Damage to the Optic Radiation in Multiple Sclerosis Is Associated With Retinal Injury and Visual Disability

Daniel S. Reich, MD, PhD; Seth A. Smith, PhD; Eliza M. Gordon-Lipkin, BS; Arzu Ozturk, MD; Brian S. Caffo, PhD; Laura J. Balcer, MD; Peter A. Calabresi, MD

Objective: To determine whether damage to the optic radiation (OR) in multiple sclerosis (MS) is associated with optic nerve injury and visual dysfunction.

Design: Case-control study.

Setting: Referral center.

Participants: Ninety referred patients with MS and 29 healthy volunteers.

Main Outcome Measures: Magnetic resonance imaging indices along the OR were reconstructed with diffusion tensor tractography. Retinal nerve fiber layer thickness and visual acuity at high and low contrast were measured in a subset of the MS group (n=36).

Results: All tested magnetic resonance imaging indices (fractional anisotropy [FA]; mean, parallel, and perpendicular $\lambda_\perp$ diffusivity; T2 relaxation time; and magnetization transfer ratio) were significantly abnormal in patients with MS. Mean retinal nerve fiber layer thickness was significantly correlated with FA ($r=0.55; P<.001$) and $\lambda_\perp$ ($r=-0.37; P=.001$). The retinal nerve fiber layer thickness in the nasal retinal quadrant was also specifically correlated with FA and $\lambda_\perp$ in the synaptically connected contralateral OR. In individuals with less severely damaged optic nerves (mean retinal nerve fiber layer thickness >80 µm), letter acuity scores at 2.5% contrast were correlated with OR-specific FA ($r=0.55; P=.004$), $\lambda_\perp$ ($r=-0.40; P=.04$), and magnetization transfer ratio ($r=0.54; P=.01$), as well as the fraction of OR volume made up of lesions ($r=-0.69; P<.001$).

Conclusions: Fractional anisotropy and $\lambda_\perp$ are potentially useful quantitative magnetic resonance imaging biomarkers of OR-specific damage in MS. Such damage is associated with retinal injury and visual disability.

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Table 1. Sample Size and Cohort Characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>All Types (n=90)</th>
<th>RRMS (n=52)</th>
<th>SPMS (n=24)</th>
<th>PPMS (n=14)</th>
<th>Healthy Volunteers (n=29)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female sex, No.</td>
<td>62</td>
<td>40</td>
<td>14</td>
<td>8</td>
<td>20</td>
</tr>
<tr>
<td>Age, median (range), y</td>
<td>45 (24-67)</td>
<td>40 (24-62)</td>
<td>51 (40-66)</td>
<td>53 (29-67)</td>
<td>31 (22-63)</td>
</tr>
<tr>
<td>Disease duration, median (range), y</td>
<td>8 (0-42)</td>
<td>6 (0-29)</td>
<td>16 (5-38)</td>
<td>5 (2-42)</td>
<td>...</td>
</tr>
<tr>
<td>Clinical history of optic neuritis, No.</td>
<td>26</td>
<td>17</td>
<td>9</td>
<td>0</td>
<td>...</td>
</tr>
<tr>
<td>EDSSa</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cases observed, No.</td>
<td>62</td>
<td>33</td>
<td>17</td>
<td>12</td>
<td>...</td>
</tr>
<tr>
<td>Score, median (range)</td>
<td>4.0 (0-8)</td>
<td>2.5 (0-6)</td>
<td>6.5 (3.5-8)</td>
<td>5.0 (1.5-6.5)</td>
<td>...</td>
</tr>
<tr>
<td>OCT/visual acuityb</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cases observed, No.</td>
<td>36</td>
<td>21</td>
<td>8</td>
<td>7</td>
<td>...</td>
</tr>
<tr>
<td>RNFL thickness, median (range), µm</td>
<td>83 (44-114)</td>
<td>83 (56-110)</td>
<td>80 (44-90)</td>
<td>89 (78-114)</td>
<td>...</td>
</tr>
<tr>
<td>Correctly named letters, median (range), %</td>
<td>91 (0-100)</td>
<td>94 (75-100)</td>
<td>88 (0-100)</td>
<td>77 (50-100)</td>
<td>...</td>
</tr>
<tr>
<td>100% contrast</td>
<td>55 (0-78)</td>
<td>58 (40-72)</td>
<td>44 (0-65)</td>
<td>33 (11-78)</td>
<td>...</td>
</tr>
<tr>
<td>2.5% contrast</td>
<td>28 (0-58)</td>
<td>34 (4-58)</td>
<td>2 (0-28)</td>
<td>18 (0-45)</td>
<td>...</td>
</tr>
<tr>
<td>1.25% contrast</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.0% contrast</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: EDSS, Expanded Disability Status Scale; ellipses, not applicable; MS, multiple sclerosis; OCT, optical coherence tomography; PPMS, primary progressive MS; RNFL, retinal nerve fiber layer; RRMS, relapsing-remitting MS; SPMS, secondary progressive MS.

aData for EDSS and visual acuity were obtained within 30 days of magnetic resonance imaging.

METHODS

PARTICIPANTS

Study participants (Table 1) were recruited from The Johns Hopkins Multiple Sclerosis Center. The OCT and visual acuity data were included if collected within 30 days of MRI. Protocols were approved by the institutional review board.

MRI ACQUISITION

Details of our MRI protocol have been published. Using a 3-T scanner (Intera; Philips Medical Systems, Best, the Netherlands), we obtained and coregistered, separately for each participant and using a rigid body transformation model, data from axial whole-brain DTI, magnetization transfer, and proton-density and T2-weighted sequences. Axial fluid-attenuated inversion recovery (FLAIR) images were also coregistered to the mean DTI map for that participant via an affine transformation.

TRACT RECONSTRUCTION

We used the DTI data and the fiber assignment by continuous tractography method to reconstruct the courses of both ORs in each participant. Within DtiStudio, regions of interest were placed over the proximal and distal OR, as visualized on coronal reconstructions of the DTI color maps (Figure 1). Reconstructed tracts were terminated when fractional anisotropy (FA) decreased to less than 0.13 or the between-voxel turning angle was more than 30º. Similar regions of interest and termination criteria have been used by other groups. Only fibers that connected the anterior and posterior regions of interest were selected for further analysis.

Across the voxels that composed the reconstructed OR, we measured median values of the following MRI indices: FA, mean diffusivity (MD), parallel diffusivity (λ1), perpendicular diffusivity (λ2), magnetization transfer ratio (MTR), and absolute T2 relaxation time. We also created tract profiles to visualize the variation in each of these MRI indices as a function of normalized position along the tract.

LESION SEGMENTATION

Lesions were identified on each section of the coregistered FLAIR images by a neuroradiologist (A.O.) blinded to participant identity and level of disability or retinal damage. They were then outlined with a region-growing technique (implemented in DtiStudio) that extended a contiguous area around hand-selected seed points until a threshold intensity was reached. The threshold was adjusted for optimal qualitative capture of the lesions with minimal inclusion of adjacent normal-appearing white matter.

OPTICAL COHERENCE TOMOGRAPHY

Our OCT data acquisition and processing methods are detailed elsewhere. We used the Fast-RNFL Thickness protocol on an OCT device (Stratus-3; Carl Zeiss Meditec, Dub-
lin, California). In both eyes, we recorded the RNFL thickness over the entire retina and, separately, the nasal and temporal quadrants.

VISUAL FUNCTION TESTING

Best-corrected visual function was assessed binocularly in the MS group using retroilluminated or nonretroilluminated Sloan letter charts (Precision Vision Inc, LaSalle, Illinois) at 100%, 2.5%, and 1.25% contrast. The results were recorded as the percentage of letters correctly identified. In the 11 participants for whom data were obtained using both charts, no significant chart type–related differences were observed (paired t tests, \( P > .05 \) at all contrast levels); thus, data from both chart types were merged.

CORRELATIONS AND STATISTICAL MODELING

Statistical calculations were performed using commercially available software (STATA; StataCorp LP, College Station, Texas). We used parametric statistics (t tests and multiple linear regression analysis) to assess the relationship of OCT abnormalities and visual function scores to OR-specific median MRI indices. For correlations of MRI indices with disability scores and OCT, we used multiple observations per participant if the observations were within 30 days of MRI. We calculated modified correlation coefficients based on subject-specific means, an approach that appropriately accounts for multiple observations per participant.\(^{26}\) We report \( P \) values directly without adjustment for multiple comparisons.
RESULTS

MRI INDICES ALONG THE ORs

Mean spatially normalized OR profiles from the MS and control groups, depicting the spatial variation in MRI indices from the LGN anteriorly to the subcortical white matter of the occipital lobe posteriorly, are shown in Figure 2. These profiles demonstrate marked abnormalities for all MRI indices examined, most prominently in the middle third of the tract. Profiles from the relapsing-remitting subgroup were less abnormal than those from the progressive subgroups. Median MRI indices along the OR were also significantly abnormal in all MS subgroups (Table 2). As expected, T2 relaxation time, MD, \( \lambda_\parallel \), and \( \lambda_\perp \) were increased, whereas FA and MTR were decreased.

LESIONS vs NORMAL-APPEARING WHITE MATTER

Lesions that overlapped the reconstructed ORs were identified on FLAIR images, and the remainder of the OR was considered normal-appearing white matter. Across the MS cohort, a median of 5.9% (range, 0%-65%) of the total OR volume consisted of lesions. Figure 3 shows mean OR profiles from the lesion and normal-appearing white matter portions of the tract. (For comparison, profiles from healthy volunteers and unsegmented profiles from patients with MS, taken from Figure 2, are reproduced herein.) These results demonstrate that MRI indices are more abnormal in lesions than in normal-appearing white matter.

ASSOCIATION WITH HISTORY OF OPTIC NEURITIS

We could demonstrate no significant association between self-reported history of optic neuritis and any of the tested MRI indices. For this analysis, we used logistic regression adjusting for age and sex (\( P > .05 \) for all comparisons).

CORRELATION WITH RETINAL DAMAGE

In the MS group (Table 3 and Figure 4A-D), we found moderate correlations of average RNFL thickness with FA and \( \lambda_\parallel \), but not MD or \( \lambda_\perp \), along the OR, controlling for age, sex, and the corresponding MRI indices along the portion of a nonvisual pathway, the corticospinal tract, that runs within the corona radiata (like the OR, a common site of MS lesions\(^27\)). These results suggest an association between RNFL and OR damage that is not primarily driven by imaging abnormalities outside the visual system. Data in Table 3 also indicate that both the lesional and normal-appearing white matter components of the OR are associated with RNFL thinning and that segregation of the 2 components generally lowers the correlation coefficients and their associated \( P \) values.

We further characterized the specificity of this association by assessing the correlation between OR-specific MRI indices and RNFL thinning in the nasal retinal quadrants, axons from which project contralaterally. To do this, we constructed a regression model that adjusts for age, sex, temporal-quadrant RNFL thickness, and the corresponding MRI index in the corona radiata portion of the corticospinal tract. Despite higher variability in quadrant-specific RNFL assessment,\(^28\) which might reduce the...
Table 2. Mean Values of Median Optic Radiation–Specific MRI Indices Across the MS and Healthy Volunteer Groups

<table>
<thead>
<tr>
<th>MRI Index</th>
<th>All Types</th>
<th>RRMS</th>
<th>SPMS</th>
<th>PPMS</th>
<th>Healthy Volunteers</th>
</tr>
</thead>
<tbody>
<tr>
<td>FA</td>
<td>0.49 (.001)</td>
<td>0.49 (.001)</td>
<td>0.48 (.001)</td>
<td>0.50 (.001)</td>
<td>0.58</td>
</tr>
<tr>
<td>MD, µm²/ms</td>
<td>0.98 (.001)</td>
<td>0.98 (.001)</td>
<td>0.98 (.001)</td>
<td>1.00 (.001)</td>
<td>0.85</td>
</tr>
<tr>
<td>λ</td>
<td></td>
<td>, µm²/ms</td>
<td>1.58 (.001)</td>
<td>1.57 (.001)</td>
<td>1.57 (.001)</td>
</tr>
<tr>
<td>T2, ms</td>
<td>102.1 (.001)</td>
<td>100.3 (.001)</td>
<td>101.9 (.001)</td>
<td>108.1 (.002)</td>
<td>82.30</td>
</tr>
<tr>
<td>MTR</td>
<td>0.42 (.001)</td>
<td>0.42 (.001)</td>
<td>0.41 (.001)</td>
<td>0.40 (.001)</td>
<td>0.46</td>
</tr>
</tbody>
</table>

Abbreviations: FA, fractional anisotropy; MD, mean diffusivity; MRI, magnetic resonance imaging; MS, multiple sclerosis; MTR, magnetization transfer ratio; PPMS, primary progressive MS; RRMS, relapsing-remitting MS; SPMS, secondary progressive MS; T2, absolute T2 relaxation time; λ||, parallel diffusivity; and λ⊥, perpendicular diffusivity.

Sample size: All available data were used; age information was unavailable for 1 patient with RRMS and 1 healthy volunteer. For healthy volunteers: n=28 for diffusion tensor imaging (DTI) indices (FA, MD, λ||, and λ⊥); 25 for T2; 23 for MTR. For RRMS: 51 for DTI indices; 43 for T2; 39 for MTR. For SPMS: 24 for all indices. For PPMS: 14 for all indices. The MS cohort was assessed both as a group and separately for each MS subtype. Differences from healthy volunteers were assessed using multiple linear regression analysis adjusting for age and sex; P values are given in parentheses.

Table 3. Correlation of RNFL Thickness With MRI Indices

<table>
<thead>
<tr>
<th>MRI Index</th>
<th>vs Entire Tract</th>
<th>vs Lesions</th>
<th>vs Normal- Appearing White Matter</th>
<th>Nasal Quadrant vs Entire Tract</th>
</tr>
</thead>
<tbody>
<tr>
<td>FA</td>
<td>0.55 (.001)</td>
<td>0.38 (.011)</td>
<td>0.49 (.001)</td>
<td>0.37 (.01)</td>
</tr>
<tr>
<td>MD</td>
<td>−0.10 (.49)</td>
<td>−0.20 (.19)</td>
<td>−0.01 (.94)</td>
<td>−0.09 (.57)</td>
</tr>
<tr>
<td>λ</td>
<td></td>
<td></td>
<td>−0.37 (.001)</td>
<td>−0.42 (.005)</td>
</tr>
<tr>
<td>T2</td>
<td>−0.23 (.13)</td>
<td>−0.21 (.17)</td>
<td>−0.11 (.46)</td>
<td>−0.17 (.27)</td>
</tr>
<tr>
<td>MTR</td>
<td>0.04 (.77)</td>
<td>−0.09 (.61)</td>
<td>−0.05 (.78)</td>
<td>−0.07 (.69)</td>
</tr>
</tbody>
</table>

Abbreviations: FA, fractional anisotropy; MD, mean diffusivity; MRI, magnetic resonance imaging; MTR, magnetization transfer ratio; RNFL, retinal nerve fiber layer; T2, absolute T2 relaxation time; λ||, parallel diffusivity; and λ⊥, perpendicular diffusivity.

Sample size: All available data were used: n=36 for diffusion tensor imaging (DTI) indices (FA, MD, λ||, and λ⊥); 35 for T2; and 32 for MTR. Weighted Pearson partial correlation coefficients between MRI indices and mean RNFL thickness, accounting for age, sex, and MRI indices along the corona radiata portion of the corticospinal tract. RNFL thickness data were collected within 30 days of MRI. Correlations between optic radiation MRI indices and RNFL thickness in the nasal quadrants were controlled for age, sex, RNFL thickness in the temporal quadrants, and MRI indices in the corona radiata portion of the corticospinal tract. P values are given in parentheses, correlation coefficients for which P<.05 are given in boldface, and comparisons represented graphically in Figure 4 are given in boldface italics.
Figure 4. Association of optic radiation magnetic resonance imaging (MRI) indices with retinal nerve fiber layer (RNFL) thickness (A-D) and visual acuity at 2.5% contrast (E-H). Only multiple sclerosis data are included, and marker size is proportional to the number of images obtained for each participant within 30 days of visual acuity or retinal testing. A, C, E, and G, Raw data. B, D, F, and H, Data adjusted for age, sex, corticospinal tract MRI indices, and, for plots of visual acuity, mean RNFL thickness. Each data point represents a single participant, and marker size is proportional to the number of observations for that participant. Weighted multiple linear regression analysis was used to derive the values in B, D, F, and H. FA indicates fractional anisotropy; MTR, magnetization transfer ratio.
Table 4. Correlation of Visual Acuity With MRI Indices

<table>
<thead>
<tr>
<th>MRI Indices</th>
<th>Lesion Fraction</th>
<th>vs Letter Acuity at 100% Contrast</th>
<th>vs Letter Acuity at 2.5% Contrast</th>
<th>vs Letter Acuity at 1.25% Contrast</th>
</tr>
</thead>
<tbody>
<tr>
<td>FA</td>
<td>0.13 (.54)</td>
<td>−0.23 (.29)</td>
<td>0.13 (.53)</td>
<td><strong>0.55 (.004)</strong></td>
</tr>
<tr>
<td>MD</td>
<td>−0.15 (.48)</td>
<td>−0.01 (.98)</td>
<td>0.12 (.58)</td>
<td>−0.37 (.06)</td>
</tr>
<tr>
<td>λ₁</td>
<td>−0.06 (.76)</td>
<td>−0.05 (.82)</td>
<td>0.23 (.28)</td>
<td>−0.34 (.99)</td>
</tr>
<tr>
<td>λ₂</td>
<td>−0.07 (.75)</td>
<td>0.04 (.84)</td>
<td>0.10 (.65)</td>
<td>−0.40 (.04)</td>
</tr>
<tr>
<td>T2</td>
<td>−0.23 (.26)</td>
<td>0.01 (.98)</td>
<td>0.03 (.87)</td>
<td>−0.27 (.19)</td>
</tr>
<tr>
<td>MTR</td>
<td>0.44 (.049)</td>
<td>0.01 (.97)</td>
<td>0.08 (.74)</td>
<td><strong>0.54 (.01)</strong></td>
</tr>
</tbody>
</table>

Abbreviations: FA, fractional anisotropy; MD, mean diffusivity; MTR, magnetization transfer ratio; OR, optic radiation; λ₁, parallel diffusivity; and λ₂, perpendicular diffusivity.

Our primary findings are as follows: (1) OR-specific MRI indices are abnormal in MS within both lesions and normal-appearing white matter; (2) structural damage to the retina, usually from optic neuritis, is associated with MRI abnormalities along the OR; and (3) OR abnormalities contribute to visual dysfunction in MS, particularly at low contrast, in individuals without severe retinal damage.

CORRELATION WITH VISUAL ACUITY

Low-contrast letter acuity is a sensitive indicator of visual dysfunction in MS, and impairment on this task can result from optic neuritis. To assess for specific associations of visual dysfunction with OR damage, we calculated partial correlation coefficients between OR-specific MRI indices and visual acuity scores, adjusting for age, sex, mean RNFL thickness (to reduce contribution from optic nerve damage), and the corresponding OR lesions along the corticospinal tract. To exclude participants with severe optic nerve damage that might dominate any contribution from the OR, we included only participants with mean RNFL of more than 80 µm.

The results (Table 4 and Figure 4E-H) show that diminished low-contrast vision is associated with lower FA, higher λ₂, and lower MTR along the OR. Separate analysis of the lesion and normal-appearing white matter portions of the OR demonstrated that these correlations were more strongly dependent on MRI indices within the normal-appearing white matter. The OR lesion fraction, but not MRI indices within lesions, was strongly associated with acuity scores.

COMMENT

Our primary findings are as follows: (1) OR-specific MRI indices are abnormal in MS within both lesions and normal-appearing white matter; (2) structural damage to the retina, usually from optic neuritis, is associated with MRI abnormalities along the OR; and (3) OR abnormalities contribute to visual dysfunction in MS, particularly at low contrast, in individuals without severe retinal damage.

RETTINAL vs OR DAMAGE

Our results demonstrate an association between RNFL thinning and OR-specific MRI abnormalities that result, at least in part, because MS is a diffuse disease that affects the entire central nervous system. By design, our study could reveal only correlations but not their causes. Adding covariates to the analysis, including MRI indices along the corticospinal tract and abnormalities in nonconnected portions of the visual system, can refine the interpretation, although these 2 covariates do not fully capture the spectrum of MS-related disease. For example, any role of other portions of the visual system such as the brainstem oculomotor pathways may not have been detected.

Thus, our finding that there is a residual correlation between retinal and brain structures that are synaptically connected at the LGN requires careful interpretation and cannot be taken as direct evidence of a transsynaptic degenerative process; such evidence would have to come from animal models or unifocal diseases. Moreover, our results give no insight into whether such transsynaptic changes, even if they are real, are anterograde (retina to OR) or retrograde.

In this context, it is worth reviewing relevant previous literature. In the visual system, transsynaptic changes in the LGN, OR, and visual cortex have been observed after acute optic neuritis. In one study, the location of the OR, reconstructed from DTI data obtained 1 year after a first episode of optic neuritis, seemed to have shifted relative to its normal location in age-matched control individuals. In a separate study, the MTR, which reflects macromolecule (typically, myelin)–bound protons in tissue, was found to be lower in the visual cortex of individuals with a history of optic neuritis at least 6 months previously, both with and without intracranial imaging abnormalities consistent with a diagnosis of MS; we found no association between MTR and RNFL thickness. However, in a DTI analysis of the prestripate visual pathway in individuals with a variety of optic nerve lesions, significant changes in MD were observed only in the affected tract and not transsynaptically.

Further investigation of the source of the observed transsynaptic correlations is warranted. The mechanisms that underlie transsynaptic changes are important.
for normal development; however, they may also contribute to disability and/or recovery in neurologic disease. For these reasons, transsynaptic changes are an attractive target for neuroprotective drugs, which might act by either preventing degeneration or enhancing function-preserving plasticity.

**VISUAL ACUITY AT HIGH AND LOW CONTRAST**

Recent work has convincingly demonstrated a link between anterior pathway abnormalities, including RNFL thinning, and visual dysfunction in MS, in particular, at low contrast.\(^{22}\) We have presented evidence for a contribution of posterior pathway abnormalities, manifested by lesion fraction and MRI index abnormalities along the OR, to visual disability. Whereas correlations with lesion fraction were noted at all contrast levels, correlations with MRI indices were more prominent at low contrast and were primarily associated with abnormalities within the normal-appearing white matter. This result agrees with previous work in which low-contrast letter acuity was found to be related to T2-weighted lesion load in the entire brain, occipital lobe white matter, and OR.\(^{3}\)

The most well-known functional correlates of OR lesions are visual field deficits, and such deficits are commonly observed after infarcts that affect the OR. Although it would be interesting to study the imaging correlates of visual field loss in MS, we did not test visual fields in the present study. Moreover, it is not clear that the OR reconstructions are complete or specific enough (see the “Study Limitations” subsection) to perform and properly interpret such a study.

Identifying the source of disability in MS is a crucial step toward developing therapeutic strategies to prevent such disability from occurring. To this end, our finding that visual dysfunction is related to quantitative MRI indices in the normal-appearing white matter of the OR, but not to corresponding values within lesions, is important. The origin of normal-appearing white matter abnormalities has been extensively discussed\(^{33,34}\) and may be related to a combination of axon degeneration distal to lesions, the presence of inflammatory lesions that are too small to be resolved using current MRI techniques (and that would confound the subjective distinctions between lesions and normal-appearing white matter that were drawn by a single neuroradiologist [A.O.] on the FLAIR images), and diffuse primary pathologic findings including inflammation and neurodegeneration. Our results cannot directly differentiate among these possibilities, although the strength of the correlations between visual acuity and OR lesion fraction suggest that distal degeneration has an important role.

**DIFFERENCES BETWEEN VISUAL AND MOTOR TRACTS**

Comparison with related work on the corticospinal tract in an overlapping cohort (most of the images used in the corticospinal tract study were also used in this study, with additional images added in the interim)\(^{23,35}\) reveals that OR-specific MRI indices are much more abnormal than their counterparts in the corticospinal tract. Possible reasons for this finding include the following: (1) anatomical differences between the 2 tracts, in particular, differences in orientation and curvature, may differentially affect susceptibility to the pathologic and reparative processes in MS and (2) the tracts may differ in their ability to recruit undamaged collateral fibers. Analysis of other tracts with different geometries and comparison with other diseases that affect these tracts might help differentiate among these possibilities.

**STUDY LIMITATIONS**

A principal limitation of the present study is the intrinsic variability of DTI-based tractography to identify tracts such as the OR.\(^{36}\) By adopting multiple anatomical constraints on the tracking algorithm, we tried to reduce the number of spurious fibers included in the analysis. In the presence of disease, this approach can lead to exclusion of some fibers most affected by disease (which may be hardest to track reliably), thereby potentially reducing the correlation with disability. We partially offset this possibility by selecting a permissive FA threshold, and the reconstructed tracts typically passed through lesions rather than stopped at them. Thus, we believe that our results cover the core of the OR.

Another limitation is that we did not obtain clinical and OCT data for all individuals scanned because of the way in which participants were recruited into the study, scheduling conflicts, and unavailability of OCT scanning at our center until 2006. Although we are not aware of any systematic difference between participants with and without OCT and visual acuity testing results, this disparity reduces our sensitivity for detection of clinical-radiologic correlations. In addition, we did not control for treatment type; such analysis would require a larger cohort but may be relevant for detecting and interpreting long-term changes in MRI indices.

In our study, the control group was substantially younger than the MS cohort, on average, although the age range was similar. Although we adjusted our regression models for age, these differences, which affect DTI indices,\(^{37}\) may still affect the comparisons between patients with MS and healthy volunteers (though not the correlations with visual disability and retinal damage, which did not include control data).

Another potentially important issue is the high false discovery rate induced by multiple comparisons. We report P values directly and limited assignment of statistical significance to the extent possible. Because many of the variables tested here are correlated with one another, applying a simple Bonferroni correction would not be appropriate. Thus, we assigned greater weight to consistent patterns, in particular, that FA and \(\lambda_1\) seem to be most associated with retinal damage and visual disability. However, validation of these indices as imaging biomarkers of clinically relevant disability will require direct prospective testing.

In conclusion, the present study demonstrates functionally relevant abnormalities of the OR in MS that are specifically related to visual disability and identifies imaging biomarkers, in particular, FA and \(\lambda_1\), that are...
worthy of further targeted study. The results may be important for understanding and preventing the propagation of neuronal dysfunction in the brain.

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Correspondence: Daniel S. Reich, MD, PhD, Department of Neurology, The Johns Hopkins University School of Medicine, 600 N Wolfe St, Phipps Bldg, Room B-112, Baltimore, MD 21287 (reichd@jhmi.edu).

Author Contributions: Dr Reich had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Reich, Smith, and Calabresi. Acquisition of data: Reich, Smith, and Gordon-Lipkin. Analysis and interpretation of data: Reich, Ozturk, Caffo, Balcer, and Calabresi. Drafting of the manuscript: Reich, Smith, Ozturk, and Calabresi. Critical revision of the manuscript for important intellectual content: Reich, Smith, Gordon-Lipkin, Caffo, Balcer, and Calabresi. Statistical analysis: Reich, Caffo, and Balcer. Obtained funding: Calabresi. Administrative, technical, and material support: Smith and Gordon-Lipkin. Study supervision: Calabresi.

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Additional Contributions: Terri Brawner, BS, Deanna Cettomai, BS, Hormouziyari Danesbrock, BA, Sheena Farrell, BS, Kathleen Kahl, Ivana Kusevic, and Mathew Pulecken, MD, assisted with data collection or analysis, and Jonathan Farrell, PhD, Richard Lawner, PhD, Susumu Mori, PhD, and Peter van Zijl, PhD, participated in helpful scientific discussions.

REFERENCES