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Leucoaraiosis, nigrostriatal denervation and motor symptoms in Parkinson's disease

Nicolaas I. Bohnen,[1,2,3] Martijn L. T. M. Müller,[1] Natalia Zarzhevsky,[1] Robert A. Koeppe,[1] Christopher W. Bogan,[1] Michael R. Kilbourn,[1] Kirk A. Frey[1,2] and Roger L. Albin[2,3]

1 Department of Radiology, Division of Nuclear Medicine, University of Michigan, Ann Arbor, MI 48105-9755, USA
2 Department of Neurology, University of Michigan, Ann Arbor, MI 48105-9755, USA
3 Neurology Service and Geriatric Research Education and Clinical Center, Veterans Administration Ann Arbor Healthcare System, Ann Arbor, MI 48105, USA

Correspondence to: Nicolaas I. Bohnen, MD, PhD,
Functional Neuroimaging,
Cognitive and Mobility Laboratory,
Departments of Radiology and Neurology,
University of Michigan,
24 Frank Lloyd Wright Drive,
Box 362, Ann Arbor,
MI 48105-9755, USA
E-mail: nbohnen@umich.edu

Leucoaraiosis is associated with motor symptoms in otherwise normal older adults. Comorbid leucoaraiosis is predicted to contribute also to motor features in Parkinson's disease but previous studies of white matter changes in Parkinson's disease show variable results. No prior studies have compared directly the effects of both leucoaraiosis and the degree of nigrostriatal dopaminergic denervation on motor features. We investigated the effect of leucoaraiosis severity on motor impairment independent of the degree of nigrostriatal dopaminergic denervation in Parkinson's disease. Seventy-three subjects with Parkinson's disease (Hoehn and Yahr stages 1–3) underwent brain magnetic resonance and [^{11}C]dihydrotetrabenazine vesicular monoamine transporter type 2 positron emission tomography imaging. Automated assessment of supratentorial fluid-attenuated inversion recovery magnetic resonance hyperintense white matter voxels was performed using cerebellar white matter as the intensity reference. White matter signal hyperintensity burden was log-transformed and normalized for brain volume. Unified Parkinson's Disease Rating Scale total and subscore ratings were assessed to determine motor impairment. Subjects receiving dopaminergic medications were examined in the clinically defined 'OFF' state. Multivariate regression analysis with measures of white matter signal hyperintensity burden and nigrostriatal denervation as independent variables demonstrated a significant overall model for total motor Unified Parkinson's Disease Rating Scale scores ($F = 11.4$, $P < 0.0001$) with significant regression effects for both white matter signal hyperintensity burden ($t = 2.0$, $\beta = 0.22$, $P = 0.045$) and striatal monoaminergic binding ($t = -3.5$, $\beta = -0.38$, $P = 0.0008$). Axial motor impairment demonstrated a robust association with white matter signal hyperintensity burden ($t = 4.0$, $\beta = 0.43$, $P = 0.0001$) compared with striatal monoaminergic binding ($t = -2.1$, $\beta = 0.22$, $P = 0.043$). White matter signal hyperintensity burden regression effects for bradykinesia had borderline significance. No significant white matter signal hyperintensity burden effects were found for rigidity or tremor subscores. White matter signal hyperintensity burden was significantly higher in the subgroup with postural instability and gait difficulties compared with the tremor-predominant subgroup despite no significant differences in age or duration of disease. These findings indicate that increased white matter signal hyperintensity burden is associated with worse motor performance independent of the degree of nigrostriatal dopaminergic denervation in Parkinson's disease. Comorbid white matter disease is a greater determinant of axial motor impairment than nigrostriatal dopaminergic denervation.

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Abbreviations: UPDRS = Unified Parkinson's Disease Rating Scale

Introduction

Areas with altered signal intensity in white matter are encountered frequently on brain imaging studies in the elderly (Gunning-Dixon and Raz, 2000). These white matter abnormalities, or leucoaraiosis, are associated commonly with small vessel cerebrovascular disease, often in the context of diabetes and cardiovascular disease (Pantoni and Garcia, 1997). White matter changes are associated with loss of vascular and reduced blood–brain barrier integrity (Young *et al.,* 2008). In otherwise normal older adults, white matter abnormalities have been associated with subtle abnormalities of balance and gait (Longstreth and Manolio, 1996; Baezner *et al.,* 2008; Soumare *et al.,* 2009; Murray *et al.,* 2010; de Laat *et al.,* 2011).

As white matter changes are associated with motor dysfunction in otherwise normal elderly adults, comorbid leucoaraiosis could be predicted to also contribute to clinical features in Parkinson's disease. It is plausible that leucoaraiosis severity is an independent contributor to motor impairments in Parkinson's disease. Previous studies, however, of motor impairment and white matter changes in Parkinson's disease show variable results with some studies indicating more severe axial motor symptoms associated with comorbid leucoaraiosis (Piccini *et al.,* 1995; Lee *et al.,* 2009). Correlations between leucoaraiosis severity and symptoms of rigidity and bradykinesia are inconsistent (Sohn and Kim, 1998; Acharya *et al.,* 2007; Slawek *et al.,* 2010). Accurate assessment of the contribution of leucoaraiosis severity to motor dysfunction in Parkinson's disease is inherently difficult in the absence of an objective marker of Parkinson's disease-specific pathology that can be used to directly assess the relative contributions of nigrostriatal denervation and white matter changes. The primary goal of the present study was to investigate the relationship between leucoaraiosis severity and motor impairments relative to the degree of nigrostriatal dopaminergic denervation in Parkinson's disease as determined by [^{11}C]dihydrotetrabenazine PET. The secondary goal was to determine if leucoaraiosis severity correlates with specific aspects of motor dysfunction in Parkinson's disease. Demonstration that leucoaraiosis severity correlates with some, but not all, motor dysfunctions in Parkinson's disease would strongly support the conclusion that leucoaraiosis severity is an independent determinant of motor impairment in Parkinson's disease.

Materials and methods

Subjects and clinical test battery

This cross-sectional study involved 73 subjects with Parkinson's disease (62 males; 11 females). Subjects met the UK Parkinson's Disease Society Brain Bank Research Centre clinical diagnostic criteria for Parkinson's disease (Hughes *et al.,* 1992). Subjects were recruited from an academic medical centre, Parkinson's disease support groups and a Department of Veterans Affairs hospital. Recruitment from the latter source, which has a predominantly male patient population, explains the male predominance in our study sample beyond that typically seen in Parkinson's disease. Diagnoses of Parkinson's disease were confirmed by the presence of nigrostriatal dopaminergic denervation on dihydrotetrabenazine PET imaging. Patients had mild-to-moderate severity of disease; two subjects were Hoehn and Yahr stage 1, two subjects were Hoehn and Yahr stage 1.5, 18 subjects were Hoehn and Yahr stage 2, 29 subjects were Hoehn and Yahr stage 2.5 and 22 subjects were Hoehn and Yahr stage 3 (Hoehn and Yahr, 1967). The mean age of subjects was 68.5 ± 8.9 years (range 51–83) with mean duration of disease 6.9 ± 3.8 years (range 0.5–17). The mean Mini-Mental State Examination score was 28.9 ± 1.7 (range 21–30) (Folstein *et al.,* 1975). The mean Clinical Dementia Rating Scale score was 0.4 ± 0.5 (range 0–2) (Hughes *et al.,* 1982). The mean Unified Parkinson's Disease Rating Scale (UPDRS) score was 26.7 ± 8.0 (range 5–46) (Fahn and Elton, 1987). UPDRS motor scores were divided into subscores for tremor (UPDRS items 20 and 21), rigidity (item 22), bradykinesia (items 23–26 and 31) and axial symptoms (items 27–30). Patients were subclassified into three subtypes by Jankovic's method (Jankovic *et al.,* 1990). Depending on the dominant motor features, the Parkinson's disease subtype was defined as (i) tremor dominant; (ii) postural instability and gait difficulty dominant; or (iii) intermediate. Subtype classification is based on the ratio of the average of UPDRS 'tremor' items (16, 20 and 21) divided by the average of 'postural instability and gait difficulty dominant' items (13–15, 29 and 30). This ratio indicates the Parkinson's disease subtypes as follows: $\leqslant 1.0$ = postural instability and gait difficulty dominant, $\geqslant 1.5$ = tremor dominant and > 1.0 and < 1.5 = intermediate.

Subjects treated with dopaminergic drugs were examined and imaged in the morning after withholding dopaminergic drugs overnight ('OFF' state). Forty patients were taking a combination of dopamine agonists and carbidopa–levodopa, 21 were taking carbidopa–levodopa alone, 10 were taking dopamine agonists alone, and two were not on dopaminergic medications. Dopamine agonist use in the subjects consisted of either short-acting ropinirole or pramipexole formulations. Levodopa equivalent dose was calculated using published conversion formulae (Tomlinson *et al.,* 2010). The mean levodopa equivalent dose was 733 ± 487 (range 150–2725) mg. Subjects with a large vessel stroke, striatal lacunar states, intracranial mass lesion, contraindication for MRI (such as deep brain stimulator), moderate to severe dementia, normal dihydrotetrabenazine PET scan, inability to lie still on a magnetic resonance or PET camera table or disabling freezing were not eligible for the study. Subjects were consecutively recruited as long as they met eligibility criteria and were willing to undergo the study procedures. Table 1 provides baseline clinical characteristics of the subjects. The study was approved by the Institutional Review Boards of the University of Michigan and Ann Arbor Veterans Administration Medical Centre for studies involving human subjects.

Imaging techniques

MRI was performed on a 3 Tesla Philips Achieva system (Philips) utilizing an 8-channel head coil and the 'ISOVOX' exam card protocol primarily designed to yield isotropic T_1 spoiled gradient (SPGR) spatial resolution. A standard T_1-weighted series of a 3D inversion

Table 1 Summary of clinical characteristics of the study subjects ($n = 73$)

	Average (SD/range or percentage)
Age (years)	68.5 ± 8.9 (51–83)
Male/female	62/11
Age of onset of motor disease	61.6 ± 9.8 (34.5–82.0)
Disease duration (years)	6.9 ± 3.8 (0.5–17)
Hoehn and Yahr Stage	2.5 ± 0.5 (1–3)
UPDRS part I (mentation, behaviour and mood) score	2.2 ± 1.5 (0–5)
UPDRS part II (activities of daily living) score	10.9 ± 4.9 (0–21)
UPDRS part III (motor) score	26.7 ± 8.0 (5–46)
Tremor-predominant motor phenotype	25/73
Postural instability and gait difficulty motor phenotype	36/73
Intermediate motor phenotype[a]	10/73
History of freezing of gait	20/73 (27.4)
History of falls	30/73 (41.1)
History of dyskinesias	14/73 (19.2)
Presence of mild hallucinations or delusions	7/73 (9.6)
Levodopa equivalent dose	733 ± 487 (150–2725)
Mini-Mental State Examination score	28.9 ± 1.7 (21–30)
Clinical dementia rating scale	0.4 ± 0.5 (0–2)
Subjects on anti-depressants	15/73 (20.5)
Subjects on benzodiazepines	8/73 (10.9)
Smoking	4/73 (5.5)
Hypertension	27/73 (37)
Diabetes mellitus	7/73 (9.6)
Hyperlipidaemia	18/73 (24.7)
History of atrial fibrillation	2/73 (2.7)
History of myocardial infarction or transient ischaemic attack	6/73 (8.2)
Subjects on warfarin anti-coagulation	3/73 (4.1)
Subjects on anti-platelet drugs	23/73 (31.5)

a Two subjects could not be classified because of a summed postural instability and gait difficulty dominant score of zero.

recovery-prepared turbo-field-echo was performed in the sagittal plane with repetition time/echo time/inversion time = 9.8/4.6/1041 ms; turbo factor = 200; single average; field of view = 240 × 200 × 160 mm; acquired matrix = 240 × 200. Slices ($n = 160$) were reconstructed to 1 mm isotropic resolution. This sequence maximizes contrast among grey matter, white matter and CSF and provides high-resolution delineation of cortical and subcortical structures. Subjects also completed a volumetric and fast fluid-attenuated inversion recovery (FLAIR) (repetition time/echo time = 9002/56 ms effective; inversion time = 2200 ms, number of excitations = 1; slice thickness = 1.5 mm) brain magnetic resonance sequence.

Vesicular monoamine transporter type 2 (VMAT2) PET imaging was performed in 3D imaging mode using an ECAT HR+ tomograph (Siemens Molecular Imaging, Inc.), which acquires 63 transaxial slices (slice thickness: 2.4 mm; intrinsic in-plane resolution: 4.1 mm full-width at half maximum over a 15.2 cm axial field-of-view). A NeuroShield (Scanwell Systems) head-holder/shielding unit was attached to the patient bed to reduce the contribution of photons originating from the body outside the scanner field-of-view (Thompson *et al.*, 2001). Prior to the dihydrotetrabenazine injection, a 5-min transmission scan was acquired using rotating ^{68}Ge rods for attenuation correction of emission data using the standard vendor-supplied segmentation and reprojection routines.

No-carrier-added (+)-[^{11}C]dihydrotetrabenazine (250–1000 Ci/mmol at the time of injection) was prepared as reported previously (Jewett *et al.*, 1997). Dynamic PET scanning was performed for 60 min beginning immediately following a bolus injection of 55% of a 666 MBq (18 mCi) total (+)-[^{11}C] dihydrotetrabenazine dose (containing $<50\,\mu g$ of cold dihydrotetrabenazine mass) over the first 15–30 s of the study, while the remaining 45% of the dose was continuously infused over the next 60 min, resulting in stable arterial tracer levels and equilibrium with brain tracer levels after 30 min (Koeppe *et al.*, 1997). A 15-frame sequence of scans over 60 min were obtained as follows: four at 30 s; three at 1 min; two at 2 min 30 s; two at 5 min; and four at 10 min. All subjects were studied supine, with eyes and ears unoccluded, resting quietly in a dimly lit room.

Positron emission tomography analysis

All image frames were spatially coregistered within subject with a rigid-body transformation to reduce the effects of subject motion during the imaging session (Minoshima *et al.*, 1993). IDL image analysis software (Research Systems, Inc.) was used to manually trace volumes of interest on the magnetic resonance scans representing the striatum, including the putamen and caudate nucleus. Total cortical volumes of interest were defined using semi-automated threshold delineation of cortical grey matter signal on the MRI scan.

Dihydrotetrabenazine images were analysed using equilibrium modelling to estimate the non-displaceable binding potential (BP_{ND}), which is equivalent to the ratio of specific (V_S) to non-displaceable (V_{ND}) binding in each imaged voxel or target volume of interest (Koeppe *et al.*, 1997). We estimated specific [^{11}C]dihydrotetrabenazine binding by subtraction of the global neocortex value, a reference region very low in VMAT2 binding sites, with the assumption that the non-displaceable distribution is uniform across the brain at equilibrium (Koeppe *et al.*, 1999).

Reference-based automated assessment of supratentorial hyperintense white matter voxels

Automated methods of magnetic resonance FLAIR-based white matter signal hyperintensity burden assessment have been developed based on the use of a reference region or tissue, such as intensity of normal grey matter (de Boer *et al.*, 2009). We previously developed a routine to define hyperintense supratentorial voxels in which we use the mean intensity of cerebellar white matter voxels on magnetic resonance FLAIR images as a reference (Bohnen *et al.*, 2009). The cerebellum was chosen as a reference region because of the clinical observation that age-associated white matter changes, unlike diseases such as multiple sclerosis, relatively spare the cerebellum (Bohnen *et al.*, 2009). In brief, spatial preprocessing and white matter segmentation was performed with standard routines and functions in the software package SPM5 (Ashburner and Friston, 1997, 2005). The total number of supratentorial white matter voxels on FLAIR images that were hyperintense relative to a cerebellar white matter intensity cut-off of mean + 1.65 SD was computed and then log transformed. Data were also normalized for supratentorial white matter volume (Bohnen *et al.*, 2009).

Statistical analysis

Multivariate regression analysis was used to evaluate regression effects of imaging variables on motor outcome variables. Total striatal VMAT-2 binding was selected as the primary PET variable to avoid denervation 'floor' effects that are present with putaminal dopaminergic levels in Parkinson's disease (Bohnen *et al.,* 2007). Analysis of covariance was used to evaluate differences among patient subgroups. Pearson's correlation coefficients were computed, except for variables that were not normally distributed which were analysed using Spearman rank correlation coefficients (*Rs*). Data were analysed using the SAS program (version 9.2, SAS Institute).

Results

All patients had a typical nigrostriatal pattern of denervation on the VMAT2 PET scans, with posterior putaminal greater than caudate denervation. The mean striatal VMAT2 volume of distribution was 1.92 ± 0.30 (range 1.39–2.86). The log normalized and volume corrected supratentorial hyperintensity white matter variable had a mean value of $10.07 \times 10^{-6} \pm 2.44 \times 10^{-6}$ (range $4.37 \times 10^{-6} - 17.35 \times 10^{-6}$). The white matter hyperintensity measure had a normal distribution (Kolmogorov–Smirnov $D = 0.06$, $P > 0.15$).

Motor analysis: total group

Increased white matter hyperintensities were associated with higher Hoehn and Yahr stages ($Rs = 0.42$, $P = 0.0002$) and higher total UPDRS motor scores ($R = 0.34$, $P = 0.0035$). Corresponding bivariate correlation coefficients for the UPDRS subscores were as follows: axial ($R = 0.49$; $P < 0.0001$); bradykinesia ($R = 0.30$; $P = 0.0088$); rigidity ($R = 0.08$; $P = 0.50$) and tremor ($R = -0.16$; $P = 0.17$). Results of multivariate regression analyses using striatal VMAT2 binding and white matter hyperintensities as independent variables and UPDRS total motor and subscores as dependent variables are shown in Table 2. There was a significant overall model for total UPDRS motor scores ($F = 11.4$, $P < 0.0001$) with significant regression effects for both white matter hyperintensities ($t = 2.0$, $\beta = 0.22$, $P = 0.045$) and striatal VMAT2 binding ($t = -3.5$, $\beta = -0.38$, $P = 0.0008$). Axial motor impairments demonstrated more robust regression for white matter hyperintensities ($t = 4.0$, $\beta = 0.43$, $P = 0.0001$) compared with striatal VMAT2 binding ($t = -2.1$, $\beta = 0.22$, $P = 0.043$). White matter hyperintensities regression effects for bradykinesia had borderline significance. No significant white matter hyperintensity effects were found for rigidity or tremor scores (Table 2). Figure 1 shows a scatter plot of white matter hyperintensities and axial UPDRS motor scores.

Motor subgroup analysis: subjects with and without motor fluctuations

To take into account possible differences of practically defined motor 'OFF' ratings between subjects with and without motor fluctuations, i.e. freezing, we performed separate subgroup analyses. First, analysis limited to the subjects without freezing ($n = 53$) demonstrated similar findings for axial motor scores, which demonstrated a significant overall model ($F = 7.4$, $P = 0.0015$) with significant regression effects for white matter hyperintensities ($t = 2.9$, $\beta = 0.37$, $P = 0.005$), and non-significant trend for striatal VMAT2 binding ($t = -1.8$, $\beta = -0.22$, $P = 0.086$). Second, analysis limited to the smaller subgroup of subjects with freezing ($n = 20$) also demonstrated a significant overall model ($F = 3.9$, $P = 0.041$) with significant regression effects for white matter hyperintensities ($t = 2.1$, $\beta = 0.47$, $P = 0.0048$), and non-significant effect for striatal VMAT2 binding ($t = -0.8$, $\beta = -0.16$, $P = 0.45$).

We performed an additional analysis for axial motor scores by entering the total levodopa equivalent dose in the regression model for the complete group. Results demonstrated a significant overall model ($F = 11.8$, $P < 0.0001$) with significant regression effects most robust for white matter hyperintensities ($t = 4.4$, $\beta = 0.46$, $P < 0.0001$), moderate significant effect for levodopa equivalent dose ($t = 2.4$, $\beta = 0.26$, $P = 0.021$) and no longer significant for striatal VMAT2 binding ($t = -0.9$, $\beta = -0.10$, $P = 0.26$). As expected, there was a significant inverse correlation between striatal VMAT2 binding and levodopa equivalent dose ($R = -0.42$, $P = 0.0002$), whereas there was no significant correlation between levodopa equivalent dose and white matter hyperintensities ($R = 0.008$, $P = 0.94$).

Motor phenotype subgroup analysis: postural instability and gait difficulty dominant versus tremor-predominant versus intermediate phenotypes

Thirty-six subjects were postural instability and were gait difficulty dominant, 25 subjects were tremor dominant and 10 subjects had

Table 2 Results of multivariate regression analysis of the UPDRS scores

Dependent variable	Striatal VMAT2 binding		White matter hyperintensities		Overall model	
	β	t (P)	β	t (P)	F	P
UPDRS total motor	−0.38	−3.5 (0.0008)*	0.22	2.0 (0.045)	11.4	<0.0001*
UPDRS axial motor	−0.22	−2.1 (0.043)	0.43	4.0 (0.0001)*	14.0	<0.0001*
UPDRS bradykinesia	−0.35	−3.1 (0.0027)	0.20	1.8 (0.08)	8.9	0.0004*
UPDRS tremor	0.0027	0.02 (0.98)	−0.16	−1.3 (0.20)	0.9	0.41
UPDRS rigidity	−0.24	−2.0 (0.053)	0.008	0.07 (0.95)	2.2	0.12

Standardized coefficients (β) are given for each independent variable with parameter *t*-value, level of significance and total model statistics.
*Significant after Bonferroni correction.

an intermediate motor phenotype. Two subjects could not be classified because of a summed postural instability and gait difficulty dominant score of zero. There were no significant differences in average duration of disease ($F = 1.6$, $P = 0.20$; 7.5 ± 4.0; 5.9 ± 3.5 and 7.6 ± 3.2 years for postural instability and gait difficulty dominant, tremor-predominant and intermediate motor phenotype subgroups, respectively) or age ($F = 1.2$, $P = 0.30$; 70.3 ± 9.0; 66.7 ± 9.7 and 67.8 ± 6.1 years for postural instability and gait difficulty dominant, tremor-predominant and intermediate motor phenotype subgroups, respectively) among the three groups. Analysis of covariance using motor phenotype as the grouping variable, striatal VMAT2 binding as covariate and white matter hyperintensities as dependent variable demonstrated a significant overall model ($F = 4.8$, $P = 0.0043$), significant white matter hyperintensities group effect ($F = 4.4$, $P = 0.039$) and significant covariate VMAT2 effect ($F = 3.7$, $P = 0.029$). Duncan subgroup analysis demonstrated significantly higher white matter hyperintensities in the postural instability and gait difficulty dominant group ($10.95 \times 10^{-6} \pm 2.38 \times 10^{-6}$) compared with the tremor-predominant group ($9.16 \times 10^{-6} \pm 2.34 \times 10^{-6}$) but each not significantly different from the intermediate motor group ($9.52 \times 10^{-6} \pm 2.08 \times 10^{-6}$).

Individual item analysis

An individual item analysis of the postural instability and gait difficulty dominant components showed robust correlations between white matter hyperintensities and the retropulsion test ($Rs = 0.40$, $P = 0.0005$), walking assessment by history ($Rs = 0.25$, $P = 0.033$) and borderline correlations with gait examination ($Rs = 0.22$, $P = 0.06$). As a related clinical finding, there was a significant correlation between white matter hyperintensities and UPDRS rating of posture ($Rs = 0.43$, $P = 0.0002$). There was a weak correlation between white matter hyperintensities and speech difficulties ($Rs = 0.23$, $P = 0.047$).

Finally, we evaluated effects of specific motor complications in relationship to white matter hyperintensities. Three separate analyses of covariance using white matter hyperintensities as the outcome parameter, presence or absence of freezing, falls or dyskinesias as grouping variable, respectively, and using striatal VMAT2 binding as covariate did not reveal main group effects for subjects with freezing ($F = 2.53$, $P = 0.12$), falls ($F = 1.33$, $P = 0.25$) or dyskinesias ($F = 0.44$, $P = 0.51$).

Discussion

Previous studies of motor impairment and leucoaraiosis severity in Parkinson's disease show variable results, with several studies indicating more severe axial motor features associated with greater comorbid white matter disease (Piccini *et al.*, 1995; Lee *et al.*, 2009). Prior reports suggest inconsistent correlations between white matter changes and symptoms of rigidity or bradykinesia (Sohn and Kim, 1998; Acharya *et al.*, 2007; Slawek *et al.*, 2010). The variable results obtained in prior studies of comorbid leucoaraiosis correlates in Parkinson's disease may relate to differences in assessment methods of white matter changes, differing patient populations and—most importantly—lack of an objective covariate marker of Parkinson's disease-specific nigrostriatal pathology. Our results indicate that comorbid leucoaraiosis is associated with worsening motor performance independent of the degree of nigrostriatal dopaminergic denervation in Parkinson's disease. In particular, comorbid leucoaraiosis is a greater predictor of axial motor impairment than nigrostriatal dopaminergic

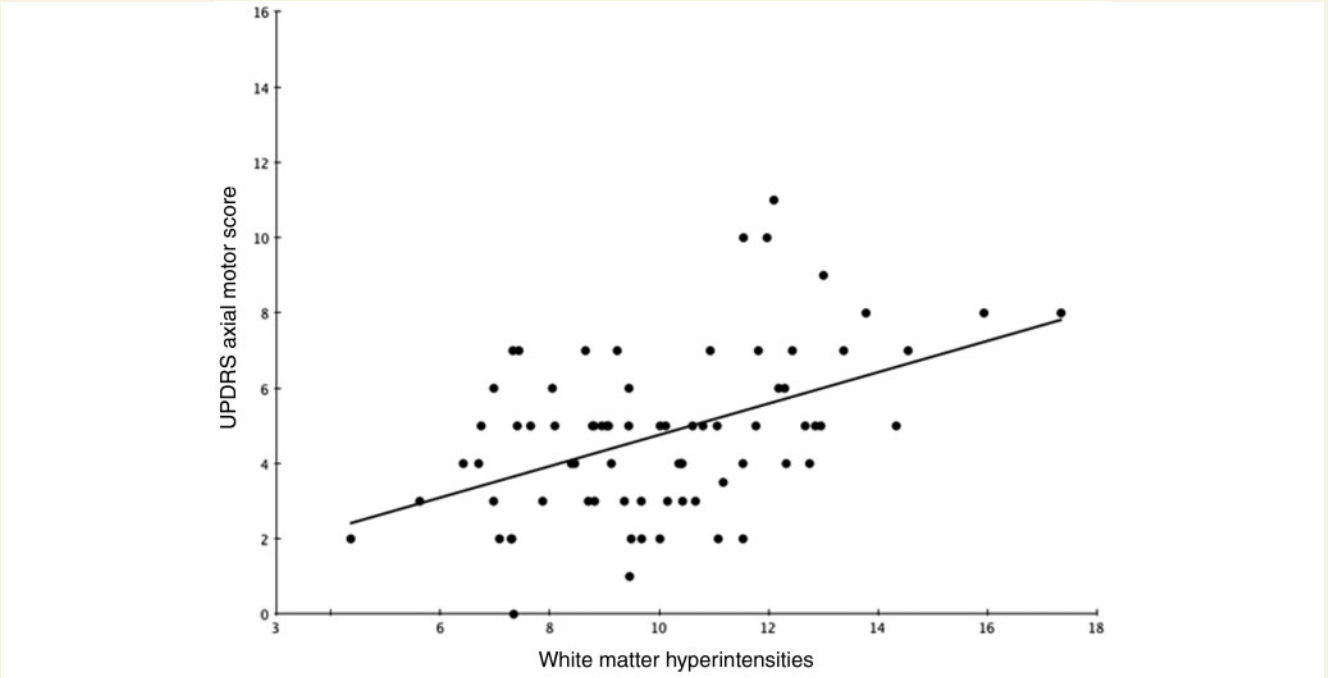

Figure 1 Scatter plot of leucoaraiosis severity ($\times 10^{-6}$) and axial UPDRS motor scores.

denervation. Independent effects of white matter hyperintensities on bradykinesia and gait had borderline significance and there were no significant independent effects of white matter changes for tremor or rigidity scores.

In comparisons of motor subtypes, severity of leucoaraiosis was significantly higher in the postural instability and gait difficulty dominant subgroup compared with the tremor-predominant subgroup. Individual motor test items with robust correlation with leucoaraiosis severity included postural instability, as defined by the retropulsion test and walking assessment by history. There was no significant leucoaraiosis correlation with the history of falls. As a related clinical finding, there was a significant correlation between white matter hyperintensities and ratings of posture. There was a borderline correlation of freezing with white matter changes. The different strength of associations between leucoaraiosis severity and different motor dysfunctions of Parkinson's disease and greater leucoaraiosis severity in the postural instability and gait difficulty dominant subtype supports the conclusion that leucoaraiosis severity is an independent determinant of motor dysfunction in Parkinson's disease.

In a large group of 141 patients with Parkinson's disease, Lee *et al*. (2009) found that leucoaraiosis was associated with the postural instability and gait difficulty dominant subgroup. These authors found significant correlations between white matter hyperintensities and ratings of bradykinesia and rigidity but that study did not control for the effects of nigrostriatal dopaminergic denervation.

Balance problems are common and disabling in Parkinson's disease and are poorly responsive to dopamine replacement therapy (Bloem *et al.,* 2003; Bohnen and Cham, 2006). Our findings support the concept that non-dopaminergic mechanisms contribute significantly to impaired balance in Parkinson's disease. Postural control is not simply a summation of static reflexes but involves complex coordination and integration of visual, somatosensory and vestibular systems. Relative weight placed on each of these sensory system inputs is dependent on the goals of the movement task and the environmental context (Horak, 2006). Comorbid white matter changes are predicted to significantly interfere with postural control systems because of the widespread and bilateral hemispheric distribution of these neural systems. It is likely that white matter changes interfere with the central and cognitive processing of sensorimotor signals, leading to impaired postural responses (Kuo and Lipsitz, 2004). White matter changes may interrupt corticocortical and long descending fibre tracts and may thus reduce the efficiency of CNS integration of postural control (Murray *et al.,* 2010).

Our findings indicate a significant relationship between burden of white matter disease and Hoehn and Yahr disease stage (Hoehn and Yahr, 1967). As disease severity ratings increase, the number of general and instrumental activities of daily living reported as being performed either with any difficulty or needing assistance, also progressively increase (Shulman *et al.,* 2008). Disability with loss of independent function is usually first reported between Hoehn and Yahr stages 2 to 3 (Shulman *et al.,* 2008). Thus, the transition from Hoehn and Yahr stage 2 to 3 marks a pivotal milestone in Parkinson's disease, characterized by the emergence of postural instability, when gait and balance impairment results in disability in many gait-dependent activities. Our results also indicate that the effects of leucoaraiosis on axial motor impairment are more robust compared with nigrostriatal dopaminergic denervation alone. Therefore, assessment of white matter disease may potentially be used as a clinically relevant biomarker in studies of disease progression in Parkinson's disease.

We found also that ratings of abnormal posture are associated strongly with increased severity of leucoaraiosis in Parkinson's disease. Stooped posture and its related displacement of the centre-of-pressure contribute to impairments of postural control (Bloem *et al.,* 1999). Some postural abnormalities seen in patients with Parkinson's disease can be reproduced in healthy subjects by mimicking a stooped parkinsonian posture (Jacobs *et al.,* 2005). None of our patients met clinical criteria for camptocormia or Pisa syndrome, which is considered a form of axial dystonia (Djaldetti *et al.,* 1999). Although stooped posture is a destabilizing posture, comorbid leucoaraiosis was not significantly associated with an increased risk of falls in our study.

An important question is whether comorbid leucoaraiosis in Parkinson's disease reflects effects of normal ageing, a disease-specific effect, or is due to other pathological processes (Levy, 2007). We found greater severity of comorbid leucoaraiosis in the postural instability and gait difficulty dominant subgroup compared with the tremor-predominant subgroup despite no significant differences in age or duration of disease, suggesting that increased leucoaraiosis in the postural instability and gait difficulty dominant group is not fully a function of normal ageing. Further research is needed to evaluate possible interaction effects between Parkinson disease and leucoaraiosis processes (Bohnen and Albin, 2011).

The specific aetiology of leucoaraiosis-white matter hyperintensities is a matter of ongoing investigation. These changes are associated with vascular disease risk factors and vascular pathology, particularly that of microvasculature, is generally regarded as a significant factor in leucoaraiosis-white matter hyperintensities (van Swieten *et al.,* 1991; Pantoni and Garcia, 1997; Brown *et al.,* 2009; Brown and Thore, 2011). Other processes may contribute to leucoaraiosis-white matter hyperintensities. Wallerian degeneration secondary to loss of cortical or grey matter neuronal perikarya is a possible cause of subcortical and deep white matter leucoaraiosis-white matter hyperintensities (Leys *et al.,* 1991). On the other hand, Wallerian degeneration could be secondary also to loss of cortical or other grey matter neuronal perikarya due to microinfarcts (Smith, 2010). The latter would be a second mechanism by which an independent pathological process, vascular disease, causes cerebral injury in Parkinson's disease (Longstreth *et al.,* 2009). Wersching *et al*. (2010) suggested that low-grade inflammation contributes to leucoaraiosis-white matter hyperintensities. Further research is needed to evaluate the roles of normal ageing, disease-specific neurodegenerative effects and independent pathological processes such as vascular disease or inflammation in comorbid white matter changes in Parkinson's disease.

Our findings support the concept that motor dysfunction in Parkinson's disease is multifactorial in origin. Prior dopaminergic imaging studies indicated that measures of bradykinesia have most robust correlation with nigrostriatal dopaminergic deficits in Parkinson's disease, whereas measures of tremor or rigidity have

no or poor correlations (Eidelberg *et al.,* 1990; Antonini *et al.,* 1995; Vingerhoets *et al.,* 1997; Pirker, 2003). These findings indicate that the spectrum of motor impairment in Parkinson's disease cannot be fully explained by presynaptic nigrostriatal dopaminergic denervation. Although leucoaraiosis severity is a robust determinant of axial motor impairment, white matter hyperintensities correlate only weakly with measures of bradykinesia and white matter hyperintensities do not significantly contribute to rigidity or tremor. We previously reported that degeneration of the pedunculopontine-thalamic cholinergic projection may contribute to increased fall risk in Parkinson's disease (Bohnen *et al.,* 2009), an inference supported by a recent pilot trial of cholinesterase inhibitors in Parkinson's disease (Chung *et al.,* 2010). The complex motor impairments of Parkinson's disease are likely caused by dopaminergic deficits, comorbid white matter disease, cholinergic system degeneration and other pathologies interacting in complex ways.

A limitation of this study is its cross-sectional nature; we cannot assess changes over time, and therefore, longitudinal studies are needed to confirm predictions from our findings. Prospective documentation of vascular risk factors, vascular risk factor treatment, markers of inflammation and use of anti-inflammatory drugs may be particularly valuable. Our subject population is not a true epidemiological sample but rather draws heavily from academic and Department of Veterans Affairs movement disorders clinics. To approximate a relatively unbiased sample, we excluded potential subjects with severe motor impairments or significant dementia. Nonetheless, it would be important to replicate our findings in a population-based sample. Another limitation is that our PET imaging technique does not allow assessment of the contribution of cortical dopaminergic or other monoaminergic system changes.

We conclude that increased severity of leucoaraiosis is associated independently with worsening axial motor impairments and stooped posture in Parkinson's disease. This conclusion is consistent with an emerging theme in neurodegeneration research; the importance of interactions between vascular, neuroinflammatory and neurodegenerative pathologies. Systematic post-mortem studies of dementias indicate that comorbid vascular pathology contributes significantly to the clinical manifestation of neurodegenerative dementias (Jagust *et al.,* 2008; Kuczynski *et al.,* 2008; Schneider and Bennett, 2010). This concept may have both immediate clinical and public health implications. Axial motor dysfunction is one of the most disabling features of advanced Parkinson's disease. It is possible that aggressive management of vascular or inflammatory risk factors in patients with Parkinson's disease could reduce the severity of axial motor dysfunction in Parkinson's disease. Similarly, aggressive treatment of vascular or inflammatory risk factors earlier in life might ameliorate the late life morbidity associated with Parkinson's disease and other neurodegenerations (Gao *et al.,* 2011).

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