Magnetic resonance imaging markers of Parkinson’s disease nigrostriatal signature

Patrice Péran,1,2 Andrea Cherubini,1 Francesca Assogna,3 Fabrizio Piras,3 Carlo Quattrocchi,1 Antonella Peppe,3 Pierre Celsis,2 Olivier Rascol,2,4 Jean-François Démonet,2 Alessandro Stefani,5 Mariangela Pierantozzi,5 Francesco Ernesto Pontieri,6 Carlo Caltagirone,3 Gianfranco Spalletta3 and Umberto Sabatini1

1 Department of Radiology, I.R.C.C.S. Foundation Santa Lucia, Rome, Italy
2 INSERM U825, Université Paul-Sabatier, Toulouse, France
3 Laboratory of Clinical and Behavioural Neurology, IRCCS Santa Lucia Foundation, Rome, Italy
4 Clinical Investigation Centre (INSERM CIC-9302) and Department of Neurosciences, University Hospital and University of Toulouse, France
5 Department of Neuroscience, Tor Vergata University, Rome, Italy
6 Sapienza University, Second Faculty of Medicine, Department of Neurology, Rome, Italy

Correspondence to: Patrice Péran,
Department of Radiology,
I.R.C.C.S. Foundation Santa Lucia,
Via Ardeatina 306,
00179 Rome,
E-mail: p.peran@hsantalucia.it

One objective of modern neuroimaging is to identify markers that can aid in diagnosis, disease progression monitoring and long-term drug impact analysis. In this study, Parkinson-associated physiopathological modifications were characterized in six subcortical structures by simultaneously measuring quantitative magnetic resonance parameters sensitive to complementary tissue characteristics (i.e. volume atrophy, iron deposition and microstructural damage). Thirty patients with Parkinson’s disease and 22 control subjects underwent 3-T magnetic resonance imaging with T2*-weighted, whole-brain T1-weighted and diffusion tensor imaging scans. The mean R2* value, mean diffusivity and fractional anisotropy in the pallidum, putamen, caudate nucleus, thalamus, substantia nigra and red nucleus were compared between patients with Parkinson’s disease and control subjects. Comparisons were also performed using voxel-based analysis of R2*, mean diffusivity and fractional anisotropy maps to determine which subregion of the basal ganglia showed the greater difference for each parameter. Averages of each subregion were then used in a logistic regression analysis. Compared with control subjects, patients with Parkinson’s disease displayed significantly higher R2* values in the substantia nigra, lower fractional anisotropy values in the substantia nigra and thalamus, and higher mean diffusivity values in the thalamus. Voxel-based analyses confirmed these results and, in addition, showed a significant difference in the mean diffusivity in the striatum. The combination of three markers was sufficient to obtain a 95% global accuracy (area under the receiver operating characteristic curve) for discriminating patients with Parkinson’s disease from controls. The markers comprising discriminating combinations were R2* in the substantia nigra, fractional anisotropy in the substantia nigra and mean diffusivity in the putamen or caudate nucleus. Remarkably, the predictive markers involved the nigrostriatal structures that characterize Parkinson’s physiopathology. Furthermore, highly discriminating combinations included markers from three different magnetic resonance parameters (R2*, mean diffusivity and fractional anisotropy). These findings demonstrate that multimodal magnetic resonance imaging of subcortical grey matter structures is useful for the evaluation of Parkinson’s disease and, possibly, of other subcortical pathologies.

© The Author (2010). Published by Oxford University Press on behalf of the Guarantors of Brain. All rights reserved.
For Permissions, please email: journals.permissions@oxfordjournals.org
Keywords: parkinsonism; iron; mean diffusivity; anisotropy; MRI
Abbreviations: DTI = diffusion tensor imaging; FLAIR = fluid-attenuated inversion recovery; LEDD = levodopa equivalent daily dose; ROC = receiver operating characteristic; UPDRS = Unified Parkinson’s Disease Rating Scale

Introduction

The diagnosis of Parkinson’s disease is based mainly on a set of clinical assessments that do not provide great accuracy (Hughes et al., 1992; Caslake et al., 2008). Conventional MRI only aids in excluding underlying pathologies (e.g. vascular lesions). One objective of modern neuroimaging is to find markers that aid in diagnosis, disease progression monitoring and long-term drug impact evaluation. The main anatomical and functional changes induced by Parkinson’s disease can be represented as a three-level system: mesencephalic (dopaminergic neuronal loss), basal ganglia (dopaminergic deafferentation) and cortical (functional reorganization) (Péran et al., 2006). Previous neuroimaging studies have generally focused on only one of these three levels. For example, nuclear medicine imaging techniques have been employed to assess the functional integrity of presynaptic nigrostriatal projections (for a review, see Pavese and Brooks, 2009).

Since MRI is non-invasive and more widely available than nuclear neuroimaging, numerous MRI studies have also been used to investigate brain modifications in Parkinson’s disease in vivo. The most common MRI approach is voxel-based morphometry, which can be used to evaluate cortical atrophy in both cortical and subcortical structures. Several studies have measured grey matter density alterations in Parkinson’s disease using this method (Burton et al., 2004; Nagano-Saito et al., 2005; Summerfield et al., 2005; Feldmann et al., 2008; Benninger et al., 2009; Ibarretxe-Bilbao et al., 2009; Wattendorf et al., 2009). These studies have revealed, for example, the greater temporal lobe atrophy of demented compared with non-demented patients with Parkinson’s disease (Ramirez-Ruiz et al., 2005; Summerfield et al., 2005; Ibarretxe-Bilbao et al., 2008). However, voxel-based morphometry investigations have not obtained consistent results regarding the differences between non-demented patients with Parkinson’s disease and healthy subjects. One voxel-based morphometry study found no difference between these groups (Price et al., 2004), while others found atrophy in different anatomical regions, such as the frontal cortex (Burton et al., 2004; Nagano-Saito et al., 2005), caudate nucleus (Brenneis et al., 2003), hippocampus or superior temporal gyrus (Summerfield et al., 2005). Furthermore, no differences between non-demented patients with Parkinson’s disease and healthy subjects were seen when changes in the grey matter were measured by volumetry from regions of interest (Schulz et al., 1999; Cordato et al., 2002; Ghaemi et al., 2002).

Parkinson-induced prefrontal cortical changes are essentially functional and result from dopaminergic deafferentation in the basal ganglia (Alexander et al., 1986). This may explain why there has been a lack of reliable results from magnetic resonance (MR) cortical atrophy studies in non-demented patients with Parkinson’s disease. Functional neuroimaging studies of patients with Parkinson’s disease performing a simple motor task clearly demonstrate reduced activation (reflected in reduced regional cerebral blood flow) at the level of the main cortical regions that receive afferents from the basal ganglia, including the supplementary motor area (Rascol et al., 1992; Sabatini et al., 2000), dorsolateral prefrontal cortex (Playford et al., 1992) and anterior cingulate gyrus (Jahanshahi et al., 1995). Taken together, these results indicate that cortical-level structural MRIs such as voxel-based morphometry have not provided substantial contributions to Parkinson’s disease diagnosis.

Other MRI techniques have demonstrated more consistent and promising results from exploring microstructural modifications at the basal ganglia and mesencephalic levels. One such way to explore brain modifications via MR is through diffusion tensor imaging (DTI). This method provides quantitative parameters (Le Bihan, 1995), such as the mean diffusivity that increases with microscopic barrier disruption and extracellular fluid accumulation, and the fractional anisotropy that provides information on the microstructural integrity of highly oriented microstructures (e.g. myelin) (Abe et al., 2002). Both mean diffusivity and fractional anisotropy are highly influenced by physiological ageing (Cherubini et al., 2009b). Furthermore, previous DTI studies on patients with Parkinson’s disease have demonstrated a decrease in fractional anisotropy in the substantia nigra compared with healthy controls (Yoshikawa et al., 2004; Chan et al., 2007; Vaillancourt et al., 2009).

Another potential MRI technique is the quantification of mineral levels in the brain. In the last decade, efforts have been made to find a sensitive, reliable method such as MR relaxometry to evaluate the brain iron content in vivo (Ordidge et al., 1994; Gorell et al., 1995; Bartzokis et al., 1997; Gelman et al., 1999; Graham et al., 2000; Jensen et al., 2006; Péran et al., 2007). Iron metabolism dysregulation and iron accumulation in various parts of the brain are implicated in the pathogenesis of many neurodegenerative diseases, including Parkinson’s disease (for a review, see Moos and Morgan, 2004; Zecca et al., 2004). Moreover, increased iron content is consistently reported post-mortem in the substantia nigra of patients with Parkinson’s disease (Dexter et al., 1987; Griffiths and Crossman, 1993; Griffiths et al., 1999). This latter observation has been confirmed in vivo by transcranial sonography (Berg, 2009) and various MRI techniques (Ordidge et al., 1994; Martin et al., 2008). The study of microstructural modifications of the substantia nigra appears to be crucial to investigating the physiopathological mechanisms of Parkinson’s disease. Indeed, the major pathologic degeneration of Parkinson’s disease is the loss of dopaminergic neurons in the substantia nigra pars compacta.

An approach measuring MR parameters sensitive to complementary tissue characteristics (e.g. volume atrophy, iron deposition and microstructural damage) in Parkinson’s disease brains could have great potential for investigating pathological changes. Recently, multimodal MRI was used to characterize the
physiological ageing of deep grey matter nuclei in healthy subjects (Cherubini et al., 2009a). This previous study revealed that physiological ageing does not influence the same parameters in the functioning anatomical region. Overall, the best predictors of physiological ageing were iron deposition in the putamen, and microstructural damage and atrophy in the thalamus. Multimodal MRI may therefore be a valid tool to measure the microstructural integrity of brain structures.

The present work was designed to overcome the limitations of previous single MR parameter studies. We simultaneously measured volume, DTI scalars and $T_2^*$ relaxation rates in six deep grey matter structures (thalamus, putamen, caudate, pallidum, substantia nigra and red nucleus) in patients with Parkinson’s disease and healthy controls. The aims of the study were (i) to assess changes occurring in idiopathic patients with Parkinson’s disease compared with controls, as well as the relationship of these changes to clinical data, and (ii) to discriminate patients with Parkinson’s disease from healthy individuals using multimodal MRI.

Materials and methods

Subjects

All participants provided written informed consent. This study was approved by the Santa Lucia Foundation ethics committee. Thirty right-handed patients with Parkinson’s disease were included. Parkinson’s disease was diagnosed by a staff neurologist, on the basis of akinesia associated with tremor and/or rigidity and responsiveness to levodopa therapy. All patients with Parkinson’s disease fulfilled the UK Parkinson’s Disease Brain Bank criteria for the diagnosis of idiopathic Parkinson’s disease. No patient had a history of neurological or psychiatric disease other than Parkinson’s disease. All patients were on anti-parkinsonian medication at the time of testing. The levodopa equivalent daily dose (LEDD) was calculated for each patient (Brodsky et al., 2003). Patient motor disabilities were evaluated using the motor part of the Unified Parkinson’s Disease Rating Scale (UPDRS). Patients who had end-of-dose motor fluctuations were always tested in the ON phase. Patients who scored <25 on the Mini Mental State Examination were excluded to avoid inclusion of demented patients with Parkinson’s disease. A control group of 22 right-handed subjects closely matched to the patients with Parkinson’s disease for age, sex and education was also included. None of these control individuals had a history of head injury, stroke or any neurological or psychiatric diseases. Subjects were recruited from community recreational centres, hospital personnel and patients’ relatives. Two expert radiologists examined all MRIs to exclude potential brain abnormalities and subjects with microvascular lesions, as apparent from conventional fluid-attenuated inversion recovery (FLAIR) and $T_2^*$-weighted images. All subjects (patients and controls) displayed normal conventional imaging results. The demographic and clinical data of the two groups are reported in Table 1.

Acquisition

Participants were examined using a 3 T Allegra MR Imager (Siemens Medical Solutions, Erlangen, Germany) with a standard quadrature head coil. All participants underwent the same MRI protocol, including whole-brain $T_2^*$-weighted, $T_1$-weighted, DTI, conventional $T_2^*$-weighted and FLAIR scanning. All planar sequence acquisitions were acquired along the anterior/posterior commissure line. Particular care was taken to centre the subject in the head coil and to restrain subject movements with cushions and adhesive medical tape.

Six consecutive $T_2^*$-weighted gradient-echo whole-brain volumes were acquired using a segmented echo-planar imaging sequence at different echo times: 6, 12, 20, 30, 45 and 60 ms (repetition time = 5000; bandwidth = 1116 Hz/voxel; matrix size $128 \times 128$; 80 axial slices; flip angle 90°; voxel size of $1.8 \times 1.8 \times 1.8 \text{ mm}^3$). Diffusion-weighted volumes were acquired using spin-echo echo-planar imaging (echo time/repetition time $= 89/8500 \text{ ms}$; bandwidth $= 2126 \text{ Hz/voxel}$; matrix size $128 \times 128$; 80 axial slices, voxel size $1.8 \times 1.8 \times 1.8 \text{ mm}^3$) with 30 isotropically distributed orientations for the diffusion-sensitizing gradients at a $b$-value of 1000 s mm$^{-2}$ and six $b=0$ images. Scanning was repeated three times to increase the signal-to-noise ratio. Since the DTI and $T_2^*$ volumes each consisted of $128 \times 128 \times 80$ identical isotropic voxels, the slice positioning and orientation of the diffusion-weighted volumes were set to be identical with the $T_2^*$ volumes to improve subsequent coregistration. Finally, whole-brain $T_2^*$-weighted images were obtained in the sagittal plane using a modified driven equilibrium Fourier transform sequence (Deichmann et al., 2004) (echo time/repetition time $= 2.4/7.92 \text{ ms}$, flip angle $15^\circ$, voxel size $1 \times 1 \times 1 \text{ mm}^3$). The total duration of the imaging protocol was 45 min.

Post-processing

Image processing was performed using FSL 4.1 (FMRIB Software Library; www.fmrib.ox.ac.uk/fsl/) and an in-house software developed in Matlab (version 6.5, The MathWorks), with procedures similar to those described earlier (Cherubini et al., 2009a; Péran et al., 2009). Anatomical regions of interest on the $T_1$-weighted images were obtained automatically with the segmentation tool FMRIB Integrated Registration and Segmentation Tool (FIRST) 1.2 integrated within

Table 1 Demographic and clinical data of patients with Parkinson’s disease and controls

<table>
<thead>
<tr>
<th>Sex (M/F)</th>
<th>Age (years)</th>
<th>Disease duration (years)</th>
<th>Hoehn and Yahr stage (I/II)</th>
<th>UPDRSm</th>
<th>UPDRSm (MAS)$^a$</th>
<th>UPDRSm (LAS)$^a$</th>
<th>LEDDb</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD</td>
<td>20/10</td>
<td>61.9 ± 11.1</td>
<td>4.5 ± 2.5</td>
<td>10/20</td>
<td>12.0 ± 5.9</td>
<td>6.5 ± 3.2</td>
<td>2.2 ± 2.6</td>
</tr>
<tr>
<td>Controls</td>
<td>11/11</td>
<td>57.4 ± 9.7</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

$^a$LEDD = [levodopa (× 1.2 if catechol-O-methyltransferase (COMT) inhibitor) + [pramipexole × 400] + [ropinirole × 400] + [cabergoline × 160] + [ergolide × 200] + [bromocriptine × 10] + [lisuride × 160]; all doses are in mg.

$^b$Limb evaluation more affected side (MAS) / limb evaluation less affected side (LAS).
the FSL software. In each subject, four deep grey matter structures (thalamus, putamen, caudate and pallidum) were segmented. The substantia nigra and red nucleus were manually segmented using mean \( T_2^* \)-weighted images by two trained radiologists who were blinded to group membership and clinical information. The images were magnified \( \times 4 \) before tracing. The substantia nigra is located in the mesencephalon, posterior (dorsal) to the crus cerebri, anterior (ventral) to the midbrain tegumentum and laterally to the red nucleus. The high \( T_2^* \) contrast-to-noise ratio of our images allowed us to discriminate the mesencephalic structures from the surrounding white matter (Péran et al., 2007). Based on previous MR studies (Massey et al., 2010), the substantia nigra was identified as the band of signal hypointensity on \( T_2^* \) in the midbrain on each slice (4–6 continuous slices). The red nucleus was also identified using signal hypointensity on a different slice (3–4 continuous slices). For each subject, voxels that were considered to be of the substantia nigra or red nucleus were those included by both radiologists. A DTI model was fit at each voxel, generating fractional anisotropy and mean diffusivity maps. The fractional anisotropy maps were then registered to brain-extracted whole-brain volumes from \( T_1 \)-weighted images using a full affine (correlation ratio cost function) alignment with nearest-neighbour resampling. The calculated transformation matrix was then applied to the mean diffusivity maps with identical resampling options.

The six \( T_2^* \)-weighted volumes were averaged to generate a mean \( T_2^* \)-weighted volume. A full affine 3D alignment was calculated between each of the six \( T_2^* \)-weighted volumes and the mean \( T_2^* \)-weighted volume. For each subject, a voxel-by-voxel nonlinear least-squares fitting of the data was acquired at the six echo times to obtain a mono-exponential signal decay curve \( S = S_0 e^{-(T/T_2^*)} \). This method, combining data acquisition and data processing of \( T_2^* \) images, demonstrated good reproducibility (Péran et al., 2007). To facilitate analysis of the relaxation results, we considered the inverse of the relaxation times (i.e. relaxation rates \( R_2^* = 1/T_2^* \)) as described earlier (Cherubini et al., 2009a, b; Péran et al., 2007, 2009). The mean \( T_2^* \)-weighted volume was registered to the \( T_1 \)-weighted volume using a full affine alignment. The calculated transformation matrix was then applied to the \( R_2^* \) maps with nearest-neighbour resampling options, as well as to the manually segmented regions (substantia nigra and red nucleus). As a result of this processing, the mean diffusivity, fractional anisotropy and \( R_2^* \) maps were corrected for head movements, and shared an identical reference space with the anatomical \( T_1 \)-weighted volumes.

For each subject and each hemisphere, the volumes of the segmented subcortical areas were calculated. To reduce the effects of inter-individual variability in head size, individual volume values were multiplied by a normalization factor obtained with the S jENAX tool (http://www.fmrib.ox.ac.uk/fsl/sienax/index.html) from the corresponding \( T_1 \)-weighted image. This normalization factor was derived from the normalizing transform; the brain image was affine-registered to a template, and a multiplying factor was calculated from the transformation matrix itself, using the skull image to determine the registration scaling.

For each subject, the region of interest segmentation results, coregistered fractional anisotropy map and coregistered \( R_2^* \) map were all superimposed onto the original \( T_1 \)-weighted volume. The resulting images were visually assessed by two trained radiologists to exclude misregistration or erroneous region of interest identification. The segmented structures defined the binary masks, where the mean values of mean diffusivity, fractional anisotropy and \( R_2^* \) were calculated for each individual. The \( T_1 \)-weighted volume was registered to the Montreal Neurological Institute template using FMRIB’s (Functional Magnetic Resonance Imaging of the Brain, University of Oxford) non-linear image registration tool (http://www.fmrib.ox.ac.uk/fsl/fnirt/index.html). This spatial transformation was applied to each subject to coregister \( R_2^* \), mean diffusivity and fractional anisotropy maps on the Montreal Neurological Institute template. To determine the probability of a particular voxel in Montreal Neurological Institute space being occupied by a structure of interest, the frequency at which the structure of interest resided at a particular voxel was assessed across the 52 data sets. Unit increments in voxel intensity correspond to an increase in the probability of encountering a particular structure at that location in 1/52=1.9% of data sets (see Supplementary Material).

**Statistical analysis**

For each of the six deep grey matter structures, the volume, \( R_2^* \), mean diffusivity and fractional anisotropy were used to perform statistical region- and voxel-based analyses.

**Region-based analysis**

The mean values of \( R_2^* \), fractional anisotropy, mean diffusivity and volume were compared using a repeated-measures multivariate analysis of covariance (MANCOVA) with three factors (group, anatomical region, lateralization) and with age as covariate. Then, a two-way MANCOVA with group and lateralization as factors and age as covariate was applied to each parameter and each structure. For each significant result, multiple regression analysis for the given anatomical region was conducted between clinical data (age, disease duration, LEDD, motor part of UPDRS) and the respective MR parameter.

**Voxel-based analysis**

The \( R_2^* \), mean diffusivity and fractional anisotropy values were compared using a two-sample unpaired t-test with age as covariate. Significant subregions were those with values above a threshold of \( P<0.01 \), based on threshold-free cluster enhancement (minimum cluster size=50). For each subregion associated with a specific MR parameter (\( R_2^* \), mean diffusivity or fractional anisotropy), the mean values were extracted for all participants. Multiple regression analysis was conducted between clinical data (age, disease duration, LEDD and motor part of UPDRS) and the respective MR parameter/subregion pair mean.

**Predictive analysis and ROC curves**

Since one of the main goals of the study was to discriminate patients with Parkinson’s disease from controls, logistic regressions and receiver operating characteristic (ROC) curves were computed using the mean of each MR parameter/subregion pair individualized from the previous voxel-based analysis. All combinations among these pairs were tested to determine the combinations with the best discriminating power. A repeated 10-fold cross-validation was performed to calculate the area under the ROC curve for each combination. Combinations with areas under the curve of \( >95\% \) are reported.

**Results**

The probabilistic map (see Supplementary Material) indicated that the identification of anatomical regions (using automatic and manual segmentation) and spatial registration were accurately assessed, according to the results of Péran et al. (2009).
Differences between patients with Parkinson’s disease and controls

Patients with Parkinson’s disease displayed significantly higher $R^2*$ values in the substantia nigra, lower fractional anisotropy values in the substantia nigra and thalamus, and higher mean diffusivity values in the thalamus than control subjects (Table 2 and Fig. 1). Voxel-based analyses confirmed the previous localizations and identified differences for the mean diffusivity in the striatum (Table 3 and Fig. 2).

Multiple regressions with clinical data

The anatomical region and subregion analyses revealed strong correlations between age and mean diffusivity values, and between age and fractional anisotropy values in the thalamus (Table 4). A positive relationship was also observed between $R^2*$ values and LEDD scores in the right substantia nigra, when the entire substantia nigra or subregions of the substantia nigra were considered.

Predictive analysis

Logistic regression analysis showed that combinations of three different markers were sufficient to obtain $\geq 95\%$ discrimination between patients with Parkinson’s disease and controls (maximum area under the curve: 98%, Fig. 3A). The markers comprising the discriminating combinations were: ($R^2*$ values in left or right substantia nigra) + (fractional anisotropy values in right substantia nigra) + (mean diffusivity in putamen or caudate nucleus) (Fig. 3B). Logistic regressions were also computed for combinations of four markers. The discriminating power of the four-marker combinations did not increase significantly (maximum area under the curve: 99%), and the distribution of markers, characterized by their percentage of presence in the discriminant combinations, confirmed the previous findings, since the markers comprising the three-marker combinations were the ones with a higher percentage of presence: fractional anisotropy/right substantia nigra: 98%, mean diffusivity/right putamen: 52%, $R^2*$/left substantia nigra: 52%, $R^2*$/right substantia nigra: 40%, mean diffusivity/right caudate nucleus1: 38%, fractional anisotropy/left putamen: 24%, mean diffusivity/right caudate nucleus2: 22%, mean diffusivity/left thalamus: 16%, mean diffusivity/left substantia nigra: 14%, fractional anisotropy/right thalamus: 12%, mean diffusivity/right thalamus2: 12%, mean diffusivity/left thalamus: 10% and mean diffusivity/right thalamus: 10%. Furthermore, it is important to note that the discriminant power in considering only one marker at a time comprises 71–83% (for the main markers: fractional anisotropy/right substantia nigra: 77%, mean diffusivity/right putamen: 83%, $R^2*$/left substantia nigra: 77%, $R^2*$/right substantia nigra: 75% and mean diffusivity/right caudate nucleus: 75%).

Discussion

We used multimodal MRI to quantify subcortical changes in patients with idiopathic Parkinson’s disease. Our work on a large group of non-demented patients with Parkinson’s disease showed mainly an increase in $R^2*$ values and a decrease in fractional anisotropy values in the substantia nigra, and a decrease in the mean diffusivity values in the striatum compared with healthy subjects. Taken together, our findings reveal that multimodal MRI is able to discriminate patients with Parkinson’s disease from healthy control subjects.

Predictive markers

The results of the ROC and cross-validated discrimination analyses demonstrated that combinations of three different markers were sufficient to obtain $\geq 95\%$ discrimination between patients with Parkinson’s disease and healthy controls. The markers comprising the discriminating combinations were the $R^2*$ and fractional

Table 2 MANCOVAs from region-based analysis: volume, $R^2*$, mean diffusivity and fractional anisotropy

<table>
<thead>
<tr>
<th>MANCOVA from region-based analysis: volume, $R^2*$, mean diffusivity and fractional anisotropy</th>
<th>Volume</th>
<th>$R^2*$</th>
<th>Mean diffusivity</th>
<th>Fractional anisotropy</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Global MANCOVA</strong></td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Main effect group</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Interaction Group × Anatomical Region</td>
<td>ns</td>
<td>$F = 1.94; P = 0.09$</td>
<td>ns</td>
<td>$F = 2.29; P &lt; 0.05$</td>
</tr>
<tr>
<td><strong>MANCOVA for anatomical region</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thalamus</td>
<td>ns</td>
<td>ns</td>
<td>$F = 11.33; P &lt; 0.05$</td>
<td>$F = 5.07; P &lt; 0.05$</td>
</tr>
<tr>
<td>Putamen</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Main effect group</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Caudate nucleus</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Main effect group</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Pallidum</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Main effect group</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Substantia nigra</td>
<td>ns</td>
<td>$F = 5.9; P &lt; 0.02$</td>
<td>ns</td>
<td>$F = 4.94; P &lt; 0.05$</td>
</tr>
<tr>
<td>Red nucleus</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Main effect group</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

ds: non significant.
anisotropy in the substantia nigra, and the mean diffusivity in the putamen or caudate nucleus (Fig. 3). Unsurprisingly, the predictive markers involved the nigrostriatal structures that characterize Parkinson’s disease physiopathology. The highly discriminating combinations were composed of markers from three different MR parameters ($R^*_2$, mean diffusivity and fractional anisotropy), suggesting that the MR parameters provide different but complementary information. Indeed, taken individually, none of the markers reached as high a discriminant power as those obtained in combination.

Two recent studies have shown MRI-guided discrimination between patients with Parkinson’s disease and controls. The first study revealed that fractional anisotropy in the substantia nigra distinguishes early-stage patients with Parkinson’s disease from healthy individuals (Vaillancourt et al., 2009). In this previous work, regions of interest were manually drawn using small circles in the substantia nigra localized from a single DTI slice and did not consider the nigral subdivisions (pars reticularis or pars compacta). In our study, the regions of interest correspond to the entire substantia nigra. The second study (Menke et al., 2009), using
driven equilibrium single pulse observation of $T_1$ to identify the substantia nigra, combined volumetry of the substantia nigra and its connectivity with the thalamus to achieve a good sensitivity (100%) and specificity (80%) for classifying patients with Parkinson’s disease. It is important to note that our MR study assessed ROC analysis with a larger number of patients with Parkinson’s disease than in these previous works. Despite the difference of DTI data acquisition and DTI data analysis with Vaillancourt’s study (2009), the results of the present study not only confirm the importance of fractional anisotropy in the substantia nigra to discriminate patients with Parkinson’s disease from healthy subjects but also demonstrate the utility of considering multiple parameters.

Iron content also played a key role in the substantia nigra and microstructural damage in the striatum of patients with Parkinson’s disease. Using the same methodology as ours, a recent study revealed that the best predictor of physiological ageing is iron deposition in the striatum (Cherubini et al., 2009). Our findings also confirm the crucial role of the substantia nigra in Parkinson’s disease. Previous MR studies confined substantia nigra quantification to area measurements within a few selected slices through the midbrain, rather than the whole substantia nigra volume (Martin et al., 2008; Vaillancourt et al., 2009). In contrast, we considered all mesencephalic structures (red nucleus and substantia nigra) identified on high-resolution T2*-weighted images (Péran et al., 2007). Since substantia nigra degeneration is not

### Table 3 Voxel-based analysis

<table>
<thead>
<tr>
<th>Clusters</th>
<th>Voxels</th>
<th>P-value</th>
<th>Mean (±SD) Patients with Parkinson’s disease</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R_2^*$/right substantia nigra and red nucleus</td>
<td>207</td>
<td>&lt;0.001</td>
<td>33.34 (±4.72)</td>
<td>29.89 (±2.08)</td>
</tr>
<tr>
<td>$R_2^*$/left substantia nigra and red nucleus</td>
<td>215</td>
<td>&lt;0.001</td>
<td>32.27 (±3.90)</td>
<td>29.02 (±2.11)</td>
</tr>
<tr>
<td>Mean diffusivity/right thalamus 1</td>
<td>1242</td>
<td>&lt;0.001</td>
<td>864.65 (±64.42)</td>
<td>802.68 (±28.36)</td>
</tr>
<tr>
<td>Mean diffusivity/right putamen</td>
<td>854</td>
<td>&lt;0.0001</td>
<td>773.72 (±79.66)</td>
<td>703.60 (±36.74)</td>
</tr>
<tr>
<td>Mean diffusivity/right cerebral nucleus</td>
<td>287</td>
<td>&lt;0.001</td>
<td>771.22 (±39.42)</td>
<td>735.95 (±19.88)</td>
</tr>
<tr>
<td>Mean diffusivity/right caudate nucleus 2</td>
<td>105</td>
<td>&lt;0.005</td>
<td>806.85 (±94.58)</td>
<td>726.86 (±39.24)</td>
</tr>
<tr>
<td>Mean diffusivity/right thalamus 2</td>
<td>83</td>
<td>&lt;0.001</td>
<td>1377.03 (±249.80)</td>
<td>1162.92 (±185.49)</td>
</tr>
<tr>
<td>Mean diffusivity/left thalamus 2</td>
<td>72</td>
<td>&lt;0.005</td>
<td>746.09 (±50.50)</td>
<td>709.76 (±18.75)</td>
</tr>
<tr>
<td>Mean diffusivity/left substantia nigra</td>
<td>53</td>
<td>&lt;0.0001</td>
<td>844.53 (±53.12)</td>
<td>776.20 (±44.90)</td>
</tr>
<tr>
<td>Fractional anisotropy/right thalamus</td>
<td>803</td>
<td>&lt;0.001</td>
<td>0.25 (±0.03)</td>
<td>0.29 (±0.020)</td>
</tr>
<tr>
<td>Fractional anisotropy/right substantia nigra</td>
<td>109</td>
<td>&lt;0.001</td>
<td>0.42 (±0.03)</td>
<td>0.46 (±0.030)</td>
</tr>
<tr>
<td>Fractional anisotropy/left putamen</td>
<td>312</td>
<td>&lt;0.001</td>
<td>0.21 (±0.03)</td>
<td>0.18 (±0.018)</td>
</tr>
</tbody>
</table>

**Figure 2** Differences between patients with Parkinson’s disease and controls from voxel-based analysis of $R_2^*$, mean diffusivity and fractional anisotropy maps. The z-position on the template is indicated under each slice. The light yellow corresponds to the investigated regions.
Table 4  Multiple regression analysis with clinical data

<table>
<thead>
<tr>
<th>Modality/ anatomical region</th>
<th>Modality/ subregion</th>
<th>Age p(β)</th>
<th>Disease duration p(β)</th>
<th>LEDD p(β)</th>
<th>Left side UPDRSm p(β)</th>
<th>Right side UPDRSm p(β)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R2* / right SN</td>
<td>0.11 (0.35)</td>
<td>0.73 (0.06)</td>
<td>&lt;0.03 (0.42)</td>
<td>0.27 (0.24)</td>
<td>0.35 (0.17)</td>
</tr>
<tr>
<td></td>
<td>R2* / left SN</td>
<td>0.20 (0.31)</td>
<td>0.67 (0.09)</td>
<td>0.34 (0.20)</td>
<td>0.37 (0.22)</td>
<td>0.32 (0.21)</td>
</tr>
<tr>
<td></td>
<td>MD / right putamen</td>
<td>&lt;0.01 (0.55)</td>
<td>0.95 (0.01)</td>
<td>0.29 (0.19)</td>
<td>0.68 (0.08)</td>
<td>0.10 (0.30)</td>
</tr>
<tr>
<td></td>
<td>MD / right thalamus 1</td>
<td>&lt;0.00 (0.84)</td>
<td>0.74 (0.05)</td>
<td>0.35 (0.13)</td>
<td>0.35 (0.15)</td>
<td>0.69 (0.05)</td>
</tr>
<tr>
<td></td>
<td>MD / left thalamus 1</td>
<td>&lt;0.00 (0.88)</td>
<td>0.13 (0.20)</td>
<td>0.82 (0.03)</td>
<td>0.14 (0.22)</td>
<td>0.67 (0.05)</td>
</tr>
<tr>
<td></td>
<td>MD / left thalamus 2</td>
<td>&lt;0.00 (0.85)</td>
<td>0.76 (0.04)</td>
<td>0.13 (0.22)</td>
<td>0.13 (0.25)</td>
<td>0.12 (0.22)</td>
</tr>
<tr>
<td></td>
<td>MD / right thalamus 2</td>
<td>&lt;0.00 (0.80)</td>
<td>0.49 (0.10)</td>
<td>0.58 (0.08)</td>
<td>0.45 (0.13)</td>
<td>0.31 (0.15)</td>
</tr>
<tr>
<td></td>
<td>MD / left SN</td>
<td>&lt;0.01 (0.49)</td>
<td>0.74 (0.06)</td>
<td>0.30 (0.17)</td>
<td>0.25 (0.22)</td>
<td>0.26 (0.19)</td>
</tr>
<tr>
<td></td>
<td>MD / right CN1</td>
<td>&lt;0.00 (0.71)</td>
<td>0.55 (0.10)</td>
<td>0.15 (0.24)</td>
<td>0.34 (0.18)</td>
<td>0.86 (0.03)</td>
</tr>
<tr>
<td></td>
<td>MD / right CN2</td>
<td>&lt;0.05 (0.47)</td>
<td>0.58 (0.11)</td>
<td>0.61 (0.10)</td>
<td>0.30 (0.25)</td>
<td>0.67 (0.09)</td>
</tr>
<tr>
<td></td>
<td>FA / right thalamus</td>
<td>&lt;0.00 (0.74)</td>
<td>0.33 (0.17)</td>
<td>0.97 (0.01)</td>
<td>0.16 (0.27)</td>
<td>0.86 (0.03)</td>
</tr>
<tr>
<td></td>
<td>FA / right SN</td>
<td>0.91 (0.03)</td>
<td>0.28 (0.24)</td>
<td>0.58 (0.12)</td>
<td>0.84 (0.05)</td>
<td>0.82 (0.05)</td>
</tr>
<tr>
<td></td>
<td>FA / left putamen</td>
<td>0.76 (0.07)</td>
<td>0.69 (0.09)</td>
<td>0.99 (0.00)</td>
<td>0.81 (0.06)</td>
<td>0.44 (0.17)</td>
</tr>
</tbody>
</table>

MD = mean diffusivity; SN = substantia nigra; Thal = thalamus; FA = fractional anisotropy; CN = caudate nucleus. Significant values are indicated in bold.

Figure 3  In the centre box (A) we show the combinations of MR parameters that were able to significantly discriminate between patients with Parkinson’s disease and controls. Colours indicate the parameters (red = $R_2^*$, green = mean diffusivity, blue = fractional anisotropy) while acronyms identify the anatomical regions of interest indicated. Combinations are numbered from I to IV based on discriminant power (95–98%). (B) ROC curves associated with each significant combination.

Homogeneous (Fearnley and Lees, 1991), a voxel-level analysis was used to address the subregions that are more susceptible to Parkinson’s disease-associated changes. In contrast to previous studies where the localization of substantia nigra subregions was manually defined prior to statistical analysis (Martin et al., 1998; Vaillancourt et al., 2009), here this region was the product of the voxel-based analysis from the $R_2^*$ and fractional anisotropy values. The subregion that emerged from our voxel-based analysis is located in the posterior-medial portion of substantia nigra, which could correspond to the anatomical localization to the substantia nigra pars compacta, particularly sensitive to Parkinson’s disease. The exact and complete segmentation of the substantia nigra and red nucleus on MRI must be considered with caution (for a review, see Massey et al., 2010). However, high-resolution images combined with voxel-based analysis in the present work as well as in a recent study (Baudrexel et al., 2010) are a promising way to study microstructural modifications in mesencephalic structures.

The gold standard of Parkinson’s disease diagnosis is neuropathology, and misdiagnosis in some clinically diagnosed patients cannot be excluded (Hughes et al., 1992; Janjiovic et al., 2000; Tolosa et al., 2006). Here, we tested a group of non-demented medicated patients with moderate Parkinson’s disease. Most diagnostic errors occur in early disease stages (Osaki et al., 2002;
Tolosa et al., 2006), and the disease duration, history and dopaminergic treatment efficacy tend to consolidate Parkinson’s disease diagnosis. Despite presenting with clinical markers of Parkinson’s disease, the conventional MR examinations of our patients were classified as normal by both radiologists. However, we achieved good individual discrimination in the Parkinson’s disease patient group, which presented with a discrete spectrum of drug treatments, disease durations and motor disabilities. As a consequence, the observed nigrostriatal signature of the Parkinson’s disease population can be considered as relatively generic and not specific to one category of patients with Parkinson’s disease. Considering the results of the present study, the multimodal MRI approach provides evidence that physiological and pathological processes can be described by different MR parameters that provide different but complementary measures of microstructural modifications.

Magnetic resonance biomarkers and Parkinson’s disease physiopathology

We utilized relaxation rates such as $R_2^*$ as an indirect measure of the iron level in the deep grey matter. Our region-based results showed an increase in $R_2^*$ only in the substantia nigra. The subregion of the substantia nigra that could correspond to the pars compacta is also more sensitive to increases in $R_2^*$ (Fig. 2). Iron-level increases in the substantia nigra pars compacta of post-mortem Parkinson’s disease patient brains were first reported in 1924 (Lehermitte and McAlpine, 1924). Since then, numerous histochemical studies (Dexter et al., 1987; Sofic et al., 1991) and MR studies (Graham et al., 2000; Martin et al., 2008; Baudrexel et al., 2010) have confirmed this specific iron accumulation. The increased substantia nigra iron content is generally thought to represent dopaminergic neuronal loss. This interpretation is consistent with the significant relationship found between the $R_2^*$ and LEDD in the substantia nigra, both throughout the whole structure and the subregion of the substantia nigra. This result must be considered with caution, since the correlation was found with only the right substantia nigra. Although further investigations are required, the link between dopaminergic drug treatment and dopaminergic loss in the substantia nigra appears promising.

Previous studies using DTI showed that fractional anisotropy in the substantia nigra is reduced in patients with Parkinson’s disease compared with healthy subjects (Yoshikawa et al., 2004; Chan et al., 2007; Vaillancourt et al., 2009). Likewise, we also observed lower mean fractional anisotropy values in the substantia nigra of patients with Parkinson’s disease compared with control subjects. Histochemical analyses of a Parkinson’s disease animal model showed that DTI can be used to assess nigrostriatal degeneration by measuring the decrease of fractional anisotropy in the substantia nigra (Boska et al., 2007).

Our results also revealed an increase in the mean diffusivity in the striatum subregions of patients with Parkinson’s disease. One possible interpretation for this alteration is the deafferentation of the nigrostriatal pathway, since striatal dopaminergic denervation induces a complex microstructural reorganization. While the observed non-correlation between striatum mean diffusivity and motor scale does not corroborate this interpretation, it should be noted that our patients were on dopaminergic medication, which masks the real state of movement disorders. Since such findings have never been reported before, further studies are required to corroborate our interpretation.

Apart from markers included in the discriminant signature, we also found other markers for which differences were found between patients with Parkinson’s disease and controls. For example, we found lower mean diffusivity values in patients with Parkinson’s disease in the thalamus from anatomical region analysis than from voxel-based analysis. However, the mean diffusivity in the thalamus also exhibited a strong relationship with age in our Parkinson’s disease population. In consequence, new investigations will be necessary to understand whether these markers play a specific role in Parkinson’s disease physiopathology.

Our study investigated patients with moderate Parkinson’s disease on medication. Longitudinal studies on large cohorts of patients with Parkinson’s disease will be crucial to confirm our results and to follow accurately the brain modifications due to Parkinson’s disease progression. Further studies will also be necessary to define potential specific signatures of different Parkinson’s disease subgroups (e.g. de novo patients, patients with predominant akinesia versus predominant tremor, patients with frozen gait) or differences between Parkinson’s disease and atypical parkinsonian syndromes such as multiple system atrophy and progressive supranuclear palsy. Another crucial investigation will be to study the effects of anti-parkinsonian medication to assess whether MR measures changes in the OFF state compared with the ON state, as well as the relationship of these changes to clinical data.

Conclusion

This study demonstrates that multimodal MRI is able to identify the nigrostriatal signature of Parkinson’s disease using specific MRI markers and to discriminate patients with Parkinson’s disease from healthy control subjects with a high accuracy. The combination of different MR biomarkers opens new perspectives to investigate pathological changes, such as disease progression and long-term drug impact, detecting non-dopaminergic degeneration and evaluating neuroprotective effects.

Funding

INSERM-DHOS 2007-2009 grant and Italian Ministry of Health grant (RF07.97.5).

Supplementary material

Supplementary material is available at Brain online.

References


