Iron Deposition on SWI-Filtered Phase in the Subcortical Deep Gray Matter of Patients with Clinically Isolated Syndrome May Precede Structure-Specific Atrophy

BACKGROUND AND PURPOSE: Increasing evidence suggests that iron deposition is present in the later stages of MS. In this study we examined abnormal phase values, indicative of increased iron content on SWI-filtered phase images of the SDGM in CIS patients and HC. We also examined the association of abnormal phase with conventional MR imaging outcomes at first clinical onset.

MATERIALS AND METHODS: Forty-two patients with CIS (31 female, 11 male) and 65 age and sex-matched HC (41 female, 24 male) were scanned on a 3T scanner. Mean age was 40.1 (SD = 10.4) years in patients with CIS, and 42.8 (SD = 14) years in HC, while mean disease duration was 1.2 years (SD = 1.3) in patients with CIS. MP-APT, NPTV, and normalized volume measurements were derived for all SDGM structures. Parametric and nonparametric group-wise comparisons were performed, and associations were determined with other MR imaging metrics.

RESULTS: Patients with CIS had significantly increased MP-APT (P = .029) and MP-APT volume (P = .045) in the pulvinar nucleus of the thalamus compared with HC. Furthermore, the putamen (P = .004), caudate (P = .035), and total SDGM (P = .048) displayed significant increases in MP-APT volume, while MP-APT was also significantly increased in the putamen (P = .029). No global or regional volumetric MR imaging differences were found between the study groups. Significant correlations were observed between increased MP-APT volumes of total SDGM, caudate, thalamus, hippocampus, and substantia nigra with white matter atrophy and increased T2 lesion volume (P < .05).

CONCLUSION: Patients with CIS showed significantly increased content and volume of iron, as determined by abnormal SWI-phase measurement, in the various SDGM structures, suggesting that iron deposition may precede structure-specific atrophy.

ABBREVIATIONS: CIS = clinically isolated syndrome; EDSS = Expanded Disability Status Scale; ETL = echo-train length; FIRST = fMRI-integrated registration and segmentation tool; Gd = gadolinium; GM = gray matter; HC = healthy controls; LV = lesion volume; MP-APT = mean phase of the abnormal phase tissue; NBV = normalized brain volume; NGMV = normalized gray matter volume; NLMV = normalized lateral ventricle volume; NPTV = normal phase tissue volume; NWMV = normalized white matter volume; pFOV = phase FOV; RRMS = relapsing-remitting MS; SDGM = subcortical deep GM
form of ferritin, influence the frequency of proton spin and cause local magnetic field changes.\textsuperscript{31} The SWI-filtered phase imaging method uses a complex-space high-pass filter to retain only biologically relevant phase shifts, to generate a metric of iron attenuation.\textsuperscript{23,31}

In this study, we compared SDGM measurements of abnormal phase, indicative of increased iron content, and normalized volume between patients with CIS and a group of HC. Moreover, we sought to investigate the relationship of these measurements with conventional MR imaging techniques.

**Materials and Methods**

**Subjects**

We prospectively enrolled 42 consecutive patients with CIS (31 women, 11 men). Inclusion criteria were a diagnosis of CIS,\textsuperscript{32} age 18–65 years, and disease duration between 0 and 5 years. Sixty-five age- and sex-matched HC were also prospectively enrolled, all of whom had normal MR imaging and physical examinations. Patients with CIS underwent full neurologic assessment, including determinations of EDSS.\textsuperscript{33} Participants were excluded if they had a relapse or were treated with steroids within the month preceding study entry, were pregnant, or had any pre-existing medical conditions known to be associated with brain pathology. The internal institutional review board approved the study protocol and written informed consent was obtained from all participants.

**Image Acquisition**

A 3T Signa Excite HD 12.0 (GE Healthcare, Milwaukee, Wisconsin) with an 8-channel head and neck (HDNV) coil was used for scanning purposes. SWI data were acquired using a 3D flow-compensated gradient recalled-echo sequence with 64 locations, 2 mm thickness, a $512 \times 192$ (frequency $\times$ phase) matrix, FOV = 25.6 cm $\times$ 19.2 cm, with $p$FOV = 0.75, resulting in an in-plane resolution of 0.5 mm $\times$ 1 mm. Other relevant parameters included flip angle = 12°, TE = 22 ms, TR = 40 ms, and acquisition time = 8 min, 46 s.\textsuperscript{23}

In addition, we acquired the following sequences: 2D planar dual FSE proton attenuation and T2-weighted imaging (TE1/TE2/ TR = 9/98/5300 ms, flip angle = 90°, and ETL = 14; FLAIR (TE/TR = 120/8500 ms, TI = 2100 ms, flip angle = 90°, ETL = 24); 3D high-resolution T1WI using fast-spoiled gradient echo with magnetization-prepared inversion recovery pulse (TE/TI/TR = 2.8/900/5.9 ms, flip angle = 10°); and spin-echo T1WI (TE/TR = 16/600 ms, flip angle = 90°). All sequences except SWI were obtained with a $256 \times 192$ matrix, FOV = 25.6 cm $\times$ 19.2 cm, pFOV = 0.75, for an in-plane resolution of 1 mm $\times$ 1 mm. Forty-eight sections were collected, resulting in isotropic resolution. All scans were prescribed in an axial-oblique orientation, parallel to the subcallosal line. One average was used for all sequences.

**Image Analyses**

Analyses were performed by operators who were unaware of the patients’ disease status.

**Abnormal Phase Identification**

Raw (k-space) SWI data were transferred to an off-line Linux workstation for postprocessing using software developed in-house and written in Matlab (MathWorks, Natick, Massachusetts). A detailed overview of SWI image acquisition, processing, analysis, and validation is provided elsewhere.\textsuperscript{23}

To segment SDGM structures, a combination of semi-automated edge-contouring and FMRIB’s FIRST (http://www.fmrib.ox.ac.uk/fsl/downloads/) on 3D T1 WI was used.\textsuperscript{34} Specifically, the thalamus, caudate, putamen, globus pallidus, hippocampus, amygdala, and nucleus accumbens were identified in this way.\textsuperscript{35} Structures not identifiable by FIRST, such as the red nucleus, pulvinar nucleus of the thalamus, and substantia nigra, were identified semi-automatically using JNMS (Xinapse Systems, Northamptonshire, United Kingdom) on the most representative section for each subject.\textsuperscript{35}

Using the SWI-filtered phase images, additional processing was performed to identify voxels likely to contain abnormal amounts of iron, based on their phase values. Reference phase values (means and SD) for each SDGM structure were determined previously using a large sample of HC.\textsuperscript{23} Only the voxels of SDGM structures with phase values lower than 2 standard deviations below the reference group were retained. This yielded structure-specific maps of voxels with abnormally low phase. Subsequently, as a measure of the level of phase decrease, the mean value of subthreshold voxels was calculated to yield the MP-APT. More negative MP-APT values indicate increased iron content within that region, and mean values are reported in radians. In addition, we determined the SDGM NPTV by subtracting the abnormal phase tissue volume of a particular structure from the total volume of the same structure.

**Global Atrophy and Lesion Analyses**

The SIENAX cross-sectional software tool (version 2.6; http://www.fmrib.ox.ac.uk/fsl/feeds/doc/index.html) was used for brain extraction and tissue segmentation, with correction for T1-hypointensity misclassification.\textsuperscript{26} As described previously,\textsuperscript{37} we acquired the following volume measures: NBV, NGMV, NWVM, and NLV. T2- and Gd-LVs and numbers were measured using a semi-automated edge detection contouring/thresholding technique, as described previously.\textsuperscript{36} Normalized volumes were obtained for all SDGM structures using FIRST.\textsuperscript{35}

**Statistical Analysis**

Analyses were conducted using PASW Statistics, version 18.0 (IBM, Somers, New York). Differences between the groups were tested using the $\chi^2$-test (sex) and Student $t$ test. Distributions of the data were tested for normality using the Shapiro-Wilk test. Because SDGM MP-APT, MP-APT volume, and NPTV were not normally distributed ($P < .001$), the Mann-Whitney $U$ test was used to evaluate group differences of these variables. SDGM volume measurements were compared using the Student $t$ test. Effect sizes are stated as Cohen $d$. We used Spearman correlation coefficients to assess the relationship between SDGM MP-APT, MP-APT volume, NPTV, and normalized volume with other MR imaging variables. Nominal $P$ values < .05 were regarded as significant, using 2-tailed testing, and $P$ values < .10 were considered a trend.

**Results**

Table 1 shows the demographic, clinical, and MR imaging characteristics of patients with CIS and HC. The mean age of patients with CIS was 40.1 years (SD = 10.4), with a mean disease duration of 1.2 years (SD = 1.3) and median EDSS score of 1.0 (range = 0–2.5). Thirty-one (73.8%) patients with CIS were female. Of the 42 patients with CIS, 22 (52.4%) were...
on disease-modifying therapy and 35 (83.3%) had a monosymptomatic onset. No significant differences were observed in age, sex, or atrophy measures between patients with CIS and HC. Of the 42 patients with CIS, 33 presented with brain T2 lesions and 9 with spinal cord T2 lesions. Of those patients with CIS and HC in the putamen (P = .004), caudate nucleus (P = .035), pulvinar nucleus of the thalamus (P = .045), and total SDGM (P = .048).

Table 3 shows differences in MP-APT volume between patients with CIS and HC. A significantly increased volume of the MP-APT was observed in patients with CIS compared with HC in the putamen (P = .004), caudate nucleus (P = .035), pulvinar nucleus of the thalamus (P = .045), and total SDGM (P = .048).

Tables 4 and 5 show normalized volume and NPTV differences between patients with CIS and HC. No significant differences between the study groups were detected. The differences between the groups were tested using the Mann-Whitney U test.

Table 3: Differences in MP-APT volume between patients with CIS and HC

| MP-APT Volume | HC | CIS | d | % Difference | P Value |
|---------------|----|-----|---|-------------|--------|
| Total SDGM   | 14.33 (2.07) | 15.21 (1.96) | .44 | 6.1 | .048 |
| Caudate      | 2.33 (5.4) | 2.6 (1.59) | .5 | 11.6 | .035 |
| Putamen      | 2.64 (8) | 3.1 (1.79) | .58 | 17.4 | .004 |
| Globus pallidus | 1.33 (25) | 1.37 (29) | .16 | 3 | .396 |
| Thalamus     | 6.43 (84) | 6.51 (87) | .09 | 1.2 | .721 |
| Hippocampus  | 1.15 (59) | 1.12 (48) | .04 | 2.6 | .861 |
| Amygdala     | .37 (2) | .41 (21) | .23 | 10.8 | .118 |
| Nucleus accumbens | .08 (9) | .08 (11) | .02 | 2.9 | .630 |
| Red nucleus  | .02 (0) | .03 (02) | .18 | 15.2 | .255 |
| Substantia nigra | .07 (03) | .07 (03) | .12 | 5.9 | .595 |
| Pulvinar     | .03 (05) | .11 (04) | .4 | 19.6 | .045 |

Note:—All values are reported in milliliters. Differences between groups were tested using the Mann-Whitney U test.

Table 4: Differences in the SDGM normalized volumes between patients with CIS and HC

| Volume | HC | CIS | d | % Difference | P Value |
|--------|----|-----|---|-------------|--------|
| Total SDGM | 45.22 (4.59) | 45.43 (4.1) | .2 | 0.5 | .818 |
| Caudate | 6.75 (99) | 6.89 (89) | .16 | 2.1 | .437 |
| Putamen | 9.59 (12) | 9.71 (11) | 1 | 1.1 | .617 |
| Globus pallidus | 3.52 (4) | 3.44 (35) | 2 | 2.3 | .329 |
| Thalamus | 15.21 (154) | 15 (153) | .12 | 1.4 | .550 |
| Hippocampus | 7.86 (74) | 7.76 (74) | .2 | 2.3 | .324 |
| Amygdala | 2.38 (36) | 2.38 (35) | .01 | 0 | .963 |
| Nucleus accumbens | .79 (18) | .82 (17) | .18 | 3.8 | .400 |
| Red nucleus | .17 (02) | .17 (03) | .04 | 0 | .870 |
| Substantia nigra | .29 (05) | .32 (05) | .6 | 10.3 | .003 |
| Pulvinar | .43 (07) | .44 (07) | .2 | 2.3 | .619 |

Note:—All values are reported in milliliters. Differences between groups were tested using the Student t test.

Table 5: Differences in the NPTV between patients with CIS and HC

| NPTV | HC | CIS | d | % Difference | P Value |
|------|----|-----|---|-------------|--------|
| Total SDGM | 20.89 (2.56) | 30.12 (3.33) | .2 | 2.5 | .203 |
| Caudate | 4.42 (65) | 4.29 (81) | .15 | 2.9 | .556 |
| Putamen | 6.95 (125) | 6.16 (26) | .27 | 5 | .189 |
| Globus pallidus | 2.2 (33) | 2.08 (36) | .34 | 5.5 | .099 |
| Thalamus | 8.77 (97) | 8.51 (93) | .27 | 3 | .120 |
| Hippocampus | 5.85 (88) | 6.04 (8) | .22 | 3.2 | .309 |
| Amygdala | 2.37 (197) | 1.97 (39) | .11 | 1.5 | .415 |
| Nucleus accumbens | .71 (19) | .73 (19) | .15 | 2.8 | .623 |
| Red nucleus | .15 (03) | .14 (03) | .08 | 6.7 | .346 |
| Substantia nigra | .23 (06) | .26 (06) | .41 | 13 | .048 |
| Pulvinar | .33 (07) | .32 (09) | .14 | 3 | .356 |

Note:—All values are reported in milliliters. Differences between groups were tested using the Mann-Whitney U test.

Table 1: Demographic, clinical, and MRI characteristics of the study groups

| Characteristic | HC (n = 65) | CIS (n = 42) | P value |
|---------------|-------------|-------------|--------|
| Female (%)    | 41 (63.1) | 31 (73.8) | .295 |
| Age in years (mean [SD]) | 42.8 (14) | 40.1 (10.4) | .288 |
| Age at onset in years (mean [SD]) | NA | 38.8 (10.8) | NA |
| Disease duration in years (mean [SD]) | NA | 1.2 (1.3) | NA |
| EDSS (median [range]) | NA | 1.0 (0–2.5) | NA |
| Presence of disease-modifying therapy (%) | NA | 22 (52.4%) | NA |
| Type of onset (%) | NA | NA | NA |
| Symptomatic | 7 (16.7%) | NA | NA |
| Monosymptomatic | 35 (83.3%) | NA | NA |
| Spinal cord syndrome | 23 (65.7%) | NA | NA |
| Optic neuritis | 8 (22.9%) | NA | NA |
| Brain stem/cerebellar | 4 (11.4%) | NA | NA |
| Gd lesion number (mean [SD]) | NA | 4 (1.1) | NA |
| T2 lesion number (mean [SD]) | NA | 12.3 (15.3) | NA |
| 1–4 lesions (%) | NA | 15 (35.7%) | NA |
| 5–8 lesions (%) | NA | 6 (14.3%) | NA |
| ≥9 lesions (%) | NA | 18 (42.9%) | NA |
| Gd lesion volume (mean [SD]) | NA | 0.8 (1.3) | NA |
| T2 lesion volume (mean [SD]) | NA | 1.7 (2.6) | NA |
| NMGV (mean [SD]) | 822.3 (63.2) | 821.5 (58.2) | .953 |
| NWMV (mean [SD]) | 775.3 (63.3) | 760.6 (40.2) | .154 |
| NIV (mean [SD]) | 1592.9 (95.6) | 1582.5 (56.5) | .321 |
| NLV (mean [SD]) | 27.9 (13.7) | 32.6 (18.9) | .145 |

Note:—All lesion and brain volumes are expressed in milliliters. Differences between groups were tested using the Mann-Whitney U test.
CIS, compared with HC ($P = .003$), and had higher NPTV ($P = .048$).

To investigate the differences in patients with CIS, they were further divided into subgroups according to median T2-LV, T2 lesion number ($\geq 9$ and $< 9$), polysymptomatic versus monosymptomatic onset, different subtypes of monosymptomatic onset, and therapy status. No significant differences were found for any of these comparisons.

Increased MP-APT volume of the thalamus was related to increased T2-LV ($P = .033$). Furthermore, significant correlations were observed between the decreased NWMV measurements, with increased MP-APT volume in total SDGM, caudate, thalamus, hippocampus, and substantia nigra ($P < .05$). Decreased SDGM volume measurements in total SDGM, caudate nucleus, putamen, globus pallidus, thalamus, hippocampus, and red nucleus volume yielded significant relationships with decreased NWMV ($P < .01$). Moreover, increased T2-LV was inversely related to the decreased volume of the thalamus and the pulvinar nucleus of the thalamus ($P < .05$). Decreased NPTV of total SDGM, thalamus, the pulvinar nucleus of the thalamus, and hippocampus was also strongly related to decreased NWMV ($P < .01$).

No significant relationship was found between increased MP-APT or MP-APT volumes and decreased normalized volumes in the same individual SDGM structures.

**Discussion**

In this study, we investigated the extent of iron deposition using SWI-filtered phase measurements in the SDGM of patients with CIS and HC. We observed increased abnormal phase and abnormal phase volume in the SDGM of patients with CIS, compared with age- and sex-matched HC. Significant increases were observed in the total SDGM, caudate nucleus, putamen, and pulvinar nucleus of the thalamus. However, no differences were detected in volumetric measures of the same regions between the 2 study groups. These findings suggest that increased iron deposition in the SDGM structures may precede structure-specific atrophy development.

Iron deposition in the brain parenchyma of patients with MS has been observed using histopathologic techniques. More recently, MR imaging techniques, such as T2 relaxometry, T2 hypointensity, magnetic field correlation, and SWI, have been used to quantify and visualize iron deposition in vivo. Looking at changes in T2 intensity is a relatively straightforward method of evaluating iron content, because increased levels of iron result in reduced T2 relaxation time, leading to hypointensity on T2-weighted images. In MS, SDGM T2 hypointensity was found to be a predictor of clinical progression, ambulatory impairment, cognitive impairment, and brain atrophy. SDGM T2 hypointensity has also been observed in patients with benign MS. There is increasing evidence that iron deposition is also present in early stages of MS, such as in patients with pediatric MS and CIS. Cerebellar atrophy, and brain atrophy. SDGM T2 hypointensity has also been observed in patients with benign MS. There is increasing evidence that iron deposition is also present in early stages of MS, such as in patients with pediatric MS and CIS. Cerebellar atrophy, and brain atrophy. SDGM T2 hypointensity has also been observed in patients with benign MS. There is increasing evidence that iron deposition is also present in early stages of MS, such as in patients with pediatric MS and CIS. Cerebellar atrophy, and brain atrophy. SDGM T2 hypointensity has also been observed in patients with benign MS.

It was recently reported that iron deposition is dominant in the pulvinar nucleus of the thalamus in a sample of adult patients with MS aged 19–35 years. In addition to changes in the basal ganglia, we also found significant increases of pulvinar MP-APT and MP-APT volume, suggesting that this structure may be exceptionally prone to iron accumulation in MS. Because iron accumulation is observed in the pulvinar nucleus of the thalamus in patients with CIS, it stands to reason that it may be one of the earliest affected SDGM structures.

An important finding of our study was that, with exception of the substantia nigra, no SDGM volume loss was observed in patients with CIS. Moreover, global and region-specific vol...
T2-LV measurements were also similar between the 2 study groups. In contrast, significant increases of MP-APT and MP-APT volume were observed in the SDGM of patients with CIS. Until now, it has been unclear whether iron deposition is merely an epiphenomenon of MS pathology or if it is an instigator of inflammation and disease development.19,51 In a recent study, Williams et al investigated the relationship between inflammation and iron deposition using an original animal model labeled as "cerebral experimental autoimmune encephalomyelitis," which develops CNS perivascular iron deposits.52 They reported that inflammatory cell infiltrates were associated with perivascular iron deposits; however, inflammatory cells were also observed without associated iron deposits. There was an association between T2-LV accumulation and increased MP-APT volume and decreased normalized volumes and NPTV in various SDGM structures, especially the thalamus and pulvinal nucleus of thalamus. Therefore, this study provides novel information indicating that iron deposition and atrophy development in basal ganglia are closely related to inflammation, as measured by T2 and Gd lesion accumulation.

The findings from this study also suggest that iron deposition may occur before detectable atrophy in the same structures. In fact, no significant relationship was found between increased MP-APT or MP-APT volumes and decreased normalized volumes in the individual SDGM structures. A previous study showed a direct relationship between increased MP-APT and structure-specific atrophy in different SDGM regions in the later stages of the disease.23 Therefore, future longitudinal studies should explore the interplay between iron deposition and atrophy development in individual SDGM structures from the first clinical onset.

Increased MP-APT, MP-APT volume, and decreased normalized volumes and NPTV in total SDGM, caudate, thalamus, pulvinal nucleus of the thalamus, putamen, globus pallidus, hippocampus, substantia nigra, and red nucleus were related with decreased NWMV but not with NGMV. These results indicate that there is a close relationship between iron deposition and WM, but not cortical GM atrophy development at the earliest stages of MS. These findings indicate that global GM atrophy may be preceded by inflammation and iron accumulation that is confined to SDGM and WM regions.

There are several potential limitations inherent in SWI-filtered phase imaging. Phase changes could be the result of changes to the underlying tissue structure or local iron distribution rather than absolute changes in iron content, and different chemical forms of iron could potentially have different SWI-phase effects.53 Patients with CIS were relatively older and had somewhat longer disease duration than those enrolled in typical clinical trials.54 Presumably, this was because some of the patients with CIS in our study were prospectively enrolled at the time of their referral to our MS specialty center. This could explain the somewhat higher disease duration than reported in clinical trials.54 However, clinical and MR imaging features of the enrolled sample of patients with CIS are representative because the observed number and volume of T2 lesions and clinical disability are similar to previously reported baseline CIS cohorts.54 In addition, the patients with CIS were approximately 2 years younger than HC. Nevertheless, future studies should investigate iron deposition in younger patients with CIS.

However, we believe that by using a large sample, and data both on abnormal phase and atrophy measures, our study provides support for the concept that iron deposition is present at the earliest MS disease stages and may contribute to disease development and brain damage. As has been shown previously in patients with MS,53 the pulvinal nucleus of the thalamus, as well as the basal ganglia, appear to exhibit the most widespread iron deposition in patients with CIS. Future prospective studies should also explore the relationship between the rate of iron deposition and conversion to clinically definite MS and development of disability in patients with CIS.

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