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FULL PAPER

Quantifying changes in nigrosomes using quantitative susceptibility mapping and neuromelanin imaging for the diagnosis of early-stage Parkinson's disease

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Objective: To quantify nigral changes with a focus on their spatial variation within the substantia nigra pars compacta (SNpc) for diagnosing early-stage Parkinson's disease (PD).

Methods: The study participants were 18 patients with early-stage PD (PD group) and 18 healthy controls (HC group) who underwent quantitative susceptibility mapping (QSM) and neuromelanin imaging. The QSM and neuromelanin values in each whole SNpc containing the entire nigrosome and dorsolateral SNpc containing nigrosome 1 were calculated. The neuromelanin area was defined as the volume with a signal-to-noise ratio higher than that of the background region. The significance of intergroup differences in the QSM value and neuromelanin area in each SNpc region was tested. Logit (p) was used to estimate the probability of PD in relation to the QSM value and the neuromelanin area,

and receiver operating characteristic analyses were performed for each value.

Results: In both SNpc, QSM values were significantly higher and neuromelanin areas were significantly lower in the PD group compared with the HC group ($p < 0.05$). The respective areas under the receiver operating characteristic curve for the two groups were 0.70/0.73 for the QSM value, 0.81/0.78 for the neuromelanin area in the whole/dorsolateral SNpc, and 0.86 for logit (p) in relation to the QSM value of the dorsolateral SNpc and the neuromelanin area of the whole SNpc.

Conclusion: Comprehensive MRI assessment of the abnormality involving the nigrosomes can yield a high diagnostic performance for early-stage PD.

Advances in knowledge: Focusing on spatial differences in nigral changes within the SNpc can increase the sensitivity of the detection of PD-related neurodegenerative changes.

INTRODUCTION

The diagnosis of Parkinson's disease (PD) is based mainly on clinical assessments,¹ and the diagnosis of early-stage PD is more difficult than that of advanced-stage PD. Therefore, confirmation using an imaging biomarker is needed for quantitatively diagnosing early-stage PD.

PD is characterized pathologically by dopaminergic neuron loss and iron overload in the substantia nigra pars compacta (SNpc).^{2–4} When examining iron and dopaminergic neurons in the SNpc in patients with PD, quantitative susceptibility mapping (QSM) can quantify iron deposition,^{5,6} and neuromelanin imaging can quantify the loss of dopaminergic neurons.⁷ QSM quantifies the iron content in tissues by removing the susceptibility effects of the surrounding brain

tissue through deconvolution of the gradient-echo MRI phase caused by iron paramagnetism.^{6–9} QSM has been demonstrated to be sensitive for detecting tissue iron changes in PD.¹⁰ Dopaminergic neurons in the SNpc contain a black pigment known as neuromelanin,¹¹ which has paramagnetic T_1 shortening effects.¹² Therefore, a T_1 weighted fast spin echo technique at 3 T MRI allows the direct visualization of the SNpc as a hyperintense area.¹³ Hence, neuromelanin T_1 W MRI is a validated technique for examining the loss of dopaminergic neurons in the SNpc of patients with PD.¹⁴

Pathology studies have demonstrated that the vulnerable region of dopaminergic neurons varies spatially in the SNpc of patients with PD. According to specific immunohistochemical staining properties, the SNpc is subdivided into

nigrosomes and the nigral matrix.¹⁵ The nigrosomes are primary subregions of the SNpc where dopaminergic neurons are lost in PD. They are subdivided into five different zones (nigrosome 1 to 5), the largest of which, nigrosome 1, is positioned in the ventrolateral SN (dorsolateral SNpc) and is most affected in patients with PD.^{16,17} Recently, the visual evaluation of changes in nigrosome 1 using MRI demonstrated its applicability as an imaging biomarker for the diagnosis of PD. A 7 T MRI study showed that the increase in iron load in nigrosome 1 results in the disappearance of a high signal intensity in the SNpc on T_2 weighted images.¹⁸ Furthermore, loss of nigrosome 1 can also be detected using three-dimensional (3D) susceptibility-weighted images at 3 T.¹⁷ These iron-sensitive MRI studies have indicated that the imaging evaluation of nigrosome 1 may be applicable in the diagnosis of PD, with high sensitivity and specificity. Therefore, we hypothesized that imaging analysis with a focus on the spatial variation of changes in nigrosomes within the SNpc can increase the sensitivity of the detection of the PD-related neurodegenerative changes.

The imaging analysis of the SNpc region requires high accuracy and reproducibility, because this region is small and is hardly detectable with conventional imaging. Therefore, we adopted a voxel-wise and region of interest (ROI) approach based on an automatic segmenting technique for the quantitative evaluation of the SNpc. Our aim was to assess the utility of quantifying nigral changes with the use of both QSM and neuromelanin imaging by focusing on its spatial variation within the SNpc in the diagnosis of early-stage PD.

METHODS AND MATERIALS

Our institutional review board approved this study, and all participants provided written informed consent.

Patients and controls

This prospective study included data from 18 patients (PD Group; 10 males, 8 females; age, 71.21 ± 6.94 years) who were diagnosed with PD between September 2014 and September 2016 by experienced neurologists (MM and HM with 18 and 30 years of experience in movement disorders, respectively) using the UK Parkinson's Disease Society Brain Bank criteria¹⁹ and the Hoehn and Yahr (HY) stages.²⁰ The number of PD patients with an HY score of 1 and 2 were 1 and 17, respectively. 18 age-matched normal volunteers (healthy controls, HC Group; 10 males, 8 females; age, 67.18 ± 4.75 years) diagnosed as lacking any neurodegenerative disorder were included as HC. The detailed demographic and clinical data of both groups are shown in Table 1.

MRI

All MRI studies were performed using a 3 T system (Discovery MR750; GE Healthcare, Milwaukee, WI) with a 32-channel head array receiving coil. QSM was generated from the complex data obtained during the gradient-echo sequence using the morphology-enabled dipole inversion technique.⁸ The images were acquired using a 3D flow-compensated multiecho spoiled gradient-echo sequence (repetition time/echo time, 40.4/36.4 ms; slice thickness, 2.4 mm; flip angle, 10°; acquisition matrix, 384×256 ;

Table 1. Demographics and clinical status of the study participants

	PD group	HC group
Female/male, n	8/10	8/10
Age, y, mean (SD)	71.2 ± 6.94	67.1 ± 4.75
Hoehn–Yahr scale (median) (range)	1.94 ± 0.24	
Disease duration, y, mean (SD)	5.39 ± 1.95	
UPDRS Part 1, mean (SD)	4.95 ± 2.31	
UPDRS Part 2, mean (SD)	9.63 ± 4.35	
UPDRS Part 3, mean (SD)	13.05 ± 7.22	
MMSE	26.38 ± 2.82	28.57 ± 1.60

HC, healthy controls; PD, Parkinson's disease; SD, standard deviation.

field of view, 220 mm). Neuromelanin imaging was acquired using T_1 weighted fast spin echo sequences (repetition time/echo time, 550/10.49 ms; slice thickness, 2.4 mm; flip angle, 180° with no intersection gaps; acquisition matrix, 256×256 ; field of view, 180 mm). 3D sections of a T_1 weighted magnetization-prepared rapid acquisition of gradient-echo sequence were obtained in a sagittal orientation as 1-mm-thick sections (repetition time/echo time, 7.20/2.75 ms; flip angle, 11° with no intersection gaps; acquisition matrix, 256×256 ; field of view, 240 mm). In addition to these imaging approaches, all patients underwent axial T_2 weighted imaging of fast spin echo sequences (repetition time/echo time, 5800/98.6 ms; slice thickness, 5 mm; flip angle, 111°; acquisition matrix, 320×320 ; field of view, 254 mm) as part of their routine clinical care to confirm that there were no other structural abnormalities.

Image analysis

All MRI data were transferred to a computer. For highly reproducible image analysis of the SNpc, we originally developed an ROI-based automated segmentation system using a voxel-based morphometric technique for calculating both the QSM and neuromelanin values. Images were preprocessed as follows: all QSM and neuromelanin images were co-registered with 3D T_1 weighted structural images and were spatially normalized to 1 mm voxel-size using Statistical Parametric Mapping 12 software and code written in MATLAB (Mathworks, Natick, MA), thus allowing highly reproducible measurements with automatic setting of the multislice ROI. The spatially normalized images of all patients were smoothed to remove image noise using an isotropic 4 mm full-width Gaussian kernel. Subsequently, an image overlay was produced from the preprocessed QSM and neuromelanin data, and finally, the dorsolateral SNpc ROI was set using commercially available software (NordicICE v. 3.3.12; Nordic Imaging Lab, NIL, Bergen, Norway). Two respective ROIs encompassing the whole and dorsolateral SNpc were created by manual segmentation based on the averaged neuromelanin images of HC (Figure 1), because a previous postmortem neuromelanin MRI study revealed that diffuse hyperintense areas in the SNpc reflect well-preserved neuromelanin-containing neurons.²¹ Finally, the QSM and neuromelanin values for both groups were obtained using each ROI.

Figure 1. Dopaminergic neurons in the SNpc contain a black pigment known as neuromelanin. MRI of this material depicted the SNpc as hyperintense areas (a). Susceptibility-weighted MRI visualized the presumed nigrosome 1 as a hyperintense area in the dorsolateral SNpc (b). The normal anatomy of the SNpc is illustrated (c). The SNpc can be subdivided into several nigrosomes that represent small clusters of dopaminergic cells. The largest nigrosome is labeled nigrosome 1 and is positioned in the dorsolateral SNpc. Based on this information, each whole SNpc ROI containing the entire nigrosome (d) and the dorsolateral SNpc ROI containing nigrosome 1 (e) was created. ROI, region of interest; SNpc, substantia nigra pars compacta.

In the measurements of the SNpc ROI values, the units for QSM value were parts per billion (ppb) and the units for the neuromelanin area were mm^3 . We adopted a neuromelanin analysis that measured the SNpc volume that had a higher signal-to-noise ratio (SNR) compared with the background region (tegmentum in the midbrain, which contains no neuromelanin). The SNR of the SNpc in the neuromelanin images was calculated based on the mean values of the automatically segmented background region.

Data analysis

In addition to both the QSM value and neuromelanin area in each SNpc ROI, we subjected the QSM value and the neuromelanin area to logistic regression analysis, established a logistic model, and optimized the diagnostic performance for PD using the QSM value and neuromelanin area. The binary response variable of interest was the diagnosis of PD (coded as 1 = yes and 0 = no). The logistic regression model aimed at predicting a transformation of the response variable, logit (p), was used. We assumed that p = the probability that the diagnosis was PD and $1 - p$ = the probability that the diagnosis was "control". The logistic regression model enables the prediction of the probability of PD in relation to the QSM value and neuromelanin area from an equation of the form: $\text{logit}(p) = \beta_0 + \beta_1 \times \text{neuromelanin area} + \beta_2 \times \text{QSM value}$, where β_0 is the intercept term and β_1

and β_2 are the coefficients in the model associated with the QSM value and neuromelanin area. We assumed that these data are linearly related to the log odds of the response. The odds ratio for PD vs HC can be estimated as the exponentiation of the associated coefficient in the model.²²

Using these data obtained from each SNpc ROI, the significance of any differences between the PD and HC groups was tested using the Mann-Whitney non-parametric U test. $p < 0.05$ was considered significant. A receiver operating characteristic (ROC) analysis was performed to compare the diagnostic performance of the QSM value and neuromelanin area using each SNpc ROI and logit (p) with the aid of the MedCalc software (v. 13.0.4.0; MedCalc, Mariakerke, Belgium).

RESULTS

The QSM values and neuromelanin areas of the SNpc ROI are shown in Figure 2. In the whole SNpc ROI, the median QSM value in the PD group (87.67 ± 27.90 ppb) was significantly higher than that detected in the HC group (67.78 ± 24.48 ppb) ($p < 0.05$). The mean neuromelanin area in the PD group ($936.79 \pm 27.90 \text{ mm}^3$) was significantly lower than that observed for the HC group ($1003.77 \pm 42.59 \text{ mm}^3$) ($p < 0.05$). In the dorsolateral SNpc ROI, the median QSM value in the PD group (85.78 ± 28.45 ppb) was significantly higher than that in the HC

Figure 2. Comparison of the QSM values and neuromelanin areas in each whole and dorsolateral SNpc ROI between the PD and HC groups. * $p < 0.05$. In the whole SNpc ROI, the median QSM value in the PD group was significantly higher than that in the HC group ($p < 0.05$), and the mean neuromelanin area in the PD group was significantly lower than that in the HC group ($p < 0.05$). In the dorsolateral SNpc ROI, the median QSM value in the PD group was significantly higher than that in the HC group ($p < 0.05$), and the mean neuromelanin area in the PD group was significantly lower than that in the HC group ($p < 0.05$). HC, healthy controls; PD, Parkinson's disease; QSM, quantitative susceptibility mapping; ROI, region of interest; SNpc, substantia nigra pars compacta.

group (52.19 ± 23.01 ppb) ($p < 0.05$). The mean neuromelanin area in the PD group (434.02 ± 29.83 mm³) was significantly lower than that detected in the HC group (461.85 ± 21.29 mm³) ($p < 0.05$).

The ROC curves for the comparison between the whole SNpc ROI and the dorsolateral SNpc ROI in each QSM value and neuromelanin area are shown in Figure 3. For the QSM value, the area under the ROC curve distinguishing the PD and HC groups was 0.70 for the whole SNpc ROI and 0.73 for the dorsolateral SNpc ROI. For the neuromelanin area, the area under the ROC curve distinguishing the PD group from the HC group was 0.81 for the whole SNpc ROI and 0.78 for the dorsolateral SNpc ROI. The ROC curves for the QSM value obtained using the dorsolateral SNpc ROI, the neuromelanin area obtained using the whole SNpc ROI, and logit (p) are shown in Figure 4. The area

Figure 3. ROC curves for the QSM value and neuromelanin area in each whole SNpc ROI and dorsolateral SNpc ROI. The area under the ROC curve of the QSM value was higher for the dorsolateral SNpc ROI than for the whole SNpc ROI. The area under the ROC curve of the neuromelanin area was higher for the whole SNpc ROI than for the dorsolateral SNpc ROI. QSM, quantitative susceptibility mapping; ROC, receiver operating characteristic; ROI, region of interest; SNpc, substantia nigra pars compacta.

under the ROC curve for logit (p) in relation to the QSM value obtained using the dorsolateral SNpc ROI and the neuromelanin area obtained using the whole SNpc ROI was 0.86.

DISCUSSION

The onset of symptomatic PD is generally considered to become apparent when more than about 50% of neurons are lost in the SNpc.²³ Thus, even at the early stages of clinical symptoms, PD-related neurodegenerative changes in the SNpc can be substantially detected by MRI. Here, QSM and neuromelanin imaging using both the whole and dorsolateral SNpc ROIs significantly discriminated patients with early-stage PD from HC, which indicates that both MRI approaches may be applicable to the diagnosis of early-stage PD. Moreover, within the SNpc, the lateral part is most affected in PD, whereas the medial part is primarily affected, and the lateral part is relatively preserved, during healthy aging,^{23,24} which indicates that the analysis of nigral changes with a focus on their spatial differences within the SNpc of patients with PD can increase the sensitivity of detection. Previous studies that used QSM and neuromelanin imaging reported the utility of the lateral part of the SNpc for evaluating early-stage PD.^{10,25}

The comparison of the PD diagnostic performance of the whole SNpc ROI with that of the dorsolateral SNpc ROI performed in the present study showed that QSM was more sensitive to iron accumulation mainly in the dorsolateral SNpc, which contains nigrosome 1, whereas neuromelanin imaging was more sensitive to diffuse dopaminergic neuronal loss throughout the whole SNpc, which contains the entire set of nigrosomes. These findings contribute to the improvement of the diagnostic performance for PD. In particular, the identification of factors for a sensitive QSM analysis technique for measuring iron deposition in the SNpc of patients with PD will be invaluable for discriminating them from normal patients. Because the large standard deviation of the QSM value indicates a high iron variation within the SNpc in patients with PD and in HC, the QSM value clearly overlaps widely between the two groups, which makes it difficult to diagnose PD.

Figure 4. ROC curves for the QSM value in the dorsolateral SNpc ROI, the neuromelanin area in the whole SNpc ROI, and logit (p). The area under the ROC curve of logit (p) was higher than that of the neuromelanin area in the whole SNpc ROI, and was significantly higher than that of the QSM value in the dorsolateral SNpc ROI ($p < 0.05$) with the DeLong test. QSM, quantitative susceptibility mapping; ROC, receiver operating characteristic; ROI, region of interest; SNpc, substantia nigra pars compacta.

According to previous reports, although the imaging of nigro-some 1 is applicable in the diagnosis of early PD stages or the assessment of clinical symptomatic laterality with high accuracy and sensitivity,²⁶ it also requires an advanced image system, such as a high-field-strength imager; e.g. a 7 T imager^{18,27,28} or high-resolution susceptibility sensitive imaging, even by a 3 T imager.^{17,29} Thus, visual assessment of a dorsolateral nigral hyperintensity reflecting nigrosome 1 can provide excellent performance as a marker for PD.²⁷ This requires high sensitivity in delineating the iron-containing pathology for the detection of abnormalities in this region. Brain MRI with QSM for assessing PD is commonly performed on a 3 T MRI machine.⁶ QSM was generated from the complex (phase and magnitude) data obtained during the gradient-echo sequence using the morphology-enabled dipole inversion technique.⁷ The phase data from gradient-echo MRI is used to derive the local magnetic field; thus, QSM is highly sensitive to iron because of its magnetic susceptibility. A previous QSM study addressed iron variation within the SN and indicated the clinical application of QSM measurement for the detection of iron accumulation in the ventrolateral tier of the SN for diagnosing PD.¹⁰ When considering the neuropathological mechanisms, high concentrations of reactive iron can increase the oxidative-stress-induced loss of dopaminergic neurons in the SNpc, which has been postulated to underlie neurodegenerative disorders, including PD.^{30,31} By contrast, significant iron accumulation in certain brain regions, including the substantia nigra, could result from healthy aging.^{29,32} Both PD and healthy aging

cause iron accumulation in the SNpc^{30,31} and exhibit interindividual variation in the content of iron deposition in the SNpc. Thus, iron-sensitive MRI analysis with a focus on the dorsolateral SNpc, which contains nigrosome 1, is important for increasing diagnostic accuracy.

Neuronal loss was uneven across the different dopaminergic cell groups of the midbrain, both within individual cases of PD and across cases. These observations confirm the findings of earlier reports.^{33,34} Age-related dopaminergic cell loss has been found mainly in the dorsal part of the SN.²³ Hence, neuromelanin imaging with total SNpc volume measurements has shown high diagnostic power,¹⁴ consistent with our results. Neuromelanin MRI studies have reported significant reductions in measures of voxel intensity, area, and volume of the total SNpc in patients with PD.^{25,35–37} Moreover, neuropathology reports have indicated that the spatial patterns of iron accumulation and dopaminergic neuron loss within the SNpc during the PD-related neurodegenerative process occur in parallel. Dopaminergic neuronal loss is highest in the nigrosome 1 region within the dorsolateral SNpc, and then spreads to other nigrosomes and the matrix within the SNpc during PD pathogenesis.¹⁶ This pattern of selective degeneration of nigrostriatal neurons is not observed during healthy aging.³⁸ Nigrosome 1 is reported to be the most sensitive to degeneration in patients with PD,^{16,17} whereas our results of neuromelanin imaging indicated that compared with the dorsolateral SNpc ROI, which contains mainly nigrosome 1, the whole SNpc ROI, which contains the entire nigrosome, is slightly superior in diagnostic performance for early-stage PD. We propose two explanations for this result. One is technical: paramagnetic T_1 shortening effects of iron deposition may affect and increase the neuromelanin signal in the dorsolateral SNpc. However, a previous neuromelanin MRI study reported that the paramagnetic iron in neuromelanin does not have significant effects on T_1 weighting in early-stage PD.⁷ The other explanation pertains to the pathogenic mechanism of PD. It seems likely that depletion is maximum in the nigrosome 1 at first, but subsequent nerve cell loss has been observed for dopamine-containing neurons in the whole SNpc, including all nigrosomes and the matrix.¹⁶ Moreover, neuromelanin imaging can quantify the loss of dopaminergic neurons directly, and its sensitivity to nigral changes is originally high.¹³ Thus, neuromelanin imaging evaluation covering the whole SNpc can improve the diagnostic performance in the symptomatic stage of PD.

Logit (p), which was calculated for achieving the best diagnostic performance using both QSM and neuromelanin imaging, revealed that the QSM value obtained using the dorsolateral SNpc ROI and the neuromelanin area obtained using the whole SNpc ROI were the optimal combination to provide a synergistic effect for increasing diagnostic performance. This result underscored the importance of knowing each technical characteristic that corresponds to nigral changes.

Limitations

There were some problems regarding the voxel-based morphometric technique. First, the presence of a coregistration error of QSM occurred in some cases during the image analysis process.

As one possible cause, image distortion of the brainstem region in QSM might occur based on the susceptibility effect in the skull-base region because of the nearby air-containing sinus. Therefore, we checked whether the image overlays between QSM and neuromelanin were produced correctly in all cases. When such image overlay errors occurred, we manually adjusted the image overlay in a visual manner. By contrast, coregistration errors of neuromelanin images did not occur in any of the cases. In addition, all images were spatially normalized to the standard Montreal Neurological Institute space, which might distort the small SNpc. In the subsequent step, images were smoothed to compensate for the inexact nature of the anatomical standardization by which voxels of the SNpc were averaged with their neighboring brainstem regions. Serial Statistical Parametric Mapping preprocessing steps might decrease the SNR and blur the boundaries of the SNpc. A general problem that may arise in voxel-based analysis of the brainstem region is the relatively small size of the smoothing kernel that is needed to obtain significant

results. We have chosen a Gaussian kernel of 4 mm half width at half maximum by reference to a proposed theory in brainstem MRI studies.^{39,40} Few studies have used a voxel-based approach to analyze QSM; therefore, further studies are necessary to identify the optimal conditions for this approach.

CONCLUSION

Both neuromelanin imaging and QSM may be useful in the diagnosis of early-stage PD. Moreover, quantifying nigral changes with a focus on their spatial differences within the SNpc can provide a high diagnostic potential for early-stage PD.

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