ORIGINAL COMMUNICATION

Tract-specific quantitative MRI better correlates with disability than conventional MRI in multiple sclerosis

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Abstract Although diffusion tensor imaging (DTI) and the magnetization transfer ratio (MTR) have been extensively studied in multiple sclerosis (MS), it is still unclear if they are more effective biomarkers of disability than conventional MRI. MRI scans were performed on 117 participants with MS in addition to 26 healthy volunteers. Mean values were obtained for DTI indices and MTR for supratentorial brain and three white matter tracts of interest. DTI and MTR values were tested for correlations with measures of atrophy and lesion volume and were compared with these more conventional indices for prediction of disability. All DTI and MTR values correlated to an equivalent degree with lesion volume and cerebral volume fraction (CVF). Thalamic volumes correlated with all indices in the optic radiations and with mean and perpendicular diffusivity in the corpus callosum. Nested model regression analysis demonstrated that, compared with CVF, DTI indices in the optic radiations were more strongly correlated with Expanded Disability Status Scale and were also more strongly correlated than both CVF and lesion volume with low-contrast visual acuity. Abnormalities in DTI and MTR are equivalently linked with brain atrophy and inflammatory lesion burden, suggesting that for practical purposes they are markers of multiple aspects of MS pathology. Our findings that some DTI and MTR indices are more strongly linked with disability than conventional MRI measures justifies their potential use as targeted, functional system-specific clinical trial outcomes in MS.

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Introduction

It has become increasingly clear that quantitative MRI
techniques are needed to measure aspects of pathologic
change in multiple sclerosis (MS) that are not adequately
explained by more conventional measures of active
inflammation (gadolinium enhancement), lesion burden,
and brain atrophy [1]. Quantitative analysis of diffusion
tensor imaging (DTI) and magnetization transfer ratio
(MTR) data has shown measurable changes in normal-
appearing white matter and lesions [2–4]. Alterations in
the diffusion properties of water, as measured by DTI,
have been proposed as markers of neurodegeneration and
demyelination in axonal pathways [5–7], and MTR has
been proposed as a marker of the myelin content of
tissues [8], although neither type of imaging is patho-
logically specific under most clinical conditions. Never-
theless, both DTI and MTR indices correlate with
disability [9–11] and thus may be clinically relevant.
Recently, we have quantified longitudinal changes in
white matter tract-specific DTI/MTR values over time
periods, with proposed effect sizes that are reasonable for
clinical trials [12].

While it is clear that these quantitative techniques are
sensitive to aspects of MS pathology not adequately cap-
tured by more conventional techniques, it is not clear if the
additional information provided actually increases our
ability to measure overall disease burden and predict dis-
ability. Demonstration of this additional predictive ability
would render DTI and MTR more useful and clinically
interpretable as clinical trial outcomes. In this study, we
performed an analysis on a cohort of participants with MS
who had been studied with conventional and DTI/MTR
MRI techniques. We aimed to examine the relationship of
DTI/MTR outcomes to conventional MRI measures and
also to determine if the addition of these newer techniques
adds to our ability to explain disability in MS.

Methods

Standard protocol approvals and patient consents

Protocols were approved by the Institutional Review
Boards at the Johns Hopkins University School of Medi-
cine and the Kennedy Krieger Institute, who comply with
all of the ethical standards of the 1964 Declaration of
Helsinki. Written, informed consent was obtained from all
participants.

Participants

Participants with MS were recruited from the Johns Hop-
kins MS Center. Individuals with diagnoses of relapsing–
remitting (RRMS), secondary progressive (SPMS), and
primary progressive (PPMS) MS were enrolled; the diag-
nosis was assigned by the treating physician. Data were
collected from April 2005 through July 2009. Scans per-
formed within 30 days of a clinical relapse or steroid
administrations were excluded. Scans from a healthy vol-
unteer population were also analyzed to demonstrate that
the DTI and MTR findings were due to MS pathology.

MRI protocol and image analysis

The MRI acquisition protocol has been fully described
elsewhere [13, 14], and only the essential details are pre-
sented here. On a 3-T Philips scanner, we obtained the
following whole-brain sequences at the specified acquired
resolution without gaps: DTI (2.2 mm isotropic); magne-
tization transfer ($1.5 \times 1.5 \times 2.2$ mm); proton-density/T2-
weighted and fluid-attenuated inversion recovery (FLAIR)
($0.8 \times 0.8 \times 2.2$ or 4.4 mm for some of the FLAIR scans)
and three-dimensional (3D) magnetization-prepared rapid
acquisition with gradient echo (MPRAGE) (1.1 mm
isotropic).

The details of our image analysis protocol also have
been previously reported [13, 14]. In brief, MTR and dif-
fusion-weighted images were coregistered, and the diffu-
sion tensor was estimated to generate maps of fractional
anisotropy (FA), mean diffusivity (MD), parallel diffusiv-
ity ($\lambda_{\parallel}$), and perpendicular diffusivity ($\lambda_{\perp}$). To reduce the
influence of distortion at the periphery, particularly the
temporal lobes, we restricted our analysis to Montreal
Neurological Institute (MNI-152) coordinates $54 \leq z \leq 124$.
This restricted the analysis to supratentorial brain only,
from the mid-temporal lobes to the centrum semiovale.
Also a small section of the anterior frontal lobes ($y \leq 37$)
was removed, because it is particularly susceptible to dis-
tortion on DTI scans. Cerebrospinal fluid was masked out
by excluding voxels with MD ≥ 1.7 μm^2/ms. DTI indices
and MTR were calculated as mean values across this entire
supratentorial area, in addition to within specific tracts of
interest. For tract analysis, the optic radiations, cortico-
spinal tracts, and corpus callosum were analyzed in an
automated fashion via linear registration to a normalized
DTI atlas. This atlas was created by conventional tractog-
raphy [manual region of interest (ROI) drawing with
tractography between these ROIs] upon 26 healthy volun-
teers with probability-based tract reconstruction, as
described previously [12, 13]. These tracts were chosen
because of their clinical and functional relevance and
because of existing reliability data for these same tracts via

the same methodology in an overlapping dataset [13]. Whole tracts (without lesions removed) were analyzed in this study, because prior work on this dataset has demonstrated that T2 lesions only account for 1–2 % of DTI-derived tract profiles, and removal of lesions in order to examine only "normal-appearing white matter" does not meaningfully alter mean tract index values or analyses performed with these values [12, 14]. As described in our prior report [12], all values obtained were adjusted to account for known data shifts over time due to scanner upgrades/modifications.

For brain segmentation, we used the Lesion-TOADS algorithm [15, 16] (http://www.nitrc.org/projects/toads-cruise/). This algorithm uses the MPRAGE and FLAIR scans to account for and segment T2 lesions while also automatically segmenting whole-brain and multiple white and gray matter structures. All segmentation results were visually inspected for errors by an experienced observer (D.M.H.). Three cases required custom parameter settings, and one case required manual editing of the lesion mask. To account for intersubject variability in head size, the segmented volumes were normalized to each individual's intracranial volume. Thus, brain volume is reported as the cerebral volume fraction (CVF), in addition to cerebral white and gray matter fractions (WMF and GMF, respectively) and normalized brain substructure and total T2 lesion volumes (T2LV).

Disability measures

All disability assessments were performed within 30 days of scan date. Neurologic examinations were performed to determine the Expanded Disability Status Scale (EDSS) [17] score. The timed 25-foot walk, 9-hole peg test, and paced auditory serial addition test with 3 second delay (PASAT-3) were administered to determine the Multiple Sclerosis Functional Composite (MSFC) score [18]. z-Scores for individual test results and the MSFC total score were calculated according to the recommendations of the National Multiple Sclerosis Society Clinical Outcomes Assessment Task Force, using the Task Force dataset for normalization [19, 20]. Lower z-scores correspond to greater disability. Low-contrast visual acuity (1.25 %), which has been demonstrated to be more sensitive to MS-related optic nerve damage than standard visual acuity [21, 22], was used as a measure of visual function.

Statistical analysis

All statistical analyses were performed in Stata 10.1 IC (StataCorp, College Station, Texas). Comparisons between subgroups for demographic, clinical, and MRI data were performed by Student's t test (means) and Mann–Whitney

test (medians). Correlation analysis was performed by examining partial correlations, adjusted for age and sex. Multivariate linear regression (also adjusted for age and sex) was used to further probe these relationships. Comparisons between subgroups for the strength of relationship between MRI and disability data was performed in a multivariable regression model with interaction terms. To determine if the values for the DTI-derived MRI indices could be best predicted by brain volume (CVF) versus lesion burden (T2LV) and WMF versus GMF, regression analysis was performed for each of these individually, and then compared (as a nested model) with a combined model via the likelihood ratio test. To determine if the DTI-derived indices could add to the ability of CVF and T2LV to predict disability, a similar nested model regression approach was used with significance assessed by the likelihood ratio test. Because of the large number of analyses performed, a significance level of $p < 0.01$ was used for the correlation and regression analyses to reduce the false discovery rate.

Results

Scans from 117 MS cases (66 RRMS, 30 SPMS, and 21 PPMS) were analyzed, in addition to 26 healthy volunteers. Demographic and clinical data are described in Table 1. The healthy volunteer population was significantly younger than the full MS population, but not distinguishable from the RRMS population. As expected, disease duration was longest in those with SPMS [mean 20.9 years, standard deviation (SD) 10.0 years], and EDSS, MSFC, and low-contrast visual acuity were all significantly worse in those with SPMS and PPMS compared with RRMS.

Mean values for normalized structural volumes, DTI indices, and MTR can be found in Supplementary Table 1. CVF and gray matter, caudate, putamen, and thalamic volumes were all reduced in those with MS compared with healthy volunteers, whereas WMF was not. All of the DTI indices (except FA in corticospinal tract) and MTR were pathologically altered (reduced FA and MTR and increased diffusivities) in comparison with the healthy volunteers. Although there were no significant differences in the supratentorial brain analysis, most tract-specific indices were more abnormal in those with SPMS compared with RRMS.

To explore if DTI and MTR abnormalities are markers of global, gray matter, white matter, and/or lesion pathology, relationships of the DTI indices and MTR with the segmented volumes were explored by correlation analysis, the results of which are provided in Table 2. MTR and all DTI indices except FA in the corticospinal tract (which was not abnormally low in MS; see Supplementary Table 1)

Table 1 Characteristics of study population

	Healthy volunteers ($n = 26$)	All MS ($n = 117$)	Relapsing–remitting MS ($n = 66$)	Secondary progressive MS ($n = 30$)	Primary progressive MS ($n = 21$)
Mean age in years (SD)	35.1 (10.3)	45.2 (11.7)*	39.8 (11.1)	52.3 (8.0)**	52.2 (9.1)**
Female (%)	69 %	68 %	76 %	66 %	48 %**
Mean disease duration in years (SD)	n/a	10.9 (10.1)	7.1 (6.8)	20.9 (10.0)**	8.3 (9.4)[†]
On treatment (%)	n/a	64 %	80 %	50 %**	35 %**
Median EDSS (range)	n/a	3.5 (0–8)	2 (0–6.5)	6 (2.5–8)**	5.5 (2.5–8)**,[†]
Mean MSFC z-score (SD)	n/a	0.01 (0.72)	0.24 (0.58)	−0.40 (0.82)**	−0.24 (0.75)**
Mean timed 25-ft walk z-score (SD)	n/a	0.22 (0.64)	0.42 (0.15)	−0.23 (1.10)**	0.13 (0.63)**
Mean 9-hole peg test z-score (SD)	n/a	−0.25 (1.20)	0.26 (1.04)	−0.94 (1.02)**	−0.91 (1.20)**
Mean PASAT-3 z-score (SD)	n/a	−0.10 (1.11)	0.048 (1.03)	−0.42 (1.22)	−0.11 (1.18)
Mean low-contrast visual acuity (1.25 %), fraction of letters correctly identified (SD)	0.42 (0.11)	0.28 (0.16)*	0.33 (0.14)	0.22 (0.15)**	0.22 (0.17)**

* $p < 0.05$ for comparison with healthy volunteers

** $p < 0.05$ for comparison with RRMS

[†] $p < 0.05$ for comparison with SPMS

demonstrated robust correlations with CVF, T2LV, and WMF, with most correlation coefficients >0.5. A visual representation of this relationship is presented in Fig. 1a, b with optic radiations MD as an example. More modest correlations were found between GMF and DTI indices (again, except for FA in the corticospinal tract) and MTR, with correlation coefficients between 0.28 and 0.58. Values for MTR and DTI indices did not correlate with caudate or brainstem volume, and only supratentorial FA correlated with cerebellum volume ($r = 0.26$). All DTI indices and MTR in the optic radiations correlated modestly with thalamus volume, as did MD and $\lambda_\perp$ in the corpus callosum.

A nested model regression analysis was performed to evaluate whether the DTI and MTR indices were most associated with measures of atrophy (CVF) or lesion burden (T2LV). For all indices except FA, $\lambda_\perp$, and MTR in the corticospinal tract, DTI and MTR values were predicted to a greater degree in a regression model containing both CVF and T2LV, as opposed to one or the other independently (Table 3).

A similar approach was used to evaluate if MTR and the DTI indices were more strongly related to gray or white matter pathology (Table 3). Although R^2 values (measure of degree of variability in DTI and MTR predicted by regression model) were generally greater for WMF, addition of GMF to the regression model added significant additional predictive ability. This was not the case for supratentorial FA and corticospinal tract $\lambda_\parallel$, $\lambda_\perp$, and MTR, for which addition of GMF to the regression model did not add significant predictive information beyond that provided by WMF. The opposite was found for corticospinal tract FA, in which addition of WMF to the regression model did

not add predictive information beyond that provided by GMF.

Most of the DTI indices and MTR in supratentorial brain, corpus callosum, and optic radiations were significantly correlated with disability in all MS patients, as measured by EDSS and MSFC scores (Table 4). A visual representation of this relationship is shown in Fig. 1c–e, with optic radiations MD as an example. In the corticospinal tract, only $\lambda_\perp$ correlated with EDSS and only MTR correlated with MSFC scores. Low-contrast visual acuity did not correlate with DTI or MTR values in supratentorial brain or corpus callosum, but did correlate with all indices except $\lambda_\parallel$ in the optic radiations. Low-contrast visual acuity also correlated with corticospinal tract FA, $\lambda_\perp$, and MTR. Subgroup analysis revealed similar patterns of correlation in those with RRMS, and to a degree, PPMS. In SPMS, the DTI-derived indices did not significantly correlate with disability measures, and in fact, a number of indices actually had trends towards reciprocal relationships to disability compared with RRMS and PPMS; For example, whereas MD in the optic radiations in RRMS and PPMS had positive correlations with EDSS ($\rho = 0.41$ and 0.47, respectively), this index negatively correlated ($\rho = -0.26$) in SPMS.

A nested model regression approach was used to determine if DTI values and MTR in relapsing–remitting MS provided additional information toward the prediction of disability beyond that provided by CVF or T2LV (Table 5; Supplementary Table 3). DTI values and MTR in the supratentorial brain and corpus callosum, in addition to most values in the optic radiations and corticospinal tract, did not add any further significant predictive value for disability prediction beyond that provided by CVF.

Table 2 Correlation between DTI indices and MTR and volumetric measures

Volumetric measurement	Supratentorial					Corpus callosum					Optic radiations					Corticospinal tract				
	FA	MD	$\lambda_\parallel$	$\lambda_\perp$	MTR	FA	MD	$\lambda_\parallel$	$\lambda_\perp$	MTR	FA	MD	$\lambda_\parallel$	$\lambda_\perp$	MTR	FA	MD	$\lambda_\parallel$	$\lambda_\perp$	MTR
CVF	0.53*	−0.76*	−0.73*	−0.76*	0.64*	0.69*	−0.76*	−0.60*	−0.79*	0.69*	0.54*	−0.68*	−0.61*	−0.69*	0.63*	0.05	−0.50*	−0.51*	−0.40*	0.44*
T2 lesion volume	−0.56*	0.69*	0.65*	0.69*	−0.50*	−0.63*	0.61*	0.46*	0.65*	−0.65*	−0.74*	0.79*	0.69*	0.80*	−0.70*	−0.14	0.59*	0.58*	0.49*	−0.60*
Cerebral WM volume	0.58*	−0.66*	−0.57*	−0.68*	0.54*	0.65*	−0.64*	−0.48*	−0.69*	0.61*	0.45*	−0.58*	−0.53*	−0.59*	0.54*	0.01	−0.45*	−0.46*	−0.34*	0.35*
Cerebral GM volume	0.33*	−0.56*	−0.58*	−0.54*	0.41*	0.46*	−0.52*	−0.43*	0.53*	0.41*	0.39*	−0.42*	−0.37*	−0.43*	0.36*	0.11	−0.32*	−0.28*	−0.28*	0.28*
Caudate volume	−0.08	−0.1	−0.16	−0.08	−0.01	−0.02	−0.16	−0.21	−0.13	0.01	−0.01	−0.11	−0.14	−0.09	0.08	−0.12	−0.07	−0.14	−0.01	−0.04
Thalamus volume	0.01	−0.06	−0.06	−0.05	0.1	0.2	−0.26*	−0.22	−0.27*	0.24	0.25*	−0.34*	−0.33*	−0.33*	0.31*	0.06	−0.19	−0.15	−0.17	0.22
Brainstem volume	0.05	−0.09	−0.07	−0.09	0.19	0.03	−0.06	−0.05	−0.05	0.15	0.06	−0.01	0.04	−0.04	0.1	0.01	−0.11	−0.08	−0.1	0.19
Cerebellum volume	−0.26*	0.21	0.16	0.22	−0.05	−0.24	0.19	0.12	0.21	−0.08	−0.15	0.11	0.08	0.12	−0.04	−0.24	0.19	0.12	0.21	−0.08

Partial correlations, adjusted for age and gender

* $p < 0.01$ for significance of correlation coefficient

Exceptions to this were optic radiation MD and $\lambda_\perp$ for prediction of EDSS and optic radiation FA and $\lambda_\perp$ and corticospinal tract $\lambda_\perp$ and MTR for prediction of low-contrast visual acuity. None of the DTI indices or MTR was able to predict EDSS or MSFC scores beyond that predicted by T2LV alone. Values for FA and $\lambda_\perp$ in the optic radiations and corticospinal tract added significant predictive value for low-contrast visual acuity to T2LV alone. None of the DTI indices or MTR was able to add significant additional predictive value for EDSS and MSFC scores beyond CVF and T2LV combined. However, optic radiation FA and $\lambda_\perp$ were able to add additional predictive value for low-contrast visual acuity beyond CVF and T2LV combined.

Discussion

This study supports the links previously seen between DTI and MTR abnormalities and disability in MS [10, 11, 13], as demonstrated by the correlations between EDSS and MSFC and most of the measured indices, and with low-contrast visual acuity for some indices in the optic radiations and corticospinal tract. We went one step further to compare the relative strength of correlation with disability for the DTI indices and MTR with conventional MRI measures. This analysis showed that DTI values in the optic radiations may improve prediction of EDSS over brain volume alone and may also improve prediction of low-contrast visual acuity over brain volume and lesion burden, both individually and combined. The fact that it was the optic radiations that stood apart from the other tracts in comparison with conventional MRI for prediction of EDSS is an interesting finding requiring future replication. While visual disability does impact the EDSS score to a degree, it is more likely that the predominantly periventricular nature of this tract may make it more likely to represent global MS disease processes better than other tracts. Regardless, the ability to predict disability to a greater extent than the individual conventional MRI measures argues for further investigation into the use of optic radiations DTI as a surrogate marker in clinical trials. Further supporting this notion is our prior report demonstrating that this analysis technique can measure longitudinal change with variability small enough to allow for reasonable sample sizes for detection of treatment effects [12]. The strong link seen between optic radiation damage and visual disability in this study supports our prior work demonstrating the ability of tract-based DTI analysis to probe structure–function relationships [9]. Integration of more disability-specific tract-based measures into clinical trials, as opposed to global markers of pathology (such as T2LV and brain atrophy), may help resolve the
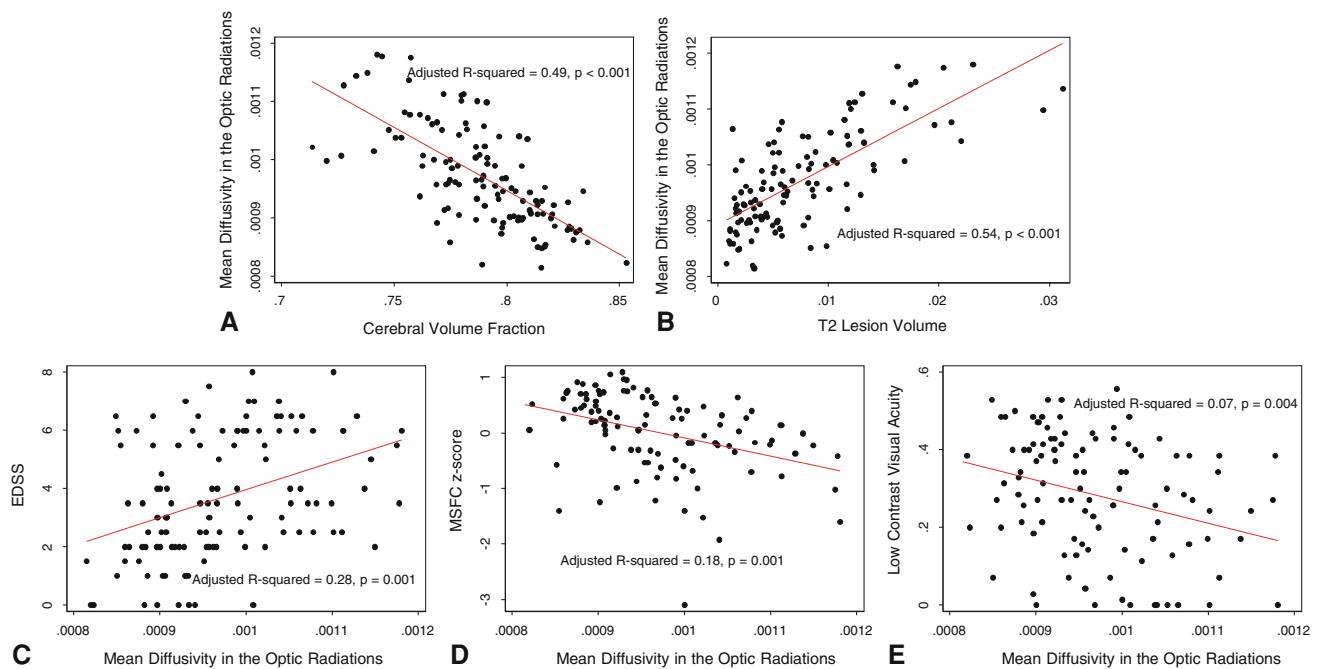
Fig. 1 Relationship between mean diffusivity in the optic radiations and atrophy, lesion load, and disability. The graphs demonstrate the relationship between mean diffusivity in the optic radiations (as an example) and cerebral volume fraction (**a**), T2 lesion volume (**b**), EDSS (**c**), MSFC (**d**), and low-contrast visual acuity (**e**). The line of best fit was obtained by plotting values as predicted by a regression model adjusted for age and sex. R^2 values represent the proportion of variability in the dependent variable explained by the independent variable

clinicoradiologic paradox noted previously in observational and therapeutic studies [1].

As opposed to conventional MRI outcomes in MS, DTI-based tract-specific measures have the particular advantage of probing structure–function relationships and may provide evidence for cellular injury along an entire functional pathway. This notion is supported by our observation that damage to all of the DTI indices and MTR in the optic radiations correlated with thalamic atrophy, and these values specifically correlated with low-contrast visual acuity. Mechanistic links between damage to thalamocortical pathways and thalamic atrophy have been previously established in MS and have been demonstrated to be independent of damage to unrelated white matter tracts [23]. Whether MS-related thalamic atrophy is primary and damage to connected pathways occurs through secondary Wallerian degeneration or vice versa has still yet to be clearly established. It is unclear if our results represent the quantification of death of neuronal cell bodies of the optic radiations originating in the thalamus, transsynaptic degeneration, or if the structure and disability correlations are simply stronger for cases in which pathology has occurred throughout the entire visual pathway. Regardless, our results demonstrate that structure–function relationships can be probed in this manner across an entire functional pathway, with clinical relevance.

Our findings also provide additional information as to what underlying disease processes may be responsible for alterations in DTI and MTR values. We were able to demonstrate robust correlations between white matter tract and supratentorial DTI and MTR and CVF, T2LV, and GMF/WMF, consistent with prior reports [10, 24]. Cluing towards the structural specificity of this type of analysis, beyond the correlations seen between thalamic volume and all indices in the optic radiations and corpus callosum MD and $\lambda_\perp$, no correlations were found with other brain substructures, such as caudate and brainstem. Further analysis demonstrated that the variability of DTI values and MTR was best predicted by a combined model including T2LV and CVF, which was superior to either independently. Also, neither white nor gray matter atrophy was superior to the other in predicting the DTI values or MTR.

These findings have implications for our understanding of the causes of DTI and MTR abnormalities in MS; For example, if MTR abnormalities were purely influenced by lesion-localized demyelination, it would be expected that values would be best predicted by lesion volume. The additional predictive value of brain atrophy towards these values, however, clues towards the additional influence of more global neurodegenerative processes and axonal injury, both of which have been shown to alter MTR in autopsy studies [8, 25]. Additionally, the fact that DTI

Table 3 Relationship of DTI indices and MTR with atrophy and lesion volume

DTI-derived indices	R^2 for prediction of DTI-derived indices by:					
	CVF	T2 lesion volume	CVF + T2 lesion volume	Cerebral WM fraction	Cerebral GM fraction	Cerebral WM + GM volume
Supratentorial FA	0.30*	0.33*	0.40†	0.37*	0.12*	0.39
Supratentorial MD	0.57*	0.46*	0.67†	0.44*	0.30*	0.61†
Supratentorial $\lambda_\parallel$	0.53*	0.42*	0.60†	0.33*	0.33*	0.54†
Supratentorial $\lambda_\perp$	0.57*	0.46*	0.66†	0.47*	0.27*	0.62†
Supratentorial MTR	0.39*	0.29*	0.44†	0.30*	0.14*	0.37†
Corpus callosum FA	0.47*	0.38*	0.55†	0.43*	0.20*	0.53†
Corpus callosum	0.57*	0.38*	0.62†	0.43*	0.27*	0.56†
Corpus callosum $\lambda_\parallel$	0.36*	0.22*	0.37†	0.24*	0.18*	0.34†
Corpus callosum $\lambda_\perp$	0.62*	0.42*	0.68†	0.48*	0.28*	0.63†
Corpus callosum MTR	0.47*	0.42*	0.59†	0.37*	0.14*	0.44†
Optic radiations FA	0.31*	0.56*	0.59†	0.24*	0.18*	0.31†
Optic radiations MD	0.49*	0.64*	0.75†	0.36*	0.24*	0.46†
Optic radiations $\lambda_\parallel$	0.39*	0.50*	0.61†	0.29*	0.19*	0.36†
Optic radiations $\lambda_\perp$	0.50*	0.66*	0.77†	0.38*	0.25*	0.47†
Optic radiations MTR	0.39*	0.47*	0.58†	0.28*	0.12*	0.33†
Corticospinal tract FA	−0.01	0.01	0.00	−0.01	0.14*	−0.01
Corticospinal tract MD	0.26*	0.35*	0.40†	0.20*	0.12*	0.25†
Corticospinal tract $\lambda_\parallel$	0.23*	0.32*	0.36†	0.19*	0.12*	0.22
Corticospinal tract $\lambda_\perp$	0.18*	0.25*	0.28	0.14*	0.08*	0.17
Corticospinal tract MTR	0.19*	0.35*	0.37	0.12*	0.06*	0.15

Values listed are R^2 values within the regression model, signifying the percentage of variability in the DTI indices and MTR predicted by atrophy and lesion volume measurements

* $p < 0.01$ for prediction of DTI-derived index value

† $p < 0.01$ for likelihood ratio test for superiority of prediction of DTI indices and MTR by the combination of CVF-TOADS and T2 lesion volume over CVF alone (or cerebral WM volume and cerebral GM volume over cerebral WM volume alone)

values are influenced by both CVF and T2LV likely means that these values are influenced by multiple processes, including demyelination, axonal transection, secondary Wallerian degeneration, and inflammation. The fact that lesion volume was not found to be the sole determinant of DTI and MTR values is consistent with multiple studies that have demonstrated DTI and MTR abnormalities in normal-appearing white matter [26–28]. Also, the observed independent predictive abilities of gray and white matter volumes toward DTI abnormalities lends credence to the idea that these values may be influenced by secondary neurodegeneration and demyelination stemming from both axonal transection in white matter lesions and primary gray matter pathology, both of which have been independently demonstrated in pathologic studies [29–31].

Also, although $\lambda_\parallel$ has been proposed as a measure of axonal degeneration and $\lambda_\perp$ has been proposed as a measure of demyelination [5–7], the fact that supratentorial atrophy, gray matter volume, white matter volume, and lesion volume all play significant independent roles in influencing these values demonstrates that these indices are

also likely not pure measures and may be influenced to varying degrees by degenerative, inflammatory, and demyelinating pathology. Although we have demonstrated here that DTI and MTR are not purely influenced by demyelination or axonal degeneration, it may turn out that these measures still prove superior to conventional measures in evaluation of future therapies aimed at demyelination or neuroregeneration [32, 33].

The pathologic alterations noted in this study in nearly all of the DTI and MTR indices are consistent with prior literature [27, 34]. These alterations were more profound in participants with SPMS when compared with RRMS, which is also consistent with prior reports [28, 35]. Despite this evidence of more significant pathology in axonal pathways, no correlations were found between these imaging outcomes and disability measures in participants with SPMS. This was not the case for those with RRMS, in whom such correlations could be found. While this may have been an issue of the smaller sample size of this subgroup, statistical comparisons found significant and mostly reciprocal differences between those with RRMS and

Table 4 Correlation of DTI indices and MTR with disability measures

	All MS			Relapsing–remitting MS			Secondary progressive MS			Primary progressive MS		
	EDSS	MSFC z-score	Low-contrast visual acuity	EDSS	MSFC z-score	Low-contrast visual acuity	EDSS	MSFC z-score	Low-contrast visual acuity	EDSS	MSFC z-score	Low-contrast visual acuity
Supratentorial FA	−0.25*	0.28*	0.12	−0.38*	0.39*	0.16	0.22	0.09[†]	−0.07	−0.41	0.16	0.18
Supratentorial MD	0.27*	−0.34*	−0.10	0.34*	−0.39*	−0.15	−0.14	−0.14	0.26	0.39	−0.32	−0.23
Supratentorial $\lambda_\parallel$	0.22	−0.31*	−0.06	0.26	−0.32	−0.10	−0.09	−0.13	0.29	0.27	−0.34	−0.21
Supratentorial $\lambda_\perp$	0.28*	−0.35*	−0.11	0.36*	−0.41*	−0.16	−0.15	−0.13	0.23	0.43	−0.31	−0.24
Supratentorial MTR	−0.21	0.32	0.09	−0.31	0.45*	0.11	0.31[†]	−0.04	−0.32	−0.52[‡]	0.40	0.51
Corpus callosum FA	−0.29*	0.33*	0.13	−0.34	0.49*	0.20	−0.05	−0.02	−0.19	−0.64	0.39	0.39
Corpus callosum MD	0.27*	−0.36*	−0.04	0.27	−0.41*	0.00	0.10	−0.13	0.14	0.33	−0.51	−0.28
Corpus callosum $\lambda_\parallel$	0.19	−0.28*	0.03	0.18	−0.26	0.09	0.07	−0.14	0.10	0.08	−0.45	−0.12
Corpus callosum $\lambda_\perp$	0.29*	−0.38*	−0.07	0.30	−0.46*	−0.06	0.11	−0.11	0.15	0.46	−0.52	−0.36
Corpus callosum MTR	−0.20	0.34*	0.14	−0.28	0.42*	0.15	0.19	0.18	−0.14	−0.53	0.40	0.52
Optic radiations FA	−0.28*	0.30*	0.31*	−0.34*	0.34*	0.38*	0.16	0.10	0.08	−0.67*,[‡]	0.48	0.43[‡]
Optic radiations MD	0.31*	−0.33*	−0.28*	0.41*	−0.40*	−0.25	−0.26[†]	−0.06	−0.11	0.47[‡]	−0.42	−0.40
Optic radiations $\lambda_\parallel$	0.24*	−0.28*	−0.20	0.37*	−0.33*	−0.14	−0.34[†]	−0.01	−0.06	0.30	−0.34	−0.31
Optic radiations $\lambda_\perp$	0.33*	−0.35*	−0.30*	0.42*	−0.41*	−0.29	−0.21[†]	−0.08	−0.12	0.54[‡]	−0.45	−0.43[‡]
Optic radiations MTR	−0.28*	0.37*	0.26*	−0.31	0.51*	0.24	0.37[†]	0.14	−0.15	−0.50[‡]	0.27	0.46[†,‡]
Corticospinal tract FA	−0.16	0.04	0.27*	−0.15	0.12	0.30	−0.06	0.02	0.63*	−0.05	−0.36	−0.17
Corticospinal tract MD	0.22	−0.19	−0.23	0.34*	−0.23	−0.28	−0.10	−0.07	−0.27	0.33	−0.08	−0.11
Corticospinal tract $\lambda_\parallel$	0.13	−0.17	−0.07	0.26	−0.16	−0.11	−0.13	−0.07	0.18	0.27	−0.27	−0.20
Corticospinal tract $\lambda_\perp$	0.24*	−0.17	−0.30*	0.33*	−0.24	−0.36*	−0.05	−0.06	−0.50	0.31	0.11	−0.03
Corticospinal tract MTR	−0.21	0.30*	0.26*	−0.25	0.41*	0.31	0.34	0.11	−0.22	−0.54[‡]	0.17	0.54[‡]

Partial correlations, adjusted for age and gender. Intergroup comparisons for magnitude and direction of this relationship performed within multivariate regression model with interaction terms

* $p < 0.01$ for correlation coefficient

[†] $p < 0.01$ for comparison with RRMS

[‡] $p < 0.01$ for comparison with SPMS

Table 5 Summary of comparison of prediction of disability by brain volume, lesion load, and DTI and MTR in RRMS

Disability measure	Conventional imaging indices in predictive model	DTI/MTR indices adding significant additional information predictive of disability
EDSS	CVF	Optic radiations MD, $\lambda_\perp$
	T2 lesion volume	None
	CVF + T2 lesion volume	None
MSFC	CVF	None
	T2 lesion volume	None
	CVF + T2 lesion volume	None
Low-contrast visual acuity	CVF	Optic radiations FA, $\lambda_\perp$; corticospinal tract $\lambda_\perp$, MTR
	T2 lesion volume	Optic radiations FA, $\lambda_\perp$; corticospinal tract FA, $\lambda_\perp$
	CVF + T2 lesion volume	Optic radiations FA, $\lambda_\perp$

Regression performed for prediction of disability measure by CVF, lesion volume or CVF and lesion volume. DTI-derived indices added to the model to determine if they provided additional predictive information to the nested model (tested by likelihood ratio test). Significant indices achieved $p < 0.01$ for likelihood ratio test for superiority of the full model over the nested model. Full data found in Supplementary Table 3

PPMS compared with SPMS in a number of the tract-specific indices and supratentorial MTR. This disconnect between more severe DTI and MTR abnormalities and disability in SPMS may represent a basement effect, in which pathologic alterations of axonal pathways in MS are responsible for disability earlier in the disease course, until a critical threshold, after which other aspects of MS pathology become more prominent determinants of disability. Thus, in SPMS, many of the axonal tracts being measured may have already lost function, yet they may continue to experience further pathologic change, either due to the disease process or age-related changes. Alternately, these reciprocal relationships may be the result of the influence of inflammation on DTI values, with increases in diffusivity occurring in the RRMS population due to inflammatory change and tissue edema, correlating with worsening disability. Conversely, because of a reduction in central nervous system (CNS) inflammation in the later stages of secondary progressive phase of MS, diffusivity values may be reducing in late SPMS due to a pseudonormalization effect. This would also correlate with worsening disability due to the expected worsening of disability seen clinically in SPMS.

It should be noted that our methodology of summary statistics derived from automated, probability-based tract identification differs from other studies in which DTI and MTR were measured by whole-brain histogram techniques, ROI analysis, or voxel-based morphometry. While our technique may provide simple, easy to interpret values in an automated manner, and has been shown to correlate well with disability and be stable enough to evaluate for longitudinal change [12, 13], it is nonetheless difficult to compare these results directly with studies of those types. Additionally, the stronger correlations with physical disability seen for the optic radiations and corpus callosum over the corticospinal tracts may have been influenced by the anatomy of these tracts, which allow for superior automated tractography. Because of their simple anatomy and sheet-like architecture, these tracts are less susceptible to registration errors, resulting in more reproducible tract identification than the corticospinal tract [13]. Lastly, definitive conclusions in regards to our MS subtype analysis should be tempered with an acknowledgement of the reduced sample sizes of the progressive subtypes compared with RRMS. Regardless, we feel that the comprehensive nature of this analysis and the large cohort analyzed mean that the results contribute substantially to our understanding of the role of DTI and MTR in measuring pathology and its relationship to disability in multiple sclerosis.

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