PAPER

Mechanisms of normal appearing corpus callosum injury related to pericallosal T1 lesions in multiple sclerosis using directional diffusion tensor and ^1H MRS imaging

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Objectives: To investigate the extent of tissue damage in a region of normal appearing corpus callosum (NACC) for different forms of multiple sclerosis (MS) using diffusion tensor and proton magnetic resonance (MR) spectroscopic imaging.

Methods: A total of 47 patients with MS and 15 controls were included. Regions of interest from the NACC were manually segmented using high resolution anatomical images. Diffusion tensor eigenvalues and metabolite ratio of N-acetyl-aspartate (NAA) to creatine/phosphocreatine (Cr) were calculated in the NACC region.

Results: Increased apparent diffusion coefficients (ADCs) and decreased anisotropy were observed in the NACC for patients with MS relative to the control subjects. These resulted from increased diffusion tensor eigenvalues perpendicular to the maximum diffusion direction. The NAA:Cr ratio was decreased in the NACC for patients with MS relative to the control subjects. Significant correlations between pericallosal T1 lesion load and MR modalities in the NACC were observed for patients with relapsing remitting/secondary progressive MS (RR/SPMS), but not for patients with primary progressive MS (PPMS).

Conclusion: This study provides further insight into changes in the ADC and diffusion anisotropy based on the diffusion tensor eigenvalues for patients with MS. The changes in the diffusion tensor eigenvalues and NAA:Cr ratio in the NACC for patients with RR/SPMS suggest axonal injury and/or dysfunction induced by wallerian degeneration. The lack of correlation between these variables in the NACC and focal MS lesions for patients with PPMS further supports intrinsic differences related to tissue injury between these subtypes of MS.

Several magnetic resonance (MR) imaging modalities have been developed for monitoring disease progression and evaluating response to therapy non-invasively in patients with multiple sclerosis (MS). While these modalities provide important information, there remains a need for more sensitive and specific markers of the biological effects of MS. This is particularly true for monitoring patients with early stage disease and evaluating differences in parameters associated with the various subtypes of the disease. The clinical pattern referred to as relapsing remitting MS (RRMS) occurs in more than 85% of patients. Within 10–15 years, approximately 50% of these patients experience gradual progression of disability with or without superimposed relapses and are classified as having secondary progressive MS (SPMS).¹ Approximately 15% of patients experience a clinical course that is gradually progressive from onset with no or very subtle acute clinical worsening and are classified as having primary progressive MS (PPMS).^{2,3} Although differences among the expressions of the disease in patients with RRMS, SPMS, and PPMS are important for monitoring disease activity and response to therapy, the underlying aetiology that distinguishes these groups remains largely unknown. Previous studies have shown that PPMS may have several distinct clinical phenotypes, and that the most common initial presentation is a progressive spastic paraparesis with, less frequently, sensory or visual disturbances.⁴ Although the mean number and volume of new gadolinium enhancing and T2-weighted brain lesions are generally less for patients with PPMS than for patients with the RRMS and SPMS, the range of these variables is quite broad in PPMS.⁵ Diffuse abnormalities in normal appearing white matter (NAWM) in the brain and spinal cord provide a possible

explanation for the increased disability in PPMS with absence of multiple focal lesions.^{6,7}

Diffusion is a microscopic random motion of molecules. Pathological processes may alter the structural barriers for water diffusion and cause abnormal water diffusivity. Diffusion tensor imaging is useful to exploit diffusion anisotropy since water diffusion is a three dimensional process and molecular mobility in NAWM is anisotropic.^{8–10} Partial voluming effect of highly diffusive cerebrospinal fluid (CSF) with brain parenchyma can be reduced with fluid attenuated inversion recovery (FLAIR) diffusion imaging.¹¹ Previous studies of diffusion weighted imaging in MS have reported increased diffusivity and decreased anisotropy in NAWM and in lesions relative to controls.^{12–15} It has also been shown that abnormal diffusion parameters correlate with MS lesion volume.^{16,17} While interesting, these results do not indicate structural changes related to specific pathology.

Diffusion tensor eigenvalues are the magnitudes of the principal vectors (eigenvectors) that describe directional diffusion. The three directions of diffusion are the direction of maximum diffusion (λ_1) and the two directions orthogonal to each other and perpendicular to the maximum diffusion (λ_2 and λ_3). The apparent diffusion coefficient (ADC) is the average of the three diffusion tensor eigenvalues. Anisotropy is a scalar invariant reflecting the variance

Abbreviations: ADC, apparent diffusion coefficient; 3D SPGR, three dimensional spoiled gradient echo; CSF, cerebrospinal fluid; Cr, creatine/phosphocreatine; EDSS, Expanded Disability Status Scale; FLAIR, fluid attenuated inversion recovery; ^1H MRSI, proton magnetic resonance spectroscopic imaging; MS, multiple sclerosis; NAA, N-acetyl-aspartate; NAWM, normal appearing white matter; PPMS, primary progressive MS; SPMS, secondary progressive MS; ROI, region of interest; RRMS, relapsing remitting MS

of the three diffusion tensor eigenvalues.¹⁸ A diffusion tensor study of corticospinal tracts following lacunar infarcts showed that the anisotropy was reduced in highly anisotropic regions because of the increased diffusion tensor eigenvalues perpendicular to the maximum diffusion tensor eigenvalue—that is, λ_2 and λ_3 . Such changes were thought to reflect wallerian degeneration, a process known to occur following a vascular infarct.¹⁹ Also in a recent study from our research group the same pattern was observed in highly anisotropic NAWM regions of RRMS patients and was interpreted as a possible in vivo signature of wallerian degeneration induced by distant MS lesions.²⁰

Proton magnetic resonance spectroscopic imaging (¹H MRSI) enables the measurement and quantification of the spatial distribution of brain metabolites such as choline-containing compounds, creatine/phosphocreatine (Cr), and *N*-acetyl aspartate (NAA) and has been used to study many brain disorders. NAA is found mainly in neurones and axons of the mature brain²¹ and its intensity relative to that of Cr has been proposed as an index of axonal damage.²² In MS, ¹H MRSI is particularly useful for determining whether axonal damage and/or dysfunction extend beyond the border of visible lesions to include regions of NAWM. Previous studies have reported decreased NAA:Cr ratio in NAWM as well as MS lesions.^{23–25} Pathological studies in MS have also shown transected axons in MS lesions,²⁶ and the loss of axons in the NAWM might be the result of the wallerian degeneration of axons transected from distant MS lesions.²⁷

The corpus callosum is an area of the brain connecting homologous regions of the right and left hemispheres. It may be very sensitive to changes in white matter tracts and thus a useful site for detection of such changes particularly in patients with MS. A previous histopathological study has shown significant loss of both total number of axons and axonal density of fibres crossing the corpus callosum in patients with RRMS and SPMS relative to non-diseased brain and their correlation with regional MS lesions.²⁸ It is also well known that MS lesions have a tendency to cluster in periventricular white matter. This suggests that the corpus callosum may be a sensitive region for detecting in vivo tissue damage induced by brain lesions for patients with MS.

This study demonstrates the potential value of in vivo diffusion tensor eigenvalues and ¹H MRSI in patients with MS for quantifying tissue injury in normal appearing corpus callosum. We hypothesised that the tissue changes measured by diffusion tensor eigenvalues and ¹H MRSI may originate from distinct pathological mechanisms in different forms of MS.

MATERIAL AND METHODS

Study population

A total of 47 patients with MS were included in this study from a cohort of patients followed at the University of California, San Francisco Multiple Sclerosis Center. These patients were included on the basis of the absence of T1- and T2-weighted visible MR imaging abnormalities in the studied corpus callosum region as examined by an experienced MS neurologist (DP). Eleven patients had RRMS and 12 patients had clinically definite SPMS as defined by the Poser criteria.²⁹ A total of 24 patients with PPMS were included. These patients were defined by (a) progressive clinical worsening from onset for 12 months or more with no episode of acute neurological exacerbation and (b) abnormal CSF as defined by the presence of two or more oligoclonal bands or elevated IgG index. Neurological evaluation included the Expanded Disability Status Scale (EDSS).³⁰ Fifteen healthy control subjects were examined using the same MR protocol. All subjects gave informed written consent. The mean age, disease duration, and EDSS for the individual subgroups are given in table 1.

Table 1 Clinical characteristics of the individual subgroups

	Controls	RR/SPMS	PPMS
No. of subjects	15	23	24
Age, years	43.2 (9.1), 3.6–57.2	44.5 (8.9), 22.2–59.0	51.6 (6.3), 39.3–66.2
Disease duration, years	N/A	12.9 (8.4), 4.5–9.2	8.7 (7.0), 1.0–25.5
EDSS	N/A	4.1 (2.50), 0.0–8.0	4.8 (1.4), 2.5–6.5
No. of subjects—DTI	15	22	22
No. of subjects— ¹ H MRSI	15	22	22

Data are mean (SD), range.

DTI, diffusion tensor imaging; EDSS, Expanded Disability Status Scale; ¹H MRSI, proton magnetic resonance spectroscopic imaging; PPMS, primary progressive multiple sclerosis; RR/SPMS, relapsing remitting/secondary progressive MS.

MR imaging examination

MR data were acquired with a 1.5 T General Electric Medical System scanner (General Electric, Milwaukee, WI) equipped with a quadrature head coil. Each MR imaging examination included oblique T2-weighted fast spin echo (TE/TR = 90/2000 ms, 256×256 matrix, 240 mm×240 mm FOV, 16 contiguous 5 mm thick slices), axial T1-weighted three dimensional spoiled gradient echo (3D SPGR) (TE/TR = 6/27 ms, flip angle = 40°, 192×256×124 matrix, 180 mm×240 mm×186 mm FOV) and axial T2-weighted (TE/TR = 80/2500 ms, 192×256 matrix, 180 mm×240 mm FOV, 3 mm interleaved 48 slices) volume images. The T2-weighted fast spin echo volume image was used as a reference for the ¹H MRSI acquisition.

Diffusion tensor imaging

An echo planar imaging spin echo FLAIR diffusion tensor pulse sequence was acquired. The FLAIR diffusion tensor imaging parameters were TE/TR/TI = 90/10000/2200 ms, 128×64 matrix, 360 mm×180 mm FOV, 28 interleaved 3 mm thick slices, *b* value = 1000 s/mm², gradient strength = 40 mT/m, gradient duration (δ) = 21 ms, and gradient separation (Δ) = 27 ms. The inversion time used in this sequence was set to suppress the signal from CSF. As a compromise between accurate mapping and scan time, we used six gradient directions. The maximum diffusion tensor eigenvalue was defined as λ_1 and the other two, perpendicular to λ_1 , were defined as λ_2 and λ_3 . All patients except three (one with RRMS and two with PPMS) underwent diffusion tensor imaging examination.

¹H MRSI protocol

Two dimensional chemical shifting imaging was applied with PRESS volume selection³¹ and 1.5 ml nominal spatial resolution using a commercially available sequence (General Electric Medical System). The PRESS volume was positioned to cover a central brain slab of approximately 90 mm×120 mm×15 mm centred at the middle of the corpus callosum (central brain). The two dimensional chemical shifting imaging parameters were TE/TR = 144/1000 ms, 24×24 phase encoding matrix, 240 mm×240 mm FOV, and 15 mm slice thickness. Automatic shimming and water suppression were applied as part of the data acquisition. All patients except three (one with SPMS and two with PPMS) underwent ¹H MRSI examination.

Post-processing

After each examination, both the images and raw spectra data were transferred to a SUN Ultra 10 workstation (Sun Microsystems, CA) for post-processing. The volumetric

T1-weighted 3D SPGR images were resampled to create high resolution images in the sagittal orientation to manually segment the corpus callosum and exclude visible MS lesions. Regions of interest (ROIs) corresponding to the normal appearing corpus callosum were drawn in a very conservative manner since it was not possible to find sharp boundaries where it merged into white matter. The normal appearing corpus callosum ROIs were saved as a three dimensional mask image in the axial orientation and resampled to correspond to the T2-weighted fast spin echo volume set, using nearest neighbour interpolation. Both T1-weighted 3D SPGR images and normal appearing corpus callosum ROIs were aligned to the T2-weighted fast spin echo images using an algorithm developed in our laboratory,³² so that the normal appearing corpus callosum ROIs and the spectral data were in the same plane. Masks with constant values in the normal appearing corpus callosum ROIs were generated for analysis of the corresponding diffusion parameters and spectral intensities.

All ¹H MRSI processing algorithms were developed inhouse and have been described previously.^{33, 34} In this study, the intensity of NAA and Cr were calculated from peak height because it has been observed that the variation in metabolite ratios obtained from peak area is larger than that from peak height. It was anticipated that any difference in line width due to variation in shimming would affect all resonance equally.³⁴ Results are expressed as the ratio of NAA to Cr since in vitro MRS analysis has demonstrated stable Cr levels in normal appearing tissue.³⁵ Voxels corresponding to the anatomic ROIs were determined by resampling the corresponding mask according to the PRESS selection and chemical shifting imaging phase encoding using inhouse software described previously.³⁴ The normal appearing corpus callosum voxels were identified by determining which had greater than 20% overlap with the normal appearing corpus callosum region. We kept the same cut-off for all the patients and controls. Metabolite levels from the normal appearing corpus callosum voxels from one patient with SPMS were excluded from the analysis because there were no voxels satisfying the criteria.

The diffusion tensor eigenvalues, ADC, and fractional anisotropy maps were calculated pixel-by-pixel based on MR signal intensity decay from diffusion tensor imaging examination. All diffusion processing algorithms were developed inhouse. All calculated maps were resampled to have the same centre as the T1-weighted 3D SPGR volume and aligned into the T2-weighted fast spin echo volume using

the same image alignment parameter used for the normal appearing corpus callosum ROIs. All aligned maps were resampled again to cover the same portion of central brain as in the ¹H MRSI. The CSF was segmented out based on the intensity of the FLAIR diffusion tensor imaging T2-weighted images without diffusion weighting (threshold method). Aligned normal appearing corpus callosum ROIs drawn from high resolution anatomic images were further refined by taking highly anisotropic regions from the functional anisotropy map (threshold method) to minimise possible alignment error between echo planar and anatomic images. Each histogram of diffusion parameters for the normal appearing corpus callosum ROIs was calculated and normalised to the total number of voxels and the histogram median calculated.

T1 lesions were drawn based on semiautomated threshold with manual editing on the axial T1-weighted 3D SPGR volume by an experienced neurologist (DP). T1 lesions hypointense relative to the white matter as well as isointense to the grey matter were included. The axial T2-weighted anatomic images acquired in the same examination confirmed that each T1 lesion was associated with a region of T2 hyperintensity. Pericallosal T1 lesions from all patients were defined as T1 lesions close to the corpus callosum but just above and below the central brain slab (total of 30 mm thickness). Pericallosal T1 lesions were considered in this study because it was expected that most of the bundles of transected axons in the focal MS lesion located at the central brain would cross the corpus callosum. Figure 1 shows an example of a pericallosal region (solid line) from a patient with RRMS (dashed line shows the ¹H MRSI PRESS volume). The total pericallosal T1 lesion load was calculated as the sum of individual lesion volumes.

Statistical analyses were performed using standard least square means (LSM) tests with adjustment for age because of the relatively younger mean age of the controls and RRMS patients with respect to the SPMS and PPMS patients in this study. The results are reported as LSM (standard error (SE)) unless otherwise stated. Non-parametric Spearman's test was used for analysing correlations of the MR modalities. The RRMS and SPMS patients were considered as one group (RR/SPMS), separate from the PPMS patients, because of the biological differences of the disease characteristics. $p < 0.05$ was regarded as significant.

RESULTS

Pericallosal T1 lesions

The average volume of total pericallosal T1 lesion load was 2.2 ml (range 0.002–12.8 ml) for the patients with RR/SPMS and 2.1 ml (range 0.06–16.2 ml) for the patients with PPMS.

Diffusion parameters in the normal appearing corpus callosum ROIs

The age adjusted mean values of the diffusion tensor eigenvalues, ADC, and functional anisotropy derived from the normal appearing corpus callosum ROIs are listed in table 2. Figure 2 shows aligned maps of the diffusion tensor eigenvalues, the ADC and functional anisotropy maps with corresponding normal appearing corpus callosum ROIs for a control subject (fig 2A) and a patient with SPMS (fig 2B). There was a significant increase of the averaged diffusion tensor eigenvalues perpendicular to the maximum diffusion—that is, $(\lambda_2 + \lambda_3)/2$, in the normal appearing corpus callosum region for both patient groups relative to the controls ($p = 0.001$ for the RR/SPMS and $p = 0.0002$ for the PPMS). The maximum diffusion tensor eigenvalue was similar to the controls for the PPMS patients ($p = 0.87$), but higher for the RR/SPMS patients relative to the controls with marginal significance ($p = 0.05$). ADCs were significantly

Figure 1 An example of a pericallosal region (solid line) from a patients with RRMS where T1 lesions were restricted to a 30 mm brain slab (pericallosal T1 lesions) surrounding the ¹H MRSI PRESS volume (dashed line, 90 mm×120 mm×15 mm).

Figure 2 Aligned maps of the maximum (λ_1), medium (λ_2) and minimum (λ_3) diffusion tensor eigenvalues, the apparent diffusion coefficients (ADC) and fractional anisotropy (FA) maps with corresponding normal appearing corpus callosum regions of interest for (A) a control subject and (B) a patient with SPMS.

increased for both patient groups ($p = 0.0002$ for the RR/SPMS and $p = 0.002$ for the PPMS), and functional anisotropy was significantly decreased for both patient groups ($p = 0.02$ for RR/SPMS and $p = 0.001$ for PPMS) relative to the controls in the normal appearing corpus callosum ROIs.

NAA:Cr ratio in the normal appearing corpus callosum ROIs

The age adjusted mean value of NAA:Cr ratio derived from the normal appearing corpus callosum voxels are given in table 2. There were highly significant reductions of NAA:Cr ratio in the normal appearing corpus callosum region for both patient groups relative to the controls ($p = 0.0003$ for the RR/SPMS and $p = 0.0002$ for the PPMS).

Relationship between pericallosal T1 lesion load, disability, and MR imaging modalities

Table 3 shows the non-parametric Spearman's correlation coefficients for the pericallosal T1 lesion load and MR imaging modalities for both MS patient groups. Figure 3 shows the distribution of pericallosal T1 lesion load and the averaged diffusion tensor eigenvalues perpendicular to the maximum diffusion ($(\lambda_2 + \lambda_3)/2$), and the NAA:Cr ratio in the NACC region for both patient groups. A significant correlation was observed between the EDSS and $(\lambda_2 + \lambda_3)/2$ for the patients with PPMS ($r = 0.60$, $p = 0.004$), but not for patients with RR/SPMS ($r = 0.28$, $p = 0.209$). Lastly, NAA:Cr ratio was moderately correlated ($r = -0.48$, $p = 0.002$) with the averaged diffusion tensor eigenvalues perpendicular to the maximum diffusion ($(\lambda_2 + \lambda_3)/2$) when the results of all the patients were combined together.

DISCUSSION

We investigated the role of directional diffusion tensor eigenvalues and ^1H MRSI for patients with MS in the normal appearing corpus callosum, a highly anisotropic region. Our first finding was a significant increase of ADC and reduction of functional anisotropy, mainly induced by an increase in averaged diffusion tensor eigenvalues perpendicular to the maximum diffusion direction, in both the RR/SPMS and the PPMS group relative to the healthy controls. The second finding was that both patients groups showed a significant decrease of the NAA:Cr ratio in the normal appearing corpus callosum relative to the controls. Thirdly, we found significant correlations between MR outcomes in the region of normal appearing corpus callosum and distant pericallosal T1 lesions for the patients with RR/SPMS, but not for the patients with PPMS.

Pierpaoli *et al* have shown increased transverse eigenvalues along corticospinal tracts injured by distant lacunar infarcts.¹⁹ Stanisz *et al* evaluated degeneration of rat sciatic nerves and showed increased diffusion transverse to the nerve fibres as a signature of demyelination and wallerian degeneration confirmed by histopathology.³⁶ A recent study from our laboratory showed that decreased anisotropy for patients with RRMS arose from changes only in regions with high anisotropy (corpus callosum, internal capsule, and corona radiata) due to significant increase of diffusion transverse to the fibres and no significant changes along the fibres.²⁰ Many diffusion studies have been done in patients with MS,^{14 15 37} but most of these reported only ADC or anisotropy and, as in the present study, found increased ADC and reduced anisotropy. We found that the origin of these differences can be better

Table 2 The mean diffusion tensor eigenvalues, ADC, functional anisotropy, and NAA:Cr ratio for patients with multiple sclerosis and controls subjects derived from the normal appearing corpus callosum regions of interest

	Controls	RR/SPMS	PPMS
λ_1 ($\times 10^{-3}$ mm ² /s)	1.55 (0.02)	1.60 (0.01) ($p = 0.05$)	1.55 (0.02) ($p = 0.87$)
$(\lambda_2 + \lambda_3)/2$ ($\times 10^{-3}$ mm ² /s)	0.42 (0.01)	0.47 (0.01) ($p = 0.001$)	0.48 (0.01) ($p = 0.0002$)
ADC ($\times 10^{-3}$ mm ² /s)	0.80 (0.01)	0.85 (0.01) ($p = 0.0002$)	0.84 (0.01) ($p = 0.002$)
FA	0.69 (0.01)	0.66 (0.01) ($p = 0.02$)	0.64 (0.01) ($p = 0.001$)
NAA:Cr	2.39 (0.07)	2.03 (0.06) ($p = 0.0003$)	1.99 (0.07) ($p = 0.0002$)

Data are the least square mean (SE) with age adjustment.
 λ_1 is the maximum diffusion tensor eigenvalue and $(\lambda_2 + \lambda_3)/2$ is the average of the diffusion tensor eigenvalues perpendicular to the maximum diffusion.
ADC, apparent diffusion coefficients; Cr, creatine/phosphocreatine; FA, functional anisotropy; NAA, N-acetyl-aspartate; normal appearing corpus callosum, normal appearing corpus callosum; for other abbreviations see table 1.

Figure 3 Distributions for the pericallosal T1 lesion load and (A) the averaged diffusion tensor eigenvalues perpendicular to the maximum diffusion $(\lambda_2 + \lambda_3)/2$ and (B) the NAA:Cr for patients with RR/SPMS (left) and PPMS (right).

understood by evaluating the diffusion tensor eigenvalues, and that changes in the diffusion in normal appearing tissue of the patients with MS are best studied by focusing on the transverse diffusion in highly ordered white matter tracts. To our knowledge, no previous studies have investigated diffusion tensor eigenvalues for patients with PPMS.

While directional diffusion provides evidence of structural tissue changes, the ^1H MRSI provides a putative marker of axonal dysfunction and/or loss. In support of the changes seen with transverse diffusion tensor eigenvalues, the present study also showed a significant reduction of NAA:Cr ratio in the normal appearing corpus callosum for all MS subgroups relative to the controls. These findings are consistent with recent neuropathological studies providing evidence of axonal damage in normal appearing white matter for MS patients,^{26–38–40} including specifically the study of the corpus callosum.²⁸ In MS, the aetiology of the normal appearing white matter tissue damage may be due to wallerian degeneration distal to transected axons in demyelinating MS plaques and/or diffuse microscopic lesions to which conventional MR images are not sensitive.⁴¹ As MS lesions may be acting as insult to bundles of distal axons that undergo wallerian degeneration, we believe that the corpus callosum is a sensitive region for detecting such tissue damage because bundles of axons are densely packed, highly aligned, and a significant number of axons travel through it from both hemispheres.

However, to support a relation between MS plaques and distant axonal injury, one would expect to find a strong

correlation between lesions visible on MR imaging and variables such as transverse diffusion eigenvalues and NAA:Cr. We found such a correlation between pericallosal T1 lesion load and both diffusion parameters and NAA:Cr ratio in the patients with RR/SPMS (see fig 3), but not in patients with PPMS. While the diffusion and ^1H MRSI data indicate degeneration in the patients with PPMS similar to that found in the patients with RR/SPMS, the absence of correlation with pericallosal T1 lesions in PPMS suggests an alternative aetiology. This could be explained by intrinsic differences related to global and diffuse axonal disease characteristics in PPMS although the presence of microscopic lesions in NAWM cannot be excluded. The results presented here further support the lack of a relation between visible lesions and overall brain tissue injury in PPMS as also suggested in our recent work showing that patients with PPMS categorised on the basis of T2 lesion volumes did not differ with regard to clinical characteristics. Yet these patients still showed reduction of both NAA:Cr ratio, derived from a central brain region, and whole brain atrophy in comparison with healthy controls.⁴²

The findings regarding the corpus callosum in this study are also pertinent to a disease such as PPMS, which often presents clinically as a progressive myelopathy, and especially since we did not take cervical MR scans and could not directly test the relation between cervical lesions and brain NAWM. One can argue that cervical MR imaging lesions could potentially cause brain NAWM injury by possible retrograde degeneration but such changes are not likely to occur in the corpus callosum itself as fibre tracts travelling in the spinal cord are quite distinct from those travelling in the corpus callosum.

The significant correlation between diffusion parameters in the normal appearing corpus callosum and EDSS for patients with PPMS but not for patients with RR/SPMS may highlight the difference between these disease subtypes. In particular, since the lesions might only determine the areas of degeneration for patients with RR/SPMS, the normal appearing corpus callosum degeneration would reflect disability in cases where the lesions are primarily pericallosal. On the other hand, the

Table 3 Non-parametric Spearman's correlation coefficients between pericallosal T1 lesion load and MR parameters for the patients with multiple sclerosis

	RR/SPMS	PPMS
$(\lambda_2 + \lambda_3)/2$ ($\times 10^{-3} \text{ mm}^2/\text{s}$)	0.78 ($p < 0.0001$)	0.32 ($p = 0.15$)
ADC ($\times 10^{-3} \text{ mm}^2/\text{s}$)	0.76 ($p < 0.0001$)	0.47 ($p = 0.03$)
FA	-0.60 ($p = 0.003$)	-0.17 ($p = 0.44$)
NAA:Cr	-0.52 ($p = 0.01$)	-0.05 ($p = 0.84$)

For abbreviations see tables 1 and 2.

degeneration measured in the normal appearing corpus callosum in patients with PPMS may reflect more diffuse and global axonopathy and therefore be consistently related to disability.

Persistent non-enhancing hypointense T1 lesions are generally thought to represent more advanced and destructive MS plaques⁴³ although the definition of T1 lesion load remains somewhat arbitrary. In this study no gadolinium contrast agents were used. Hypointense T1 lesions were based on high resolution T1-weighted 3D SPGR images, which may include both chronic and acute lesions. In the RR/SPMS patients we assume that chronic and acute T1 lesions may be related to distant axonal damage and/or dysfunction as reported in De Stefano *et al.*⁴⁴ In PPMS contrast enhancing lesions are very rare⁴⁵ and should only contribute marginally to the overall T1 lesion load.

CONCLUSION

This study demonstrates that it is possible to measure significant in vivo tissue damage in the normal appearing corpus callosum in patients with MS using non-invasive MR imaging multimodalities: diffusion tensor imaging and ¹H MRSI. A significant increase of the ADCs and decreased anisotropy were observed in the normal appearing corpus callosum induced by increased diffusion tensor eigenvalues perpendicular to the maximum diffusion directions. A significant decrease of the NAA:Cr ratio was observed in the same ROIs. Strong correlations were found between pericallosal T1 lesions and both the average of the diffusion tensor eigenvalues perpendicular to the maximum diffusion direction and the NAA:Cr ratio in the normal appearing corpus callosum for the patients with RR/SPMS, but not for the patients with PPMS. In RR/SPMS, tissue injury in normal appearing corpus callosum can be explained partly by the result of degeneration of axons transected from distant MS plaques, but in PPMS alternative aetiology should be considered.

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