

## Gray and White Matter Imaging: A Biomarker for Cognitive Impairment in Early Parkinson's Disease?

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**ABSTRACT: Background:** The aim of this work was to investigate the cortical and white matter changes that underlie cognitive impairment in patients with incident Parkinson's disease (PD) disease using voxel-based morphometry and diffusion tensor imaging.

**Methods:** Newly diagnosed nondemented PD (n = 125) and control subjects (n = 50) were recruited from the Incidence of Cognitive Impairment in Cohorts with Longitudinal Evaluation in Parkinson's Disease Study and completed cognitive assessments and 3T structural and diffusion tensor MR imaging. Voxel-based morphometry was performed to investigate the relationship between gray matter volume and cognitive ability. Microstructural white matter changes were assessed with diffusion tensor imaging measures of fractional anisotropy and mean diffusivity using tract-based spatial statistics.

**Results:** Increased mean diffusivity was observed bilaterally in subjects with PD, relative to controls ( $P = 0.019$ ). Increased mean diffusivity was associated with performance on the semantic fluency and Tower of London tasks in frontal and parietal white matter tracts, including the cingulum, superior longitudinal fasciculus,

inferior longitudinal fasciculus, and inferior fronto-occipital fasciculus. There was no difference in total gray matter volume between groups; however, bilateral reductions in frontal and parietal gray matter volume were associated with reduced performance on measures of executive function in PD subjects.

**Conclusions:** At the earliest stages of PD, regionally specific increases in central white matter mean diffusivity are present and suggest early axonal damage. Such changes are not accompanied by significant gray matter volume loss and are consistent with proposed models of pathological progression of the disease. Structural MRI, especially diffusion tensor imaging analysis, offers potential as a noninvasive biomarker reflecting cognitive impairment in PD. © 2015 International Parkinson and Movement Disorder Society

**Key Words:** Parkinson's disease/parkinsonism; MCI (mild cognitive impairment); Parkinson's disease with dementia; magnetic resonance imaging; DWI

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Early identification of Parkinson's disease (PD) patients at high risk of developing PD dementia (PDD) is of prognostic importance. Additionally, it enables clinical trials of disease-modifying interventions targeted at dementia to be instituted early in the disease process before extensive neuronal loss. Mild cognitive impairments occur in 20% to 40% of patients with newly diagnosed PD<sup>1-4</sup>; these patients may be at highest risk of subsequently developing PDD.<sup>5</sup> This process is driven by disruption of dopaminergic, cholinergic, and serotonergic neurotransmitter systems secondary

to abnormal alpha synuclein ( $\alpha$ -Syn) aggregation; however, the contribution of amyloid, tau, and vascular pathologies can also be factors.<sup>6,7</sup>

Structural MRI is an established biomarker in observational and interventional studies of Alzheimer's disease (AD). Most studies using region of interest (ROI), voxel-based morphometry (VBM), and cortical thickness analysis approaches have not found significant gray matter (GM) loss in patients with early, cognitively intact PD (PD-NC), although mild posterior atrophy was reported in one series.<sup>8-12</sup> GM loss involving parietal, temporal, and occipital regions is a consistent finding in PDD.<sup>8,11,13-18</sup> More-limited GM loss has been reported in subjects with PD with mild cognitive impairment (PD-MCI).<sup>8,11,17,19,20</sup> In a 2-year longitudinal study, PD-MCI subjects who developed dementia had lower GM density at baseline in the prefrontal cortex, insular cortex, and caudate nuclei than PD-MCI patients who did not subsequently convert.<sup>21</sup>

Diffusion tensor imaging (DTI) is a sensitive technique for detecting microstructural white matter (WM) pathology. Through quantifying the magnitude and directionality of the motion of water molecules, it provides an *in vivo* surrogate measure of the integrity of tissue microstructure. Degeneration of structural barriers, such as myelin and cell membranes, increases mean diffusivity (MD) and lowers the directionality of its flow, measured as fractional anisotropy (FA). Tract-based spatial statistics (TBSS) interrogates the integrity of WM tracts without the limits of the operator-dependent ROI approach.<sup>22</sup> Changes in FA and MD have been reported in both PDD<sup>12,23,24</sup> and PD-MCI.<sup>23</sup>

We sought evidence of alterations in regional GM volume and integrity of the principal WM tracts in patients with newly diagnosed PD. We hypothesized that because increased MD is a sensitive marker of WM damage, changes in MD would be detectable before reduced FA<sup>23,25</sup> and significant GM loss.

## Patients and Methods

### Subjects

All patients and controls were enrolled prospectively as part of the Incidence of Cognitive Impairment in Cohorts with Longitudinal Evaluation in Parkinson's Disease (ICICLE-PD) Study.<sup>4,26</sup> All patients with newly diagnosed PD attending movement disorder, neurology, and geriatric medicine clinics in Newcastle and Gateshead between 1 June 2009 and 31 December 2011 were invited to participate. Enrolled subjects fulfilled the UK Brain Bank Criteria for idiopathic PD.<sup>27</sup> Healthy and unrelated control subjects were recruited from the local community.

Exclusion criteria comprised: patients with parkinsonism diagnosed before onset of the study; insuffi-

cient working knowledge of English; significant cognitive impairment or dementia at presentation; and use of antipsychotic medication. Patients were reviewed after 18 months to ensure that other causes of parkinsonism were excluded.

The study was approved by the Newcastle and North Tyneside Research Ethics Committee. All subjects provided written informed consent.

### Clinical and Neuropsychological Assessment

Clinical, neuropsychological, and imaging assessments were completed for each participant within a 4 month period. Assessments included a standardized neurological examination, H & Y staging,<sup>28</sup> the International Parkinson Movement Disorder Society (MDS)-revised MDS-UPDRS part III,<sup>29</sup> and the Geriatric Depression Scale (GDS-15).<sup>30</sup> Patients were assessed while taking their normal regimen of dopaminergic and other medications. Dopaminergic medication doses were standardized and are presented as levodopa equivalent daily dose (LEDD).<sup>31</sup>

Global cognitive function was assessed with the Mini-Mental State Examination (MMSE)<sup>32</sup> and Montreal Cognitive Assessment (MoCA).<sup>33</sup> Attention was measured using tests from the Cognitive Drug Research battery. Scores of simple reaction time, choice reaction time, and digit vigilance were summed to produce a composite power of attention (PoA) score.<sup>34</sup> Memory was assessed with the spatial recognition memory, pattern recognition memory, and paired associates learning subsets from the Cambridge Neuropsychological Test Automated Battery (CANTAB).<sup>35</sup> Executive function was measured using tests of phonemic fluency (words beginning with F, A, and S for 60 seconds each)<sup>36</sup> and semantic fluency (animals named in 90 seconds)<sup>37</sup> and with the Tower of London (TOL) task from the CANTAB battery.<sup>38</sup>

### MRI Acquisition

All images were acquired using a 3T Intera Achieva scanner (Philips Medical Systems, Eindhoven, The Netherlands) with an eight-channel receiver head coil in a single session. A standard sagittal T1-weighted volumetric scan was acquired covering the whole brain using a magnetization prepared rapid gradient echo sequence: echo time (TE) = 4.6 ms; repetition time (TR) = 9.6 ms; flip angle = 8 degrees, SENSE factor = 2; in-plane field of view (FOV) = 240 × 240 mm; slice thickness = 1.2 mm; voxel size = 1.15 × 1.15 mm. DTI acquisitions were based on a two-dimensional diffusion-weighted, spin-echo, echo planar imaging sequence with 59 slices: TR = 6,100 ms; TE = 70 ms; flip angle = 90 degrees; voxel size = 2.1 × 2.1 mm; slice thickness = 2.1 mm; FOV = 270 × 270 mm. Diffusion weighting was performed in 64 uniformly distributed directions (diffusion b = 1,000 s/

mm<sup>-2</sup>) and in six acquisitions without diffusion weighting ( $b = 0$  s/mm<sup>-2</sup>).

### MRI Preprocessing

Images were inspected for any artefacts or gross abnormalities and were processed using Statistical Parametric Mapping (SPM8; <http://www.fil.ion.ucl.ac.uk/spm>) and MATLAB software (7.14; MathWorks, Natick, MA).<sup>39</sup> T1-weighted images were segmented into GM, WM, and cerebral spinal fluid (CSF) using the standard unified segmentation sequence in SPM8. These images were also inspected for segmentation classification errors. A study-specific GM template was created from all patients and controls using the diffeomorphic anatomical registration through exponentiated Lie algebra (DARTEL) toolbox in SPM8.<sup>40</sup> GM data were spatially normalized and warped in DARTEL and transformed to Montreal Neurological Institute (MNI) space (<http://www.mni.mcgill.ca>). Images were Jacobian modulated to preserve the relative volumes of GM after normalization. An 8-mm full-width half maximum Gaussian kernel was used to smooth the images. The smoothed, modulated, and normalized GM data sets were used for statistical analyses. Total intracranial volume (TIV) was calculated by summing the total tissue assignments to GM, WM, and CSF from probability maps generated in the initial segmentation step.

### DTI Preprocessing

Preprocessing was performed using TBSS in FSL (<http://fsl.fmrib.ox.ac.uk/fsl/tbss>).<sup>22</sup> To correct for distorting effects of eddy currents, a modified version of the technique described by Shen et al.<sup>41</sup> was used with an affine registration to register pairs of diffusion-weighted images together. The diffusion-weighted images were then coaligned with a rigid body registration to the  $b = 0$  s/mm<sup>-2</sup> image. MD and FA maps were calculated using the FSL tensor analysis of the aligned diffusion-weighted images at each brain voxel. First, individual FA images from all subjects were each nonlinearly aligned to the predefined FSL FMRIB58 FA map using a resolution of 1 mm in the standard MNI152 space. Data were checked visually to ensure accuracy of the nonlinear transformation process. A mean FA image was created and thinned to create a mean FA skeleton, which represented the centers of all tracts common to the group. Finally, the aligned FA and MD image for each subject was projected onto the constructed skeleton. Voxel-wise statistical analyses of FA and MD data were performed using TBSS to compare group differences between the WM skeletons.

### Statistical Analyses

Clinical and neuropsychological data were analyzed with Statistical Package for Social Sciences software (version 19; SPSS, Inc., Chicago, IL). Independent  $t$  tests were used to compare normally distributed continuous variables, and Mann-Whitney's  $U$  test was used for continuous data without a normal distribution. Pearson's chi-squared tests were used to compare categorical data, except where the number in a category was less than 5, in which case Fisher's exact test was used. Significant results were reported at a value of  $P < 0.05$ . Bonferroni's adjustment was performed to correct for multiple testing of neuropsychological data, yielding a  $P < 0.007$  level of significance. Consistent with recent recommendations,<sup>42</sup> and to make this study comparable with the work of others,<sup>43,44</sup> a test score was considered impaired if it was 1.5 standard deviations (SDs) below the mean score of the control subjects.

GM volume differences were assessed using one-way analyses of variance in the SPM8 General Linear Model based on random Gaussian field theory. An absolute threshold mask of 0.1 was used in all GM analyses. Age, TIV, and education were included as covariates in all multiple regression analyses. MDS-UPDRS-III score was included as a covariate for PD subjects. The relationship between GM volumes and cognitive test score was examined in the PD group. Mean regional GM volume differences between groups (control vs. PD-NC, PD-NC vs. PD-<sub>impaired-test</sub>, control vs. PD-<sub>impaired-test</sub>) were compared by analysis of covariance in SPM8, with impaired being defined as test performance below 1.5 SD the normative mean. Reverse contrasts were performed for all analyses. Significant clusters were identified using a voxel-wise uncorrected threshold of  $P < 0.001$ . Clusters were regarded as significant if their extent exceeded 100 voxels. To control for multiple comparisons, a family-wise error (FWE) threshold of  $P_{\text{FWE-corr}} < 0.05$  was applied. Anatomical location was determined using the Talairach daemon (<http://talairach.org/>).

Differences in MD and FA were compared between groups (control vs. PD-NC, PD-NC vs. PD-<sub>impaired-test</sub>, control vs. PD-<sub>impaired-test</sub>) with a permutation-based, nonparametric test, two-sample, unpaired  $t$  test in the FSL "randomize" program. Age and education were included as covariates. Separate FA and MD models excluding controls assessed PD subjects (PD-NC and PD-<sub>impaired-test</sub>) with the same covariates plus MDS-UPDRS-III score. For each contrast, 5,000 permutations of the data were generated producing statistical maps uncorrected and FWE-corrected for multiple comparisons. Statistical maps were interrogated using a corrected threshold of  $P < 0.05$ . The threshold-free cluster enhancement (TFCE) algorithm was used to identify significant clusters and control for multiple

**TABLE 1.** Demographic and clinical characteristics of the PD and control subjects

	Control (n = 50)	PD (n = 125)	P Value
<b>Demographics</b>			
Age, years	65.8 ± 8.0	66.0 ± 10.5	0.88 <sup>a</sup>
Gender, male (% male)	29 (58)	85 (68)	0.21 <sup>b</sup>
Education, years	11.5 (3–24)	12 (3–24)	0.36 <sup>c</sup>
<b>Clinical information</b>			
Disease duration, months	—	6.15 (4.66)	—
MDS-UPDRS-III	—	26.8 ± 11.1	—
H & Y stage	—	2 (1–3)	—
LEDD, mg/day	—	175 (0–590)	—
GDS-15 (0–15)	0 (0–7)	2 (0–12)	<0.001 <sup>c</sup>
<b>Vascular risk factors, n (%)</b>			
Diabetes	1 (2)	10 (8)	0.18 <sup>d</sup>
Ischemic heart disease	3 (6)	13 (10.4)	0.56 <sup>d</sup>
Hypertension	13 (26)	38 (30.4)	0.56 <sup>b</sup>
Hypercholesterolaemia	10 (20)	16 (12.8)	0.23 <sup>b</sup>
Transient ischemic attack	0 (0)	10 (8)	0.06 <sup>d</sup>
Ever smoked	27 (54)	55 (44)	0.26 <sup>b</sup>

Values are mean ± SD, except median (range) for disease duration, education, H & Y stage, GDS, and number (%) of vascular risk factors. Comparisons between groups performed using <sup>a</sup>Student *t* test, <sup>b</sup>chi-square, <sup>c</sup>Mann-Whitney's U test, or <sup>d</sup>Fisher's exact test.

comparisons.<sup>4,5</sup> Regions showing significant differences between groups were located and labeled by mapping the statistical map to the John Hopkins University DTI WM atlas within FSL.

## Results

### Participant Characteristics

Table 1 shows that subject groups were similar in age, gender, and education. Although those with PD reported more depressive symptoms on the GDS-15 compared to controls, there was no difference in prevalence of clinically diagnosed depression. Nor were there significant differences in the prevalence of cardiovascular disease risk factors.

Results of cognitive testing are shown in Table 2. After Bonferroni's correction for multiple testing, subjects with PD had small, but significant, reductions in performance on tests of global cognition and all domain-specific cognitive tests, compared to controls.

### VBM

There was no significant difference in total or regional GM volumes between the entire PD group and control subjects ( $P = 0.58$ ), nor after correction for total intracranial volume ( $P = 0.42$ ). Reduced GM volume in frontal, parietal, and temporal areas in PD subjects was associated with poorer performance on the semantic fluency task ( $P_{\text{FWE-corr}} < 0.05$ ; Table 3; Fig. 1A). Reduced GM volume in frontal and parietal areas was evident in PD subjects with impaired semantic fluency (PD-<sub>impaired-SF</sub>), when compared to PD subjects with normal semantic fluency performance and

control subjects. A relationship between reduced GM volumes in frontal and parietal areas and poor performance on the executive TOL task was also observed in PD subjects (Fig. 1B). However, no mean differences were noted between groups (control vs. PD-NC, PD-NC vs. PD-<sub>impaired-ToL</sub> or control vs. PD-<sub>impaired-ToL</sub>). There was no significant association between GM volume and performance on any of the other cognitive tests.

### DTI

Averaged across the entire WM, PD subjects had greater MD than controls ( $0.758 \times 10^{-3} \text{ mm}^2/\text{s}$  [range, 0.717–0.957] vs.  $0.752 \times 10^{-3} \text{ mm}^2/\text{s}$  [range, 0.701–0.893];  $P = 0.019$ ). Interrogation with TBSS showed MD increases bilaterally in frontal and parietal subcortical tracts, including the forceps minor, cingulum, superior longitudinal fasciculus, inferior longitudinal fasciculus, inferior fronto-occipital fasciculus, corticospinal tract, corpus callosum, and internal capsule (Fig. 2A,B). These changes were also observed in PD subjects with impaired semantic fluency (PD-<sub>impaired-SF</sub>), relative to PD-NC and controls (Fig. 2C). There were no areas where the controls had higher MD values than the subjects with PD. There was no difference in total FA between control and PD subject groups (0.42 [range, 0.37–0.48] vs. 0.42 [range, 0.32–0.48];  $P = 0.255$ ), nor was there any

**TABLE 2.** Cognitive test data of the PD and control subjects

	Control (n = 50)	PD (n = 125)	P Value <sup>a</sup>
<b>Global cognitive testing</b>			
MMSE (0–30)	30 (26–30)	29 (24–30)	0.003 <sup>a</sup>
MoCA (0–30)	28 (21–30)	26 (16–30)	<0.001 <sup>a</sup>
<b>Cognitive tests</b>			
Power of attention, ms	1,242 ± 116	1,363 ± 198	<0.001 <sup>b</sup>
Impaired at 1.5 SD, n (%)	2 (4)	28 (22.6)	0.003 <sup>c</sup>
Semantic fluency (words)	24.4 ± 6.3	21.2 ± 6.8	0.004 <sup>b</sup>
Impaired at 1.5 SD, n (%)	4 (8.0)	19 (15.2)	0.22 <sup>c</sup>
Phonemic fluency (words)	41.6 ± 12.9	33.0 ± 12.4	<0.001 <sup>b</sup>
Impaired at 1.5 SD, n (%)	3 (6)	20 (16)	0.13 <sup>c</sup>
TOL (0–20)	17 (9–20)	15 (1–20)	<0.001 <sup>a</sup>
Impaired at 1.5 SD, n (%)	3 (6.0)	25 (20.0)	0.07 <sup>c</sup>
Spatial recognition memory (0–20)	17 (3–20)	16 (9–20)	<0.001 <sup>a</sup>
Impaired at 1.5 SD, n (%)	1 (2)	29 (23.2)	0.004 <sup>c</sup>
Pattern recognition memory (0–24)	22 (15–24)	20 (11–24)	0.001 <sup>a</sup>
Impaired at 1.5 SD, n (%)	2 (4)	25 (20.0)	0.030 <sup>c</sup>
Paired associates learning	1.63 (1.13–3.57)	1.88 (1–7)	0.002 <sup>b</sup>
Impaired at 1.5 SD, n (%)	3 (6)	17 (13.6)	0.35 <sup>c</sup>

Values are median (range) for MMSE, MoCA, TOL, spatial recognition memory, pattern recognition memory, and paired associates learning, except for PoA, semantic, and phonemic fluency, which are mean ± SD.

Comparisons between groups performed using <sup>a</sup>Mann-Whitney's U test, <sup>b</sup>Student *t* test, or <sup>c</sup>Fisher's exact test.

<sup>a</sup>Following Bonferroni's correction for multiple neuropsychological tests,  $P < 0.007$  was considered a significant difference in test scores between subjects with PD and controls.

**TABLE 3.** Anatomical location of clusters of reduced GM volume associated with cognitive task performance

Brain Region	Cluster-wise Value ( $P_{FWE-corr}$ )	Cluster Size (k)	Peak Voxel T, Z	MNI Coordinates (mm)		
				X	Y	Z
<b>(a) Association between GM volume and semantic fluency scores</b>						
R insular cortex	0.001	3,092	5.73, 5.37	45	-12	10
R precentral gyrus	0.002	1,697	5.26, 4.97	59	12	10
R middle frontal gyrus	0.037	871	4.60, 4.40	30	59	1
L insular cortex	0.003	1,605	5.07, 4.81	-48	-18	16
L cingulate gyrus	0.002	1,792	4.23, 4.07	-3	-9	45
<b>(b) Controls &gt; PD<sub>-impaired-semantic fluency</sub></b>						
R inferior parietal lobule	0.040	888	4.66, 4.31	63	-19	27
L transverse temporal gyrus	0.013	1,217	4.47, 4.16	-51	-21	12
<b>(c) PD-NC &gt; PD<sub>-impaired-semantic fluency</sub></b>						
R inferior parietal lobule	0.001	2,189	4.57	64	-19	27
L insular cortex	0.028	1,006	4.17, 4.01	-42	-18	12
L cingulate gyrus	0.035	947	4.13, 3.98	-4	-10	45
<b>(d) Association between GM volume and performance on TOL task</b>						
R insular cortex	0.002	1,848	5.61, 5.26	41	21	-0
R insular cortex	0.020	1,070	4.55, 4.35	39	-12	15
R cingulate gyrus	0.031	956	4.38, 4.20	9	-30	45
L insular cortex	0.005	1,524	5.08, 4.81	-39	17	3

Location and peak significance of significant reduction in GM volume in: (a) patients with PD where GM volume was associated with performance on the semantic fluency task; (b) controls and those PD subjects impaired on the semantic fluency task; (c) PD subjects with normal performance against those with impaired performance; and (d) PD patients where GM volume was associated with performance on the executive TOL task. For each peak the table shows cluster-level significance ( $P_{FWE-corr}$ ), spatial extent (k), t and z scores, MNI coordinates, and anatomical region. L, left; R, right.

significant association between FA and cognitive test performance.

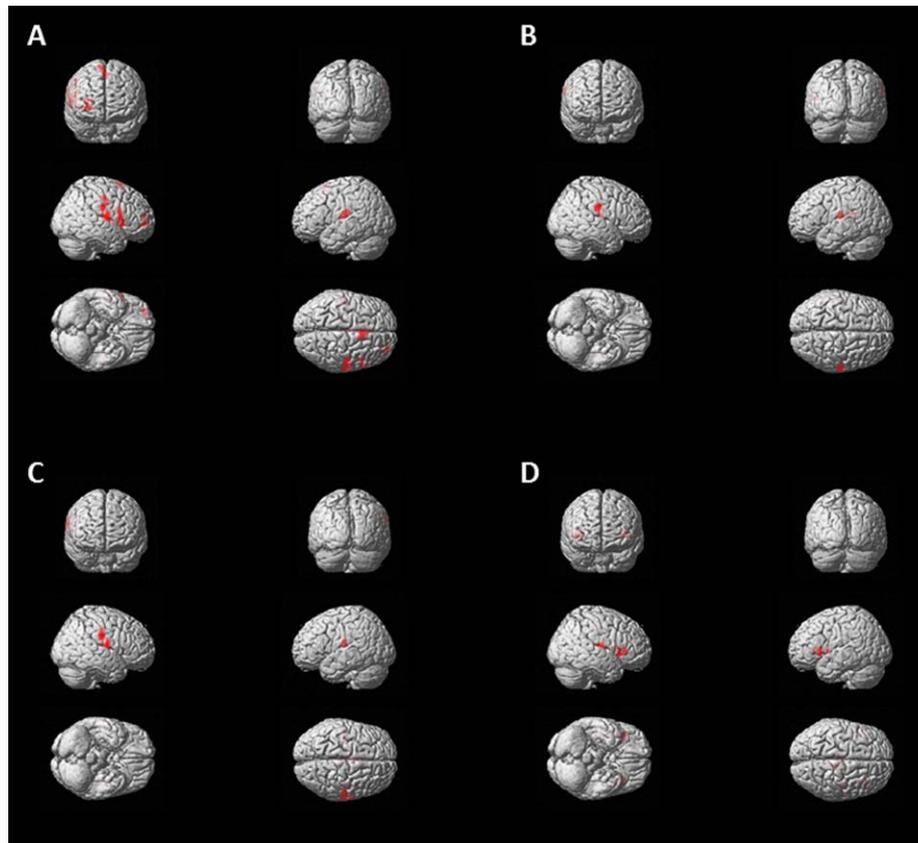
## Discussion

In this large series of patients with newly diagnosed PD, we found that altered MRI measures of WM integrity and GM volume correlated with cognitive decline. The principal findings were: (1) Increases in the MD of central WM tracts were detectable in the absence of reductions in either FA or GM volume; and (2) increased MD in frontal and parietal tracts and reduced GM volume in frontal, parietal, and temporal areas correlated with poor performance on the semantic fluency and executive TOL tasks.

These findings in our cohort of patients with newly diagnosed PD extend the work of Melzer et al.,<sup>23</sup> who observed increased MD in the absence of significant reductions in FA in patients with established PD targeting frontal and parietal tracts with involvement of the external capsule, corticospinal tract, corpus callosum, inferior occipital fasciculus, and inferior longitudinal fasciculus. Together, these results indicate that degeneration of central WM tracts occurs early in PD and may underlie early cognitive dysfunction. Widespread increases in MD involving the major WM tracts have been reported in PDD.<sup>23</sup> TBSS has detected reductions in FA in the superior longitudinal fasciculus, inferior longitudinal fasciculus, inferior fronto-occipital fasciculus, uncinata fasciculus, and cingulum in PDD, relative to age-matched controls.<sup>12</sup> In the same study, patients

with PDD had lower FA values in the superior longitudinal fasciculus, inferior longitudinal fasciculus, inferior fronto-occipital fasciculus, uncinata fasciculus, cingulum, and corpus callosum, compared to PD-NC subjects. However, these changes were not present in PD-NC subjects, supporting our findings and suggesting that decreases in FA appear later than rises in MD during the disease. Increases in MD may occur with minimal, or even absent, alterations in FA and have been observed in studies of both AD and PD.<sup>23,25</sup> Although the pathological substrate underlying DTI changes is unclear, such changes may result from similar proportional variations in the measured tensor dimensions within the diffusion ellipsoid caused by the neurodegenerative process,<sup>25</sup> and not solely changes in the longitudinal diffusivities. The pathogenesis of WM damage in PD is not well understood. Lewy neurites are the axonal manifestation of  $\alpha$ -Syn pathology and may be associated with impaired axonal transport with subsequent microstructural changes in the axon or surrounding myelin. Indeed, there is now evidence to suggest that PD is primarily a result of synaptic dysfunction with early axonal transport problems leading to subsequent cell death.<sup>46</sup>

Our finding of normal cortical volumes in newly diagnosed PD-NC patients is in line with other VBM studies of early PD<sup>10,11</sup> and Braak neuropathological staging of the disease.<sup>47</sup> We are aware of only one other study of PD-NC patients and this reported a small cluster of GM loss in the occipital lobe, relative to controls.<sup>8</sup> In PD-MCI, GM loss is evident in temporal, parietal, and frontal regions.<sup>11,19,21</sup> Subsequently,



**FIG. 1.** Anatomical location of clusters of reduced GM volume associated with cognitive task performance. Three-dimensional surface renders showing clusters of cortical GM loss (red) in those with PD: (A) significant association between poorer performance on the semantic fluency task with GM loss in the insular cortex, precentral gyrus, and middle frontal gyrus in the right hemisphere, and the insular cortex and cingulate gyrus in the left hemisphere; (B) in PD impaired on the semantic fluency task relative to controls showing GM loss in the right inferior parietal lobule and left transverse temporal gyrus; (C) in PD impaired on the semantic fluency task compared with PD not impaired showing GM loss in the right inferior parietal lobule, left insular cortex, and left cingulate gyrus; and (D) association with performance on TOL task with areas of reduced GM in the right and left insular cortex and the right cingulate gyrus. All results are presented at a cluster-wise threshold corrected  $P_{FWE-corr} < 0.05$ .

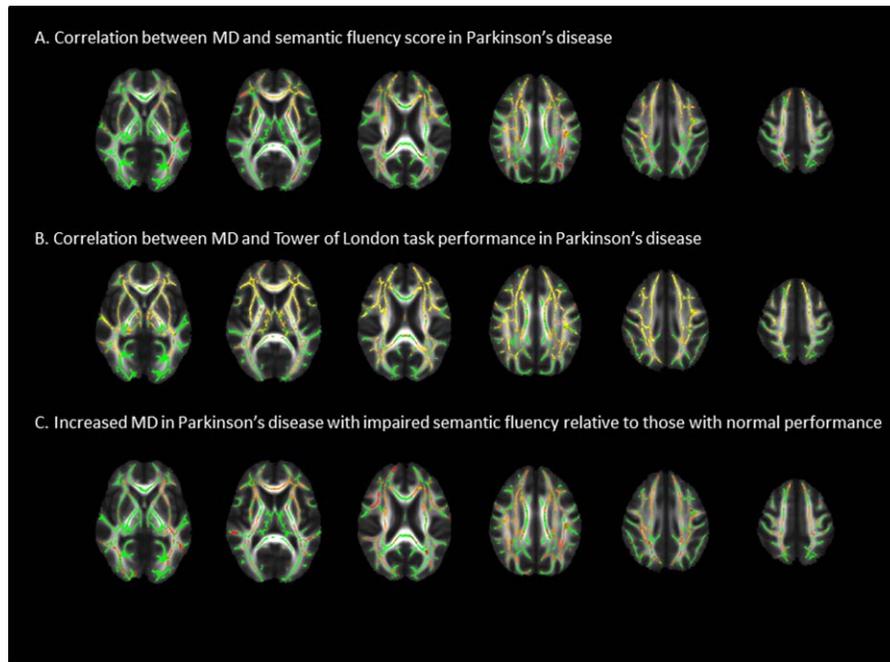
with disease progression and onset of dementia, diffuse GM loss becomes evident.<sup>11,13</sup>

The association of raised MD and reduced frontal GM volume changes with impaired semantic fluency in early PD is a novel finding. In established PD, GM density changes in the inferior and middle frontal gyrus and in the temporal lobe have been reported to correlate with semantic fluency performance.<sup>48</sup> Semantic fluency is more than a measure of executive function and is closely related to temporal lobe function. In PD, it is reported to be more significantly impaired relative to phonemic fluency.<sup>49</sup> This may indicate that the cognitive speed and retrieval of semantic items may represent an additional dysfunction of semantic memory.<sup>49</sup> Longitudinal studies of cognition in PD report that impaired semantic fluency may herald the development, or be an early feature, of PDD.<sup>50,51</sup>

Involvement of the cingulate gyrus and its WM connections to the thalamus and entorhinal cortex by the cingulum in PD is noteworthy. The cingulate is critical to regulation of mood and autonomic function; moreover, it plays a key role in working and planning memory, attention, and visuospatial skills, all of which may

be impaired in early PD. It is reported to be particularly vulnerable to Lewy pathology and may be one of the earliest sites of limbic involvement in PD. MRI studies report cingulate GM loss in PDD, and changes in this structure detectable with MRI may prove a useful marker of early disease progression.<sup>11,15</sup>

Strengths of our study include a large, well-characterized incident cohort of patients with early PD. All subjects were imaged using the same 3T scanner. The inclusion of a control group, well matched for age, gender, and level of education, permitted the generation of appropriate normative cognitive reference data. We employed validated clinical and cognitive assessments that have previously been adopted by observational and interventional studies of PD and cognition. Because the prognostic implications and neuroanatomical mechanisms of cognitive decline in PD are poorly described, we avoided a generic PD-MCI classification and examined performance on each cognitive test separately. In doing so, we hoped to gain a better appreciation of the neuroanatomical correlates and temporal progression of changes associated with cognitive decline in PD.



**FIG. 2.** Associations between MD and performance on the semantic fluency and TOL tasks. TBSS map showing areas of increased MD (yellow–red) in the WM in PD overlaid on the study specific mean FA skeleton (green): (A) significant association between increased MD and lower semantic fluency score; (B) significant association between increased MD and poorer performance on the executive TOL task; and (C) in PD impaired on the semantic fluency task compared with PD not impaired. All results are  $P < 0.05$ , corrected for multiple comparisons using TFCE.

Our large cohort permitted the generation of study specific templates/skeletons for the imaging analyses, without reliance upon the standard templates included within analysis software packages, which are generated from younger subjects. The advantage of using TBSS for analyzing diffusion data is that it combines the ability to interrogate all WM without the limitations of an a priori ROI approach. Through enhanced alignment of the central WM during the registration and skeletonization steps, the use of nonparametric statistics and the specific TFCE method, TBSS, has been shown to be more sensitive than voxel-based approaches. However, TBSS is biased toward the central WM tracts and less sensitive to changes in peripheral WM tracts; therefore, we may have been able to detect fewer MD and FA changes in these regions.<sup>52</sup>

Our adoption of the UK Brain Bank criteria and use of a longitudinal study design should have minimized possible misdiagnoses.<sup>26,27</sup> Only a small proportion of patients were drug naïve, however, as reflects current clinical practice, MDS guidance, and other published work.<sup>8,11,21,23,42</sup>

By performing analysis of cortical GM volumes and measuring the integrity of the underlying WM, we have shown that MRI has the potential to become a biomarker that is associated with cognitive function in PD. Our results suggest that increased diffusivity is detectable before changes in GM volume. Future work will be aimed at establishing the temporal nature of GM and WM abnormalities in PD and how these and

the baseline changes correlate with subsequent cognitive decline. ■

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