The demographic and clinical data are summarized in Table 1. Although there were no significant differences in age or gender between the overall PD group and control subjects or among controls and the PD subgroups, there were more males in the PD group and more females in the controls, reflecting the fact that PD is more common in males and many controls were spouses of PD patients. Because the exact effects of gender on PD and disease

progression are unknown, we have adjusted for gender in all means comparisons as reported below.

DTI results

The PD subjects overall had significantly reduced FA values in both rostral and caudal segments of the SN compared to control subjects, the FA decrease in the caudal region seemed to be more robust than the rostral region [$p=0.020$ for rostral SN, $p<0.001$ for caudal SN, Table 2]. In addition, FA values showed a different pattern of change between the rostral and caudal regions of the SN across the PD subgroups. Namely, FA value decreases did not reach significance until the very late stage of PD (PD_{LS} subgroup) for the rostral SN, whereas in the caudal region significant decreases were evident in the very early stage of the disease (PD_{ES} subgroup, see Table 2). Although there appears to be a linear trend of FA decreasing across PD subgroups (Figure 2), there was no statistically significant difference among PD subgroups. The results of analyses of MD, AD, and RD values are presented in Supplemental Table 1, and indicated that only RD in the caudal SN showed a significant increase in the overall PD group and the PD_{LS} subgroup when compared to Controls.

R2* results

PD subjects, as a whole, had significantly increased R2* values in both rostral and caudal segments of the SN compared to control subjects [$p<0.001$ in both segments, Table 2]. In contrast to the FA results, R2* values showed a similar pattern in both the rostral and caudal regions of the SN in PD subgroup analyses (see Figure 2). Namely, there was only a marginal increase in R2* values in the PD_{ES} subgroup [$p=0.050$ for rostral SN, $p=0.094$ for caudal SN], that then reached significance in the PD_{MS} subgroup [$p=0.012$ for rostral SN, $p=0.004$ for caudal SN], and was even more significant in the PD_{LS} subgroup [$p<0.001$ for both SN regions]. There also were significant differences between PD_{ES} and PD_{LS} [$p=0.013$] subgroups in the caudal region of the SN, with a trend observed in the rostral region [$p=0.053$].

Relationship of imaging and clinical values

None of the clinical measurements were associated with FA values (third and fourth rows in Figure 3). Conversely, R2* values from the caudal region of the SN were significantly correlated with all three clinical measures (second row graphs in Figure 3), whereas R2* in the rostral area were correlated only with UPDRS-III scores (first row graphs in Figure 3).

Discussion

In the past, DTI and R2* in the SN have each been hypothesized to be useful in assisting with the diagnosis of PD and gauging its progression.³⁻⁷ We previously found a lack of a correlation between R2* and FA, although combined R2* and FA measurements in the SN may increase the discrimination between PDs and Controls.¹⁰ One implication of these prior data is that these two MRI measurements reflect different pathological processes in the SN. The results of the current study are consistent with this hypothesis, and are the first to show that SN FA and R2* changes follow different temporal and spatial (dorsal and caudal) patterns in PD. Namely, FA changes in the caudal SN were present at the time of disease diagnosis, but did not capture the clinical progression thereafter. Conversely, R2* changes in both SN regions were not obvious until later disease stages, but correlated significantly with all clinical measures.

Histopathological studies have shown that there is a specific temporal and spatial pattern of SN neuron loss in PD. Temporally, this loss follows an exponential course that gradually decreases with disease progression.¹ Spatially, more than 90% of cells in the caudal region

are lost at the onset of motor symptoms, whereas only ~50% are lost in the rostral region.^{1,2} In the present study, the FA changes are consistent with the spatial and temporal pattern of dopaminergic cell loss reported in pathological studies. Namely, FA changes were evident very early in the disease (around the time of diagnosis), most significantly in the caudal region of the SN, and seemed not to capture disease progression. The spatial finding of FA changes in early-stage PD is consistent with the results of a previous imaging study by Vaillancourt et al.⁷ that showed decreased FA values in the caudal, but not rostral, area of the SN in early-stage PD patients. In our study, SN FA changes did not correlate significantly with any clinical measurements as was noted earlier.⁹ The lack of a correlation between FA values and clinical measurements of disease progression may be due to the asymptotic nature of cell loss, and/or the limited FA sensitivity to detect subtle changes related to progressive loss of the remaining ~10% of neurons in the caudal region. Collectively, these data underscore the need for future testing of the hypothesis that FA changes in the SN may be a reflection of neuronal loss and consequential tissue microstructure damage in the SN, and may have potential for pre-symptomatic screening.

Our R2* data in the SN strongly discriminated PD and controls, consistent with previous studies.^{6,9,10} More interestingly, we found a significant correlation between SN R2* values and all clinical measurements of PD. This is consistent with the hypothesis that R2* in the SN may be used as a quantitative marker for disease progression.^{6,9} Unlike FA changes, the R2* changes in the SN did not follow the temporal and spatial patterns reported for dopaminergic cell death as delineated in past pathological studies.^{1,2} Temporally, R2* changes were not obvious in early-stage PD subjects, but only became significant later in the disease. Spatially, similar R2* changes were observed in both caudal and rostral regions of the SN. This pattern discrepancy between R2* and dopaminergic cell loss suggests that R2* values may not be a good marker for dopaminergic cell loss.

Although the exact mechanism of the R2* changes in PD is unknown, there are two potential explanations for the R2* value changes and its clinical associations in PD. First, it is possible that the R2* value may be an indicator of the oxidative stress related to excessive iron deposition,²⁹⁻³² suggesting that R2* may be a surrogate marker for PD-related etiological mechanism(s). Alternatively, R2* is known to be altered by complex factors (e.g., ferric iron and amyloid plaques, ferritin and hemosiderin, and calcium deposition) that create magnetic field inhomogeneity.³³⁻³⁶ The presence of a significant correlation between SN R2* values and all clinical measurements after diagnosis also may suggest that R2* reflects iron homeostasis determined by PD-related iron accumulation although we cannot exclude epiphenomena such as treatment-induced metabolic changes. Increased iron content in the SN has been observed in the most severe PD cases, but not in milder or presymptomatic PD cases.^{37,38} The fact that we detected significant SN R2* changes only in later disease stages subjects is consistent with the hypothesis that R2* reflects SN iron accumulation. Neuromelanin plays an important role in iron homeostasis³⁹ and an iron-neuromelanin interaction in the SN is observed in PD postmortem brain tissue.^{40,41} T1-weighted MRI was suggested to reflect SN neuromelanin content, and recently reported to change in a disease stage-dependent manner in PD.⁴² Combining these two MRI measurements in future studies may provide insight on the *in vivo* dynamic changes in iron homeostasis in the PD process.

Unlike previous studies,^{6,7} we developed a semi-automated protocol to define SN subregions on both R2* and FA images, one that provided a robust method for reducing the variability introduced by manual segmentation in different MRI modalities. Definition of the SN in MR images remains controversial.^{6,43-45} Instead of separating the SN into the pars compacta and pars reticulata, we delineated the rostral and caudal SN because this is most comparable to previous pathology studies.^{1,2} Moreover, in the data analysis, PD subjects

were divided into three disease duration subgroups to capture better the known pattern of PD-related dopaminergic cell progression,^{1,20} thus providing increased power for detecting potential temporal correlations between these two MRI measurements and PD progression.

There are some limitations in the present study. For example, the sample size of the current study is relatively small for a cross-sectional observational study. The spatial resolution of the DTI images in the study is 2×2×2 mm, lower than that for the R2* images (1×1×1 mm). Therefore, the power of FA values in predicting PD may be underestimated, and may explain why we did not achieve as great a discrimination between PD and controls as did Vaillancourt et al.⁷ Emerging 7T MRI data may provide better spatial resolution and increased sensitivity to detect iron in the SN. Indeed, 7T SWI recently was effective in differentiating PDs from controls,⁴⁶ and may be integrated into MRI biomarker research once it becomes more accessible.

In summary, this study is consistent with the hypothesis that FA and R2* values in the SN reflect different aspects of PD-related pathology and/or concomitant epiphenomena. As FA is thought to reflect the microstructural integrity of a region, its change in the caudal SN in early PD may indicate existing and significant PD-related microstructural alterations in this area, and may be useful for early PD detection. R2* values, on the other hand, may reflect iron homeostasis and/or other metabolic/biochemical changes occurring during disease progression in PD and may be useful as an objective marker for PD progression. Further studies with drug-naïve subjects, larger sample size and longitudinal design, and histopathological correlations are warranted to test these hypotheses and investigate the detailed pathological underpinnings of these two *in vivo* MRI markers in PD and their utility in clinical practice and research studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Procedure used to segment the rostral and caudal regions of the SN. A: Illustration of the position of slices used for the definition of ROIs. B and C: Illustration of the three dimensional relationship of the ROIs and the separation of the rostral and caudal regions of the SN. D(1-6): Illustration of the exact locations of the SN ROI.

Figure 2. Correlations between clinical measurements and MRI measurements. The scatterplots depict the age-adjusted residuals. The shaded areas represent the 95% confidence interval for each regression line.

Figure 3.

Comparison of R2* and FA values in different segments of the SN in PDs and Controls.

Error bars represent the SEM. **PD_{ES}** - “early-stage,” are newly diagnosed PD subjects within the first year of diagnosis; **PD_{MS}** - “mid-stage,” are those that are in the rapidly progressing stage, 2-5 years following diagnosis; and **PD_L** - “late-stage,” are PD subjects who are >5 years since initial diagnosis.. Significant differences between PD and control subjects are represented as: *: $p<0.05$; ***: $p<0.001$.

Table 1

Demographics of PD and control subjects.

	Controls	PD				P-values	
		Total	PD _{ES} Early stage (1 yr)	PD _{MS} Mid-stage (2 to 5 yrs)	PD _{LS} Later stage (6 yrs)	Group ⁺	Subgroup [‡]
Male/Female	13/15	23/17	7/8	8/6	8/3	0.131	0.486
Age (years)	59.8±7.0	60.8±8.2	60.2±10.1	59.2±6.0	63.4±7.9	0.602	0.548
Hoehn-Yahr stage (I/II/III)	-	13/22/4	8/5/1	4/9/1	1/8/2	-	-
Disease duration (years)	-	4.2±4.7	0.5±0.5	3.3±1.1	10.4±4.3	-	-
LEDD [*] (mg/day)	-	528±401	278±225	456±199	960±444	-	-
UPDRS-III scores	-	23.5±15.1	17.1±9.4	21.6±11.0	34.6±20.3	-	-

Age, UPDRS-III scores, disease duration and LEDD values represent by mean ± SD.

P-values are from two-simple t-tests, one-way ANOVA, and/or Fisher's exact tests as appropriate.

^{*} LEDD = levodopa-equivalent daily dosage.

⁺ Group difference between PD and Controls.

[‡] Group difference between subgroups of PD and Controls.

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Table 2

FA and R2* values in Controls and PD patients

		Mean (SD)					P-values					
		Controls	PD _T	PD _E	PD _M	PD _L	Controls vs PD _T	Controls vs PD _{ES}	Controls vs PD _{MS}	Controls vs PD _{LS}	PD _{ES} vs PD _{MS}	PD _{ES} vs PD _{LS}
DTI FA	Rostral	0.518 (0.051)	0.485 (0.057)	0.493 (0.055)	0.488 (0.057)	0.472 (0.064)	0.020	0.167	0.082		0.720	0.377
	Caudal	0.533 (0.044)	0.488 (0.048)	0.497 (0.055)	0.490 (0.045)	0.475 (0.043)	<0.001	0.022	0.007		0.669	0.369
R2* (s ⁻¹)	Rostral	30.2 (4.30)	35.0 (5.58)	33.4 (4.89)	34.4 (6.09)	37.7 (5.26)	<0.001	0.050	0.012		0.568	0.053
	Caudal	27.2 (4.07)	31.8 (5.25)	29.8 (4.78)	31.7 (5.49)	34.7 (4.56)	<0.001	0.094	0.004		0.246	0.013

PD_T: total PD patients; PD_{ES}: early stage subgroup; PD_{MS}: mid-stage subgroup; and PD_{LS}: later stage subgroup.

Significant p-values are highlighted by italic bold font.

P-values are from separate ANCOVA with adjustment for age and gender.