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Multi-layer analysis of quantitative 7T MRI in the cortex of MS patients reveals pathology associated with disability

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Abstract

Background.—Cortical demyelination is a relevant aspect of tissue damage in multiple sclerosis (MS). Microstructural changes may affect each layer in the cortex differently.

Objectives.—To evaluate the sensitivity of quantitative MRI (qMRI) measurements on cortical layers as clinically accessible biomarkers of grey matter (GM) pathology.

Methods.—45 participants with MS underwent 7T MRI of the brain. Magnetization prepared 2 rapid acquisition gradient echoes (MP2RAGE) was processed for T₁-weighted images and a T₁-map. Multi-echo gradient echo images were processed for quantitative susceptibility and R₂^{*} maps. Cortical GM volumes were segmented into four cortical layers and relaxometry metrics were calculated within and between these layers.

Results.—Significant correlations were found for disability scales and multilayer metrics, e.g. Expanded Disability Status Scale (EDSS) and peak height (PH) in the subpial (T₁ $\rho = -0.372$, $p < 0.050$) and inner (R₂^{*}: $\rho = -0.359$, $p < 0.050$) cortical layers. Multivariate regression showed interdependency between atrophy and cortical metrics in some instances, but an independent relationship between cortical metrics and disability in others.

Conclusions.—Cortical layer 7T qMRI analyses reveal layer-specific relationships with disability in MS and allow emergence of clinically relevant associations that are hidden when analyzing the full cortex.

Keywords

Multi-layering; 7 Tesla; qMRI; Cortex; Multiple sclerosis

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Introduction

Multiple sclerosis (MS) produces both inflammatory and degenerative pathology of the central nervous system (CNS) and usually leads to chronic disability.(1) MS is classically regarded as a white matter (WM) disease, but histopathological studies reveal that grey matter (GM) is also affected.(2) These studies show neuronal loss and demyelination in the cortical grey matter (cGM) and deep GM and that these changes are independent of WM lesions and are related to disability.(3)

Visualization and quantification of these changes to the cortex are necessary for a greater understanding of MS-related pathology. Quantitative MRI (qMRI) methods produce images and quantitative relaxometry maps that are more reproducible and more independent of acquisition details than weighted images, along with providing information that is a more direct correlate of *in vivo* histopathology.(4,5) The relationship between qMRI methods and histopathology have promise towards better understanding how MS affects varying depths of cortex – of importance due to the varying functions and levels of myelination seen between layers I – VI of human cortex. Nevertheless, applications of qMRI to the cortex at standard field strengths can be limited by the thin nature of the cortex, its gyrification, and partial volume averaging with adjacent WM and cerebrospinal fluid (CSF). These problems may be overcome using ultra-high field 7-tesla (7 T) acquisitions at high resolution, along with layer-based segmentation.(6)

The purpose of this study was to evaluate the sensitivity of qMRI measurements in multi-layered, segmented cortex from 7 T MRI as clinically-relevant biomarkers of cGM pathology in MS. Quantitative MRI measures in segmented cortical layers, along with the difference of these measures between layers, were compared to similar measures across the full cortical thickness on the hypothesis that analysis of segmented layers may reveal clinically relevant disability relationships hidden when summarizing qMRI values across the full cortical thickness.

Methods

Study Participants

Protocols were approved by the Institutional Review Boards at the University of Maryland School of Medicine, the Johns Hopkins University School of Medicine, and the Kennedy Krieger Institute. Written informed consent was obtained from all participants. Volunteers aged 18 – 65 with diagnoses of relapsing-remitting multiple sclerosis (RRMS), secondary progressive (SPMS) and primary progressive multiple sclerosis (PPMS) according to revised 2010 McDonald Criteria were recruited.(7) Participant study visits included demographic and clinical data collection and a neurological examination for calculation of the Expanded Disability Status Scale (EDSS) score,(8) timed 25-foot walk (T25FW), 9-hole peg test (9HPT). The Brief International Cognitive Assessment for Multiple Sclerosis (BICAMS) battery was also obtained at each visit, which includes the California Verbal Learning Test (CVLT), Symbol Digit Modalities Test (SDMT) and Brief Visuospatial Memory Test (BVMT).(9)

MRI acquisition, processing and analysis

Detailed MRI acquisition parameters and details on processing and analysis can be found in the Supplement. In brief, participants underwent MRI in a 7.0 Tesla (7T) Philips Achieva scanner (Philips Healthcare, Best, The Netherlands) with a volume transmit/32-channel receive head coil (Novamedical, Wilmington, MA, USA). Images underwent segmentation for various brain compartments, WM lesions, and cGM. The cGM volume was divided into four layers (Figure 1 and 2) (10) and the related masks for each layer were applied to T_1 , R_2^* and relative susceptibility (χ_R) maps for the whole cohort and metrics were calculated.

Statistical Analysis

The intensity distribution for T_1 and R_2^* relaxation times and χ_R were calculated in Matlab and histograms were made for each subject, for the full cortex and each cortical layer. Distributions were normalized and divided into bins of width 0.25 percent units (p.u.). Smoothing by convolution with a Gaussian kernel was performed over the histograms and to correct the difference of tissue volume between subjects, each histogram was normalized by dividing it by the volume related to each layer or full cortex. Its arithmetic calculations were performed using FSL (Centre for Functional Magnetic Resonance Imaging of the Brain, University of Oxford, UK) and Matlab.

Spearman rank-order correlation coefficient was used to assess associations between T_1 , R_2^* and χ_R histogram metrics (median, skewness and peak height) along with their inter-layer differences, and disability scales: EDSS, T25FW, 9HPT, CVLT, SDMT and BVMT. Bonferroni-adjusted alpha (0.050/1) correction was used. Multivariable linear regressions were also performed to assess the influence of other factors (age, disease duration, volume layer fraction) on the relationship between qMRI metrics and disability as a dependent variable. Patients were divided into two groups according to its EDSS scale (EDSS median and EDSS > median) in order to perform histogram comparisons. One-way analysis of variance (ANOVA) was used to assess the significant difference of the cortical multi-layer metrics. All statistical analyses were performed with SPSS for Windows (v. 17, SPSS Inc., Chicago, IL).

Results

Analysis was performed on scans from forty-five patients with MS (33 RRMS, 4 SPMS and 8 PPMS). Median (range) age was 48.7 (22.6, 63.5) years and disease duration was 8.3 (0.7, 38.4) years. A full description of the clinical characteristics of the study group is seen in Table 1. Briefly, median EDSS score was 3.0 (1.0, 6.5), median T25FW was 4.6 (3.5, 90.9) seconds and median SDMT was 54.0 (35.0, 83.0).

Histogram metrics for T_1 and R_2^* maps, and to a lesser degree χ_R , showed significant inter-layer differences (Figure 1, Supplemental Table 1). Median T_1 and R_2^* significantly varied between layers, whereas χ_R did not ($p = 0.462$). T_1 relaxation time increased in each subsequent layer towards the subpial layer, whereas R_2^* increased towards the inner layer. Histogram peak height (PH) for T_1 and R_2^* significantly decreased towards the subpial

layer, whereas χ_R significantly increased (Figure 1, Supplemental Table 1). Skewness also significantly varied between layers for T_1 R_2^* and χ_R .

qMRI metrics measured across the full cortex only had mild relationships with disability. For example, skewness of T_1 correlated with EDSS scores ($\rho = 0.360$, $p < 0.050$) and SDMT ($\rho = -0.350$, $p < 0.050$) and skewness of R_2^* correlated with SDMT scores ($\rho = 0.366$, $p < 0.050$) (Figure 3 and 4, Supplemental Table 2 and 3).

Segmented cortical layer analyses revealed significant correlations with multiple disability scores and T_1 and R_2^* in individual layers (Figure 3 and 4, Supplemental Table 2 and 3) many of which were not observed in full cortex analyses. For example, PH of T_1 correlated with EDSS ($\rho = -0.372$, $p < 0.050$) and SDMT ($\rho = 0.355$, $p < 0.050$) in the outermost layer (4th layer). Skewness of 4th layer R_2^* correlated with EDSS ($\rho = -0.361$, $p < 0.050$) and SDMT ($\rho = 0.367$, $p < 0.050$), whereas the full cortex did not. The ability of segmented layer analyses to reveal relationships with disability not seen in full cortex measurements did not appear to extend to χ_R (Figure 5, Supplemental Table 4). Multivariate regression was performed to determine if these relationships were independent of age, disease duration, and layer-specific atrophy (Supplemental Table 5). Although some relationships were no longer present, significant relationships remained between SDMT scores and T_1 metrics in multiple layers and R_2^* in each of the four layers and T25FW scores.

Participants were stratified into those with low ($\leq$ median) EDSS and high ($>$ median) EDSS, with comparative histograms shown in Figure 9. These distributions show that the negative correlation between 4th layer T_1 PH and skew with EDSS is due to participants with higher levels of disability having less voxels clustered around the median with a shift of those to a higher T_1 value. Similarly, correlations between EDSS and R_2^* distributions are likely due to more heterogeneity of voxel intensity values (thus less clustered around the mean).

The difference in qMRI values between consecutive layers were also calculated, and difference values were evaluated for correlations with disability. These analyses revealed significant correlations for all quantitative maps (Figure 6, 7 and 8, Supplemental Table 6, 7 and 8). The difference between inner layers correlated with disability to a greater degree than a difference between middle layers.

Analysis of cortical tissue volumes and thickness (Table 2) revealed significant correlations with EDSS for the full cortex ($\rho = -0.494$, $p = 0.001$) and each segmented cortical layer (e.g., $\rho = -0.503$, $p < 0.001$, for the subpial layer), but lack of such a relationship for brain parenchyma fraction ($\rho = -0.228$, $p = 0.113$).

Discussion

These results suggest that stratification of cGM into layers and measurement of layer-specific qMRI may be more sensitive to pathology responsible for disability in MS than measurement of qMRI indices across the entire cortex. This supports the integration of cortical layer segmentation into future studies of cGM in MS and reinforces the importance

of the use of 7 T imaging for the study of cGM – given the imaging resolution needed for reliable segmentation of cortex into individual layers.

Detailed examination of the cGM is necessary in MS, as cortical pathology is the likely substrate of clinical symptoms and cognitive impairment in this condition (11-13) Our most robust findings were for multiple cortical layer T1 metrics and the SDMT score – a validated measure of cognitive impairment in MS.(14) Our results also found clinically relevant T₁ abnormalities in the outer (subpial) layer. T₁ is affected by myelin content, axonal density and by gliosis.(15) This is in accordance with previous measurements of magnetization transfer ratio (MTR) imaging in which the largest change in MTR in MS subjects was in the superficial cortical layer.(16)

Studies of microscopic myeloarchitecture carried out in parallel to classical cytoarchitectonic investigations confirm a six-layer pattern of myelin density corresponding to the known cellular layers of the cortex.(17) Although these patterns vary depending on anatomic location, inner cortical layers (V, VI) generally contain a higher density of myelinated fibers (interneurons and axonal projections to non-cortical regions), whereas outer layers (I, II) generally contain a higher density of dendritic connections and less myelin.(18) Since anatomic cortical layers have varying architecture, degrees of myelination, and cellular contents, it should be expected that individual layers may be affected in MS differently. Proximity to MS-related inflammation in the meninges may result in neuronal, oligodendrocyte, and astrocyte loss in layers close to the pial surface, which may be reflected in our findings of altered T₁ and R₂* in the outermost layer and its relationship with disability.(19) Conversely, given proximity to WM and higher levels of intracortical myelination, inner cortical layers may be directly affected by demyelination independently or in the setting of leukocortical lesions.(20) The varying sources of pathology leading to inner and outer layer relaxometry alterations in MS are likely responsible for the opposing directions for correlation coefficients in inner versus outer layers with disability scales seen in this study. This difference was especially notable for T₁ relaxometry metrics. The inner layer showed a higher PH for the more disabled group of patients, whereas a lower PH in the subpial layer (Figure 9). This might be the result of the different impact of leukocortical and subpial lesions. In the inner layer, leukocortical lesions are like WM lesions, being relatively uniform. The related abnormalities might have similar T₁ values, thus clustering around a peak. In the outer layer, where subpial lesions tend to be more diffuse, the damage related to subpial demyelination may result in T₁ values shifted towards higher values reflecting a gradient of abnormality in T₁. Given opposing signs for correlations seen between inner and outer layers, the counterbalance of these changes likely explains why in such situations metrics full cortex metrics did not significantly correlate with disability. In this sense, our data suggests that averaging of relaxometry metrics across the full cortex in MS may lead to pseudo-normalization and masking of clinically relevant pathology due to differences in how the surface and inner layers are affected in MS.

In addition to the differences in how MS affects the underlying histopathologic substrate of cortical layers, the associations noted in this study may also represent the varying function of cortical layers. The relationships seen between inner layer metrics and disability

scales (i.e. T_1 and R_2^* PH with EDSS and T25FW) may be due to the importance of the interruption of cortico-thalamic, cortico-brainstem, and callosal projections in anatomic layers V/VI in the physiologic functions measured by these scales. Similarly, the relationships between outer layer metrics and disability scales (i.e. T_1 PH with 9HPT and CVLT and R_2^* skewness with EDSS, T25FW, 9HPT, and SDMT) may represent the importance of dendritic connections in anatomic layers I/II towards the functions measured by these scales.

These data may be used on conjunction with other MRI metrics to better explain MS-related pathology. As expected, our results also show a significant relationship between cortical GM atrophy, including layer-specific atrophy, and disability, and these relationships far exceeded any relationship between overall brain atrophy or WM lesions and disability. We also performed a multivariable linear regression to evaluate if our findings were entirely contingent on this layer-specific atrophy (Supplemental Table 5). In this analysis, some relationships seen in univariate correlation between qMRI metrics and disability remained, some did not, and new relationships not previously seen emerged. This mixed result likely indicates that in some instances the relationship between qMRI metrics within a particular layer are intertwined with the processes that cause atrophy, whereas in other cases the qMRI indices are independent of atrophy. More importantly, the revelation of new relationships shows that in some cases both atrophic and other pathologic processes (i.e. demyelination) are both necessary to explain disability – justifying evaluation of both atrophy and qMRI metrics in MRI research.

The difference between layers for χ_R showed an ability to discriminate levels of disability, despite a lack of sensitivity for individual layer assessment. Similar differences were not seen for R_2^* . On the mesoscopic scale, the intracortical variation of iron concentration is responsible for much of the changes of MR signal phase reflecting the changes of χ_R . (21) Our χ_R results may reflect such sensitivity to iron mapping because different cortical layers have different iron content. (22) In addition, the lipid molecules composing myelin have an overall diamagnetic susceptibility and thus are counteracting the paramagnetic contribution of iron. It seems plausible that different levels of demyelination between the inner layers contributed to the higher sensitivity of differences in χ_R . Although in general more correlations were seen between disability measures and T_1 and R_2^* metrics, the differential sensitivity of T_1 , R_2^* , and QSM to disability in this study and myelin and iron in others likely suggest the need for continued use of multi-modal analyses.

This study is limited by its cross-sectional nature. It is unclear from our data if qMRI in segmented cortex would reveal changes over time indicative of MS pathology and/or responsible for disability worsening. Future work could look to follow a large cohort of MS subjects over time by this method. Further, our results could be influenced by partial volume averaging inherent to segmentation of the cortical layers. For example, the contribution of focal atrophy of the subpial cortex could explain the shift noted to higher T_1 in the high EDSS group for the 4th layer (Figure 9) due to a greater CSF contribution to each voxel in this layer. Similar effects could occur at the inner layers due to WM lesions. In order to reduce this effect, our study utilized ultra-high field, high resolution images, and segmentation methods validated for more robust segmentation of the cortex based on such

images.(23) Further, the segmentation was performed based on an equi-volume algorithm, which reduces partial volume effects in locations with a high curvature, and since it is based on volume, it should not be influenced by intensity characteristic changes induced by focal cortical lesions.(24) We also tried to address this possibility by re-evaluating correlations in a multi-variate model that included layer-specific atrophy as a co-variate. Although some of the correlations noted in univariate analysis were no longer significant, others remained, and new relationships emerged. We also have previously demonstrated the lack of a linear relationship between cortical depth and qMRI metric distribution, which would be expected if our results were due to partial volume averaging with CSF alone.(25) These data together suggest that our results cannot be explained by layer-specific atrophy and partial volume averaging alone, and more likely both atrophy and underlying tissue alterations (i.e. demyelination, oligodendrocyte loss, etc.) that lead to qMRI shifts represent parts of an interrelated disease process in the cortex causative of disability. Finally, the utilization of GRE-based parametric maps (R_2^* and QSM) translated to MP2RAGE space could have introduced noise to these measurements. The need to establish one space as native and registration of other parametric maps into this space is a necessary step in any multi-parametric neuroimaging study. Although there are drawbacks to this, previous work demonstrates that the impact on statistical analyses performed using translated qMRI maps is negligible.(26,27)

In conclusion, cortical multi-layer qMRI analyses at ultra-high field 7 T reveal layer-specific relationships with disability in MS and allow the rise of clinically relevant links that are hidden when analyzing the full cortex. We therefore suggest consideration of cortical layer stratification in future studies in MS, including those attempting to measure changes in myelination of the cortex and to monitor treatment response.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Cortical segmentation. (A) The image corresponds to an axial view from a MS patient for the MP2RAGE T₁-weighted scan and the T₁, R₂^{*} and χ_R maps. The boundaries of the full cortex (left) and our four layers segmentation (right) are shown after the application of the Volumetric Layering Module of CBS Tools. (B) Average multi-layer histograms across all patients of T₁, R₂^{*} and χ_R . Details of the metrics are shown on the 4th layer of R₂^{*}: an arrow shows the peak height and a dashed line the median value in a positively skewed distribution. One-way ANOVA revealed significant differences in median T₁ and R₂^{*} between layers, but not for χ_R .

Figure 2.

Coronal images with cortical multilayer overlay. (A) T_1 , (C) R_2^* and (E) quantitative susceptibility maps are shown with detailed multilayer segmentation overlay (B), (D) and (F). Cubic interpolation was used in the quantitative images and layers. The 4th (outer, subpial) layer can be seen in red, 3rd layer in yellow, 2nd in green and the 1st (inner) layer is in blue.

Figure 3. Magnitude of correlations for quantitative T₁ map values and disability scales in full cortex and individual layers.

Spearman rank-order correlation coefficients are represented as bar graphs. P values were corrected for multiple comparisons. *** P < 0.001; ** P < 0.010; * P < 0.050.

EDSS = Expanded Disability Status Scale, T25FW = timed 25-foot walk, 9HPT = 9-Hole Peg Test, CVLT = California Verbal Learning Test, SDMT = Symbol Digit Modalities Test, BVMT = Brief Visuospatial Memory Test. 4th = fourth layer or subpial cortical layer, 3rd = third layer, 2nd = second layer, 1st = first layer or inner cortical layer.

Unit for median: second, skewness and peak height: no unit.

Figure 4. Magnitude of correlations for quantitative R_2^* map values and disability scales in full cortex and individual layers.

Spearman rank-order correlation coefficients are represented as bar graphs. P values were corrected for multiple comparisons. *** $P < 0.001$; ** $P < 0.010$; * $P < 0.050$. EDSS = Expanded Disability Status Scale, T25FW = timed 25-foot walk, 9HPT = 9-Hole Peg Test, CVLT = California Verbal Learning Test, SDMT = Symbol Digit Modalities Test, BVMT = Brief Visuospatial Memory Test. 4th = fourth layer or subpial cortical layer, 3rd = third layer, 2nd = second layer, 1st = first layer or inner cortical layer.

Unit for median: hertz, skewness and peak height: no unit.

Figure 5. Magnitude of correlations for quantitative χ_R map values and disability scales in full cortex and individual layers.

Spearman rank-order correlation coefficients are represented as bar graphs. P values were corrected for multiple comparisons. *** P < 0.001; ** P < 0.010; * P < 0.050.

EDSS = Expanded Disability Status Scale, T25FW = timed 25-foot walk, 9HPT = 9-Hole Peg Test, CVLT = California Verbal Learning Test, SDMT = Symbol Digit Modalities Test, BVMT = Brief Visuospatial Memory Test. 4th = fourth layer or subpial cortical layer, 3rd = third layer, 2nd = second layer, 1st = first layer or inner cortical layer.

Unit for median: parts per million, skewness and peak height: no unit.

Figure 6. Magnitude of correlations between interlayer differences in quantitative T₁ map values and disability scales.

Spearman rank-order correlation coefficients are represented as bar graphs. P values were corrected for multiple comparisons. *** P < 0.001; ** P < 0.010; * P < 0.050.

EDSS = Expanded Disability Status Scale, T25FW = timed 25-foot walk, 9HPT = 9-Hole Peg Test, CVLT = California Verbal Learning Test, SDMT = Symbol Digit Modalities Test, BVMT = Brief Visuospatial Memory Test. 4th = fourth layer or subpial cortical layer, 3rd = third layer, 2nd = second layer, 1st = first layer or inner cortical layer.

Unit for median: second, skewness and peak height: no unit.

Figure 7. Magnitude of correlations between interlayer differences in quantitative R_2^* map values and disability scales.

Spearman rank-order correlation coefficients are represented as bar graphs. P values were corrected for multiple comparisons. *** $P < 0.001$; ** $P < 0.010$; * $P < 0.050$.

EDSS = Expanded Disability Status Scale, T25FW = timed 25-foot walk, 9HPT = 9-Hole Peg Test, CVLT = California Verbal Learning Test, SDMT = Symbol Digit Modalities Test, BVMT = Brief Visuospatial Memory Test. 4th = fourth layer or subpial cortical layer, 3rd = third layer, 2nd = second layer, 1st = first layer or inner cortical layer.

Unit for median: hertz, skewness and peak height: no unit.

Figure 8. Magnitude of correlations between interlayer differences in quantitative χ_R map values and disability scales.

Spearman rank-order correlation coefficients are represented as bar graphs. P values were corrected for multiple comparisons. *** $P < 0.001$; ** $P < 0.010$; * $P < 0.050$.

EDSS = Expanded Disability Status Scale, T25FW = timed 25-foot walk, 9HPT = 9-Hole Peg Test, CVLT = California Verbal Learning Test, SDMT = Symbol Digit Modalities Test, BVMT = Brief Visuospatial Memory Test. 4th = fourth layer or subpial cortical layer, 3rd = third layer, 2nd = second layer, 1st = first layer or inner cortical layer.

Unit for median: parts per million, skewness and peak height: no unit.

Figure 9.

Average multi-layer and full cortex T_1 , R_2^* and susceptibility histograms for patients with low (blue) EDSS (median) and high (red) EDSS (> median).

Table 1:

Summary of clinical and imaging characteristics of patient cohort (n = 45).

Characteristic	Value
RRMS (n, %)	33 (73.3%)
SPMS (n, %)	4 (8.9%)
PPMS (n, %)	8 (17.8%)
Age (years)	48.7 (22.6, 63.5)
Sex (F/M)	(28, 17)
Disease Duration (years)	8.3 (0.7, 38.4)
Relapses within last year (n)	0 (0, 3)
WM lesion volume (ml)	3.33 (0.05, 21.16)
WMLF	3.2e-3 (4.5e-5, 1.8e-2)
BPF	0.85 (0.76, 0.89)
cGMF	0.38 (0.32, 0.42)
L1F	0.10 (0.09, 0.11)
L2F	0.10 (0.09, 0.11)
L3F	0.10 (0.08, 0.11)
L4F	0.09 (0.07, 0.11)
Cortical thickness (mm)	3.51 (0, 6)
EDSS	3.0 (1.0, 6.5)
T25FW (sec)	4.6 (3.5, 90.9)
9HPT (sec)	22.3 (16.1, 402.7)
CVLT score	49.0 (28.0, 70.0)
SDMT score	54.0 (35.0, 83.0)
BVMT score	40.0 (1.0, 70.0)

Median (range) values unless otherwise indicated.

Abbreviations: WM = white matter, RRMS = relapsing remitting MS, SPMS = secondary progressive MS, PPMS = primary progressive MS, M = Male, F = Female, WMLF = white matter lesion fraction, BPF = brain parenchyma fraction, cGMF = cortical grey matter fraction, L1F = first layer or inner cortical layer fraction, L2F = second layer fraction, L3F = third layer fraction, L4F = fourth layer or subpial cortical layer fraction, EDSS = Expanded Disability Status Scale, T25FW = timed 25-foot walk, 9HPT = 9-Hole Peg Test, CVLT = California Verbal Learning Test, SDMT = Symbol Digit Modalities Test, BVMT = Brief Visuospatial Memory Test.

Table 2.

Correlation of normalized volumes and cortical thickness with disability scales.

	BPF	WMLF	cGMF	L1F	L2F	L3F	L4F	Cortical thickness
EDSS	-0.228	0.197	-0.494**	-0.398**	-0.487**	-0.469**	-0.503***	-0.446**
T25FW	-0.005	0.121	-0.447**	-0.306*	-0.415**	-0.410**	-0.470**	-0.380*
9HPT	-0.373*	0.409**	-0.490**	-0.432**	-0.467**	-0.468**	-0.471**	-0.510***
CVLT	0.108	-0.155	0.400**	0.300*	0.329*	0.362*	0.423**	0.444**
SDMT	0.306*	-0.399**	0.409**	0.385**	0.400**	0.391**	0.359*	0.394**
BVMT	0.123	-0.195	-0.068	-0.092	-0.086	-0.072	-0.067	0.013

All volumes were normalized to intracranial volume and are thus reported as fractions. Spearman rank-order correlation coefficients. P values were corrected for multiple comparisons.

P < 0.001

**
P < 0.010

*
P < 0.050.

EDSS = Expanded Disability Status Scale, T25FW = timed 25-foot walk, 9HPT = 9-Hole Peg Test, CVLT = California Verbal Learning Test, SDMT = Symbol Digit Modalities Test, BVMT = Brief Visuospatial Memory Test. BPF = brain parenchyma fraction, WMLF = white matter lesion fraction, cGMF = cortical grey matter fraction, L1F = first layer or inner cortical layer fraction, L2F = second layer fraction, L3F = third layer fraction, L4F = fourth layer or subpial cortical layer fraction.