Increase in the Iron Content of the Substantia Nigra and Red Nucleus in Multiple Sclerosis and Clinically Isolated Syndrome: A 7 Tesla MRI Study

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Purpose: To study iron deposition in the substantia nigra (SN) and red nuclei (RN), in patients with clinically isolated syndrome (CIS) and relapsing remitting MS (RRMS) and healthy controls (HC).

Materials and Methods: Iron deposition was assessed using susceptibility maps and T2*-w images acquired at high resolution MRI at 7 Tesla (T). Mean intensities were calculated within circular regions of interest in the SN (d/v, dorsal/ventral) and RN on high resolution T2*-w, quantitative susceptibility maps and their product for: RRMS, CIS and HC (N = 14, 21, 27, respectively).

Results: Magnetic susceptibility was significantly greater in SNd and RN in RRMS compared with HC (P = 0.04 [0.001, 0.48] and P = 0.01 [0.005, 0.05]), with intermediate values for the CIS group. 1/T2*-w did not show significant inter-group differences (for SNv, SNd, RN, respectively: P = 0.5 [−0.352, 0.976], P = 0.35 [−0.208, 0.778], P = 0.16 [−0.114, 0.885] for RRMS versus HC) and the T2*-susceptibility product maps showed the difference only for RN (P = 0.01, [0.009, 0.062]). Changes were independent of EDSS and disease duration.

Conclusion: MR changes consistent with iron accumulation occurring in the SN and RN of CIS patients can be identified using susceptibility mapping; this may provide an additional method of monitoring early MS development.

Key Words: multiple sclerosis; clinically isolated syndrome; magnetic resonance imaging; gray matter; substantia nigra; red nucleus

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nucleus, globus pallidus, and dentate nucleus (10,16,21,22). However results of iron deposition in the substantia nigra (SN) and red nucleus (RN) remain inconsistent: while some studies reported a difference between MS and healthy subjects in both structures (8,11,12), others found increased iron only in the SN (23,24) or only in the RN (6) and some studies reported no difference in either (5,9). The brainstem is commonly involved in MS and is associated with poor prognosis possibly due to its engagement in motor control (20,25). Lesions of the RN including demyelinating plaques within it or affecting its connection to the cerebellum may cause a characteristic rubral tremor (26). In addition, involvement of the SN in MS has been demonstrated through the detection of lesions on transcranial sonography (27) and decreased levels of proteins associated with myelin, neuronal development, and axonal growth indicating demyelination of the SN (24).

In clinically isolated syndrome (CIS, a condition indicating the earliest clinical stage of demyelinating disease), previous MRI studies of iron accumulation in dGM structures often excluded the SN and RN (8,10). We have recently reported significant differences in susceptibility mapping based measures of iron content of dGM structures (caudate nucleus, putamen, globus pallidus, thalamus, and pulvinar) between CIS patients and healthy controls, but failed to include the brain stem regions in that study (21).

Here, we extend a previous study to investigate changes in iron deposition in the SN and RN in two groups of patients, with relapsing-remitting MS (RRMS) and CIS, hypothesizing that iron deposition in the SN and RN might ultimately provide a marker of the progression of CIS to MS. The aim of this work was to assess iron content in the SN and RN of patients with CIS and RRMS compared with healthy controls (HC), using susceptibility mapping (8) which is sensitive to absolute iron content and T2*-weighted signals which depend on the compartmentalization of iron. We acquired the data using a 7 Tesla (T) MR scanner with 16-channel receive coil and head-only volume transmit coil. A T2*-weighted three-dimensional (3D) GRE sequence was acquired with repetition time/echo time (TR/TE) = 150/20 ms, flip angle 14°, SENSE factor 2, echo planar imaging (EPI) factor 3, number of acquisitions 1, scan time 8.5 min. Four interleaved stacks parallel to the AC-PC line with coverage 192 × 164 × 25 mm were acquired for each scan, where the overlap between stacks was 5 mm. Spatial resolution of the data was 0.5 × 0.5 × 0.5 mm.

To overcome the problem of nonlocal effects in phase maps, susceptibility maps were generated from the phase data using the thresholded k-space division method (17). For each subject, the five slices of each T2*-w image in which both structures were most visible were chosen and then circular regions of interest (ROIs) of diameter 3 mm were drawn by a physicist trained in the dGM anatomy using ITK-Snap software (http://www.itksnap.org), separately on the left and right RN, ventral and dorsal SN (Fig. 1). All of the SN ROIs were positioned to avoid touching ventral and medial boundaries of the structure. Two further ROIs of 5 mm diameter were placed on the white matter (WM) in the same slices and used to provide reference values for the susceptibility measurements.

Matlab (The MathWorks Inc., 2010) was used for further processing of the data. Mean intensities were measured on T2*-w modulus images and susceptibility maps for SNv, SNd, and RN ROIs, and the values obtained from the left and right sides were averaged together. The results were normalized using the mean intensity of the WM region, taking the difference between the ROI and WM region for susceptibility and ratio of the ROI to the WM region for T2*-w. Moreover the product of the negative logarithm of the normalized T2*-w signal (which should be linear in 1/T2* and hence iron concentration) and normalized susceptibility signal (Susceptibility-T2* product) was determined for each structure, to increase sensitivity in a similar manner to the SWI (by combining magnitude and phase information which are independent physical measures but have reinforcing effects in the

**MATERIALS AND METHODS**

Fourteen patients with relapsing-remitting multiple sclerosis (RRMS: age = 42.4 ± 11.3; range, 20–56 years old) and 21 with clinically isolated syndrome (CIS: age = 37.2 ± 8.8; range, 24–57 years old) together with 27 age-matched healthy controls (HC; age = 36.4 ± 8.8; range, 21–58 years old) were recruited from Nottingham University Hospital in accordance with local ethics committee approval. Expanded Disability Status Scale (EDSS) assessment giving values between 0 and 10 (death due to MS) was performed by a trained physician for all of the patients.

MR images were acquired on a 7 T Philips Achieva scanner with 16-channel receive coil and head-only volume transmit coil. A T2*-weighted three-dimensional (3D) GRE sequence was acquired with repetition time/echo time (TR/TE) = 150/20 ms, flip angle 14°, SENSE factor 2, echo planar imaging (EPI) factor 3, number of acquisitions 1, scan time 8.5 min. Four interleaved stacks parallel to the AC-PC line with coverage 192 × 164 × 25 mm were acquired for each scan, where the overlap between stacks was 5 mm. Spatial resolution of the data was 0.5 × 0.5 × 0.5 mm.

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Normality was checked using the Kolmogorov-Smirnov test. One-way analysis of variance (ANOVA) tests with the Tukey-Kramer correction for multiple comparisons were performed to examine differences in susceptibility and $1/T2^*$-w values between the CIS, RRMS patients, and HC for each structure. Left and right parts of the structures were also compared using one-way ANOVA. Scatter plots of susceptibility and $1/T2^*$-w signal were overlaid with ellipses indicating the HC, CIS and RRMS population distributions ($\pm 1.5$ SD). Dependence of the $1/T2^*$-w, susceptibility and product values on age was tested for healthy controls and dependence on the EDSS score and disease duration for the CIS and RRMS patients.

RESULTS

Mean EDSS results obtained for the two groups of patients were $2.8 \pm 0.7$ for RRMS and $1.6 \pm 1.0$ for CIS.

There was a trend for susceptibility in all of the structures to increase between HC, CIS, and RRMS patients (Fig. 2a) and the differences between HC and RRMS in SNd and RN were significant ($P = 0.04$, confidence intervals [0.001, 0.048] and $P = 0.01$ [0.005, 0.05] for SNd and RN, respectively). No difference was seen in SNv although this may be because the data for this region were more scattered. The $1/T2^*$-w signal in both parts of the SN and RN was unchanged for CIS patients compared with HC and higher for RRMS patients, although differences were not significant (for SNv, SNd, RN: $P = 0.99$, $P = 0.6$, $P = 0.84$, confidence intervals $[-0.55, 0.60]$, $[-0.60, 0.26]$, $[-0.54, 0.33]$ for HC versus CIS and $P = 0.5$, $P = 0.35$, $P = 0.16$, $[-0.35, 0.98]$, $[-0.21, 0.78]$, $[-0.11, 0.89]$ for HC versus RRMS, Fig. 2b). The Susceptibility-$T2^*$ product increased the separation of RRMS versus HC ($P = 0.01$, [0.009, 0.062]) and RRMS versus CIS groups ($P = 0.03$, [0.003, 0.057]) in RN; however, the difference between the groups for SNd was no longer significant ($P = 0.06$, [-0.001, 0.057]) for RRMS versus HC and $P = 0.31$ [-0.012, 0.048] for RRMS versus CIS).

Scatter plots in Figure 3 show that there is relatively weak correlation between susceptibility and $1/T2^*$-w signal in all ROIs for all subject groups (correlation coefficients: 0.16, 0.34, 0.44 for SNv, SNd, and RN, respectively).

Figure 2. Mean susceptibility (a), $1/T2^*$-w (b), and product (c) in SNv (○), SNd (×), and RN (△) for HC, CIS, and RRMS patients; mean values calculated within each group plotted in black and connected by lines. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
Susceptibility, 1/T2*-w, and the product did not show a significant correlation either with the patients’ EDSS score (correlation coefficients and P-values for SNv, SNd, RN: 0.05, 0.05, 0.21, and 0.77, 0.77, 0.23 for susceptibility, 0.21, 0.31, 0.20, and 0.23, 0.07, 0.26 for 1/T2*-w and 0.1, 0.16, 0.23, and 0.46, 0.36, 0.23 for product) or disease duration as well as with age for HC (correlation coefficients and P-values for SNv, SNd, RN: −0.01, −0.15, 0.11 and 0.98, 0.48, 0.62 for susceptibility, 0.30, 0.15, 0.39, and 0.17, 0.51, 0.07 for 1/T2* and 0.09, −0.05, 0.25, and 0.69, 0.82, 0.25 for product) and no significant differences between the left- and right side- structures were found (Fig. 4).

**DISCUSSION**

Changes consistent with increased iron content in the SNd and RN were found in RRMS patients compared with HC. There was a trend for the CIS patients to have higher values than HC in all three ROIs, although the differences were not significant. These results suggest a relationship between iron deposition and progression from CIS to RRMS, although because this was a cross-sectional study, it is not possible to be certain if this relates directly to conversion to RRMS. These results concord with previous work applying the same quantitative susceptibility mapping method to the other dGM structures for the same CIS patients (21). The combined measure, Susceptibility-T2* product, was affected by the difference in trends occurring in 1/T2*-w signal and susceptibility between HC and patients groups. The values of this product increased the separation of the RRMS and HC groups in comparison to susceptibility, but did not show significant differences for the CIS versus HC (as may be expected from the results on 1/T2*-w alone). However, there remained considerable overlap between the three groups for all measures, with similar spread observed for the healthy controls as for both patient groups. Nonetheless, the combined measure of iron concentration could have an advantage over susceptibility alone in monitoring CIS and MS patients.

The effects of iron accumulation on susceptibility maps and T2*-w images are different. Susceptibility will depend on the total iron content within a voxel (13). On the other hand T2*-w signal decay will depend on both the total iron content of the tissue and also its distribution (i.e., whether or not the iron is aggregated into “clumps”). Therefore, a trend for susceptibility to change earlier in demyelinating diseases (CIS) than T2*-w might suggest that iron accumulation is uniform initially but becomes more heterogeneous later. However, susceptibility and T2*-w signal also depend on myelin content (13); thus, demyelination may also explain the observed differences in the changes in T2*-w and susceptibility between groups. Myelin is diamagnetic (negative susceptibility), whereas iron is paramagnetic (positive susceptibility). Hence, both demyelination and increased iron content would be manifested as a net increase in susceptibility. In contrast, myelin generally reduces T2 so demyelination will cause a decrease in 1/T2*-w where increased iron will cause an increase in 1/T2*-w. Further work is required to separate these effects but either way the early changes may make susceptibility mapping potentially useful in the future for monitoring progression of CIS.

We have recently reported that the nigrosome 1, the biggest of five subgroups of dopaminergic neurons located in medial part of the SN previously identified in histology (29), is visible on 7T MRI (30) of healthy controls but not of Parkinson’s disease (PD) patients (30,31). Post hoc examination of the data acquired here found no change in frequency of nigrosome detection between RRMS and CIS patients and controls.

Interestingly, in our study greater differences in susceptibility were found in SNd compared with SNv. Variation across the SN may partially explain the lack of consistency in previous studies using images acquired at lower field strengths, as high resolution imaging at

![Figure 4. Values of susceptibility (a), 1/T2*-w (b), and product (c) in SNv (×), SNd (x), and RN (+) plotted against their EDSS scores together with lines of the best fit. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]](image)
Iron Increase in the SN & RN in MS & CIS

7T allowed specific ROIs to be defined within the SN. It could also suggest a similar pattern of the iron accumulation in SN to that previously found in Parkinson’s disease in histology (28) and T2*-w images (32) which is in agreement with the gradient of neurodegeneration found in SN in PD patients using MRI (33). Larger scatter of the SNv values compared with SNd can be possibly explained by the individual differences in the vasculature within that region.

In PD, it has previously been shown in histology (29) and in vivo MRI (30,31) that iron is preferentially deposited in nigrosome 1 and that nigrosome 1 cannot be detected in vivo in patients. On the other hand, the presence of nigrosome 1 at the same rate in both healthy subjects and MS patients does not support that hypothesis, as a previous study showed the absence of this structure in Parkinson’s patients (30).

The lack of variation in iron content observed over the age range used in our study (20–58 years) agrees with previous results that suggested significant changes only in the first 2 decades (34). As in previous studies of dGM iron in MS patients, we also found no correlation between iron and EDSS, possibly due to a relatively low range of EDSS scores (8,9).

The quantitative susceptibility mapping used in this work is a robust method providing a more direct measure of the spatial iron distribution by the actual susceptibility values overcoming the problem of nonlocal relationship between the field distortion (and hence phase of the magnetization) and susceptibility (17). Although the susceptibility maps are more sensitive to noise, the increase of the signal-to-noise ratio in the images obtained from 7T magnetic field is sufficient to acquire good quality data. The need for a reference region will increase scatter in the data, and could potentially bias the results if there were a systematic change in the WM. However, as cerebrospinal fluid is affected by flow, WM is the most robust choice of normalization region.

Limitations of this study include the low number of subjects, especially considering certain level of intragroup heterogeneity within the patients groups. Further work should also include longitudinal analysis taking into account conversion from CIS to RRMS on the later stages of the disease. Quantitative measurement of R2* values (to replace 1/T2*-w used in this work) would give a more accurate insight in changes of iron concentration in the analyzed structures, and additional measures such as R2, the field-dependent rate increase technique, or magnetization transfer could provide further information about the changes occurring.

In this study we used quantitative susceptibility mapping to analyze iron accumulation in SN and RN in CIS and RRMS patients. Future work will include R2* and magnetization transfer measurements in longitudinal studies, will investigate whether these measures can predict conversion from CIS to RRMS.

ACKNOWLEDGMENTS

Grant support: Morris, Bowtell, Francis, Glover, Gowland, Kockenberger, Auer, Jackson, Krumbholz, Lid-


