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## Diffusion Tensor Imaging of the Optic Nerve in Multiple Sclerosis: Association with Retinal Damage and Visual Disability

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### Abstract

**Background/Purpose**—There is a well-known relationship between multiple sclerosis (MS) and damage to the optic nerve, but advanced, quantitative magnetic resonance imaging (MRI) methods have not been applied to large cohorts. Our objective was to determine if a short imaging protocol (<10 minutes), implemented with standard hardware, could detect abnormal water diffusion in the optic nerves of MS patients.

**Methods**—We examined water diffusion in human optic nerves via diffusion tensor MRI (DTI) in the largest multiple sclerosis cohort reported to date (104 individuals, including 38 optic nerves previously affected by optic neuritis). We also assessed if such abnormalities are associated with loss of visual acuity (both high and low contrast) and damage to the retinal nerve fiber layer (assessed via optical coherence tomography).

**Results**—The most abnormal diffusion was found in the optic nerves of patients with secondary progressive MS, especially in optic nerves previously affected by optic neuritis (19% drop in fractional anisotropy). DTI abnormalities correlated with both retinal nerve fiber layer thinning (correlation coefficient 0.41) and loss of visual acuity, particularly at high contrast and in nerves previously affected by optic neuritis (correlation coefficient 0.54). However, diffusion abnormalities were overall less pronounced than retinal nerve fiber layer thinning.

**Conclusion**—Diffusion tensor imaging is sensitive to optic nerve damage in patients with MS, but a short imaging sequence added to standard clinical protocols may not be the most reliable indicator of optic nerve damage.

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## Introduction

Impaired vision is extremely common in multiple sclerosis (MS) and may arise from damage (demyelination and axonal loss) to different components (e.g., optic nerve, tract, and radiation) of the visual system. Of these, the optic nerve is the best-characterized, as optic neuritis (ON) is the presenting symptom in many cases of MS (1;2). Nevertheless, recent research suggests that visual dysfunction accumulates even without prior ON. This dysfunction can be detected clinically using low-contrast letters to assess visual sensory function (3); optical coherence tomography (OCT) to assess damaged or destroyed visual axons by measuring peripapillary retinal nerve fiber layer (PRNFL) thickness and total macular volume (TMV) (4); and visual evoked potentials (VEPs) to assess speed and amplitude of impulse conduction (5).

Magnetic resonance imaging (MRI) can detect visual pathway damage and has been extensively investigated. MRI shows T2-weighted hyperintensities in optic nerves with prior ON, whereas in acute cases, post-contrast T1-weighted hyperintensities are appreciated (6). Recently, quantitative MRI techniques such as magnetization-transfer (7–9) and diffusion-weighted (10–16) imaging have revealed time-evolving abnormalities following an attack of ON. Unfortunately, both of these techniques are time consuming, prohibiting clinical application.

Diffusion tensor imaging (DTI) uses information contained in the diffusion properties of water to probe tissue microstructural abnormalities such as demyelination and axonal damage, both of which occur in ON. The most commonly reported DTI-derived indices are fractional anisotropy (FA), mean diffusivity (MD), and diffusion parallel ( $\lambda_{\parallel}$ ) and perpendicular ( $\lambda_{\perp}$ ) to the long axis of specific white-matter tracts (17). DTI studies of affected optic nerves in patients with a remote history of ON reveal reduced FA and elevated diffusivities as well as correlations with concurrent measurements of acuity, VEPs, and PRNFL thickness (10;14;16). However, sample sizes in these studies have been low due to technical hindrances related to the small diameter ( $\leq 3\text{mm}$ ) and mobility of the optic nerves and their proximity to the paranasal sinuses, which induce susceptibility-related artifacts and interfere with conventional DTI. Additionally, lengthy acquisition times actually may accentuate the effects of optic-nerve motion.

The goals of this study were: (1) To develop and evaluate a rapid optic-nerve DTI sequence performed with standard coils in a clinically acceptable time frame ( $<10$  minutes); and (2) To relate abnormal water diffusion in the optic nerve to measures of visual sensory function and retinal structure in the largest MS cohort reported to date. This cohort includes patients who reported an attack of acute ON more than 6 weeks prior to scanning; patients without a history of clinically recognized ON; and healthy volunteers. Thus, we examined optic-nerve abnormalities in a representative MS cohort rather than a cohort dominated by patients with ON. We hypothesized that DTI indices would distinguish MS optic nerves affected by prior ON from MS optic nerves without a history of ON, and healthy optic nerves. Furthermore, we hypothesized that, at least in MS, DTI-derived measurements would correlate with visual sensory dysfunction, PRNFL thinning, and loss of TMV.

## Methods

### Participants

Individuals with MS were recruited from the Johns Hopkins MS Center after the examining neurologist confirmed the diagnosis and the absence of confounding ophthalmologic or neurologic diseases. Healthy volunteers were recruited from the community. Participants were not selected based on history of ON; however, scans performed less than 6 weeks

following the onset of acute ON were excluded. OCT and visual acuity testing using both high- and low-contrast (Sloan) letters were performed on all participants within 30 days of MRI. The median time between MRI and OCT/visual acuity testing was 0 days (range 0–23). Prior to testing, all participants gave signed, informed consent, and all studies were approved by the institutional review board.

### OCT and Visual Acuity Testing

PRNFL thickness, TMV, and both high- and low-contrast visual acuity scores were measured as previously described (4). PRNFL and TMV measurements were obtained using an OCT-3 scanner (Stratus; Carl Zeiss Meditec, Dublin, CA) with the “Fast RNFL Thickness” and “Fast Macular Thickness” protocols, respectively. Participants were encouraged to use their corrective lenses when performing contrast-acuity testing with Sloan letter charts (Precision Vision, La Salle, IL) at 100%, 2.5%, and 1.25% contrast. Results were recorded as the percentage of letters, out of 70, correctly identified.

### MRI Acquisition

MRI data were obtained on a Philips 3T Achieva (Philips Health Care, Best, The Netherlands) using the quadrature body coil for transmission and a 16-channel neurovascular coil for reception. Data were acquired during a 442-day period between March 26, 2008, and June 11, 2009. Multi-slice, fat-saturated, double-echo proton-density and T2-weighted ( $TR/TE_1/TE_2 = 3000/10/80\text{ms}$ , echo-train length 100, nominal acquired resolution  $0.67\text{mm} \times 0.88\text{mm}$  in plane, matrix  $224 \times 170$ , slice thickness  $3.35\text{mm}$ , SENSE factor 2.5, 2 averages) and T1-weighted ( $TR/TE = 774/13\text{ms}$ , echo-train length 2, nominal acquired resolution  $0.58\text{mm} \times 0.74\text{mm}$  in plane, matrix  $260 \times 204$ , slice thickness  $3\text{mm}$ , SENSE factor 2, 2 averages) sequences were used to provide anatomical information. In the MS cohort, the T1-weighted scans were performed after the intravenous administration of  $0.1\text{ mmol/kg}$  gadopentetate dimeglumine (Magnevist; Bayer HealthCare, Leverkusen, Germany). The slices were oriented coronal to the head, which was oblique to the orbital portion of the optic nerves in some cases.

DTI was performed in the same plane as conventional MRI, with a pulsed-gradient, spin-echo with single-shot, echo-planar-imaging readout. The nominal acquired voxel size was  $1.18\text{mm} \times 1.18\text{mm} \times 2.5\text{mm}$ , and the data were zero-padded in k-space to achieve a reconstructed in-plane resolution of  $0.28\text{mm} \times 0.28\text{mm}$ . The field of view was  $80\text{mm} \times 80\text{mm}$ , and 25 slices were obtained covering the anterior visual pathway from the globe to the optic chiasm. A high parallel-imaging factor (SENSE factor 3) enabled reduced TE, and high-order shims were combined with outer-volume-suppression to minimize susceptibility-related artifacts arising from the paranasal sinuses and tissues lateral to the optic nerves, respectively. Other parameters were:  $TR/TE = 5300/55\text{ms}$ ,  $b\text{-value} = 500\text{s/mm}^2$ , 15 gradient directions uniformly distributed about a sphere, and 5 minimally diffusion-weighted acquisitions (“ $b=0$ ”; actual  $b\text{-value} \sim 33\text{s/mm}^2$ ) that were averaged on the scanner. Two separate DTI acquisitions were obtained and entered into the tensor calculation as independent measurements without pre-averaging. No ocular fixation was used. The total scan time for the DTI acquisitions was 9min 22sec.

### MRI Data Analysis

All diffusion data were processed using CATNAP (18). Diffusion-weighted images were coregistered to the first  $b=0$  scan using a 6 degree-of-freedom registration algorithm supplied by AIR (19). Diffusion weighting was corrected for the rotational component of the registration, and the tensor was calculated in the standard fashion. Maps of FA, MD,  $\lambda_{\perp}$  and  $\lambda_{\parallel}$  were calculated (17).

The investigative team, which includes a neuroradiologist, two neuroophthalmologists, and four neurologists, determined that the MD maps displayed the best contrast between the optic nerves and the surrounding cerebrospinal fluid (CSF). Therefore, in each slice where the optic nerve was visible, a trained neuroophthalmologist (ZRW) placed regions of interest (ROIs) circumscribing the optic nerve on the MD images, as the MD images showed the greatest contrast between nerve and surrounding CSF. To minimize partial-volume effects, the outermost voxels of the ROIs were automatically removed using the erode function in Matlab (The Mathworks, Natick, MA). Diffusivity values that were spuriously (i.e., due to noise) negative were set to 0, and FA values that were spuriously greater than 1 were set to 1. This truncation prevents non-physiologic results while reducing bias by removing those slices entirely. DTI indices were then averaged across all slices, weighting the slice by the number of included voxels. These averaged values entered into the statistical calculations.

## Statistics

Statistical calculations were performed in Stata 9.0 (Stata LP, College Station, TX). Stability of the quantitative measurements was assessed over the study's 442-day period using linear regression. There was no significant change in PRNFL thickness, TMV, visual acuity scores, expanded-disability-status-scale (EDSS) score (20), mean MS-severity score (MSSS) (21), or age ( $p>0.05$  in all cases). However, diffusivity values gradually trended upward over time ( $p=0.05$  for MD,  $p=0.01$  for  $\lambda_{\parallel}$ , and  $p=0.07$  for  $\lambda_{\perp}$ ), or approximately 16% per year for MD, 14% for  $\lambda_{\parallel}$ , and 22% for  $\lambda_{\perp}$ . Scatter plots (not shown) revealed the relationship to be linear. Therefore, data were preprocessed by adjusting all DTI-indices to their expected value at the center date of the study (November 7, 2008), based on the slope of a mixed-effects regression using data from both optic nerves. There was no significant change in FA over time ( $p=0.36$ ), but the FA values were adjusted similarly for consistency.

## Intra-rater and Inter-rater Reliability

A subset of 10 consecutive data sets was analyzed. The same neuroophthalmologist analyzed 10 cases at two time points separated by  $>12$  weeks; a second rater (JNR, MS neurologist) separately analyzed the same 10 cases. DTI indices were averaged across the right and left optic nerves, yielding a single measurement of each DTI index for each case. Bland-Altman analysis compared the results within the same rater as well as between the raters. Intraclass and interclass correlation coefficients were also calculated.

To assess differences between MS cases and healthy volunteers and to quantify the correlations between DTI indices and PRNFL thickness, TMV and visual acuity, mixed-effects regression models were used to account for multiple observations (i.e., from two optic nerves per person), with a p-value of 0.01 denoting statistical significance.

## Results

Cohort characteristics are presented in Table 1. There was no difference in the age distribution between MS and healthy-volunteer groups ( $p=0.11$ , chi-squared test), although there was a higher proportion of women among the healthy volunteers. The overall disability level in our cohort was moderate: median EDSS score = 3.5, mean MSSS score = 4.8. In a linear mixed-effects model accounting for age and gender, individuals with MS had decreased PRNFL thickness ( $p=0.01$ ), decreased TMV ( $p=0.004$ ), and worse monocular visual acuity at 1.25% ( $p=0.03$ ) compared with healthy volunteers. Monocular visual acuity was normal at 100% ( $p=0.41$ ) and 2.5% ( $p=0.10$ ) contrast in both groups. None of the optic nerves in the MS cases demonstrated enhancement on post-contrast T1-weighted images.

Figure 1 shows T2-weighted and DTI-index maps for the right (OD, left panel) and left (OS, right panel) optic nerves in a healthy volunteer. The optic nerve is clearly visible on all images. It is brighter than the surrounding CSF in the FA and darker in all other maps and on the T2-weighted images. Images from an MS patient with remote left (OS) ON are shown in the right panels. As is typical in ON, the T2-weighted signal is increased within the affected optic nerve (circle). In the same nerve, FA is lower and all directional diffusivities are higher. These results are consistent with data from prior studies of remote ON (10–16).

### Statistical Reliability

Reliability was assessed using Bland-Altman analysis (22). Differences among values derived by the same rater across both sessions are presented in Supplementary Table 1 (top panel). For all DTI indices, the difference between each session was less than 3%, and the 95% confidence interval for the difference overlapped 0. The 95% limits of agreement indicate a threshold for detecting real differences between pairs of values for each index and were <10% for FA and approximately 15–20% for diffusivities. Intraclass correlation coefficients were close to 1 for all indices, indicating excellent agreement.

Bland-Altman analysis of the difference between raters is shown in the bottom panel of Supplementary Table 1. As with the intra-rater analysis, all of the Bland-Altman 95% confidence intervals for the difference overlapped 0, indicating a non-significant deviation. The percent difference between raters was larger, however, up to 9.4% for  $\lambda_{\perp}$  but less than 5% otherwise. Inter-class correlation coefficients were again close to 1. The 95% limits of agreement were slightly larger for the inter-rater analysis and indicate that differences on the order of 10% for FA and 20% for the diffusivities can be considered significant.

### Cohort Analyses

We obtained usable data from 226 of the 238 (95%) optic nerves; analysis of the other 12 nerves was marred by noise. We found no associations between any of the DTI indices and age, gender, or disease duration ( $p>0.1$ ), so we made no adjustments for these variables.

Figure 2 and Supplementary Table 2 present DTI indices for optic nerves with and without previous ON. The lowest FA and highest diffusivities were found in optic nerves with previous ON in patients with secondary progressive MS (SPMS). However, in mixed-effect models accounting for age and gender, only lower FA in SPMS compared with healthy volunteers ( $p=0.001$ ) and participants with relapsing-remitting MS (RRMS) ( $p=0.01$ ) was significant. These differences were persistent ( $p=0.005$  and  $p=0.03$ , respectively) even when the analysis was restricted to optic nerves without prior ON, suggesting that damage to the optic nerves in SPMS may reflect diffuse neurodegeneration in addition to prior inflammation.

Neither regression analysis in the full MS cohort nor paired t-tests comparing optic nerves in individuals with prior unilateral ON revealed differences in DTI indices between optic nerves with and without prior ON. This contrasts with findings for measures of retinal structure and low-contrast visual acuity, in which prior ON plays a major role. Specifically, mixed-effects regression analysis in eyes with prior ON revealed PRNFL thinning ( $p<0.001$ ), loss of TMV ( $p<0.001$ ), and decreased visual acuity at 2.5% ( $p<0.001$ ) and 1.25% ( $p=0.001$ ) contrast (but not at 100% contrast;  $p=0.16$ ).

Mixed-effects regression analysis assessed the correlation between DTI-indices and OCT and visual acuity. The results are shown in Table 2 and reveal moderately strong correlations, particularly for PRNFL thickness vs. FA (Figure 3A) and  $\lambda_{\perp}$ . We found

correlation coefficients of similar magnitudes in the individual MS subgroups, but due to lower sample size, the corresponding  $p$ -values were higher.

Table 2 reveals that lower FA and higher diffusivities were associated with PRNFL thinning, TMV loss, and impaired visual acuity. Of the DTI indices, FA generated the strongest correlations whereas  $\lambda_{\parallel}$  generated the weakest. Correlations with visual acuity scores were generally weaker than with PRNFL thickness and TMV and were strongest at high contrast. These correlations weakened substantially when PRNFL was included as a covariate (Supplementary Table 3).

Finally, correlations of DTI indices with PRNFL thickness did not strongly depend on prior history of ON. For visual acuity scores, however, stronger correlations were the trend with a known history of ON (e.g. FA vs. 100% contrast visual acuity; see Figure 3B); this trend was also present for TMV. However, even the strongest of these correlations - between 100% contrast visual acuity and FA in optic nerves previously affected by ON - fell after adjusting for PRNFL thickness (Supplementary Table 3)

## Discussion

This study describes the largest reported cohort of healthy and MS optic nerves investigated with DTI. We developed a rapid DTI protocol that was implemented using standard MRI hardware. We show DTI-derived indices that are consistent with previous reports (10;13–16;23). Contrary to many previous reports, but not all (12), we did not find many significant differences between MS (with or without history of ON) and healthy volunteer optic nerves, although trends were apparent. Consistent with these trends, DTI indices in MS optic nerves correlated with measures of retinal structural damage and visual acuity scores. In this section, we interpret these results in the context of literature reports and make some concluding remarks about the potential future uses of optic nerve DTI.

### Technique and technical challenges

Technical challenges abound with DTI of the optic nerve *in vivo*, and many investigators have attempted to address these by using highly customized sequences with purpose-designed surface coils that require long acquisition times and extensive post-processing (11;13;16;23–25). Those sequences were designed to minimize image distortions and maximize the signal-to-noise ratio, thus providing optimal results. Our goal was to devise a DTI acquisition that could be applied immediately on high-field clinical scanners with standard multi-channel, phased-array head coils within a clinically acceptable time frame. We therefore recognized that some image and data compromises would be necessary.

Our analysis procedure was straightforward, and initial processing of the images, including tensor and DTI-index calculation, followed a standard pipeline previously implemented in the brain (26;27). As shown in Figure 1, we obtained high-quality images even without explicit  $B_0$  correction (15). Rather than using tractography, we drew ROIs on individual images and then eroded those ROIs to limit the effects of partial-volume averaging. We recognize, however, that given the oblique orientation of the optic nerves relative to the slice as well as the small cross-sectional diameter of the nerves, we were unable to eliminate fully partial-volume effects.

Compared with previous studies, our optic nerve DTI index values were generally in agreement. Standard deviations were slightly lower than those obtained in major cerebral white-matter tracts, including the corpus callosum and optic radiations (28;29), but higher than those measured in the optic tract (30); all of these values were recorded in overlapping, but not identical, cohorts. Note, however, that DTI indices can vary across structures and are



susceptible to imaging sequence parameters (26). Thus, we believe that our results represent reasonable estimates of the true DTI indices within the optic nerves and can be used to compare cohorts and assess relationships with non-DTI measurements.

### Interpretation

Reduced anisotropy and increased diffusivity are expected in extralesional MS white matter (31–33), and these findings do not indicate specific types of tissue damage (34;35). Such abnormalities also have been found in optic nerves following ON (36). Thus, our observation of a trend in the DTI indices in this direction is not surprising. Because we only studied optic nerves that either were not previously affected by ON or in which ON had occurred at least 6 weeks earlier, we found no evidence of decreased parallel diffusivity, as has been observed in the earliest stages of acute ON (16).

Contrary to our expectations and those in the literature, we did not observe extensive DTI abnormalities in optic nerves previously affected by ON (10;14). It is well established that following an episode of ON, affected optic nerves show a persistent increase in T2-weighted signal and are atrophic (6;8;37;38;39); thus, T2-weighted imaging remains the clinical standard for detecting previous ON. Nevertheless, it is clear from our correlations that there is a connection with underlying optic nerve pathology. Abnormal DTI indices are associated with PRNFL thinning, macular volume loss, and, to a lesser extent, impaired visual acuity at high contrast. This last finding was contrary to our expectations, as low-contrast acuity is known to be particularly affected in patients with MS (3).

Portions of the two optic nerves merge following their partial decussation at the optic chiasm, so a comparison of the results obtained here with our previous measurements in the optic tracts (30) must be interpreted with caution. In the optic tract study, using a whole brain DTI protocol, we found that optic tract MD and  $\lambda_{\perp}$  were abnormally elevated and that FA was associated with both PRNFL thinning and TMV loss (but not visual acuity). Because the optic nerves and tracts are anatomically linked, it is not surprising that we found similar results with similar correlation strengths in this study.

### Shortcomings

Because of the limited spatial resolution and SNR of our acquisition, coupled with slices that were oblique rather than perpendicular to the serpentine optic nerves, we reported summary rather than slice-wise measures. Thus, data from nerves previously affected by ON show an average of potentially severely damaged segments with data from other, undamaged, or less-affected segments. Moreover, the most damaged segments may have been systematically de-emphasized because we included only data from slices where we could confidently identify and demarcate the optic nerve. We suspect these factors contributed to our lack of sensitivity for detecting differences between unaffected and affected optic nerves.

Two additional limitations of our study were the lack of high-resolution anatomical data for assessing cross-sectional area and volume as well as the lack of VEP measurements. Both have been shown to correlate with each other and with DTI indices (10;16) and are useful for a comprehensive analysis of optic nerve damage in MS.

A future study would rectify these shortcomings by: (1) unilateral rather than bilateral nerve imaging to minimize obliqueness of the imaging slice relative to the nerve axis; and (2) transitioning to non-EPI acquisitions or to a multi-shot EPI acquisition with phase correction.

## Final Remarks

We suggest that optic nerve DTI, as a rapid, clinical adjunct to conventional T1- and T2-weighted imaging, is less sensitive to MS-induced tissue damage than OCT and low-contrast visual acuity measurements. Although DTI indices correlated with impaired visual acuity, much of that correlation could be accounted for by PRNFL thinning. Our results thus cast some doubt on the ultimate utility of DTI techniques derived from brain acquisitions as a tool in clinical care, at least in the chronic setting.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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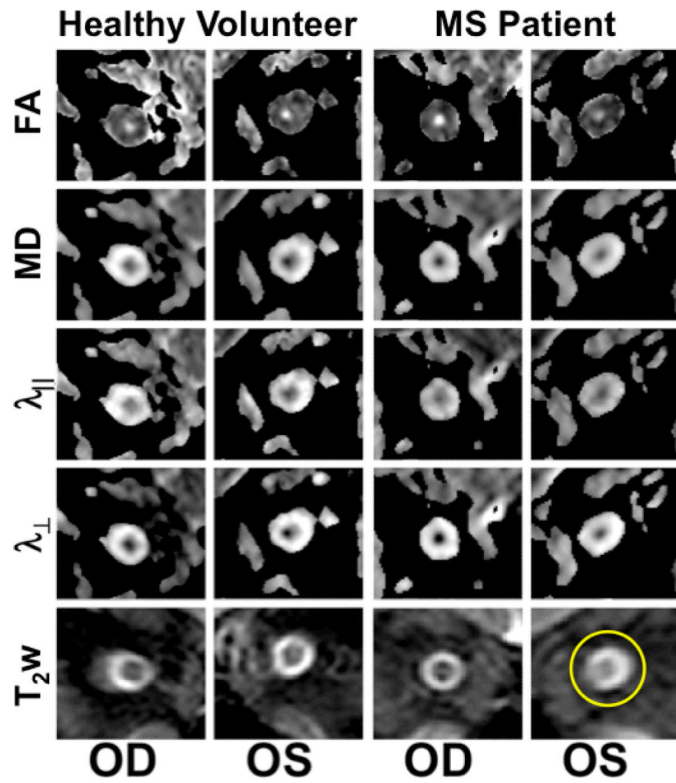
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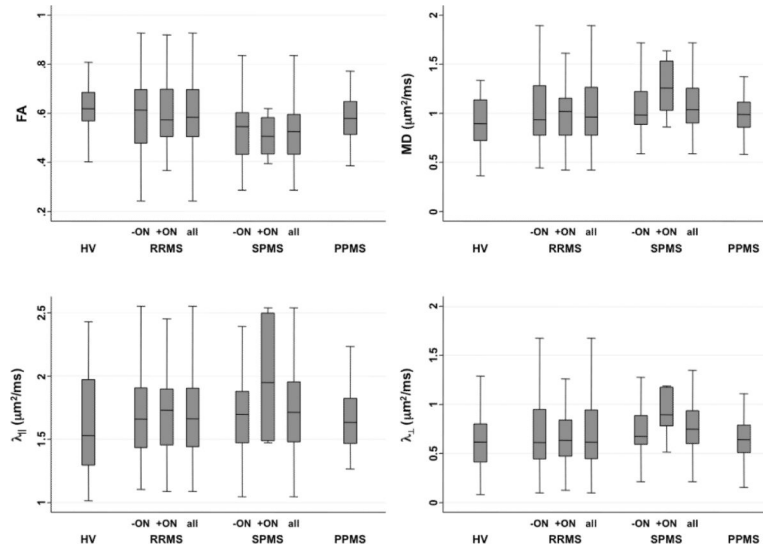


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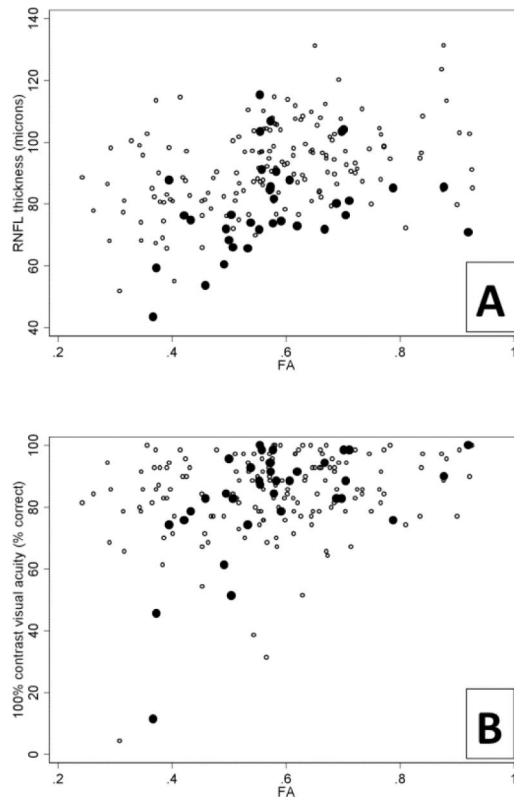


**Figure 1.** Optic nerve DTI indices and T2-weighted images ( $T_2w$ ) for a healthy volunteer and an individual with MS with previous left (OS) optic neuritis. Note the high signal on the  $T_2w$  in the left optic nerve (circle). FA, fractional anisotropy; MD, mean diffusivity;  $\lambda_{||}$ , parallel diffusivity;  $\lambda_{\perp}$ , perpendicular diffusivity; OD, right eye.



**Figure 2.**

Box plots showing DTI indices in optic nerves from healthy volunteers (HV) and relapsing-remitting (RRMS), secondary progressive (SPMS), and primary progressive (PPMS) MS, in optic nerves without (–ON) and with (+ON) previous optic neuritis. FA, fractional anisotropy; MD, mean diffusivity;  $\lambda_{\parallel}$ , parallel diffusivity;  $\lambda_{\perp}$ , perpendicular diffusivity.



**Figure 3.** Scatter plots showing the association between fractional anisotropy (FA) and peripapillary retinal nerve fiber layer (PRNFL) thickness (A) and high contrast visual acuity (B) in optic nerves without (4) and with (3) previous optic neuritis.

Table 1

Cohort characteristics.

	all MS	RRMS	SPMS	PPMS	healthy
# participants (# nerves)	104 (197)	63 (120)	23 (42)	18 (35)	15 (29)
# women (%)	67 (64%)	44 (71%)	14 (64%)	9 (50%)	13 (87%)
mean age, years (range)	46 (20–67)	40 (20–62)	54 (45–67)	53 (40–66)	38 (23–56)
mean disease duration, years (range)	11 (0–44)	8 (0–25)	21 (2–39)	10 (1–44)	N/A
median EDSS (range)	3.5 (0–7.5)	2 (0–6.5)	6 (2.5–7)	5.5 (2.5–7.5)	N/A
mean MSSS (sd)	4.8 (2.6)	3.9 (2.5)	5.8 (2.1)	7.0 (1.6)	N/A
prior optic neuritis, # individuals (# eyes)	35 (38)	27 (29)	7 (8)	1 (1)	0
Mean PRNFL thickness, $\mu\text{m}$ (sd)	90 (14)	91 (15)	86 (13)	92 (13)	102 (14)
mean TMV, $\text{mm}^3$ (sd)	6.5 (0.5)	6.5 (0.4)	6.3 (0.5)	6.6 (0.4)	6.9 (0.4)
mean monocular 100% contrast visual acuity, % correct (sd)	84 (14)	87 (13)	82 (9)	77 (19)	88 (11)
mean monocular 2.5% contrast visual acuity, % correct (sd)	40 (17)	42 (17)	35 (15)	37 (18)	49 (14)
mean monocular 1.25% contrast visual acuity, % correct (sd)	16 (15)	18 (16)	12 (12)	14 (14)	27 (16)

Summary statistics (except for medians) are derived from all data and account for multiple observations per participant (i.e., right vs. left eye and across scans). For the MS cohort, optical-coherence-tomography and visual-acuity data were obtained within 30 days of each MRI scan. For the healthy-volunteer cohort, we did not require temporal proximity.

Abbreviations: MS, multiple sclerosis; RRMS, relapsing-remitting MS; SPMS, secondary progressive MS; PPMS, primary progressive MS; EDSS, expanded disability status scale; MSSS, MS severity score; PRNFL, peripapillary retinal nerve fiber layer; TMV, total macular volume. N/A, not applicable.



**Table 2**  
Correlation of MRI indices with optical-coherence-tomography and visual-acuity measurements.

	FA		MD		$\lambda_{  }$		$\lambda_{\perp}$	
	±	-	±	-	±	-	±	-
<i>PRNFL thickness</i>	<b>0.41</b> §	<b>0.45</b> §	<b>-0.35</b> ‡	<b>-0.39</b> ‡	<b>-0.29</b> *	<b>-0.30</b> *	<b>-0.39</b> §	<b>-0.42</b> §
<i>TMV</i>	<b>0.25</b> ‡	<b>0.27</b> *	<b>-0.26</b> *	<b>-0.44</b> *	<b>-0.24</b> *	<b>-0.20</b> *	<b>-0.25</b> *	<b>-0.27</b> *
<i>100% acuity</i>	<b>0.29</b> ‡	0.17	<b>0.54</b> ‡	-0.09	-0.07	-0.07	<b>-0.20</b> *	-0.10
<i>2.5% acuity</i>	<b>0.19</b> *	0.13	0.36	-0.10	-0.13	-0.11	-0.18	-0.08
<i>1.25% acuity</i>	0.13	0.13	0.10	-0.14	-0.14	-0.21	-0.16	0.05

Correlation coefficients derived from mixed-effects regression analysis, accounting for correlations between optic nerves in the same individuals. Statistical significance is as follows:

Prior optic neuritis status is denoted:

Abbreviations: FA, fractional anisotropy. MD, mean diffusivity.  $\lambda_{||}$ , parallel diffusivity.  $\lambda_{\perp}$ , perpendicular diffusivity. PRNFL, peripapillary retinal nerve fiber layer. TMV, total macular volume.

\* ( $p < 0.01$ )

‡ ( $p < 0.0001$ )

§ ( $p < 0.00001$ )

± all optic nerves (197 nerves, 104 individuals)

- no prior optic neuritis (158 nerves, 96 individuals)

+ prior optic neuritis (35 nerves, 32 individuals).