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Magnetic Susceptibility Contrast Variations in Multiple Sclerosis Lesions

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Abstract

Purpose—Recent MRI studies have revealed heterogeneous magnetic susceptibility contrasts in multiple sclerosis (MS) lesions. Due to its sensitivity to disease related iron and myelin changes, magnetic susceptibility-based measures may better reflect some pathological features of MS lesions. Hence we sought to characterize MS lesions using combined R2* mapping and quantitative susceptibility mapping (QSM).

Materials and Methods—Three hundred and six MS lesions were selected from 24 MS patients who underwent 7T MR. Maps of R2*, frequency and quantitative susceptibility were calculated using acquired multi-echo gradient echo (GRE) phase data. Lesions were categorized based on their image intensity or their anatomical locations. R2* and susceptibility values were quantified in each lesion based on manually drawn lesion masks and compared between lesion groups showing different contrast patterns. Correlations between R2* and susceptibility were also tested in these lesion groups.

Results—In 38% of selected lesions the frequency map did not show the same contrast pattern as the susceptibility map. While most lesions (93%) showed hypointensity on R2*, the susceptibility contrast in lesions varied; with 40% being isointense and 58% being hyperintense in the lesion core. Significant correlations ($r=0.31$, $p<0.001$) between R2* and susceptibility were found in susceptibility hyperintense lesions, but not in susceptibility isointense lesions. In addition, a higher proportion (74%) of periventricular lesions was found to be susceptibility hyperintense as compared to subcortical (53%) or juxtacortical (38%) lesions.

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Conclusion—Combining $R2^*$ and QSM is useful to characterize heterogeneity in MS lesions.

Keywords

multiple sclerosis; lesion; $R2^*$; quantitative susceptibility mapping (QSM); myelin; iron

Introduction

Multiple sclerosis (MS) is an autoimmune, demyelinating disease of the central nervous system. Evaluation of MS white matter lesion burden using conventional MRI correlates only weakly with clinical disability levels (1), possibly because T1 or T2 measures do not reveal heterogeneity in MS pathology (2). Recent MRI studies on MS lesions using effective relaxation rate ($R2^*$) (3), phase imaging (3-5), susceptibility weighted imaging (SWI) (6) and quantitative susceptibility mapping (QSM) (7), especially those at high field strength and with high spatial resolution, have revealed different contrast patterns and longitudinal susceptibility changes in MS lesions. These studies suggest magnetic susceptibility could be a potential imaging biomarker that better reflects MS lesion heterogeneity. Indeed, the underlying tissue mechanisms of magnetic susceptibility contrast have been shown to be sensitive to tissue iron levels and myelin content (8-10), which are known to be altered during MS lesion formation after the initial inflammation-mediated tissue damage (2, 11). Therefore, using MR images with susceptibility-based contrasts, it may be possible to evaluate in MS lesions the contributions of different pathological processes such as demyelination, iron deposition or iron loss (12).

Combined use of $R2^*$ and susceptibility mapping may help estimate specific changes in myelin and iron content in MS lesions. One possible source of such specificity can be found in the different effects myelin has on tissue $R2^*$ and susceptibility; i.e. increased myelin content increases $R2^*$ but decreases susceptibility due to its diamagnetic nature. On the contrary, iron has similar effects on $R2^*$ and susceptibility; i.e. increased iron content leads to increases in both $R2^*$ and susceptibility. In addition, if iron is the dominant source of susceptibility contrast mechanism in lesions, strong direct correlations between $R2^*$ and susceptibility are expected, similar to those generally observed in iron-rich deep gray matter. In comparison, if myelin content is high, no correlations or weak inverse correlations are expected, as observed in normal white matter (13).

Previous postmortem studies have shown that combined $R2^*$ and phase imaging patterns in MS lesions correlate well with iron and myelin measures from histology (3, 14). Yet since MR phase is a parameter that can be affected by the magnetic susceptibility of adjacent tissues, head orientation and lesion shape, phase contrast may give a false representation of the real susceptibility contrast pattern and geometry of MS lesions (15). Such false representation of susceptibility contrast could be especially misleading for rim structures around MS lesions (3, 4, 6). Recent technical developments for QSM have made it possible to reliably map the internal tissue magnetic susceptibility source distribution from MR phase measurements (13, 16-22). After deconvolution of the field change generated from neighboring voxels, the susceptibility map obtained by QSM can give a more accurate

measure of lesion susceptibility, and thus better represents the geometry and susceptibility contrast of MS lesions.

In this study, we aimed to qualitatively and quantitatively characterize MS lesions *in vivo* using combined R2* and magnetic susceptibility mapping. Our purpose was to better characterize the susceptibility contrast patterns in MS lesions in terms of the occurrence rate and anatomical location dependence of these patterns and, using the combined quantitative R2* and susceptibility measures, to better interpret the observed magnetic susceptibility contrast variations in MS lesions.

Materials and Methods

Patients

Twenty-four patients (12 males and 12 females) with relapsing remitting (RRMS), secondary progressive (SPMS) and primary progressive MS (PPMS) were recruited. Among them, the 12 female MS patients had a mean age of 44.7 ± 11.3 years with 10 RRMS, 1 SPMS and 1 PPMS. The 12 male patients had a mean age of 43.8 ± 9.0 years with 11 RRMS and 1 SPMS. Participants were excluded if they had experienced an MS relapse in the prior 30 days or if they were experiencing symptoms of a major depressive episode. The Institutional Review Boards at the Johns Hopkins University School of Medicine and the Kennedy Krieger Institute approved protocols. Written informed consent was obtained from all participants. The patient population of this study was moderately disabled with a median Expanded Disability Status Scale (EDSS) score of 3.0 in the range of 1.5 – 6.5. The cohort is the same as in another paper under review relating R2* and susceptibility measures to clinical phenotypes.

MR Imaging Protocol

All patients underwent MRI scans at 7T (Philips Healthcare, Best, Netherlands) using a 32-channel head coil (Novamedical). Dielectric padding was used to improve B1 homogeneity. A 3D multi-echo gradient recalled echo (GRE) sequence was used to acquire susceptibility weighted imaging data, with 1.0 mm isotropic resolution, $220 \times 220 \times 110$ mm³ field of view (FOV) with axial slab orientation, matrix size of $224 \times 224 \times 110$, TR/TE1/ TE=68/4/2 ms, 8 echoes, SENSE factor of $2.5 \times 1 \times 2$, flip angle of 9°, and scan duration of 7'14". Fat suppression was also accomplished using a water-selective ProSet 121 pulse. Whole-brain 3D MPFLAIR (magnetization prepared fluid attenuated inversion recovery) images were acquired with 1.0mm isotropic resolution, TR/TI/TE=8000/2175/302 ms, flip angle 90°, TSE factor 115, SENSE factor $2 \times 1 \times 3$, scan time of 8'14". A T1 MPRAGE image was also acquired with 1 mm isotropic resolution, TR/TE=4.1/1.83 ms, flip angle 7°, TFE factor of 352, SENSE factor $2 \times 1 \times 2$, scan time of 2'20".

Image Analysis

Both MPRAGE and MPFLAIR images were first coregistered to the 3D GRE magnitude image at TE of 12 ms through linear transformation using FSL FLIRT (23). The acquired 3D GRE data was processed to generate R2*, frequency and susceptibility maps. A brain mask was obtained by skull-stripping the GRE magnitude image collected at TE of 12 ms

using FSL's brain extraction tool (BET, FMRIB Oxford, UK) (24). Using the 3D GRE magnitude images acquired at all the echo times, the effective relaxation rate $R2^*$ was estimated by fitting a monoexponential curve to the squared magnitude signal decay using the power method (13, 25). MR phase data collected at TE of 12 ms was used to generate frequency and susceptibility maps in order to get consistent measure of frequency and susceptibility with good signal to noise ratio (SNR) and minimum nonlinear phase evolution effect in white matter (26, 27). First, phase unwrapping was performed using Laplacian based phase unwrapping (28). The unwrapped phase images were then divided by $2\pi \cdot TE$ to obtain an image of frequency shift in the unit of Hz. Subsequently, background field was removed by using the V-SHARP method (19, 21, 29). For this we used a variable spherical kernel size with a maximum radius of 4mm and a singular value decomposition (svd) threshold of 0.05. After that, dipole inversion was calculated to obtain susceptibility images using the LSQR method (28).

For quantitative susceptibility values, the central cerebral spinal fluid (CSF) region in the lateral ventricles was selected for each patient as a reference region using a multi-atlas segmentation method based on the coregistered T1 contrast (30). Choroid plexus regions that generally show hyperintensity in the FLAIR images were excluded from the CSF reference region. To avoid possible partial volume effects, 3 voxels were eroded from the selected CSF mask to generate the final CSF reference region. Quantitative susceptibility values are then considered relative to the mean susceptibility value of all the voxels in this reference region.

Lesion Identification And Classification

MS lesions were selected based on their hyperintensity on MPFLAIR and hypointensity in T1 MPRAGE images. The analysis was performed by a radiologist (HL) and a neurologist (DH) and a consensus sample of MS lesions was chosen from each scan. Sample lesions were selected if they were ovoid in appearance and had discrete borders (not part of lesion complexes or confluent hyperintensity). A total of 306 lesions were selected for analysis with a median of 12 lesions (range 2 – 29) per patient. Among all selected lesions, 263 were from the 21 RRMS patients and 43 were from the 3 PPMS/SPMS patients. Each lesion was manually segmented (by DH) in three dimensions using a semi-automated region of interest (ROI) growing tool in the software MIPAV (<http://mipav.ct.nih.gov>). In addition, perilesional normal appearing white matter (PL-NAWM) regions around each lesion were automatically segmented out based on an enlarged lesion mask (6 voxel dilation) and a normal appearing white matter mask generated by segmenting the coregistered T1 image using FSL FAST (31).

For each lesion, co-registered $R2^*$, frequency, and susceptibility images were reviewed by the neurologist (DH). Based on their image intensity, lesions were labeled as being hypointense, isointense, or hyperintense relative to local white matter. If a lesion was found to have a rim (by DH) with intensity visibly different from the lesion core, this was also noted and the intensity of the rim was described as hypointense or hyperintense relative to the lesion core and adjacent white matter. Lesions were thus split into categories based on their core and rim intensity pattern. Lesion pattern labels were compared between frequency

and susceptibility maps automatically to determine if the same pattern was noted on both maps for each lesion. Similar comparisons were also done on a subset of lesions selected from the subset of patients with progressive phenotypes. According to its anatomical location, each lesion was categorized as either periventricular (immediately adjacent to the ventricles), subcortical (within white matter but neither periventricular or juxtacortical) or juxtacortical (adjacent to cortical gray matter or subcortical U-fibers). Lesion size was calculated automatically based on the manually defined lesion mask and known imaging voxel size.

Statistics And Quantitative Comparison Of Lesion Susceptibility Measures

Lesion size difference between multiple lesion groups was tested using two-sample two-way t-test with significance level of 0.05 after Bonferroni correction. In addition, differences in the proportion of specific contrast patterns among multiple lesion groups was tested using the proportion z-test with significance level of 0.05 after Bonferroni correction.

Since the MR phase or frequency shift is known to be a non-local measure that would be affected by magnetic susceptibility of surrounding tissue (16), quantitative comparisons were performed only with $R2^*$ and susceptibility values. Besides the original $R2^*$ value and the magnetic susceptibility referenced to CSF, local changes as represented by the $R2^*$ difference or magnetic susceptibility difference between each lesion and its PL-NAWM were also calculated. Since the manually drawn lesion mask based on MPFLAIR contrast did not separate out the lesion core and possible lesion rims, for quantitative comparison, 50 lesions (16.3%) that had visible rim structures observed on either $R2^*$ or QSM were excluded. Quantitative comparison of $R2^*$ or susceptibility values between lesion groups with different contrast patterns were tested using one-way analysis of covariance (ANCOVA) controlling for lesion size with significant level of 0.05. In addition, Pearson's partial correlations between $R2^*$ and susceptibility controlling for lesion size were tested in different lesion groups. Similarly Pearson's correlations between $R2^*$ and susceptibility were also tested in PL-NAWM surrounding different lesion groups.

Results

Susceptibility Contrast Patterns In Lesions

Contrary to their consistent hyperintensity on GRE magnitude images, the selected MS lesions showed various contrast patterns on $R2^*$, frequency and susceptibility maps. Some example susceptibility contrast patterns observed in the present study are shown in Fig. 1. In Fig. 1a, one lesion (arrow with circular tail) exhibited an isointense core with a hypointense rim on $R2^*$, a hyperintense core with a hypointense rim on frequency map, and a hyperintense core without obvious rim on susceptibility map. In comparison, another lesion in the same patient (arrow with square tail) showed a hypointense core without a rim on $R2^*$ and a hyperintense core with a hyperintense rim on both frequency and susceptibility maps. As shown in Fig. 1b, another commonly seen contrast pattern in some MS lesions (arrows) was a hypointense appearance on $R2^*$ and an isointense appearance without any visible rim (thus invisible) on frequency and susceptibility images.

Since the frequency image represents the map of local magnetic field change or local resonance frequency shift induced by the underlying and surrounding tissue magnetic susceptibility sources, the frequency contrast and susceptibility contrast in MS lesions do not need to agree. The number of lesions with different core and rim intensity patterns observed on frequency images and QSM are summarized in Table 1. Among the 306 selected lesions, there were 191 (62.4%) lesions (diagonal entries marked by dark gray) in which the frequency contrast agreed with the susceptibility contrast, while there were disagreements in the other 115 (37.6%) lesions (bold font in Table 1). Such disagreement was driven predominantly by lesions with a hyperintense core (80 lesions, 26.1%) and no rim on QSM that showed various contrast patterns on frequency images. On frequency images, there were two major commonly seen contrast patterns: isointense without rim (thus invisible, 42.2%) and hyperintense with no rim (29.4%). Among all lesions, rim structures were observed in 61 (19.9%), with 43 of them being hyperintense. Similar contrast patterns were also observed on QSM, with most lesions being either isointense without rim (34.0%) or hyperintense without rim (51.6%). However there were only 38 (12.4%) lesions in which a rim structure was observed on QSM, with 31 of the rims being hyperintense. If a lesion had a hyperintense rim on QSM, it was highly likely (30 lesions, thus 96.8%) to also have a hyperintense rim on phase/frequency images. Analysis on a subset of lesions selected from PP/SP patients (43 lesions) revealed about the same rate of agreement (28 lesions, 65.1%) and disagreement (34.9%) between frequency and QSM images. Most lesions from PP/SP patients also fell into two dominant contrast patterns on QSM, i.e. isointense without rim (34.9%) or hyperintense without rim (34.9%). However, PP/SP patients showed more lesion rims (27.9%) on QSM.

Compared to QSM, the $R2^*$ contrast patterns varied less. The lesion counts of combined contrast patterns on $R2^*$ and QSM are summarized in Table 2. Among all selected lesions, most (92.8%) showed a hypointense core on $R2^*$ images, including 87.6% without a rim and 5.2% with a hyperintense rim. There were 18 (6%) lesions with an isointense core without a visible rim (thus invisible) on $R2^*$ images. No lesions were seen with a hyperintense core on $R2^*$. $R2^*$ hyperintensity was only observed in the rims of 18 lesions (6%)- in which all the susceptibility contrast was either isointense or hyperintense. In a similar way, all lesions from PP/SP patients had hypointense $R2^*$ of which 93.0% were without a rim and 7.0% with a hyperintense rim.

In terms of its anatomical location dependence, there was little difference among lesions at different anatomic locations in terms of their $R2^*$ contrast (Table 3). However, there were significantly more QSM hyperintense lesions (73.6%) in periventricular areas than in subcortical (52.8%, $p<0.01$) and juxtacortical (38.4%, $p<0.001$) areas. On the contrary, there were significantly less QSM isointense lesions (24%) in periventricular areas than in subcortical (47.3%, $p<0.01$) and juxtacortical (57.0%, $p<0.001$) areas. It is also found out that the size of periventricular lesions was significantly larger than the size of subcortical or juxtacortical lesions.

Quantitative Comparison

As shown in Table 2, the vast majority of the selected lesions without any rim had a hypointense core on R2*, but had either an isointense core (104 lesions) or hyperintense core (146 lesions) on QSM. Comparing lesions that were hyperintense versus isointense on QSM revealed no significant difference in R2* between the two groups (left panel in Fig. 2a). Both lesion groups had a mean R2* of about 24 Hz (24.2 ± 0.5 Hz and 23.7 ± 0.5 Hz, mean $\pm$ standard error, for QSM hyperintense and isointense lesions, respectively) and had a decrease of about 10 Hz in R2* as compared to PL-NAWM (9.6 ± 0.4 Hz and 10.1 ± 0.5 Hz, respectively). The magnetic susceptibility values differed significantly ($p < 0.001$) between these two groups (right panel in Fig. 2a). However, it was also noticed that in both lesion groups, the mean quantitative susceptibility values as referenced to CSF were negative, i.e. -5.8 ± 1.4 ppb and -20.5 ± 1.6 ppb (mean $\pm$ standard error) in the QSM hyperintense and QSM isointense group, respectively. As compared to PL-NAWM, their magnetic susceptibility changes were 18.2 ± 1.1 ppb and -1.2 ± 1.1 ppb in the two groups respectively.

For all the lesions without any rim, partly due to partial volume effects, it was found that lesion R2* correlated inversely with lesion size ($r = -0.24$, $p < 0.001$), while lesion susceptibility correlated directly with lesion size ($r = 0.23$, $p < 0.001$). Therefore when testing the correlation between lesion R2* and susceptibility, partial correlation controlling for lesion size was used. A significant positive correlation was found between R2* and relative susceptibility in QSM hyperintense lesions ($r = 0.31$, $p < 0.001$), while no significant correlation was found in QSM isointense lesions (Fig. 2b left panel). The significant correlation between R2* and susceptibility in QSM hyperintense lesions was mainly driven by the significant correlation in lesions that are hyperintense on QSM and hypointense on R2* ($r = 0.29$, $p < 0.001$) (Table 4). In PL-NAWM around QSM isointense lesions, a weakly negative correlation ($r = -0.22$, $p < 0.05$) was found between R2* and relative susceptibility (Fig. 2b right panel). No significant correlation was found in PL-NAWM around QSM hyperintense lesions or all PL-NAWM as a single group.

The varying effects of myelin and iron on tissue R2* and susceptibility can be used to qualify lesions; i.e. demyelination decreases R2* but increases magnetic susceptibility, while iron deposition would increase both R2* and magnetic susceptibility. Using this classification approach, the interpreted (but not validated) pathological changes in myelin and iron content associated with the observed susceptibility contrast patterns in MS lesions were summarized in Table 5. The quantitative R2* and susceptibility measures obtained in this study were also taken into account for this interpretation (See discussion below). The most common contrast pattern seen in this study with hypointense R2* and hyperintense magnetic susceptibility (43%) was interpreted to come from major or moderate demyelination with minor changes in iron level. The other most commonly seen contrast pattern with hypointense R2* and isointense magnetic susceptibility (33%), was interpreted to be a consequence of both demyelination and iron loss, which results in opposite effects on magnetic susceptibility (thus cancellation) and additive effects on R2*. Some other contrast patterns we observed with lower occurrence rate are also listed in Table 5.

Discussion

Consistent with previous reports in the literature (3, 12, 14), we found heterogeneous susceptibility contrast patterns in MS lesions using high field MRI. Two dominant contrast patterns were observed using combined $R2^*$ and QSM. The reason why the lesion contrast patterns fell into these two dominant groups is believed to originate from the sensitivity of the imaging contrast to tissue myelin and iron content and the demyelinating nature of the disease. In MS lesion cores, most possibly there is demyelination. While myelin content may be preserved at the rim/edge of the lesion, it is unlikely that the myelin content will increase. On the other hand, tissue iron content may either decrease through the loss of iron containing oligodendrocytes (14) or increase through the increase of iron-positive macrophage/microglia usually at the edge/rim of the lesions (11, 12).

Complementary to previous imaging and histological studies on MS lesions (3, 14), the combined quantitative $R2^*$ and susceptibility measures from this study may help to better interpret observed magnetic susceptibility contrast variations in MS lesions. Assuming the susceptibility value of CSF is close to that of water in saline, the negative mean quantitative susceptibility values as referenced to CSF in both QSM hyperintense lesions and QSM isointense lesions seem to suggest that neither lesion type is completely demyelinated. Exceptions were a few lesions with positive magnetic susceptibility values in the QSM hyperintense group, for complete demyelination could have happened. In addition, the comparable $R2^*$ values in these two dominant lesion types seem to suggest slightly higher myelin content (thus less demyelination) in QSM isointense lesions, in which iron loss also occurs. This interpretation is further supported by the different correlation levels between $R2^*$ and susceptibility in these lesions. Normally, in white matter with high myelin content, weak or slightly negative correlations between $R2^*$ and susceptibility are expected (13). The significant positive correlation observed in those QSM hyperintense lesions therefore suggest that there is more iron or less myelin content in these lesions as compared to QSM isointense lesions.

In terms of the anatomical location dependence of the observed susceptibility contrast patterns, we observed more of the periventricular lesions to have hyperintense susceptibility, which, as discussed before, is linked to higher iron concentrations or lower myelin content (more demyelination) in comparison to QSM isointense lesions. The underlying cause of this is not very clear, but several mechanisms may contribute. First, tissue iron concentration has known anatomic variation in white matter depending on location, with the highest iron concentration appearing at the white matter-cortical gray matter junction (14). Iron content in the deep white matter is usually low to moderate. In addition, as compared to juxtacortical areas, periventricular white matter is generally in the form of large and well-organized fiber bundles with higher fiber density. Therefore, demyelination in a periventricular location may show a more profound alteration of the susceptibility signal compared to regions of lower axonal density. In the case of juxtacortical regions, where myelinated fiber density may be less, alterations in iron signal may be more influential. Second, the different vascular structures in these areas may also play a role. Being closer to larger venous structures may allow for more intense inflammatory infiltration due to blood-brain barrier breakdown, resulting in more inflammatory byproducts and iron debris, though the macrophage/

microglia contained iron is usually located at the lesion boundaries and seldom seen in the lesion core (11, 12).

Our data also supports the notion that caution must be taken when interpreting lesion features based on phase/frequency imaging or susceptibility weighted imaging (SWI) (6). As observed in the present study, phase/frequency in MS lesions showed larger contrast variance than QSM, showing for example much more rim structures around lesions. It is known that the white matter lesion contrast appearance on phase/frequency images may deviate from the underlying susceptibility contrast and thus be more variable than QSM, because it is affected by the susceptibility of surrounding structures such as the ventricles or iron-rich nuclei. In addition, as illustrated in a recent study (15), the presence of a shell or rim structure around MS lesions observed in phase imaging may be consequential to dipole field changes induced by solid lesions depending on orientation and geometry. Quantitative susceptibility mapping, on the other hand, is not susceptible to orientation and geometry effects in the same manner. Accurate representation of the presence or absence of a rim surrounding MS lesions is necessary because of the potential clinical and pathological relevance of these structures. Rim structures observed on phase/susceptibility images around MS lesions, especially with increased susceptibility and $R2^*$, are usually inferred as iron deposition caused by the increase of iron-positive macrophage/microglia at the edge of chronically demyelinated lesions (11, 14) or possible inflammatory byproducts of blood-brain-barrier opening in acute lesions (4). Note here that, because of the different conventions of defining frequency shift directions used by different scanner manufacturers, the hyperintense contrast on phase/frequency reported in this study may correspond to the opposite hypointense contrast reported in other studies (3).

As shown in a recent longitudinal study (7), MS lesions of early to intermediate age (0.5-3 years) have rapid increases in magnetic susceptibility to about 40 ppb higher than its PL-NAWM. After this initial peak magnetic susceptibility gradually reduces to levels similar to PL-NAWM over the course of months to years. According to that study, the QSM hyperintense lesions observed in the present study are likely to be of intermediate age, while QSM isointense lesions are likely older lesions. Besides the lesion age difference, it might also be possible that different pathological processes may account for differences in the two lesion groups, i.e. one with mainly demyelination and the other with both demyelination and iron loss possibly caused by damage/loss of oligodendrocytes.

In the present study, the interpretations of possible myelin and iron changes in MS lesions is based mainly on previous postmortem studies of histology correlated with quantitative $R2^*$ and susceptibility measures acquired *in vivo*. Yet, it should be noted that only a qualitative estimation of the myelin and iron changes could be made here. Getting quantitative maps of either iron or myelin specifically using $R2^*$ and QSM would be of great interest but still seems quite challenging partly because of the difficulty of estimating the quantitative relationship between all the variables *in vivo* (32). In addition, due to white matter fiber microstructures and anisotropic susceptibility of myelin lipid molecules, the macroscopic $R2^*$ and magnetic susceptibility of white matter fibers are anisotropic, i.e. they depend on the white matter fiber orientations relative to the main magnetic field (33-35). This of course makes the underlying biophysical model even more complicated. Estimation of those

orientation independent measures such as mean magnetic susceptibility (MMS) using susceptibility tensor imaging (STI) may help us better estimate the iron and myelin contents, but STI is still hard to perform in clinical practice due to its requirement of scanning patients at different head orientations (35-39)

Several other limitations of this study include the lack of contrast administration or longitudinal follow-up MRI scans. Therefore little is known about the inflammatory status and age of the selected lesions, yet most of them are likely to be chronic considering the majority of the patients were RRMS in stable phase (no recent relapses). In addition, because of technical difficulties in segmenting lesion rims from lesion cores at the current resolution, quantitative comparisons of $R2^*$ and QSM were not performed in lesions with any observed rims. However, this can be considered in future work using MR scans with even higher resolution. Further evaluation of this data in light of clinical phenotypes and disability measures will be addressed in future reports on this data set.

In conclusion, combining $R2^*$ and QSM data may help to better define the heterogeneity in MS lesions. Quantitative measures of $R2^*$ and magnetic susceptibility can further help to interpret various lesion contrast patterns.

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FIG. 1.

(a) Example image slices from a 55 y/o, female RRMS patient showing FLAIR, T1 MPRAGE, GRE magnitude, R2*, frequency and susceptibility contrasts in some selected lesions. While all the selected lesions showed consistent hyperintensity on GRE magnitude images, their contrast varied on R2*, frequency and susceptibility maps. (b) Image slices from a 46 y/o, female RRMS patient showing some example lesions (marked by arrows) that are hypointense with no rims on R2* and isointense without a rim (thus invisible) in frequency and susceptibility contrasts.

FIG. 2.

(a) Group comparison of the quantitative $R2^*$ and magnetic susceptibility values between lesions categorized as QSM hyperintense and those categorized as QSM isointense. Lesions showing any rim structures on either $R2^*$ or QSM were excluded. Error bar in the graph indicates inter-lesion standard error; *** marks significant group difference with $p < 0.001$. Left panel in (b) shows the scatter plot of the original $R2^*$ values and susceptibility values with respect to (w.r.t.) CSF of the two lesion groups. A significant correlation was found between the $R2^*$ and susceptibility values in QSM hyperintense lesions (blue line, $r = 0.31$, $p < 0.001$), while no significant correlation was found in QSM isointense lesions. Right panel in (b) shows similar scatter plot in the corresponding peri-lesional normal appearing white matter (PL-NAWM) surrounding those two lesion groups. Significant inverse correlation between $R2^*$ and susceptibility was only found in PL-NAWM around QSM isointense lesions (red line, $r = -0.22$, $p < 0.05$).

Table 1
Comparison of lesion contrast patterns on frequency and susceptibility maps

Lesion appearance on frequency map			Lesion appearance on QSM						Total
	Hypointense			Isointense			Hyperintense		
	Hypo Rim	No Rim	Hyper Rim	Hypo Rim	No Rim	Hyper Rim	Hypo Rim	No Rim	Hyper Rim
Hypointense	Hypo Rim	0	0	0	0	0	0	0	0
	No Rim	0	2/0 (0.7%)	0	8/0 (2.6%)	1/0 (0.3%)	0	15/3 (5.0%)	0
	Hyper Rim	0	0	1/0 (0.3%)	0	1/1 (0.3%)	1/0 (0.3%)	4/0 (1.3%)	3/0 (1.0%)
Isointense	Hypo Rim	0	0	0	0	0	0	9/0 (2.9%)	0
	No Rim	0	1/0 (0.3%)	0	91/15 (29.7%)	0	0	36/3 (11.8%)	0
	Hyper Rim	0	0	0	0	15/6 (4.9%)	0	7/2 (2.3%)	7/2 (2.3%)
Hyperintense	Hypo Rim	0	0	0	0	0	1/0 (0.3%)	8/0 (2.6%)	0
	No Rim	0	3/1 (1.0%)	0	5/0 (1.6%)	0	3/2 (1.0%)	78/7 (25.5%)	0
	Hyper Rim	0	0	0	0	0	0	1/0 (0.3%)	3/0 (1.0%)
Total	0	6/1 (2.0%)	1/0 (0.3%)	2/1 (0.7%)	104/15 (34.0%)	17/7 (5.6%)	5/2 (1.6%)	158/15 (51.6%)	13/2 (4.3%)

Dark gray area indicates contrast appearance agreement between frequency and susceptibility maps. Bold font marks lesions that have different appearance on frequency and susceptibility maps. For each entry, the number before the slash is the number of selected lesions with certain contrast pattern from all patients, while the number after the slash denotes the number of selected lesions from patients with progressive phenotypes. All percentages were calculated based on selected lesions from all patients.

Table 2
Comparison of lesion contrast patterns on R2* and susceptibility maps

Lesion appearance on R2* map		Lesion appearance on QSM										Total	
		Hypointense					Isointense						
		Hypo Rim	No Rim	Hyper Rim	Hypo Rim	Hyper Rim	Hypo Rim	No Rim	Hyper Rim	Hypo Rim	No Rim		Hyper Rim
Hypointense	Hypo Rim	0	0	0	0	0	0	0	0	0	0	0	0
	No Rim	0	6/1 (2.0%)	1/0 (0.3%)	101/15 (33.0%)	2/1 (0.7%)	12/4 (3.9%)	131/15 (42.8%)	5/2 (1.6%)	10/2 (3.3%)	0	268/40 (87.6%)	
	Hyper Rim	0	0	0	0	0	5/3 (1.6%)	8/0 (2.6%)	0	3/0 (1.0%)	0	16/3 (5.2%)	
Isointense	Hypo Rim	0	0	0	0	0	0	0	0	0	0	2/0 (0.7%)	
	No Rim	0	0	0	3/0 (1.0%)	0	0	15/0 (4.9%)	0	0	0	18/0 (5.9%)	
	Hyper Rim	0	0	0	0	0	0	2/0 (0.7%)	0	0	0	2/0 (0.7%)	
Total		0	6/1 (2.0%)	1/0 (0.3%)	104/15 (34.0%)	2/1 (0.7%)	17/7 (5.6%)	158/15 (51.6%)	5/2 (1.6%)	13/2 (4.3%)	0	306/43 (100%)	

For each entry, the number before the slash is the number of selected lesions with certain contrast pattern from all patients, while the number after the slash denotes the number of selected lesions from patients with progressive phenotypes. All percentages were calculated based on selected lesions from all patients.

Table 3
Lesion characteristics based on anatomic location

Lesion location		Periventricular	Subcortical	Juxtacortical
Lesion number		129	91	86
Average lesion size (mean $\pm$ standard error) in mm ³		264 $\pm$ 22	139 $\pm$ 22, ***	120 $\pm$ 11, ***
Contrast on R2 [*]	Hypointense	121 (93.8%)	79 (86.8%)	84 (97.7%) §
	Isointense	8 (6.2%)	12 (13.2%)	2 (2.3%)
	Hyperintense	0	0	0
Contrast on QSM	Hypointense	3 (2.3%)	0	4 (4.7%)
	Isointense	31 (24.0%)	43 (47.3%) **	49 (57.0%) ***
	Hyperintense	95 (73.6%)	48 (52.8%) **	33 (38.4%) ***

* indicates significant group or proportion difference as compared to periventricular lesions with p<0.05;

** indicates significant difference with p<0.01;

*** indicates significant difference with p<0.001.

§ indicates significant proportion difference as compared to subcortical lesions with p<0.05.

Table 4
Correlations between R2* and quantitative susceptibility values in different lesion types

Lesion Contrast Pattern		N	r	p
<i>Contrast on R2*</i>	<i>Contrast on QSM</i>			
Hypointense	Isointense	101	-0.10	0.32
Hypointense	Hyperintense	131	0.29	<0.001
Hypointense	Any	238	0.12	0.066
Isointense	Hyperintense	15	0.16	0.59
Isointense	Any	18	0.46	0.062
Any	Isointense	104	-0.086	0.39
Any	Hyperintense	146	0.31	<0.001
Any	Any	256	0.18	<0.01

N is the number of lesions; r is the Pearson's partial correlation coefficient controlling for lesion size; p is the p-value; Lesions with any kind of rim, observed either on R2* or QSM, and lesion groups with sample size N<10 were excluded. Significant correlations are printed in bold.

Table 5
Hypothesized pathological changes in myelin and iron content associated with observed susceptibility contrast patterns in MS lesions

Occurrence rate	R2* Intensity	QSM Intensity	Hypothesized changes in myelin	Hypothesized changes in iron
<i>High</i>	<i>Hypo</i>	<i>Hyper</i>	<i>Moderate or major demyelination</i>	<i>Minor</i>
<i>High</i>	<i>Hypo</i>	<i>Iso</i>	<i>Moderate demyelination</i>	<i>Moderate iron loss</i>
Rare	Hypo	Hypo	Minor	Moderate or major iron loss
Low	Iso	Hyper	Moderate demyelination	Moderate iron deposition
Rare, at lesion rim	Hyper	Hyper	Minor	Moderate or major iron deposition

Occurrence rate of “high” refers to 30% or more in the current study; occurrence rate of “low” refers to 5%-30%; occurrence rate of “rare” refers to less than 5%.