Relationships Between Retinal Axonal and Neuronal Measures and Global Central Nervous System Pathology in Multiple Sclerosis

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Objective: To determine the relationships between conventional and segmentation-derived optical coherence tomography (OCT) retinal layer thickness measures with intracranial volume (a surrogate of head size) and brain substructure volumes in multiple sclerosis (MS).

Design: Cross-sectional study.

Setting: Johns Hopkins University, Baltimore, Maryland.

Participants: A total of 84 patients with MS and 24 healthy control subjects.

Main Outcome Measures: High-definition spectral-domain OCT conventional and automated segmentation-derived discrete retinal layer thicknesses and 3-T magnetic resonance imaging brain substructure volumes.

Results: Peripapillary retinal nerve fiber layer as well as composite ganglion cell layer + inner plexiform layer thicknesses in the eyes of patients with MS without a history of optic neuritis were associated with cortical gray matter (P = .01 and P = .04, respectively) and caudate (P = .04 and P = .03, respectively) volumes. Inner nuclear layer thickness, also in eyes without a history of optic neuritis, was associated with fluid-attenuated inversion recovery lesion volume (P = .007) and inversely associated with normal-appearing white matter volume (P = .005) in relapsing-remitting MS. As intracranial volume was found to be related with several of the OCT measures in patients with MS and healthy control subjects and is already known to be associated with brain substructure volumes, all OCT–brain substructure relationships were adjusted for intracranial volume.

Conclusions: Retinal measures reflect global central nervous system pathology in multiple sclerosis, with thicknesses of discrete retinal layers each appearing to be associated with distinct central nervous system processes. Moreover, OCT measures appear to correlate with intracranial volume in patients with MS and healthy control subjects, an important unexpected factor unaccounted for in prior studies examining the relationships between peripapillary retinal nerve fiber layer thickness and brain substructure volumes.


Optical coherence tomography (OCT) is a high-resolution imaging technique enabling the quantitative estimation of peripapillary retinal nerve fiber layer (pRNFL) thickness. In addition, modern high-definition spectral-domain OCT renders high-resolution images, from which the individual retinal layers can also be objectively and precisely quantified. These include the macular RNFL (mRNFL), ganglion cell layer (GCL), inner nuclear layer (INL), and outer nuclear layer (ONL) (Figure 1).1-4 The RNFL is the innermost layer of the retina and comprises unmyelinated axons. These axons, which are derived from the ganglion cell neurons located in the GCL below the RNFL (Figure 1), coalesce at the optic discs to form the optic nerves and subsequently exit through the lamina cribrosa to become myelinated.5,6

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Multiple sclerosis (MS) has a predilection to affect the optic nerves clinically (e.g., from optic neuritis [ON]) and subclinically, such that 94% to 99% of patients with MS demonstrate demyelinating optic nerve lesions on postmortem examination. Optic nerve demyelination is thought to result in retrograde degeneration of the constituent axons of the optic nerve, leading to RNFL and GCL atrophy. Consistent with postmortem findings, OCT demonstrates lower pRNFL and GCL thicknesses in MS eyes compared with those of healthy control subjects, irrespective of a history of ON. Although reduced RNFL and GCL thicknesses in MS are thought to derive from the same pathologic process (optic neuropathy), GCL thickness appears to correlate better than pRNFL thickness with visual function and disability in MS. This observation may represent, at least in part, the superior reproducibility of GCL over RNFL thickness measurements.

In addition to inner retinal (RNFL and GCL) pathology, deeper retinal (INL and ONL) pathology also occurs in MS. Consistent with electroretinographic and postmortem findings, OCT segmentation demonstrates quantitative INL and ONL abnormalities in MS. A 3-dimensional macular volume cube generated by Cirrus HD-OCT from the macular region denoted by the red box in panel A from the same healthy control subject. Note the individual layers of the retina are readily discernible, except for the ganglion cell layer (GCL) and inner plexiform layer (IPL), which are difficult to distinguish. During the segmentation process (performed in 3-dimension), the segmentation software identifies the outer boundaries of the macular retinal nerve fiber layer (RNFL), IPL, and outer plexiform layer (OPL), as well as the inner boundary of the retinal pigment epithelium (RPE), which is identified by the conventional Cirrus HD-OCT algorithm. The identification of these boundaries facilitates OCT segmentation, enabling determination of the thicknesses of the macular RNFL, GCL + IPL, the inner nuclear layer (INL) + OPL, and the outer nuclear layer (ONL) including the inner and outer photoreceptor segments. C, Illustration of the cellular composition of the retinal layers depicted in panel B. ELM indicates external limiting membrane; ILM, inner limiting membrane; IPS, inner photoreceptor segments; OPS, outer photoreceptor segments; PR, photoreceptors.
ies used time-domain OCT, which has lower reproducibility and resolution than newer spectral-domain devices. The association between spectral-domain OCT-derived pRNFL thickness and brain substructure volumes in MS remains largely unexplored. Moreover, because OCT segmentation was unavailable at the time of these studies, the associations between GCL, INL, and ONL thicknesses with brain substructure volumes in MS were not examined. Finally, brain substructure volumes correlate with intracranial volume (ICV), a surrogate of head size. Consequently, individual brain substructure volumes are conventionally normalized or adjusted for ICV to account for these relationships. Because the retina is part of the CNS, it is plausible that retinal layer thicknesses may also correlate with ICV, although, to our knowledge, this has not been previously assessed. The identification of a relationship between OCT measures and ICV would suggest the need to adjust examined OCT–brain substructure relationships for ICV.

Because RNFL and GCL pathology in MS are related, it is plausible that INL and ONL pathologies may be unrelated to optic neuropathy, it is plausible that INL and ONL thicknesses in MS may reflect global CNS processes distinct from those reflected by RNFL and GCL thicknesses. It is also unclear whether INL and ONL pathology in MS may reflect the same or different global CNS processes as one another.

The principal objectives of this cross-sectional study were (1) to determine whether OCT-derived thicknesses and intracranial volume are related, so that these associations, in addition to the known associations between brain substructure volumes and ICV, may be accounted for when examining OCT–brain substructure relationships; (2) to determine the relationships of OCT-derived RNFL and GCL thicknesses with NAWM, WM lesion, and cortical GM volumes in MS, and whether these relationships are similar or different; and (3) to determine the relationships of OCT-derived INL and ONL thicknesses with NAWM, WM lesion, and cortical GM volumes in MS, and whether these relationships are similar or different.

An exploratory objective was to ascertain whether OCT-derived retinal thicknesses were related with other brain substructure volumes (eg, deep GM structures, cerebellum, and brainstem), perhaps reflecting other aspects of MS-related neurodegeneration.

**METHODS**

**PATIENTS**

Johns Hopkins University institutional review board approval was obtained for all study protocols, and written informed consent was obtained from recruited participants. Subjects with MS, recruited by convenience sampling from the Johns Hopkins Multiple Sclerosis Center, had their diagnosis confirmed by the treating neurologist (P.A.C.) based on 2010 revised McDonald criteria. Multiple sclerosis disease subtype was classified as relapsing-remitting MS (RRMS), secondary progressive MS (SPMS), or primary progressive MS (PPMS). Expanded Disability Status Scale (EDSS) scores were determined by a Neurostatus-certified EDSS examiner within 30 days of OCT and magnetic resonance imaging (MRI) examinations. Patient medical records were reviewed to determine MS disease duration and history of ON including date and side. Subjects with refractive errors of less than −6 diopters or greater than +6 diopters, other neurologic disorders, known ocular pathology, and/or diabetes mellitus were excluded from the study. To minimize confounding of OCT measurements and MRI-derived brain substructure volumes, patients within 3 months of ON and 1 month of steroid therapy were excluded. Healthy control subjects were recruited from among Johns Hopkins University staff.

**OPTICAL COHERENCE TOMOGRAPHY**

Retinal imaging was performed with spectral-domain Cirrus HD-OCT (model 4000 version 3.0; Carl Zeiss Meditec), as described in detail elsewhere. Briefly, peripapillary and macular data were obtained with the Optic Disc Cube 200 × 200 protocol and Macular Cube 312 × 128 protocol, respectively. Optical coherence tomography scanning was performed by experienced technicians, and scans were monitored to ensure fixation was reliable. Scans with signal strength less than 7/10 or with artifact were excluded from analyses.

Macular cube scans were analyzed in a blinded fashion using segmentation software, as described in detail elsewhere. Briefly, segmentation performed in 3-dimension identifies the inner limiting membrane, the outer boundaries of the mRNFL, the outer plexiform layer (IPL), outer plexiform layer, and the inner boundary of the retinal pigment epithelium (Figure 1). Following the identification of these boundaries, thicknesses of the mRNFL, GCL + IPL, INL + outer plexiform layer, and ONL (including inner and outer photoreceptor segments) were calculated in an annulus of an inner radius of 0.54 mm and outer radius of 2.4 mm, centered on the fovea. This segmentation protocol has been shown to be reproducible in patients with MS and healthy control subjects (intrarater intraclass correlation coefficients, 0.91-0.99, for all segmentation measurements described).

**MAGNETIC RESONANCE IMAGING**

Brain MRI was performed with a 3-T Philips Intera scanner (Philips Medical System). Two axial whole brain sequences without gaps were used: multislice fluid-attenuated inversion recovery (FLAIR; acquired resolution, 0.8 × 0.8 × 2.2 mm or 0.8 × 0.8 × 4.4 mm; echo time, 68 ms; repetition time, 11 seconds; inversion time, 2.8 seconds; SENSE factor, 2; averages, 1) and 3-dimensional magnetization-prepared rapid acquisition of gradient echoes (acquired resolution, 1.1 × 1.1 × 1.1 mm; echo time, 6 milliseconds; repetition time, approximately 10 milliseconds; inversion time, 835 milliseconds; flip angle, 8°; SENSE factor, 2; averages, 1).

Acquired images were analyzed with topology-preserving anatomy-driven segmentation (Lesion-TOADS), a validated, automated, segmentation method described in detail elsewhere. This technique computes ICV and parcellates the brain into its component structures, yielding the following brain substructure volumes: FLAIR WM lesions, ventricular cerebrospinal fluid, sulcal cerebrospinal fluid, cortical GM, total cerebral WM (including lesions), cerebellar GM, cerebellar WM, caudate, putamen, thalamus, and brainstem. Normal-appearing cerebral white matter volumes were calculated by subtracting FLAIR lesion volumes from total cerebral WM volumes. Cerebral volume fraction, analogous to brain parenchymal fraction, was calculated by dividing (normalizing) the summed volume of brain substructures (excluding ventricular and sulcal cerebrospinal fluid) by ICV. All segmentations were verified by a trained observer.
### Table 1. Summary of Demographics and Clinical Characteristics

<table>
<thead>
<tr>
<th></th>
<th>All MS</th>
<th>RRMS</th>
<th>SPMS</th>
<th>PPMS</th>
<th>Healthy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eyes, No.</td>
<td>84 (161)</td>
<td>58 (114)</td>
<td>18 (33)</td>
<td>8 (14)</td>
<td>24 (48)</td>
</tr>
<tr>
<td>Age, y</td>
<td>43.6 (13.0)</td>
<td>37.4 (10.2)</td>
<td>58.3 (4.9)</td>
<td>55.0 (9.0)</td>
<td>36.0 (11.1)</td>
</tr>
<tr>
<td>Female, No. (%)</td>
<td>61 (73)</td>
<td>44 (76)</td>
<td>14 (78)</td>
<td>3 (38)</td>
<td>18 (75)</td>
</tr>
<tr>
<td>Eyes with ON history, No. (%)</td>
<td>37 (23)</td>
<td>32 (26)</td>
<td>5 (15)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disease duration, y</td>
<td>11.5 (8.7)</td>
<td>7.5 (6.4)</td>
<td>21.7 (8.4)</td>
<td>15.8 (13.1)</td>
<td></td>
</tr>
<tr>
<td>pRNFL, µm</td>
<td>84.5 (13.0)</td>
<td>84.9 (13.4)</td>
<td>81.0 (10.9)</td>
<td>89.6 (12.1)</td>
<td>91.9 (9.4)</td>
</tr>
<tr>
<td>GCL + IPL, µm</td>
<td>70.6 (9.6)</td>
<td>70.8 (10.0)</td>
<td>68.8 (9.1)</td>
<td>73.3 (5.9)</td>
<td>81.9 (4.9)</td>
</tr>
<tr>
<td>mRNFL, µm</td>
<td>28.3 (5.3)</td>
<td>28.3 (5.7)</td>
<td>27.4 (4.2)</td>
<td>29.0 (2.8)</td>
<td>33.7 (3.4)</td>
</tr>
<tr>
<td>INL + OPL, µm</td>
<td>64.9 (4.6)</td>
<td>65.2 (4.7)</td>
<td>63.9 (4.2)</td>
<td>66.0 (3.6)</td>
<td>65.0 (4.8)</td>
</tr>
<tr>
<td>ONL, µm</td>
<td>118.8 (8.1)</td>
<td>119 (7.9)</td>
<td>117 (7.5)</td>
<td>126.3 (7.7)</td>
<td>119.8 (5.9)</td>
</tr>
<tr>
<td>CVF</td>
<td>0.655 (0.04)</td>
<td>0.662 (0.03)</td>
<td>0.640 (0.02)</td>
<td>0.633 (0.06)</td>
<td>0.682 (0.03)</td>
</tr>
<tr>
<td>Normalized total WM volume</td>
<td>0.267 (0.02)</td>
<td>0.269 (0.02)</td>
<td>0.263 (0.02)</td>
<td>0.258 (0.04)</td>
<td>0.275 (0.02)</td>
</tr>
<tr>
<td>Normalized total GM volume</td>
<td>0.377 (0.02)</td>
<td>0.382 (0.02)</td>
<td>0.366 (0.02)</td>
<td>0.365 (0.03)</td>
<td>0.393 (0.02)</td>
</tr>
<tr>
<td>EDSS score, median (IQR)</td>
<td>3 (2-6)</td>
<td>2.50 (1.50-3.25)</td>
<td>6.0 (6.0-6.5)</td>
<td>6.25 (6.0-6.75)</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: CVF, cerebral volume fraction; EDSS, Expanded Disability Status Scale; GCL + IPL, ganglion cell layer + inner plexiform layer; GM, gray matter; INL + OPL, inner nuclear layer + outer plexiform layer; IQR, interquartile range; mRNFL, macular retinal nerve fiber layer; MS, multiple sclerosis; ON, optic neuritis; ONL, outer nuclear layer (including the inner and outer photoreceptor segments); PPMS, primary progressive MS; pRNFL, peripapillary retinal nerve fiber layer; RRMS, relapsing-remitting MS; SPMS, secondary progressive MS; WM, white matter.

### Statistical Analyses

Statistical analyses were completed on STATA version 11 (StataCorp). Because OCT measures from both eyes of participants were included in the analyses, mixed effects multivariable linear regression (ME-MLR) models were used to account for within-subject, intereye correlations. Relationships between OCT measures and ICV were assessed using ME-MLR, adjusting for age and sex (and disease duration in MS). Partial correlations adjusting for age and sex (and disease duration in MS), but not accounting for within-subject intereye correlations, were also performed to illustrate the relationships between OCT measures and ICV. Because we found significant relationships between OCT measures and ICV in patients with MS and health control subjects (see “Results” section), ME-MLR models were adjusted for ICV, in addition to age, sex, and disease duration, when assessing the relationships between OCT measures and MRI-derived brain substructure volumes in MS. This means that the relationships between OCT measures and brain substructure volumes were also adjusted for ICV.

In comparisons of MRI-derived brain substructure volumes between patients with MS and healthy control subjects, and in the assessment of MRI-derived brain substructure volume relationships with EDSS score, individual brain substructure volumes were normalized to ICV. For comparative analyses of OCT measures and normalized brain substructure volumes with EDSS score, ME-MLR adjusting for age, sex, and disease duration was used. To assess associations between OCT measures and normalized brain substructure volumes with EDSS score, ME-MLR adjusting for age, sex, disease duration, and ON history was used. Statistical significance was defined as $P < .05$. As this was an exploratory study, reported $P$ values should be interpreted as descriptive. Accordingly, correction for multiple comparisons was not performed.

### Results

Eighty-four patients with MS (161 eyes; 7 eyes were excluded as their OCT scans had inadequate signal strength) and 24 healthy control subjects (48 eyes) were included in the study (Table 1). The mean age of the MS cohort was 37 years, and most of the cohort had RRMS (62%). The mean age of the healthy control cohort was 36 years. Twenty-six percent of RRMS eyes (n = 32) and 28% of SPMS eyes (n = 5) had a history of ON.

### Associations Between OCT Measures and ICV

Intracranial volume was associated with GCL + IPL ($P = .008$), mRNFL ($P = .004$), and ONL ($P = .005$) thicknesses, but not pRNFL ($P = .27$) or INL ($P = .36$) thicknesses in MS (Table 2, Figure 2A). Partial correlation coefficients between ICV and OCT thickness measures in MS were as follows: pRNFL: $r = 0.11$; GCL + IPL: $r = 0.11$.
In accordance with published data,\textsuperscript{2,4} pRNFL and GCL + IPL thicknesses were lower in patients with MS than healthy control subjects ($P = .01$ and $P < .001$, respectively). Likewise, mRNFL thickness was lower in all MS subtypes relative to healthy control subjects ($P < .001$), consistent with the lower pRNFL and GCL + IPL thicknesses (Table 1). Eyes with a history of ON had lower pRNFL, GCL + IPL, and mRNFL thicknesses than eyes without a history of ON ($P < .001$ for all).

Cerebral volume fraction and normalized total GM volume were lower in patients with MS than healthy control subjects ($P = .02$ and $P = .01$, respectively), whereas normalized total WM volume was not different between the 2 groups ($P = .19$). Compared with healthy control subjects, patients with MS demonstrated lower normalized thalamic (mean [SD], $-0.001 [0.0003]$; $P = .003$) and normalized caudate (mean [SD], $-0.0007 [0.0001]$; $P < .001$) volumes. Normalized cerebellar WM (mean [SD], $-0.001 [0.0004]$; $P = .008$), normalized cerebellar GM (mean [SD], $-0.003 [0.001]$; $P = .006$), and normalized brainstem (mean [SD], $-0.001 [0.003]$; $P = .001$) volumes were also lower in patients with MS than healthy control subjects.
Table 3. Relationships Between OCT Measures and Brain Substructure Volumes in MS


<table>
<thead>
<tr>
<th></th>
<th>Cortical GM</th>
<th>NAWM</th>
<th>Caudate</th>
<th>Thalamus</th>
<th>Lesions</th>
<th>Brainstem</th>
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</thead>
<tbody>
<tr>
<td>All eyes (n = 161)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pRNFL</td>
<td>.005&lt;sup&gt;b&lt;/sup&gt;</td>
<td>.98&lt;sup&gt;c&lt;/sup&gt;</td>
<td>.05</td>
<td>.18</td>
<td>.78</td>
<td>.10</td>
</tr>
<tr>
<td>GCL + IPL</td>
<td>.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>.65</td>
<td>.005&lt;sup&gt;b&lt;/sup&gt;</td>
<td>.29</td>
<td>.91</td>
<td>.04&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>mRNFL</td>
<td>.44</td>
<td>.73</td>
<td>.007&lt;sup&gt;b&lt;/sup&gt;</td>
<td>.09</td>
<td>.73&lt;sup&gt;c&lt;/sup&gt;</td>
<td>.28</td>
</tr>
<tr>
<td>INL</td>
<td>.93&lt;sup&gt;c&lt;/sup&gt;</td>
<td>.01&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>.52&lt;sup&gt;c&lt;/sup&gt;</td>
<td>.67&lt;sup&gt;c&lt;/sup&gt;</td>
<td>.18</td>
<td>.99</td>
</tr>
<tr>
<td>ONL</td>
<td>.32</td>
<td>.38&lt;sup&gt;c&lt;/sup&gt;</td>
<td>.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>.18</td>
<td>.29&lt;sup&gt;c&lt;/sup&gt;</td>
<td>.62</td>
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<tr>
<td>Non-ON eyes (n = 124)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>pRNFL</td>
<td>.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>.84&lt;sup&gt;c&lt;/sup&gt;</td>
<td>.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>.12</td>
<td>.88</td>
<td>.18</td>
</tr>
<tr>
<td>GCL + IPL</td>
<td>.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>.78&lt;sup&gt;c&lt;/sup&gt;</td>
<td>.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>.13</td>
<td>.73&lt;sup&gt;c&lt;/sup&gt;</td>
<td>.22</td>
</tr>
<tr>
<td>mRNFL</td>
<td>.53</td>
<td>.68&lt;sup&gt;c&lt;/sup&gt;</td>
<td>.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>.50&lt;sup&gt;c&lt;/sup&gt;</td>
<td>.72</td>
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<tr>
<td>INL</td>
<td>.90&lt;sup&gt;c&lt;/sup&gt;</td>
<td>.02&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>.65&lt;sup&gt;c&lt;/sup&gt;</td>
<td>.62&lt;sup&gt;c&lt;/sup&gt;</td>
<td>.16</td>
<td>.81</td>
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<tr>
<td>ONL</td>
<td>.46</td>
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<td>.11</td>
<td>.25</td>
<td>.28&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>pRNFL</td>
<td>.43</td>
<td>.61&lt;sup&gt;c&lt;/sup&gt;</td>
<td>.49</td>
<td>.56</td>
<td>.32</td>
<td>.10</td>
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<tr>
<td>GCL + IPL</td>
<td>.94</td>
<td>.58</td>
<td>.24</td>
<td>.95</td>
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<tr>
<td>mRNFL</td>
<td>.60&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>.78&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>INL</td>
<td>.42</td>
<td>.83&lt;sup&gt;c&lt;/sup&gt;</td>
<td>.78</td>
<td>.89&lt;sup&gt;a&lt;/sup&gt;</td>
<td>.69</td>
<td>.61&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>ONL</td>
<td>.19</td>
<td>.80&lt;sup&gt;c&lt;/sup&gt;</td>
<td>.09</td>
<td>.27</td>
<td>.27&lt;sup&gt;c&lt;/sup&gt;</td>
<td>.97</td>
</tr>
</tbody>
</table>

Abbreviations: GCL + IPL, ganglion cell layer + inner plexiform layer; GM, gray matter; INL, inner nuclear layer; mRNFL, macular retinal nerve fiber layer; MS, multiple sclerosis; NAWM, normal-appearing white matter; OCT, optical coherence tomography; ON, optic neuritis; OLN, outer nuclear layer (including the inner and outer photoreceptor segments); pRNFL, peripapillary retinal nerve fiber layer.

<sup>a</sup>P value entries generated with mixed effects linear regression accounting for within-subject intereye correlations, adjusting for age, sex, disease duration, and intracranial volume.

<sup>b</sup>Significant results.

<sup>c</sup>Indicates inverse relationships.

Ganglion cell layer + IPL (P = .04) and mRNFL (P = .01) thicknesses, as well as normalized NAWM volume (P = .03), were inversely associated with EDSS scores in RRMS. Of OCT and MRI measures, only INL thickness was associated (inversely) with EDSS scores in SPMS (P = .003) and PPMS (P = .003).

RELATIONSHIPS BETWEEN OCT MEASURES AND BRAIN SUBSTRUCTURE VOLUMES IN MS

Peripapillary RNFL and GCL + IPL thicknesses were associated with cortical GM volume in the entire MS cohort (P = .005 and P = .04, respectively) (Table 3). These associations were predominantly driven by relationships in eyes without a history of ON. Similarly, pRNFL (P = .04) and GCL + IPL (P = .03) thicknesses in non-ON eyes were also associated with caudate volume. Inner nuclear layer thickness and NAWM volume were inversely associated in the entire MS cohort (P = .01; Figure 2C), and again this association was predominantly driven by relationships in eyes without a history of ON.

In subgroup analyses, most of the previously mentioned relationships were observed in RRMS (Figure 2D, Table 4), with pRNFL and GCL + IPL thicknesses also associated with brainstem and cerebellar WM volumes, and INL thickness in eyes without a history of ON also associated with FLAIR lesion volume (P = .007). Of OCT measurements, OLN thickness alone was only significantly associated with cerebellar WM volume in RRMS eyes with a history of ON (P = .03). However, ONL thickness in RRMS eyes with a history of ON also trended toward significant associations with cortical GM (P = .05), caudate (P = .08), thalamus (P = .08), and brainstem (P = .10) volumes, as well as an inverse association with FLAIR lesion volume (P = .08).

In further subgroup analyses, ONL thickness was associated with cortical GM volume in SPMS (P = .04) and brainstem volume in PPMS (P = .01). Although OCT measures and brain substructure volumes were generally not correlated in SPMS, several associations, consistent with those demonstrated in RRMS, were also observed in PPMS (eTable, http://www.jamaneurol.com). In addition to cortical GM volume (P = .002), pRNFL thickness was associated with NAWM volume in PPMS (P = .001).

COMMENT

The results of this study suggest inner and outer retinal layer thicknesses, measured with spectral-domain OCT in MS, may reflect global and potentially distinct CNS processes, and that OCT may be a useful complementary in vivo technique to MRI in evaluating MS. In particular, lower pRNFL and GCL + IPL thicknesses appear to reflect lower cortical GM volume in MS, suggesting that measurements obtained with OCT, a relatively inexpensive, noninvasive, reproducible, rapid, and well-tolerated investigation may partially represent clinically relevant processes known to affect the cortical GM compartment in MS. Furthermore, pRNFL and GCL + IPL thicknesses may also reflect caudate volume in MS, as well as brainstem and cerebellar WM volumes in RRMS. Moreover, INL and ONL thicknesses, particularly in RRMS, may reflect global, although somewhat different,
CNS processes than those reflected by RNFL and GCL + IPL thicknesses. Interestingly, greater INL thickness may be associated with higher FLAIR lesion volume and lower NAWM volume. These findings indicate the possibility that the potential mechanism underlying the proposed occurrence of primary retinal pathology affecting the INL in MS may be inflammatory, such as related to retinal periphlebitis. Retinal periphlebitis is known to occur in up to 20% of patients with MS, and it has previously been shown that active retinal periphlebitis and disruption of the blood-brain barrier tend to occur simultaneously in MS. Moreover, the presence of retinal periphlebitis in patients with MS has been shown to be a risk factor for the subsequent development of relapses and gadolinium-enhancing lesions.

Outer nuclear layer thickness also appears to have a relationship with brain substructure volume. Lower ONL thickness appears to reflect lower cerebellar WM volume and, moreover, several additional trends suggesting potential associations between lower ONL thickness and lower cortical GM, caudate, thalamic, and brainstem volumes, as well as higher FLAIR lesion volume, were observed, indicating the possibility that ONL thickness may reflect the global nature of neurodegeneration in MS.

A conspicuously novel finding in this study, unaccounted for in prior studies assessing retinal-brain relationships, was the apparent relationship between OCT thickness measures with ICV. Because brain substructure volumes correlate with ICV, individual brain substructure volumes are conventionally normalized or adjusted for ICV to account for these associations. Our finding that OCT measures and ICV may be related suggests that OCT measures should also be adjusted for ICV when assessing OCT–brain substructure relationships. Of note, most of the OCT measures examined in this study were associated with ICV in MS, and the correlation coefficients between OCT measures and ICV in healthy control subjects were greater than in MS, supporting the plausibility of a biological relationship. These findings merit further investigation in future studies.

As the optic nerve is frequently affected both clinically and subclinically in MS, the anterior visual system has been proposed as a model within which to study MS-related neurodegeneration. Demyelinated, yet intact, retrobulbar axons may be potentially remyelinated by viable oligodendrocytes, protecting against axonal (RNFL) and neuronal (GCL) degeneration. Optical coherence tomography has several compelling characteristics, including significant structure-function correlations (with vision and disability), which make it useful for detecting and monitoring neurodegeneration in MS and for documenting potentially neuroprotective and/or neurorestorative effects of therapeutic agents. Because our results suggest retinal layer thicknesses in MS may reflect global CNS pathology, it is possible that neuroprotective and/or neurorestorative effects detected in the anterior visual pathway with OCT might partially represent more global CNS effects, making OCT a potentially useful adjunct to MRI outcome measures in clinical trials.

Associations between pRNFL, mRNFL, and GCL + IPL thickness measures and brain substructure volumes were similar and most pronounced in eyes without a history of ON, suggesting that subclinical optic neuropathy may be an ideal surrogate of global neurodegenera-
tion in MS. Although similar subclinical processes also likely occur in eyes with a history of ON, disproportionate local tissue degeneration following ON may potentially obscure these relationships. Higher INL thickness in non-ON eyes was associated with higher FLAIR lesion volume and lower NAWM volume, indicating the possibility that an inflammatory process, such as retinal periphlebitis, may be operative in the INL in MS eyes. However, determination of the underlying pathobiology of either INL or ONL changes was beyond the scope of this study. Nonetheless, our findings highlight that INL and ONL thicknesses in MS may reflect global CNS processes that differ from those reflected by RNFL and GCL + IPL thicknesses, which is consistent with our previous work. Interestingly, INL and ONL thicknesses did not appear to reflect the same global CNS processes, indicating the possibility that INL and ONL abnormalities in MS may be pathophysiologically distinct.

Outer nuclear layer thickness in eyes with a history of ON was associated with cerebellar WM volume, and several trends were detected between ONL thickness and brain substructure volumes in RRMS eyes with ON history. The lack of significance for some of these results is likely in part attributable to the underpowering of eyes with a history of ON. Because selective photoreceptor pathology of undetermined etiology may occur in optic neuropathies, our observations indicate the possibility that this process may likewise occur following MS-related ON, and its occurrence may be a harbinger of increased susceptibility to neurodegeneration. Inner nuclear layer and ONL pathologies in MS warrant more focused and detailed study in the future.

This preliminary cross-sectional study has several limitations. First, most of the patients included had RRMS. Thus, more accurate characterization of the relationships between OCT measures and brain substructure volumes by MS subtype, which will require enrollment of greater numbers of patients with SPMS and PPMS as well as with RRMS, is imperative. Second, limited enrollment of patients with MS and healthy control subjects, in part related to the cost of MRI, and underpowering may have contributed to the lack of significance for some of our analyses, particularly in the progressive cohorts.

Third, as this was an exploratory study, we did not adjust for multiple comparisons, thus it is conceivable that the identified relationships could have occurred by chance alone. However, because our results are consistent with and expand on prior studies demonstrating relationships between pRNFL thickness and whole brain volume (brain parenchymal fraction), it is highly unlikely that all of our findings were spurious, and most of the observed associations likely reflect true biological relationships. Nonetheless, our findings require further confirmation with other OCT devices and segmentation techniques, as well as in other MS cohorts; they also need to be examined longitudinally.

Finally, this preliminary study does not explain the mechanisms underlying the relationships observed between OCT measures and brain substructure volumes. For example, it is unclear why pRNFL and GCL + IPL thicknesses may reflect caudate, brainstem, or cerebellar WM volumes. While this could simply represent a reflection of global neurodegeneration, these potential relationships warrant further study and may be illuminating to study longitudinally.

In summary, retinal axonal and neuronal layer thicknesses in MS may reflect global CNS processes. Lower pRNFL and GCL + IPL thicknesses related to subclinical optic neuropathy predominantly reflect lower cortical GM and caudate volumes. Inner nuclear layer and ONL thicknesses in MS may reflect global CNS processes somewhat distinct from one another and those reflected by RNFL and GCL + IPL thicknesses. Greater INL thickness in non-ON eyes (potentially reflecting inflammation) may reflect higher FLAIR lesion and lower NAWM volumes, while ONL thickness in eyes with a history of ON may reflect global neurodegeneration. Optical coherence tomography measures may also correlate with ICV, finding with potential implications for future studies examining retinal-brain relationships. Although our observations require verification, they provide support for the potential use of OCT as an adjunctive outcome measure in MS clinical trials.

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