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Free-Water Improves Detection of Changes in the Substantia Nigra in Parkinsonism: A Multi-Site Study

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Abstract

Background—Imaging markers that are sensitive to parkinsonism across multiple sites are critically needed for clinical trials.

Objective—To evaluate changes in the substantia nigra using single- and bi-tensor models of diffusion magnetic resonance imaging in PD, MSA, and PSP.

Methods—The study cohort (N=425) included 107 healthy controls, 184 PD, 63 MSA, and 71 PSP patients from 3 movement disorder centers. Bi-tensor free-water, free-water corrected fractional anisotropy, free-water corrected mean diffusivity, single-tensor fractional anisotropy and single-tensor mean diffusivity were computed for anterior and posterior substantia nigra. Correlations were computed between diffusion MRI measures and clinical measures.

Results—In the posterior substantia nigra, free-water was greater for PSP than MSA, PD, and controls. PD and MSA both had greater free-water than controls. Free-water corrected fractional anisotropy values were greater for PSP when compared to controls and PD. PSP and MSA single-tensor mean diffusivity values were greater than controls and single-tensor fractional anisotropy values were lower for PSP than healthy controls. The parkinsonism effect size for free-water in the posterior substantia nigra was 0.145 and for single-tensor mean diffusivity was 0.072. The

direction of correlations between single-tensor mean diffusivity and free-water values and clinical scores were similar at each site.

Conclusions—Free-water values in the posterior substantia nigra provide consistent pattern of findings across PD, MSA and PSP in a large cohort across three sites. Free-water in the posterior substantia nigra relates to clinical measures of motor and cognitive symptoms in a large cohort of parkinsonism.

Introduction

Loss of dopaminergic neurons in the substantia nigra is common to several forms of parkinsonism, including Parkinson's disease (PD), multiple system atrophy (MSA), and progressive supranuclear palsy (PSP)^{1–3}. Neuronal loss has been documented to have more widespread damage in PSP patients than PD or MSA patients⁴. In addition, several studies have indicated that MRI-derived values can provide utility in detecting structural differences between parkinsonian syndromes. A reliable and reproducible surrogate marker of degeneration within the substantia nigra on clinical MRI scanners is of great interest in terms of clinical studies and long-term management of disease severity. A recent multi-site study has shown that free-water derived from diffusion MRI can detect differences in the substantia nigra between PD and healthy controls⁵. However, other studies have shown across sites that diffusion MRI metrics such as fractional anisotropy may not be useful in detecting differences in the substantia nigra between parkinsonian syndromes and healthy controls^{6,7}. The lack of convergence across studies most likely arises from differences in the pulse sequence used in the diffusion acquisition, analysis methods, diffusion metric of choice, and location of the regions within the substantia nigra where these measures are computed.

Diffusion MRI measures water motion within a voxel, which is affected by the cellular, axonal, and extracellular environment. Models of the diffusion signal help determine the underlying biological structures that determine the signal intensity. A single-tensor model approximates the data as a 3-dimensional Gaussian diffusion processes. In instances of pathology, single-tensor models cannot distinguish from the many cases of pathology. For example, crossing nerve bundles or fluid contamination may alter the diffusion signal in the same way or underestimating the unique orientation contribution of the structures comprising a particular voxel. To deal with fluid contamination, a bi-tensor model addresses the limitations of the single-tensor model to include a free-water compartment that originates in the extracellular space⁸. Using a bi-tensor model, it was shown that free-water (i.e. fractional volume of the free-water compartment) in the substantia nigra is increased in PD compared with control subjects in both a single site cohort and the multi-site cohort from the Parkinson's Progressive Marker Initiative (PPMI)⁵. Also, it was found that free-water in the substantia nigra increases over time in PD⁹, and is elevated in MSA and PSP¹⁰. While there is much promise in using a bi-tensor model, these sample sizes have been small, and there has not been a study directly comparing the utility of a bi-tensor model with the single-tensor model in the substantia nigra for PD, MSA, and PSP patients across several sites. The goal of the current study was to compare directly the two diffusion MRI analysis methods, namely the conventional single-tensor model and the bi-tensor model⁸ in the largest cohort

of subjects with parkinsonism to date. We quantified diffusion metrics in the substantia nigra across 425 individuals acquired at three imaging sites. This is a retrospective study, and the pulse sequence was not matched across sites. Each site included healthy controls (HC), PD, MSA, and PSP cohorts. We tested the following two hypotheses: 1) free-water in the substantia nigra will be elevated in the substantia nigra of Parkinsonism across sites, and 2) free-water in the posterior substantia nigra estimated with a bi-tensor model correlates with clinical symptoms across sites.

Methods

This study includes 425 subjects across 4 groups including HC, PD, MSA, and PSP. Subjects were enrolled in studies at the Medical University Innsbruck (MUI) and Parkinson's Disease Biomarker Program (PDBP). The PDBP for MR data includes sites at the University of Florida (UFLORIDA) and Penn State University Hershey Medical Center (PSHMC) (Table 1). Healthy controls were individuals over the age of 40 who did not have a prior history of major psychiatric or neurological disease. Patients were recruited and diagnosed by movement disorder specialists at each site using established criteria¹¹⁻¹³. Disease severity was assessed using the Unified Parkinson's Disease Rating Scale (UPDRS) part III, the Movement Disorders Society Unified Parkinson's Disease Rating Scale (MDS-UPDRS) part III¹⁴, and the Hoehn-Yahr scale. UPDRS III scores were converted to MDS-UPDRS III scores¹⁵. Patients at MUI and PSHMC were examined on regular medication, whereas patients at UFLORIDA were examined after over-night withdrawal of anti-Parkinsonian medications. All individuals also underwent clinical assessment across sites that measured depression (Beck Depression Inventory¹⁶ and Hamilton Depression Rating Scale¹⁷), and cognition (Montreal Cognitive Assessment¹⁸ and Mini-mental state examination¹⁹). All healthy controls with a UPDRS part III greater than 10 or MoCA less than 23 were excluded from further analyses.

Data Acquisition

For the UFLORIDA cohort, diffusion-weighted images were acquired on a 3T Philips MRI scanner (Achieva, Best, Netherlands) using a 32-channel head coil. For the MUI and PSHMC cohorts, diffusion-weighted images were acquired on a 3T Siemens MR system (Verio at MUI and Trio at PSHMC, respectively; Siemens Magnetom; Erlangen, Germany) with an 8-channel head coil. The UFLORIDA imaging acquisition protocol consisted of the following scan parameters: directions=64; repetition time=7748ms; echo time=86ms; b-values: 0; 1000s/mm²; field of view = 224mmx224mm; in-plane resolutions=2mmx2mm; flip angle=90°; number of contiguous slices=60; slice thickness=2mm; acceleration factor p=2, averages=none. The MUI imaging acquisition protocol consisted of the following scan parameters: directions=20; repetition time=8200ms; echo time=83ms; b-values: 0; 1000s/mm²; field of view=230mmx230mm; in-plane resolution 2mmx2mm; number of contiguous slices=45; slice thickness=3mm; averages=none. The PSHMC imaging acquisition protocol consisted of the following scan parameters: directions=42; repetition time=8300ms; echo time=82ms; b-values: 0, 1000s/mm²; field of view=256mmx256mm, in-plane resolution=2mmx2mm, flip angle=90°, number of contiguous slices=65, slice thickness=2mm, acceleration factor p=2; and averages=7 (See Supplementary Table 1).

Diffusion imaging analyses

Data preprocessing was performed with the FMRIB Software Library and Unix scripts. Each brain was corrected for eddy-currents and head rotations using FSL (reference volume at 0), and gradient directions were rotated based on eddy current corrections. MUI data was resampled to a $2 \times 2 \times 2$ resolution similar to the resolution of the other two sites. Non-brain tissue was removed from the diffusion volumes using the FSL Brain Extraction Tool (BET).

Single-tensor diffusion metrics, free-water, and free-water corrected diffusion metrics were calculated for all the diffusion imaging data. The single-tensor metrics were fractional anisotropy (FA_U) and mean diffusivity (MD_U). The bi-tensor metrics were free-water, corrected fractional anisotropy (FA_T), and corrected mean diffusivity (MD_T). Computational routines for single-tensor and bi-tensor measures have been published elsewhere^{8,20}. A linear rigid transformation was performed between the B0 image and a T2 template in MNI space, and then a diffeomorphic non-linear transformation called the Symmetric Normalization (SyN) transformation model was performed²¹. Briefly, SyN uses a gradient-based iterative convergence using diffeomorphisms to converge on an optimal solution based on a similarity metric (e.g., cross-correlation). This technique has been shown to perform greater compared to linear and other non-linear registrations. The transform matrices were then applied to all the free-water, FA_U , MD_U , FA_T , and MD_T maps.

ROI analyses

Region of interest (ROI) analyses were performed. First, the ROIs were manually drawn using an established method on the individual b-zero maps in MNI space⁹, blinded to the other diffusion maps and diagnosis. Four ROIs represented the left and right anterior and posterior substantia nigra. Each ROI was 8 voxels in size. The substantia nigra was identified in accordance with previous work where inter-rater reliability has been high^{5,10}. To assess inter-rater reliability in this study, novice raters who were blind to group diagnoses drew ROIs of 10 subjects from each group across each site.

Statistical analysis

Clinical measures of age, sex, MDS-UPDRS III, cognitive status, disease duration, and Hoehn-Yahr were compared using ANOVA within each site, and after combining all data in one large cohort. The mean values for the bilateral anterior and posterior substantia nigra ROIs were calculated for free-water, FA_T , MD_T , FA_U , and MD_U across all sites. Multivariate ANOVAs were conducted on each unique ROI-dependent measure combination controlling for age, sex, and site, with group status as the fixed factor. Post-hoc tests for the group status were evaluated using Bonferroni pairwise comparisons. Inter-rater reliability for the segmentation procedure of the ROIs was determined by an intraclass correlation coefficient within site using a two-way mixed model with absolute agreement. Statistical threshold was set at $p < 0.05$.

Correlational analyses

A priori, we decided to correlate a specific diffusion metric with MDS-UPDRS III scores, Hoehn-Yahr scores, and global cognitive status within each site, if there was a significant

group effect for the specific diffusion metric. Correlation coefficients were calculated using Spearman's rho statistic, and p-values were FDR corrected for multiple comparisons.

Results

Demographics and clinical data

Supplementary Tables 2 and 3 show the clinical and demographic data across and between sites. Across sites, there was a significant between-group effect for age, sex, MDS-UPDRS III, cognitive status, disease duration, and Hoehn-Yahr scores (p-values<0.05). Post-hoc tests revealed more males in the PD cohort and the PD cohort had longer disease duration (ie. time since diagnosis) than the MSA and PSP groups. Post-hoc test for MoCA yielded differences across groups (PSP<MSA<PD<HC) (p-values<0.05). The group effect for MDS-UPDRS III scores and Hoehn-Yahr scale scores resulted in the MSA and PSP cohorts having higher scores than the PD cohort. PD subjects also had significantly higher MDS-UPDRS III scores than HC (p-values<0.05, Supplementary Table 2).

Diffusion analyses

Intraclass correlation coefficients (ICCs) revealed strong agreement between the two raters. The average ICCs for free-water ranged from 0.88 to 0.99 (p-values<0.001), ICCs for average bi-tensor FA_T ranged from 0.90 to 0.99 (p-values<0.001), ICCs for average bi-tensor MD_T ranged from 0.91–0.99 (p-values<0.001), ICCs for single-tensor FA_U ranged from 0.87 to 0.94 (p-values<0.001), and ICCs for single-tensor MD_U ranged from 0.81 to 0.99 (p-values<0.001).

Anterior ROIs

A multivariate group effect was found for anterior free-water, single-tensor FA_U and bi-tensor FA_T, with no other group effects found for the other diffusion metrics. Post-hoc tests revealed that this effect resulted from PSP values higher than HC and PD for free-water and FA_T, and PSP with lower values than HC for single-tensor FA_U (p-values<0.05, Table 2).

There was a covariate effect of age for free-water (Table 2), and a site effect for free-water, bi-tensor FA_T, and bi-tensor MD_T (Table 2). Sex had no influence on any of the dependent metrics for the anterior ROIs (Table 2). Detailed site effects are shown in Supplemental Table 5 for each group.

Posterior ROIs

A multivariate group effect was found for free-water, bi-tensor FA_T, single-tensor FA_U and single-tensor MD_U (p-values<0.05, Table 2). Post-hoc tests revealed PSP subjects had greater free-water than MSA, PD, and HC subjects. Although no differences were observed between PD and MSA subjects for free-water, PD and MSA subjects both had greater free-water than HC. This pattern was consistent across all sites (Figure 1). Partial η^2 was 0.145 for free-water and 0.072 for single tensor MD_U. Post-hoc tests also demonstrated increased bi-tensor FA_T for PSP when compared to HC and PD subjects. In addition, the post-hoc tests revealed that PSP and MSA single-tensor MD_U values were greater than HC (Figure 1). Post

hoc tests also revealed that PSP had lower single-tensor FA_U values than HC (p-values <0.05).

A significant covariate effect of age was found for free-water and single-tensor FA_U (p-values <0.05) (Table 2). A site covariate effect was found for free-water, bi-tensor FA_T , and bi-tensor MD_T (Table 2). Supplementary Table 5 shows detailed site effects for each group. A sex covariate effect was found for free-water (Table 2).

Correlation analyses

Correlation analyses revealed that posterior free-water was significantly correlated with MDS-UPDRS III, Hoehn-Yahr, and cognitive status at all sites (Table 3). The direction of the correlation was positive for MDS-UPDRS III and Hoehn-Yahr and negative for cognitive status. Posterior single-tensor MD_U was significantly correlated with MDS-UPDRS III and Hoehn-Yahr at UF and PSHMC sites; however there were no significant correlations at the MUI site for posterior single-tensor MD_U . The anterior nigra free-water values for the MUI site were significantly correlated with motor severity and Hoehn-Yahr scores. Posterior FA_T correlations also revealed significant moderate correlations across clinical scores for the UF and MUI sites, but not the PSHMC site.

Discussion

Our findings in the largest cohort of parkinsonism patients to date demonstrate the utility of a bi-tensor model for detecting differences in the substantia nigra in parkinsonism. We examined the diffusion properties of the substantia nigra in 425 subjects and determined differences among healthy controls and patients with PD, MSA, and PSP. We found that free-water values derived from the bi-tensor model were elevated in subjects presenting with parkinsonism, and the key observation was the same pattern (PSP>MSA>PD>HC) at each site (Figure 1). The effect size for distinguishing between healthy controls and neurodegenerative parkinsonism of the bi-tensor free-water measure was 0.145 and the effect size of the single-tensor MD_U measure was 0.072. The direction of correlations between clinical measures and free-water and single-tensor MD_U were similar. Therefore, the results provide direct evidence that bi-tensor metrics for diffusion-weighted imaging are able to detect changes in parkinsonian substantia nigra across sites.

The finding for elevated free-water in the substantia nigra of PD, MSA, and PSP subjects across multiple sites extends prior work that detected microstructural changes between healthy controls and PD patients in a single-site and multi-site study⁵. We also found decreased single-tensor FA and increased bi-tensor FA_T in PSP when compared to other groups. Further, free-water elevation was more pronounced in PSP than PD and MSA. This pattern of results extends a previous single-tensor model analysis at a single-site²². PD and MSA free-water values were also greater than healthy controls. Previous reports have shown that putaminal MD_U can be useful to distinguish the parkinsonian variant of MSA from PD but not from PSP²³. Diffusivity changes of the middle cerebellar peduncle, however, may provide additional information^{10,22}. Our findings of changes in the posterior nigra are in line with pathological studies that report degeneration in the ventro-lateral tier of the substantia nigra in patients with degenerative parkinsonism¹.

The posterior substantia nigra contains cells that progressively degenerate as one ages or presents with parkinsonian symptoms. In order to detect *in-vivo* changes, one must delineate this region using indirect landmarks to outline or find the substantia nigra^{24,25}. This may be the reason why some voxel-based analyses fail to detect changes in this region when examining changes between PD patients and healthy controls⁷. The hand-segmented ROI approach used here seems to provide utility in detecting changes in single- and bi-tensor diffusion metrics for the substantia nigra^{6,7,25}. Some studies have not found changes in single-tensor FA in the substantia nigra, whereas other studies have^{6,24}. The single-tensor FA measure reflects numerous mechanisms. When more unconstrained diffusion is in the voxel, this drives the single-tensor FA_U measure lower. In contrast, high myelination and densely packed axons will lead to higher single-tensor FA_U values. Since single-tensor FA_U can be increased due to gliosis but reduced due to inflammation or fluid in the extracellular space, these mechanisms may cancel each other out on the single-tensor FA_U measure. The bi-tensor derived measures can provide more information because the bi-tensor model provides separate measures for the extracellular space (i.e., free-water) and tissue-specific measures (e.g., bi-tensor FA_T).

Increased bi-tensor FA_T and decreased single-tensor FA_U was found for both the anterior and posterior substantia nigra regions in the PSP cohorts. Previous reports have found altered diffusion metrics in the substantia nigra of PSP^{26–29}; single-tensor FA_U was increased in one study²⁹ and reduced in another²⁸. Increased bi-tensor FA_T has been suggested to reflect gliosis, where initially FA_U was low in the single-tensor model^{30,31}. At pathology, PSP patients have increased gliosis and inclusion body pathology in the substantia nigra compared to PD patients³². We found decreased FA in the single-tensor and bi-tensor models for PSP patients. Increased bi-tensor FA_T when single-tensor FA_U is originally low has been suggested to reflect cellular shrinkage or atrophy³³. It is unclear whether the increased FA_T findings for PSP from the current study reflect increased glial pathology, cellular swelling, or other pathologies.

The variability in the methods used to analyze diffusion MRI data for PD and other movement disorders range from ROI approaches to voxel-based statistics. Further, the type of scanner, field strength, and scan sequence used also vary depending on the study⁷. Multi-site studies are important for increasing the number of subjects, and to generalize clinical findings that are not dependent on site. We have found in the current paper that the site effect for the diffusion metrics was greatest for the controls and PD patients across sites and this may be due to clinical information, pulse sequence, and/or head coil differences at each site. Some multi-site studies have tried to minimize the variance due to type of scanner, although this has not led to promising results to detect differences between healthy controls and PD patients using single-tensor FA⁶. The current paper has shown that the bi-tensor calculation of free-water in the substantia nigra distinguishes healthy controls from parkinsonism patients regardless of site or scanner, even when the pulse sequence is not matched. Clearly, matching the pulse sequence is preferred and should be done in future large-scale multi-site studies, albeit the pattern of change in Figure 1 demonstrates that effects are robust even with different hardware and pulse sequences. Single-tensor MD_U also was able to distinguish between PD and MSA and healthy controls, but did not differ for PD versus controls. Due to the similarity in the pattern of correlations with single-tensor MD_U, free-

water and clinical scores, it would seem that changes in the extracellular space are a factor influencing diffusivity changes reported in the literature.

A potential limitation in this study could be that patients were on medication at the Innsbruck and Pennsylvania sites but off medication at the Florida site. Despite this limitation, we still observed similar patterns of elevated free-water in the substantia nigra across parkinsonism at each site (Figure 1). The medication differences could also be a reason why the pattern of correlation results are not similar across sites. Since this was a retrospective study, the bi-tensor model was estimated from single-shell b-value data. The accuracy of the bi-tensor fit may be improved when multiple b-values are acquired³⁴. This may improve precision and accuracy of the metrics for classification between parkinsonian disorders.

In conclusion, the present study demonstrates that free-water is elevated in the posterior substantia nigra of PD, MSA, and PSP patients when compared to healthy controls. This extends previous work that has shown utility of free-water in detecting PD from healthy controls in multi-site cohorts. Further, free-water in substantia nigra relates to clinical measures of motor and cognitive symptoms in a large cohort of parkinsonism. This is the first study to directly compare efficacy of single-tensor and bi-tensor metrics for parkinsonism. Our current findings, in combination with recent work⁹, suggest that bi-tensor metrics may provide utility in monitoring disease severity in multi-site studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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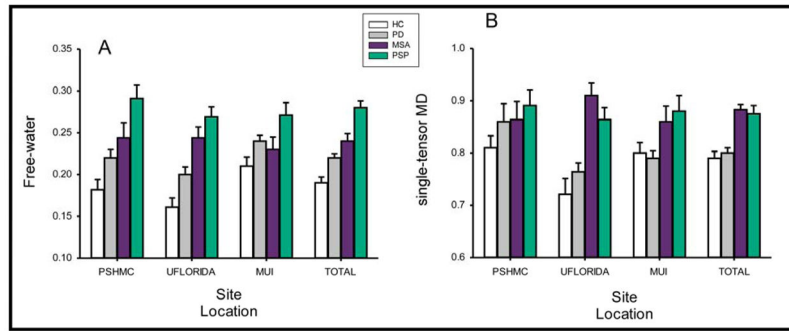


Figure 1. Free-water and single-tensor mean diffusivity across sites. The bar graphs indicate the levels of free-water and single-tensor mean diffusivity for healthy controls, Parkinson’s disease (PD), multiple systems atrophy (MSA), and progressive supranuclear palsy (PSP) subjects across all sites and within each site.

Table 1

Study Sites

Site	# Subjects	Age	Sex (M/F)	Cohort Enrollment	Data Obtained
Penn State Hershey Medical Center, Hershey, PA, USA (PSHMC)	108	68.2 (9.5)	75/33	30 HC, 45 PD, 14 MSA, 19 PSP	dMRI, MOCA, Hoehn-Yahr, HDRS1, MDS-UPDRS3
University of Florida, Gainesville, FL, USA (UFFLORIDA)	149	65.6 (9.2)	85/64	36 HC, 54 PD, 28 MSA, 31 PSP	dMRI, MOCA, Hoehn-Yahr, MDS-UPDRS3, BDI
University of Innsbruck, Innsbruck, Austria (MUJ)	168	64.4 (8.9)	101/67	41 HC, 85 PD, 21 MSA, 21 PSP	dMRI, MMSE/MoCA, Hoehn-Yahr, UPDRS
Total	425	65.8 (9.3)	161/365	107 HC, 184 PD, 63 MSA, 71 PSP	

Data are either count or mean (±SD). Abbreviations: BDI = Beck Depression Inventory, dMRI = diffusion magnetic resonance imaging, F = female, HC = healthy controls, HDRS = Hamilton Depression Rating Scale, M = male, MDS-UPDRS3 = Motor section of the Movement Disorder Society-Sponsored Revision of the Unified Parkinson's Disease Rating Scale, MMSE = Mini-Mental State Examination, MoCA = Montreal Cognitive Assessment, MSA = multiple system atrophy, PD = Parkinson's disease, PSP = progressive supranuclear palsy, UPDRS3 = Motor section of the Unified Parkinson's Disease Rating Scale.

Table 2

Diffusion Metrics

Groups/Statistics	Free-Water	Bi-Tensor FA	Bi-Tensor MD	Single-Tensor FA	Single-Tensor MD
<i>Anterior Substantia Nigra</i>					
HC	0.19 (.006)	0.57 (.009)	0.56 (.005)	0.50 (.008)	0.76 (.015)
PD	0.19 (.005)	0.58 (.007)	0.56 (.003)	0.48 (.006)	0.76 (.011)
MSA	0.19 (.008)	0.60 (.011)	0.57 (.006)	0.48 (.011)	0.77 (.019)
PSP	0.22 (.008)	0.61 (.011)	0.57 (.006)	0.46 (.010)	0.79 (.019)
Full model F statistic	7.96	3.93	4.53	2.58	3.19
Group F statistic	3.80	3.59	2.10	3.55	0.98
Partial η^2	0.027	0.025	0.015	0.025	0.007
Significant Covariate	Age, Site	Site	Site	N/A	N/A
Group P-value	0.01	0.014	0.10	0.015	0.40
P-value corrected	0.02	0.0214	0.13	0.021	0.44
Post Hoc	PSP > HC PD	PSP>PD HC	N/A	PSP<HC	N/A
<i>Posterior Substantia Nigra</i>					
HC	0.19 (.007)	0.60 (.009)	0.57 (.005)	0.49 (.008)	0.79 (.013)
PD	0.22 (.005)	0.59 (.006)	0.57 (.004)	0.48 (.006)	0.80 (.010)
MSA	0.24 (.008)	0.62 (.011)	0.57 (.007)	0.45 (.010)	0.88 (.017)
PSP	0.28 (.008)	0.65 (.011)	0.57 (.006)	0.45 (.009)	0.88 (.016)
Full model F statistic	21.51	5.41	6.08	4.25	8.11
Group F statistic	23.64	9.26	0.40	4.11	10.86
Partial η^2	0.145	0.062	0.003	0.029	0.072
Significant Covariate	Age, Sex, Site	Site	Site	Age	N/A
Group P-value	3.75E-14	6E-6	0.75	0.007	6.98E-07
P-value corrected	3.75E-13	2e-5	0.75	0.018	3.49E-06
Post Hoc	PSP>MSA PD>HC	PSP>PD HC	N/A	PSP MSA<HC	PSP MSA>PD HC

ROI data represent the mean (standard error) adjusted for age and sex, with the statistics for the comparisons and significant covariates listed below. Abbreviations: FA = fractional anisotropy, HC = healthy controls, MD = mean diffusivity, MSA = multiple system atrophy, PD = Parkinson's disease, PSP = progressive supranuclear palsy.

Table 3

Correlations between diffusion metrics and clinical scores of all parkinsonian patients across sites

Variable	PSHMC		UFLORIDA		MUI	
	rho (ρ)	P-value	rho (ρ)	P-value	rho (ρ)	P-value
UPDRS-III						
aSN FW	0.15	0.12	0.14	0.10	0.22	0.004
aSN FA _U	-0.17	0.07	-0.18	0.025	-0.20	0.01
pSN FA _U	-0.14	0.14	-0.28	0.001	0.09	0.27
pSN FA _T	0.11	0.26	0.23	0.005	0.03	0.67
pSN FW	0.40	1.6E-5	0.57	4.8E-15	0.18	0.02
pSN MD _U	0.24	0.012	0.50	1.05E-10	0.11	0.18
Hoehn-Yahr						
aSN FW	0.17	0.07	0.12	0.15	0.32	2E-5
aSN FA _U	-0.17	0.09	-0.22	0.008	-0.24	0.002
pSN FA _U	-0.12	0.23	-0.29	2.9E-4	-0.12	0.14
pSN FA _T	0.15	0.13	0.23	0.005	0.21	0.005
pSN FW	0.40	2E-5	0.58	1.2E-14	0.28	2.4E-4
pSN MD _U	0.20	0.03	0.53	2.9E-12	0.30	7E-6
Cognitive Status						
aSN FW	-0.02	0.81	-0.07	0.37	-0.08	0.30
aSN FA _U	0.02	0.84	0.13	0.11	0.18	0.02
pSN FA _U	0.18	0.06	0.27	0.001	0.03	0.72
pSN FA _T	-0.17	0.08	-0.17	0.04	-0.23	0.002
pSN FW	-0.38	6.3E-5	-0.46	4.8E-9	-0.14	0.07
pSN MD _U	-0.29	0.002	-0.42	8.2E-8	-0.14	0.06

The correlation coefficient (rho) and the corresponding p value are listed for each association. Cognitive status was measured by MoCA and MMSE. Abbreviations: aSN = anterior substantia nigra, FAT = bi-tensor fractional anisotropy, FA_U = uni-tensor fractional anisotropy, FW = free-water, MDU = single-tensor mean diffusivity, pSN = posterior substantia nigra.