

# Substantia Nigra Volumetry with 3-T MRI in De Novo and Advanced Parkinson Disease

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Supported by the Italian Ministry of Health (5 per mille, years 2014–2015) and University of Pavia.

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Conflicts of interest are listed at the end of this article.

Radiology 2020; 296:401–410 • <https://doi.org/10.1148/radiol.2020191235> • Content code: **NR**

**Background:** Magnetization transfer–prepared T1-weighted MRI can depict a hyperintense subregion of the substantia nigra involved in the degeneration process of Parkinson disease.

**Purpose:** To evaluate quantitative measurement of substantia nigra volume by using MRI to support clinical diagnosis and staging of Parkinson disease.

**Materials and Methods:** In this prospective study, a high-spatial-resolution magnetization transfer–prepared T1-weighted volumetric sequence was performed with a 3-T MRI machine between January 2014 and October 2015 for participants with de novo Parkinson disease, advanced Parkinson disease, and healthy control participants. A reproducible semiautomatic quantification analysis method that entailed mesencephalic intensity as an internal reference was used for hyperintense substantia nigra volumetry normalized to intracranial volume. A general linear model with age and sex as covariates was used to compare the three groups.

**Results:** Eighty participants were evaluated: 20 healthy control participants (mean age  $\pm$  standard deviation, 56 years  $\pm$  11; 11 women), 29 participants with de novo Parkinson disease (64 years  $\pm$  10; 19 men), and 31 participants with advanced Parkinson disease (60 years  $\pm$  9; 16 women). Volumetric measurement of hyperintense substantia nigra from magnetization transfer–prepared T1-weighted MRI helped differentiate healthy control participants from participants with advanced Parkinson disease (mean difference for ipsilateral side, 64 mm<sup>3</sup>  $\pm$  14,  $P < .001$ ; mean difference for contralateral side, 109 mm<sup>3</sup>  $\pm$  14,  $P < .001$ ) and helped distinguish healthy control participants from participants with de novo Parkinson disease (mean difference for ipsilateral side, 45 mm<sup>3</sup>  $\pm$  15,  $P < .01$ ; mean difference for contralateral side, 66 mm<sup>3</sup>  $\pm$  15,  $P < .001$ ) and participants with de novo Parkinson disease from those with advanced Parkinson disease (mean difference for ipsilateral side, 20 mm<sup>3</sup>  $\pm$  13,  $P = .40$ ; mean difference for contralateral side, 43 mm<sup>3</sup>  $\pm$  13,  $P = .004$ ).

**Conclusion:** Magnetization transfer–prepared T1-weighted MRI volumetry of the substantia nigra helped differentiate the stages of Parkinson disease.

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Online supplemental material is available for this article.

A priority in Parkinson disease research is the identification of a reliable in vivo marker for supporting early diagnosis, monitoring disease progression, and driving therapeutic interventions. Diagnosis of Parkinson disease is based on clinical criteria (1) and can be supported by functional nuclear medicine neuroimaging of dopamine radioligands. Indeed, in clinical practice, iodine 123–2β-carbomethoxy-3β-(4-iodophenyl)-N-(3-fluoropropyl)-nortropine (<sup>123</sup>I-FP-CIT) SPECT (DaTSCAN; GE Healthcare, Chicago, Ill) is mostly used as a confirmatory test (2). These studies use radioactive tracers, are influenced by physiologic or pharmacologic mechanisms (3), and are expensive; thus, their use as markers of disease progression is limited. The role of MRI in the diagnosis of Parkinson

disease is limited to the exclusion of other primary or secondary parkinsonisms.

Recent developments show that MRI can help assess the substantia nigra, the primary site of neurodegeneration in Parkinson disease. On the basis of seminal studies showing that melanin has particular iron-binding properties leading to T1 relaxation shortening (4,5), Sasaki et al (6) developed what they called a neuromelanin-sensitive scan using a short-echo T1-weighted sequence at 3 T. The scan depicted the substantia nigra and locus coeruleus in vivo as hyperintense areas in the mesencephalon and showed a reduced hyperintense area in participants with Parkinson disease compared with healthy control participants. Adding a magnetization transfer (MT) pulse to the sequence, Schwarz et al (7) confirmed a decreased substantia nigra

## Abbreviations

AUC = area under receiver operating characteristic curve, HSN = hyperintense substantia nigra, MDS-UPDRS = Movement Disorder Society–sponsored revision of the Unified Parkinson's Disease Rating Scale, MT = magnetization transfer, 3D = three-dimensional

## Summary

Magnetization transfer–prepared T1-weighted MRI volume measurement of the substantia nigra supports differentiation of stages of Parkinson disease.

## Key Results

- Volumetric measurement of hyperintense substantia nigra on magnetization transfer–prepared T1-weighted MRI helped differentiate healthy control participants from participants with advanced Parkinson disease (in advanced Parkinson disease, mean measurement was  $109 \text{ mm}^3 \pm 14$  smaller;  $P < .001$ ), healthy control participants from participants with de novo Parkinson disease (in de novo Parkinson disease, mean measurement was  $66 \text{ mm}^3 \pm 15$  smaller;  $P < .001$ ), and participants with de novo Parkinson disease from those with advanced Parkinson disease (in advanced Parkinson disease, mean measurement was  $43 \text{ mm}^3 \pm 13$  smaller;  $P = .004$ ).
- Normalized hyperintense substantia nigra volume contralateral to the most affected limb was negatively correlated with disease duration ( $r = -0.37$ ) and levodopa equivalent dose ( $r = -0.45$ ).

hyperintense area in participants with Parkinson disease compared with healthy control participants. Moreover, an exploratory subgroup analysis showed a further reduction of the area in four participants with advanced Parkinson disease compared with six participants with early-stage Parkinson disease, although this result was not replicated in large-population studies (8,9), even with use of improved spatial resolution with a three-dimensional (3D) sequence (10). Therefore, the ambiguity of results warrants further studies to determine the sensitivity and specificity of substantia nigra volumetry in large clinical cohorts.

The purpose of this study was to evaluate a semiautomated quantitative measurement of substantia nigra volume by using MRI to support early clinical diagnosis and staging of Parkinson disease. The quantitative measurement of substantia nigra volume could be used to support clinical diagnosis and staging of Parkinson disease.

## Materials and Methods

### Study Participants

The local ethics committee approved this study, and all participants gave written informed consent. Sixty-seven consecutive participants with Parkinson disease, diagnosed according to the criteria of the United Kingdom Parkinson's Disease Society Brain Bank, were prospectively and consecutively recruited between January 2014 and October 2015 by the Movement Disorders Unit at the C. Mondino Foundation Hospital; extrapyramidal signs were evaluated according to the Movement Disorder Society–sponsored revision of the Unified Parkinson's Disease Rating Scale (MDS-UPDRS) part III. The Vasser reduced clinical subscale of the motor score was considered for each limb (left or right), after exclusion of postural and action tremor.

Dopamine transporters deficit was demonstrated with  $^{123}\text{I}$ -FP-CIT SPECT. Atypical parkinsonian disorders, such as multiple-system atrophy, progressive supranuclear palsy, or corticobasal degeneration, and other neurologic conditions were considered exclusion criteria. No participants were excluded for radiologic incidental findings; seven participants with Parkinson disease (two with de novo Parkinson disease, five with advanced Parkinson disease) were excluded for not completing the MRI examination or for motion artifacts; therefore, 60 participants with Parkinson disease were included in the final analysis (Fig 1).

During clinical and MRI evaluation, participants were classified according to these further inclusion criteria: Twenty-nine participants with untreated de novo Parkinson disease had symptom onset about 1 year before the first neurologic visit; 31 participants with advanced Parkinson disease were part of the core assessment program for surgical interventional therapies in Parkinson disease, with eligibility for deep brain stimulation if they met the following criteria: at least 5 years of disease duration, Hoehn and Yahr disability scale score less than 4, no cognitive impairment, and levodopa treatment (medication washout).

To create reference metrics, 20 healthy control participants were recruited among relatives of the participants with Parkinson disease. None of the healthy control participants had a history of neurologic diseases. Five further healthy control participants underwent imaging to develop the imaging processing steps.

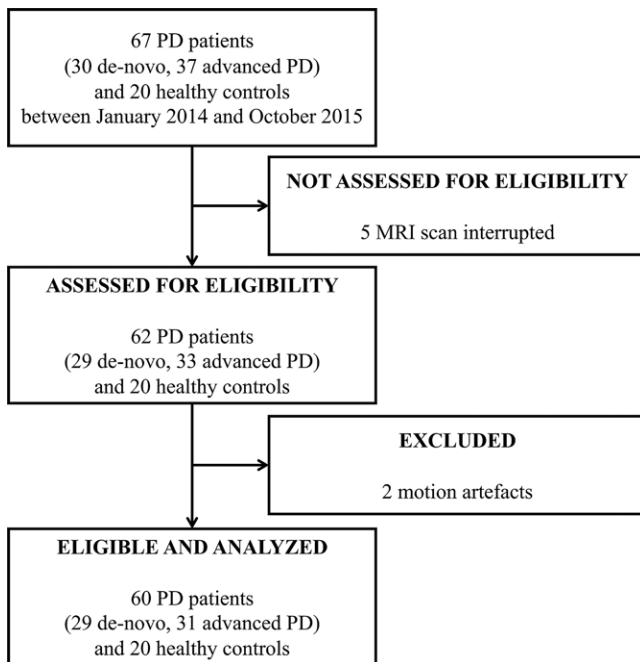
### MRI Protocol

All participants underwent 3-T MRI (Skyra, Syngo MR D13C version; Siemens, Erlangen, Germany) with a 32-channel head coil. A MT-prepared T1-weighted 3D fast low-angle shot sequence was set up as follows: repetition time msec/echo time msec, 33/4.74; flip angle, 20°; field of view,  $120 \times 82.5$  mm; voxel size,  $0.94 \times 1.2 \times 0.9$  mm reconstructed to  $0.9 \times 0.9 \times 0.9$  mm; and total acquisition time, 5 minutes 31 seconds. The sections were oriented perpendicular to the fourth ventricle floor, with the median line of the slab placed at the center of the mesencephalon. Diffusion-weighted imaging, fast low-angle inversion recovery, dual-echo T2-weighted images, T2\*-weighted images, and 3D T1-weighted volumetric images covering the whole brain were obtained in all participants to exclude other brain abnormalities.

### Image Analysis

Image analysis was developed by using a semiautomated segmentation freeware program (Mango, version 3.5.1; <http://ric.uthscsa.edu/mango>).

**Step 1: definition of multiplication factor.**—A multiplication factor was introduced to normalize the intensity of the substantia nigra with respect to the mean mesencephalic intensity. The value of this parameter depends on the pulse sequence and MRI machine; thus, it must be calculated for any new setting. MT-prepared T1-weighted images from five healthy control participants were thresholded by two independent operators (M.I.P.,



**Figure 1:** Participant flowchart. PD = Parkinson disease.

G.G.; 4 years of relevant experience) to visually distinguish the hyperintense signal of the substantia nigra. The mean mesencephalic intensity was measured from three oval regions of interest positioned over the whole mesencephalon, on three consecutive sections in the craniocaudal direction, starting from the first visualization of the separation of the mammillary bodies from the cerebral peduncles (Fig 2, Movie 1 [online]). For each participant and for each operator (M.I.P., G.G.), the threshold was divided by the mean mesencephalic intensity to define a participant-specific ratio. The standard multiplication factor was defined as the mean of the participant-specific ratio over 18 healthy control participants.

**Step 2: definition of participant-specific thresholds.**—A specific threshold was defined by multiplying the standard multiplication factor by the specific mean mesencephalic intensity of each participant.

**Step 3: segmentation of hyperintense substantia nigra.**—For each side of the substantia nigra, all voxels with intensity greater than this participant-specific threshold were selected semiautomatically section-wise in the craniocaudal direction, starting from the first mesencephalic section delineating the hyperintense substantia nigra (HSN) (Figs 3, 4; Movie 2 [online]). For each section, the operator manually drew a region much larger than the HSN, and the software automatically selected only voxels inside the region with signal intensity greater than the participant-specific threshold. This segmentation was performed with clinical information blinded from the operator.

**Step 4: volume measurement.**—The absolute HSN volume (step 2) was calculated for each side. The total intracranial vol-

ume was extracted from the 3D T1-weighted volumetric image (11) and used to calculate the normalized HSN volume.

### Intra- and Interrater Reproducibility

Five healthy control participants were randomly chosen for intra- and interrater reproducibility (12,13). Both operators analyzed the MRI scans from the five healthy control participants, as described previously, twice per participant, with an interval between the first and second time of 12 days. The intraclass correlation coefficient was calculated from the HSN volumes of the five participants recruited to assess reproducibility.

### Statistical Analysis

Statistical analysis was performed by using SPSS software (SPSS, version 21.0.0; IBM, Armonk, NY). Demographic characterization was obtained by using a  $\chi^2$  test for sex and onset and one-way analysis of variance with Bonferroni correction for multiple pairwise comparisons for other variables.

Because of the absence of a significant difference between right and left metrics in healthy control participants (Mann-Whitney  $U$  test,  $P < .05$ ), left and right HSN volumes were averaged together only for healthy control participants. The presence of a sex effect on HSN volumes was evaluated by using an independent  $t$  test between male and female healthy control participants. A multivariable general linear model ( $P < .05$ ) with age and sex as covariates was used to separately compare the HSN volumes of both sides (ie, contra- and ipsilateral to the most affected limb at onset) of participants with Parkinson disease with the mean HSN volumes of healthy control participants. All pairwise comparisons were Bonferroni corrected for multiple comparisons. Receiver operating characteristic curves were obtained for each pair of groups (ie, healthy control participants vs participants with de novo Parkinson disease, healthy control participants vs participants with advanced Parkinson disease, and participants with de novo Parkinson disease vs participants with advanced Parkinson disease). Area under the receiver operating characteristic curve (AUC), cutoffs, sensitivity, and specificity were also calculated.

Correlations between HSN volumes and demographic or clinical data, including age, disease duration, and levodopa equivalent dose, were explored with a nonparametric linear regression analysis ( $P < .05$ ). Full MDS-UPDRS III and motor examination subscale scores were then matched to HSN measurements obtained contra- and ipsilateral to the most affected limb at onset, respectively.

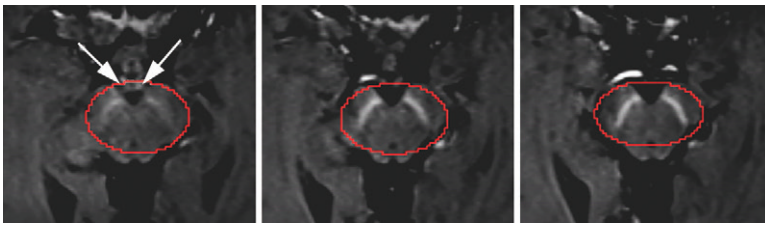
All statistical analyses were performed as previously described for normalized HSN volumes.

The power of the study and the sample size for future works were calculated by using OpenEpi (version 3.01; Open Source Epidemiologic Statistics for Public Health; <http://www.openepi.com>).

## Results

### Study Participants

From the initial 67 participants with Parkinson disease, the final numbers of participants were 29 with de novo Parkinson



**Figure 2:** Measurement of mean mesencephalic intensity at magnetization transfer–prepared T1-weighted MRI. Three oval regions of interest were positioned over the mesencephalon on three consecutive sections in the craniocaudal direction, starting from the first visualization of the separation of the mammillary bodies from the cerebral peduncles.

disease, 31 with advanced Parkinson disease, and 20 healthy control participants. Demographic and clinical characteristics of the study participants are reported in Table 1. The mean ages were 56 years  $\pm$  11 (standard deviation) for healthy control participants; 64 years  $\pm$  10 for participants with de novo Parkinson disease, and 60 years  $\pm$  9 for participants with advanced Parkinson disease. The mean disease durations were 0.6 year  $\pm$  0.8 for participants with de novo Parkinson disease and 9.5 years  $\pm$  4.7 for those with advanced Parkinson disease. The mean MDS-UPDRS III scale scores were 17.7  $\pm$  9.2 for participants with de novo Parkinson disease and 31.6  $\pm$  13.0 for those with advanced Parkinson disease.

No differences were observed in age ( $P = .07$ ) and sex ( $P = .25$ ) among the three groups. Spearman linear regression was used to confirm no correlation between normalized HSN volume and age in all participants (contralateral:  $r = 0.12$ ,  $P = .30$ ; ipsilateral:  $r = 0.11$ ,  $P = .34$ ) and healthy control participants ( $r = 0.36$ ,  $P = .12$ ). A correlation between contralateral normalized HSN volume and age was highlighted in all participants ( $r = 0.34$ ,  $P = .009$ ) and participants with advanced Parkinson disease ( $r = 0.39$ ,  $P = .03$ ), whereas ipsilateral normalized HSN volume correlated with age only in participants with de novo Parkinson disease ( $r = 0.38$ ,  $P = .04$ ).

#### Multiplication Factors and Intrarater Reproducibility

Individual multiplication factors to normalize the intensity of the substantia nigra with respect to the mean mesencephalic intensity for healthy control participants ranged between 1.060 and 1.179, with a median of 1.093 and a mean of 1.096  $\pm$  0.027.

The intraclass correlation coefficient calculation showed high intrarater (0.87 and 0.80 for the first and second operators, respectively) and interrater (0.82) reproducibility of the HSN volume measurement.

**Descriptive statistics.**—The Mann-Whitney  $U$  test performed on healthy control participants showed no differences ( $P = .51$ ) in the normalized HSN volume between the right and left sides (left mean normalized HSN volume, 262 mm<sup>3</sup>  $\pm$  56; right mean normalized HSN volume, 253 mm<sup>3</sup>  $\pm$  60; mean normalized HSN volume, 257 mm<sup>3</sup>  $\pm$  52).

The normalized HSN volume of participants with de novo Parkinson disease was lower than that of healthy control participants, both for the side contralateral to the most affected limb at

onset (198 mm<sup>3</sup>  $\pm$  51) and the ipsilateral side (219 mm<sup>3</sup>  $\pm$  51). In participants with advanced Parkinson disease, normalized HSN volumes were also lower (contralateral normalized HSN, 153 mm<sup>3</sup>  $\pm$  52; ipsilateral normalized HSN, 197 mm<sup>3</sup>  $\pm$  44) (Fig 5, Table 2). These ipsilateral and contralateral normalized HSN volumes were compared in participants with Parkinson disease, demonstrating that in de novo Parkinson disease there is no asymmetry of the substantia nigra volumes ( $P = .12$ ), whereas in participants with advanced Parkinson disease there is a different involvement of the substantia nigra depending on the side considered ( $P = .001$ ).

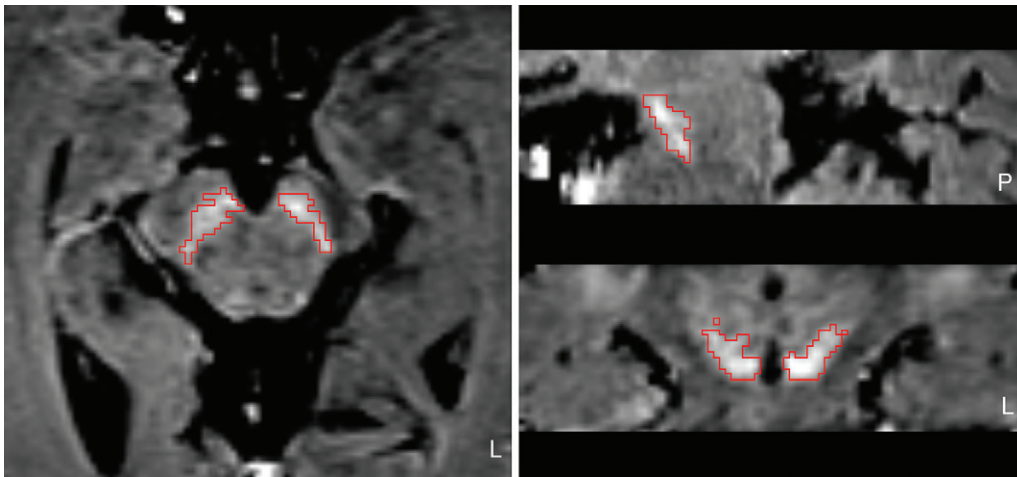
**Multivariable analysis.**—The multivariable general linear model (Bonferroni corrected with age and sex as covariates) showed that normalized HSN volumes differed among the three groups ( $P < .001$ ) (Table 2). The greatest difference was found in the part of the substantia nigra contralateral to the most affected limb at onset (mean difference: 66 mm<sup>3</sup>  $\pm$  15 for healthy control participants vs participants with de novo Parkinson disease,  $P < .001$ ; 109 mm<sup>3</sup>  $\pm$  14 for healthy control participants vs participants with advanced Parkinson disease,  $P < .001$ ; 43 mm<sup>3</sup>  $\pm$  13 for participants with de novo Parkinson disease vs participants with advanced Parkinson disease,  $P = .004$ ). When the part of the substantia nigra ipsilateral to the most affected limb at onset was considered, these differences were smaller (participants with de novo Parkinson disease vs those with advanced Parkinson disease: mean difference, 20 mm<sup>3</sup>  $\pm$  13;  $P = .40$ ) but still significant (healthy control participants vs participants with de novo Parkinson disease: mean difference, 45 mm<sup>3</sup>  $\pm$  15;  $P = .012$ ; healthy control participants vs participants with advanced Parkinson disease: mean difference, 64 mm<sup>3</sup>  $\pm$  14;  $P < .001$ ) (Table 2).

Results of the receiver operating characteristic curve analysis are shown in Figure 6 and Table 3. The best differentiation between pairs of groups was found by using the part of the substantia nigra contralateral to the most affected limb at onset. Healthy control participants and participants with advanced Parkinson disease were discriminated with a sensitivity of 29 of 31 cases (mean, 93.5%  $\pm$  8.6) and a specificity of 14 of 20 cases (mean, 70.0%  $\pm$  20.1) at a cutoff value of 225 mm<sup>3</sup> (AUC, 0.94); healthy control participants and participants with de novo Parkinson disease were discriminated with a sensitivity of 26 of 29 cases (mean, 89.7%  $\pm$  11.1) and a specificity of 11 of 20 cases (mean, 55.0%  $\pm$  21.8) at a cutoff value of 244 mm<sup>3</sup> (AUC, 0.82); and participants with de novo Parkinson disease and participants with advanced Parkinson disease were discriminated with a sensitivity of 23 of 31 cases (mean, 74.2%  $\pm$  15.4) and a specificity of 20 of 29 cases (69.0%  $\pm$  16.8) at a cutoff value of 180 mm<sup>3</sup> (AUC, 0.75).

#### Clinical Data and HSN Volumes

Mean values of clinical data for participants with de novo Parkinson disease and those with advanced Parkinson disease are reported in Table 1. The Mann-Whitney  $U$  test revealed differences





**Figure 3:** Semiautomated segmentation of the left and right hyperintense substantia nigra region at magnetization transfer–prepared T1-weighted MRI. Left, axial view; top right, sagittal view; bottom right, coronal view. L= left, P = posterior.

between participants with de novo Parkinson disease and participants with advanced Parkinson disease for total MDS-UPDRS III ( $P < .001$ ) and for MDS-UPDRS III of the less affected limb at onset ( $P < .001$ ). The normalized HSN volume contralateral to the most affected limb showed negative correlation with disease duration and levodopa equivalent dose, whereas the ipsilateral normalized HSN volume showed negative correlation only with disease duration (Table 4, Fig 7). Correlations with MDS-UPDRS III and subscale score were not significant.

#### Power of Study and Sample Size

The power of study, calculated by using a post hoc analysis on the contralateral normalized HSN to the most affected side, was 99% for the comparison on healthy control participants and participants with de novo Parkinson disease, 97% for participants with de novo Parkinson disease versus those with advanced Parkinson disease, and 100% for healthy control participants versus participants with advanced Parkinson disease.

For future studies, a sample size (80% power,  $P = .05$ ) of 29 participants with de novo Parkinson disease and 29 healthy control participants and of 75 participants with de novo Parkinson disease and 75 participants with advanced Parkinson disease was calculated on the basis of the ipsilateral normalized HSN to the most affected side.

#### Discussion

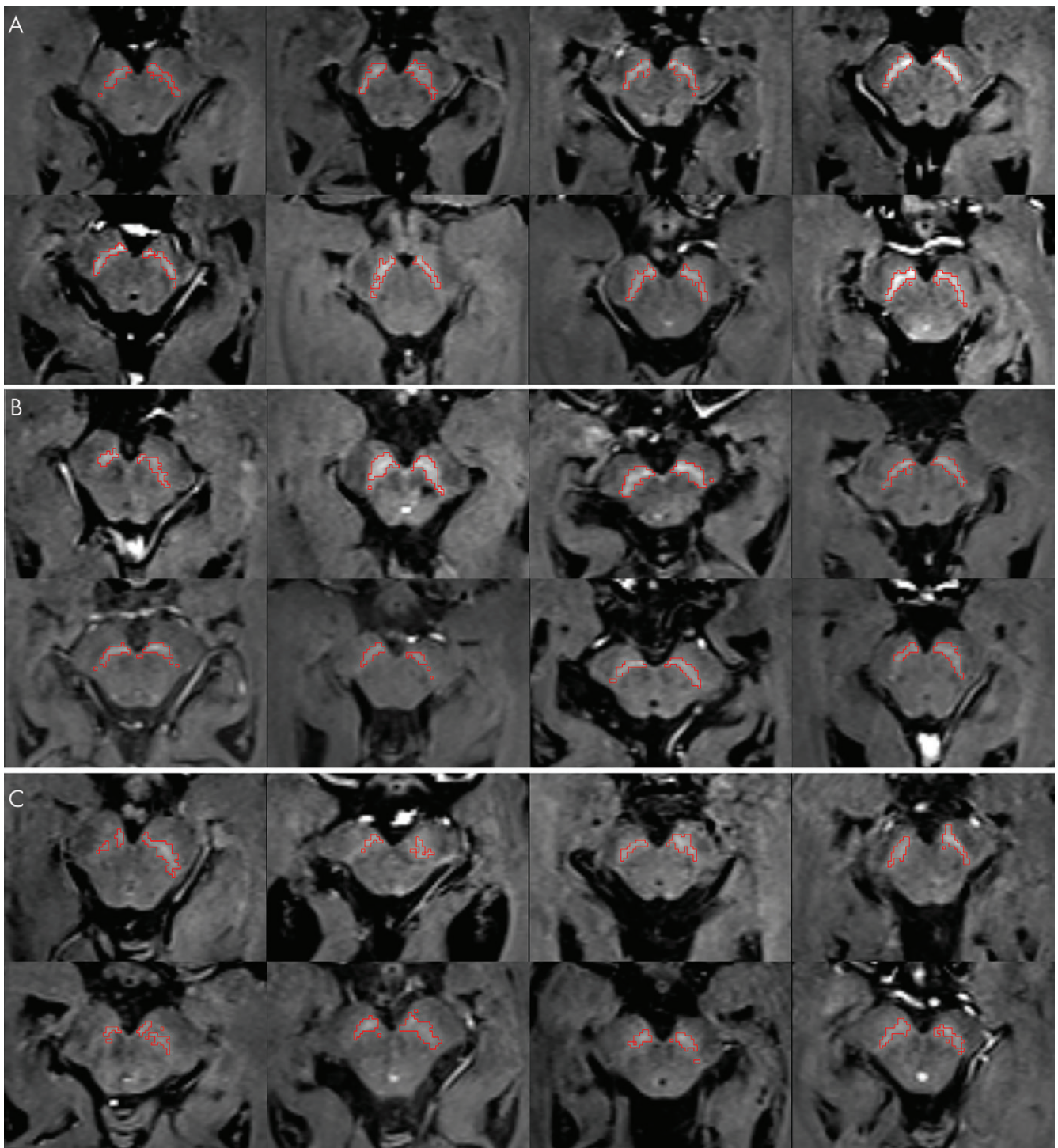
The aim of this study was to propose a quantitative measure of the substantia nigra by using MRI as a potential in vivo marker to support early clinical diagnosis and differentiate disease stages. This cross-sectional study established the diagnostic value of a high-temporal-resolution MT-prepared T1-weighted volumetric sequence that takes advantage of a combined sensitivity to macromolecules and iron content in the context of Parkinson disease pathophysiology. Participants with de novo Parkinson disease were differentiated from healthy control participants (the volumetric measurement was smaller in participants with de novo Parkinson disease by a mean difference of  $66 \text{ mm}^3 \pm 15$ ,  $P < .001$ ) and participants with advanced

Parkinson disease (the volumetric measurement was smaller in participants with advanced Parkinson disease by a mean difference of  $43 \text{ mm}^3 \pm 13$ ,  $P = .004$ ). To our knowledge, the level of significance is superior to that seen in other in vivo imaging studies published to date (6,8,9). Although statistically significant, the observed differences in substantia nigra volume were small. This reflects the relatively small measurable differences in volume between the two groups.

The presented method shows sensitivity of 70% or greater and specificity of 50%–70%, depending on the group comparison, even between participants with different disease stages. These values are similar to reported  $^{123}\text{I}$ -FP-CIT SPECT results (2). The proposed MT-prepared T1-weighted sequence takes 5 minutes and can be optimized on clinical imagers to support diagnosis. Moreover, quantification of HSN volumes can show stage-dependent degeneration, offering a potential noninvasive in vivo imaging marker of Parkinson disease.

A pilot study of six participants with early Parkinson disease and four participants with advanced Parkinson disease (7) showed differences in agreement with our study but was not replicated in larger study samples (8,9). Variability in results may depend on participant selection and differences in acquisition and analysis methods.

The observed changes in the substantia nigra contrast have been attributed to pathophysiologic changes in neuromelanin content associated with loss of dopaminergic neurons (14). Neuromelanin is paramagnetic and has a high affinity for transition metals, maintaining these metals in an oxidative inactive reduced form (15). However, cell pellets of the melanotic cell line have shown higher MRI signal intensity with increasing iron concentration, whereas those of the amelanotic cell line did not, regardless of iron concentration (5). Thus, it can be speculated that the iron content per se is not sufficient to explain the hyperintensity of the substantia nigra. Moreover, a histopathology and MRI correlation study demonstrated high correspondence ( $P < .05$ ) between the neuromelanin signal intensity and the pigmented neuron density in the substantia nigra in a healthy participant; in Parkinson disease, the loss of signal intensity was parallel to a



**Figure 4:** Axial sections of the left and right hyperintense substantia nigra regions of interest at magnetization transfer-prepared T1-weighted MRI of the first consecutive eight cases for each group. A, Healthy control participants, B, participants with de novo Parkinson disease, and, C, participants with advanced Parkinson disease.

decrement in neuronal density but independent of iron deposition (16). Factors affecting MT-prepared T1-weighted signal include neuronal density, myelin content, and paramagnetic substances, thereby limiting the association between the observed MRI changes and neuromelanin content only.

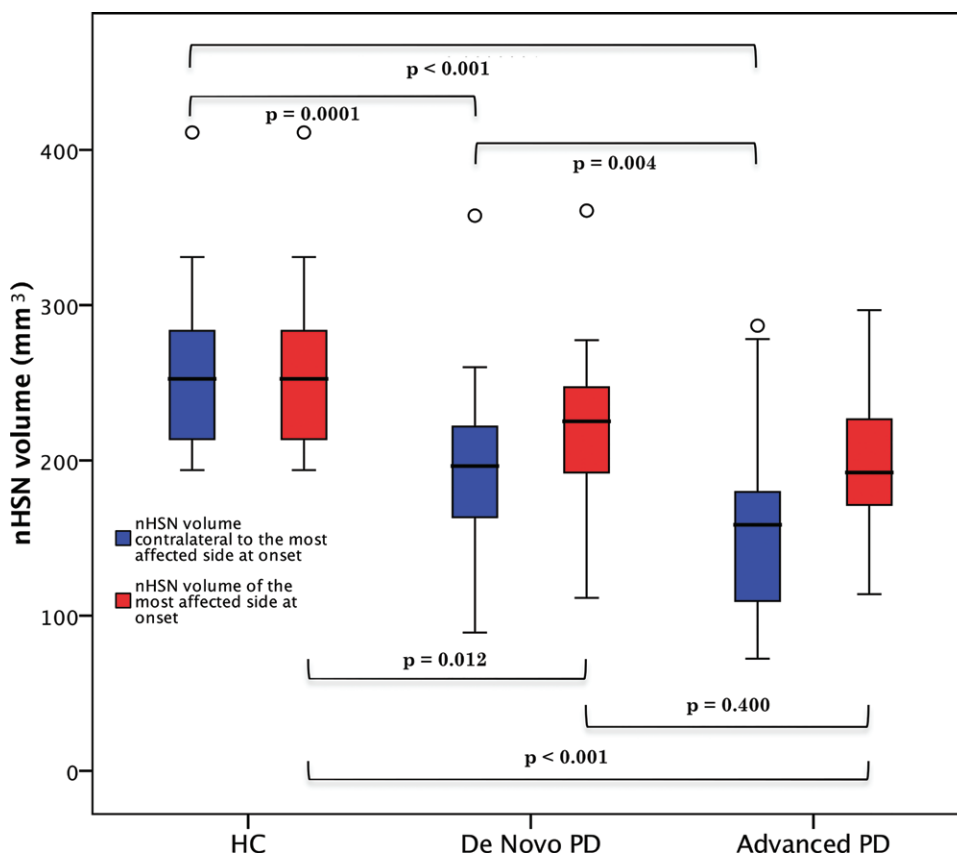
The anticorrelation found between normalized HSN volume and both disease duration and levodopa-equivalent dose indicates that there is a possible link between the disease progression

and the perpetual loss of neuromelanin in the substantia nigra that should be investigated further in future longitudinal studies. Clinical-radiologic correlations were not found for the MDS-UPDRS III motor scale for either de novo or advanced Parkinson disease. Such correlation was previously reported in only a small study sample (7). Interestingly, in a study specifically evaluating the effects of levodopa and Parkinson disease progression, clinical data suggested that levodopa slowed the progression of Parkinson

**Table 1: Demographic and Clinical Data of Study Participants**

Characteristic	HC Participants (n = 20)	Participants with De Novo PD (n = 29)	Participants with Advanced PD (n = 31)	P Value
Age (y)	56 ± 11	64 ± 10	60 ± 9	.07
Women (%)	55.0	34.5	51.6	.25
Onset on left side (%)	...	58.6	74.2	.61
Disease duration (y)	...	0.6 ± 0.8	9.5 ± 4.7	<.001
Treatment duration (y)	...	0	8.4 ± 4.3	...
Levodopa-equivalent dose (mg)	...	0	1011 ± 323	...
MDS-UPDRS III score	...	17.7 ± 9.2	31.6 ± 13.0	<.001
MDS-UPDRS III subscale score for onset side	...	8.8 ± 4.8	10.6 ± 5.1	.20
MDS-UPDRS III subscale score for contralateral side	...	2.0 ± 3.3	8.6 ± 4.5	<.001
Hoehn and Yahr scale score	...	1.63 ± 0.4	2.5 ± 0.5	NA

Note.—Unless otherwise indicated, data are mean ± standard deviation. Disease and treatment duration refer to the interval from symptom onset to MRI time. MDS-UPDRS = Movement Disorder Society–sponsored revision of the Unified Parkinson's Disease Rating Scale; NA = not applicable; PD = Parkinson disease.



**Figure 5:** Box plots of normalized hyperintense substantia nigra (nHSN) volumes of the sides ipsilateral and contralateral to the most affected one (at onset) in participants with de novo Parkinson disease (PD) and participants with advanced Parkinson disease. For healthy control (HC) participants, one mean value has been reported. Plots indicate median, and boxes indicate the upper and lower quartiles. Whiskers are defined as the standard deviation values. Outliers outside standard deviation range are shown as circles. P values were obtained with the Mann-Whitney U test.

disease or had a prolonged effect on disease symptoms (17). These observations could possibly explain the dissociation between the MDS-UPDRS scores and the neuromelanin volume. In the same study, neuroimaging data assessed by using striatal dopamine-transporter density with use of [ $^{123}\text{I}$ ] $\beta$ -CIT uptake suggested that

levodopa accelerates the loss of nigrostriatal dopamine nerve terminals, similar to the present normalized HSN volume loss.

In our study, great importance was given to both acquisition and analysis set-ups. A key aspect of our work is that a 3D T1-weighted gradient-echo sequence prepared with a magnetization transfer pulse was acquired with a reduced field of view, focusing on the substantia nigra, adapted from a protocol implemented originally on a Philips scanner (10). Previous protocols used two-dimensional T1-weighted turbo spin-echo sequences with high in-plane resolution at the expense of thicker sections (6–9,18). With use of a gradient-echo sequence rather than a spin-echo sequence, images are potentially more sensitive to susceptibility changes. Furthermore, the 3D high-spatial-resolution (1-mm<sup>3</sup>) volumetric acquisition improved accuracy of HSN volume selection through a semiautomatic segmentation method in comparison with previous results obtained by

using two-dimensional acquisition. Our procedure is easy to implement and requires manual intervention only for selection of the initial threshold. Interrater variability is low (intraclass correlation coefficient, 0.8 [ie, >0.5]) and is translated in a reproducible selection of the HSN volumes. Moreover, the use of

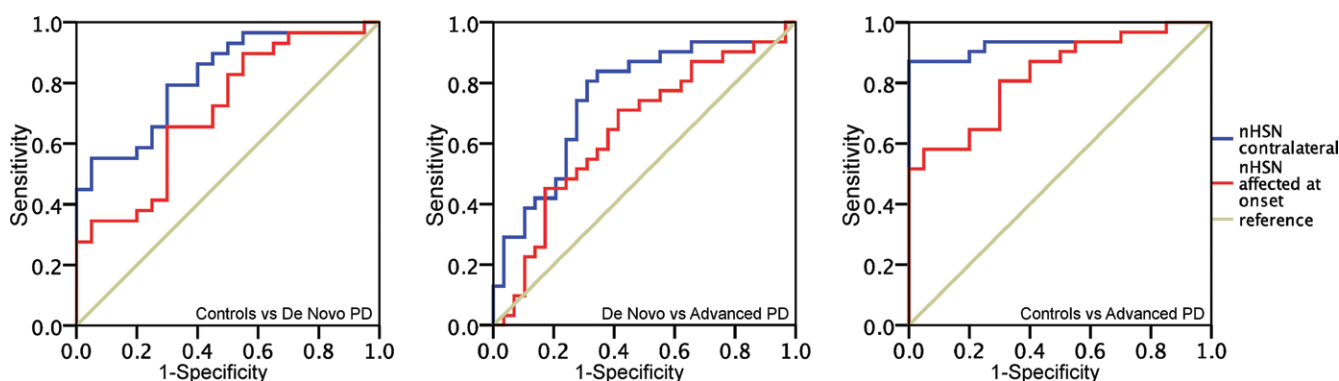


**Table 2: Normalized HSN Volumes**

Normalized HSN Volume	Group			P Value		
	HC (n = 20)	De Novo PD (n = 29)	Advanced PD (n = 31)	HC vs De Novo PD	De Novo PD vs Advanced PD	HC vs Advanced PD
<b>Ipsilateral (mm<sup>3</sup>)</b>						
Mean ± SD	257 ± 52*	219 ± 51	197 ± 44	.01	.40	<.001
SEM	12	9	8	...	...	...
95% CI	233, 282	200, 238	181, 213	...	...	...
<b>Contralateral (mm<sup>3</sup>)</b>						
Mean ± SD	257 ± 52*	198 ± 51	153 ± 52	<.001	.004	<.001
SEM	12	9	9	...	...	...
95% CI	233, 282	179, 217	134, 172	...	...	...

Note.—P values were calculated with a multivariable general linear model with age and sex as covariates and Bonferroni correction for multiple comparisons. *Ipsilateral* and *contralateral* refer to position in relation to the most affected limb at onset. CI = confidence interval, HC = healthy control participants; HSN = hyperintense substantia nigra, PD = Parkinson disease, SD = standard deviation, SEM = standard error of the mean.

\* Mean volume between left and right normalized HSN.



**Figure 6:** Receiver operating characteristic curves of normalized hyperintense substantia nigra (nHSN) volumes show that the most significant area under the curve (AUC) was always found by using the part of the substantia nigra contralateral to the most affected side at onset. Left: Healthy control participants versus participants with de novo Parkinson disease (PD) showed AUC of 0.82, with sensitivity of 89.7% and specificity of 55.0%. Middle: Participants with de novo PD versus those with advanced PD showed AUC of 0.75, with sensitivity of 74.2% and specificity of 69.0%. Right: Healthy control participants versus participants with advanced PD showed AUC of 0.94, with sensitivity of 93.5% and specificity of 70.0%.

**Table 3: Summary of Receiver Operating Characteristic Curve Analysis**

Variable	AUC	Normalized HSN Cutoff (mm <sup>3</sup> )	Sensitivity*	Specificity*
<b>Normalized HSN ipsilateral to most affected limb at onset</b>				
HC vs de novo PD	0.71	261	24/29 (82.8 ± 13.7)	9/20 (45.0 ± 21.8)
De novo vs advanced PD	0.64	215	20/31 (64.5 ± 16.8)	17/29 (58.6 ± 17.9)
HC vs advanced PD	0.83	243	27/31 (87.1 ± 11.8)	11/20 (55.0 ± 21.8)
<b>Normalized HSN contralateral to most affected limb at onset</b>				
HC vs de novo PD	0.82	244	26/29 (89.7 ± 11.1)	11/20 (55.0 ± 21.8)
De novo vs advanced PD	0.75	180	23/31 (74.2 ± 15.4)	20/29 (69.0 ± 16.8)
HC vs advanced PD	0.94	225	29/31 (93.5 ± 8.6)	14/20 (70.0 ± 20.1)

Note.—Data in parentheses are percentages and are mean ± 95% confidence interval AUC = area under the receiver operating characteristic curve, HC = healthy control participants, HSN = hyperintense substantia nigra, PD = Parkinson disease.

\* Data are proportions were used to calculate sensitivity and specificity.

a simple freeware program makes the proposed analysis available with minimal technical support.

Normalization of the individual HSN volumes to the total intracranial volume, commonly used for hippocampal

volumetry (19,20), allowed further improvement in the characterization of healthy control participants and participants with de novo or advanced Parkinson disease. Furthermore, the use of the intensity normalization step in

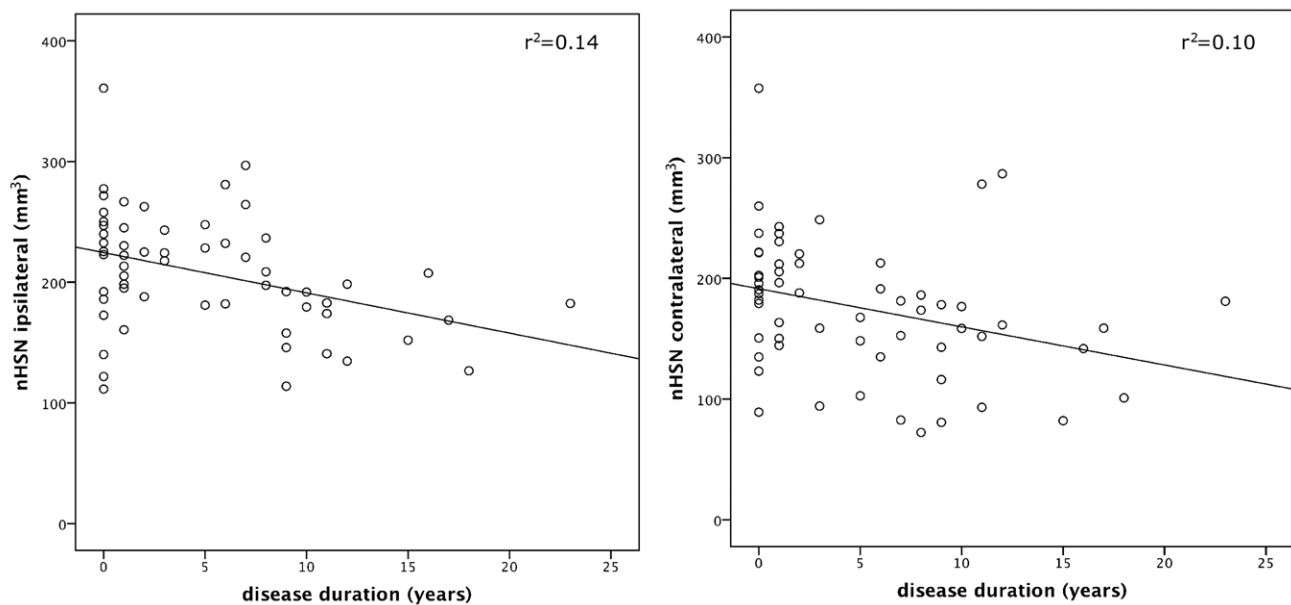


**Table 4: Correlations between Clinical Data and Normalized HSN Volumes**

Variable	Contralateral Normalized HSN	Ipsilateral Normalized HSN
<b>MDS-UPDRS III scale score</b>		
Correlation ( <i>r</i> value)	−0.08	−0.04
<i>P</i> value	.55	.80
<b>Disease duration</b>		
Correlation ( <i>r</i> value)	−0.37	−0.38
<i>P</i> value	.004	.003
<b>Levodopa-equivalent dose</b>		
Correlation ( <i>r</i> value)	−0.45	−0.23
<i>P</i> value	<.001	.09

Note.—Unless otherwise indicated, data are Spearman correlations between clinical data and normalized volumes in participants. HSN = hyperintense substantia nigra; MDS-UPDRS = Movement Disorder Society–sponsored revision of the Unified Parkinson's Disease Rating Scale.

\**P* < .01.



**Figure 7:** Negative relationship between disease duration and normalized hyperintense substantia nigra (nHSN) volumes of the side ipsilateral ( $r^2 = 0.14$ , left) and contralateral ( $r^2 = 0.10$ , right) to the most affected one (at onset) in participants with de novo Parkinson disease and those with advanced Parkinson disease.

our analysis helped eliminate, at first approximation, some sources of variability linked to the imager's preimaging routines and coil loading. This aspect is particularly beneficial for potential multicenter studies, where multiple imagers would be involved, likely from different vendors or using different head coils.

The substantia nigra contra- and ipsilateral to the most affected limb were compared separately (21,22). Indeed, the contralateral part of substantia nigra consistently presented smaller volumes in participants with de novo Parkinson disease and those with advanced Parkinson disease, supporting the evidence that cell loss in substantia nigra and nigrostriatal dysfunction are often asymmetric (23,24) and lateralized extrapyramidal symptoms are a typical feature of Parkinson disease, which persists, although reduced, through the whole disease course (25).

The main limitation of this study is the relatively small number of participants included. In fact, the proposed thresholds and cutoffs could depend on the study sample. A full validation would require a larger study sample, as suggested by the calculated sample size. Another important limitation of the study is the lack of a reference standard; therefore, lack of biomarkers for Parkinson disease diagnosis and progression prompted us to perform this study.

In conclusion, this study demonstrated the sensitivity of a clinically feasible and highly reproducible MRI method for substantia nigra volumetry in Parkinson disease. It met the needs of distinguishing de novo Parkinson disease from healthy individuals and from advanced Parkinson disease, thus promising to be a potential *in vivo* imaging marker of progression.

**Acknowledgments:** We thank Egidio D'Angelo for his substantial support to the Brain MRI 3T Mondino Research Center, Luigi Zecca for discussions on substantia

nigra and neuromelanin, and Ioannis Isaias for valuable suggestions in writing this article.

**Author contributions:** Guarantors of integrity of entire study, P.V., M.I.P., G.G., N.A., R.Z.; study concepts/study design or data acquisition or data analysis/interpretation, all authors; manuscript drafting or manuscript revision for important intellectual content, all authors; approval of final version of submitted manuscript, all authors; agrees to ensure any questions related to the work are appropriately resolved, all authors; literature research, P.V., M.I.P., G.G., A.F., P.F., B.M., C.P.; clinical studies, P.V., M.I.P., G.G., A.F., N.A., B.M., R.Z., C.P.; statistical analysis, M.I.P., P.F., G.G., A.F., B.M.; and manuscript editing, P.V., M.I.P., P.F., G.G., A.F., N.A., B.M., C.P., C.A.M.G.W.

**Disclosures of Conflicts of Interest:** P.V. disclosed no relevant relationships. M.I.P. disclosed no relevant relationships. P.F. disclosed no relevant relationships. G.G. disclosed no relevant relationships. A.F. disclosed no relevant relationships. N.A. Activities related to the present article: disclosed no relevant relationships. Activities not related to the present article: is on the board of Bracco Diagnostics, gave lectures for Bracco Diagnostics and Bayer Healthcare. Other relationships: disclosed no relevant relationships. P.F. disclosed no relevant relationships. B.M. disclosed no relevant relationships. R.Z. disclosed no relevant relationships. C.P. disclosed no relevant relationships. C.A.M.G.W. disclosed no relevant relationships.

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