Characteristics of lesional and extra-lesional cortical grey matter in relapsing-remitting and secondary progressive multiple sclerosis: A magnetisation transfer and diffusion tensor imaging study.

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ABSTRACT

Background: In multiple sclerosis(MS), diffusion tensor and magnetisation transfer(MT) imaging are both abnormal in lesional and extra-lesional cortical grey matter(GM), but differences between clinical subtypes and associations with clinical outcomes have only been partly assessed.

Objective: To compare mean diffusivity(MD), fractional anisotropy(FA), MT ratio(MTR) in cortical GM lesions(detected using phase-sensitive inversion recovery(PSIR) imaging) and extra-lesional cortical GM, and assess associations with disability in relapse-onset MS.

Methods: Seventy-two people with MS(46 relapsing-remitting(RR), 26 secondary-progressive(SP)) and 36 healthy controls were included in this study. MTR, MD and FA were measured in lesional and extra-lesional cortical GM.

Results: Mean FA was higher and MTR lower in lesional compared with extra-lesional cortical GM. In extra-lesional cortical GM mean FA and MTR were lower, and MD higher in the MS group compared with controls. Mean MTR was lower and MD higher in lesional and extra-lesional cortical GM in SPMS when compared with RRMS. These differences were independent of disease duration. In multivariate analyses, MTR in extra-lesional more so than lesional cortical GM was associated with disability.

Conclusion: MR abnormalities in lesional and extra-lesional cortical GM are greater in SPMS than RRMS. Changes in extra-lesional compared with lesional cortical GM are more consistently associated with disability.

INTRODUCTION

Cortical grey matter (GM) pathology can be substantial in multiple sclerosis (MS) and is recognised as a major factor contributing to neurological and cognitive disability. Cortical demyelinating lesions are seen after the first symptomatic episode in people who later develop MS, and extensive cortical lesions found in people with progressive MS subtypes.[1-5] Extra-lesional (sometimes described as 'normal appearing') cortical GM is also abnormal, with reductions in both neuronal and oligodendrocyte densities reported.[6]

Few *in vivo* studies have compared cortical lesion features between MS clinical subtypes. Using double inversion recovery (DIR) obtained at 1.5T Calabrese *et al.* found that cortical GM lesions were significantly more numerous and extensive in SPMS than RRMS.[7] Using a 3T MRI system, we confirmed this using DIR and phase-sensitive inversion recovery (PSIR) imaging.[8] Both magnetisation transfer (MT) imaging and diffusion tensor imaging (DTI) measures are abnormal in lesional compared with extra-lesional GM,[13,14,29] but there are only two in-vivo studies investigating differences in MTI and DTI characteristics of GM lesions between RRMS and SPMS.[14,29]

Abnormalities in extra-lesional GM have been found *in vivo* using MTI [10-12] and DTI.[13,14] However, extra-lesional GM defined on MRI is not the same as extra-lesional GM examined in histopathological studies. Of histopathologically confirmed intracortical lesions (lesions only involving cortical GM), 5% or less are detected using proton density (PD)/T2-

weighted scans obtained at 1.5T [30] and with DIR scans at 1.5T about 10% are identified.[30] As such, most cortical GM lesions will fall within MRI-defined extra-lesional GM, and both GM lesions and abnormalities within truly extra-lesional GM may contribute to the MT and DTI abnormalities previously found in MRI-defined extra-lesional GM.[10-14] In previous work aiming to improve GM lesion detection, we found about three times as many intracortical GM lesions on the PSIR compared with DIR scans, both obtained using the same 3T MRI machine in the same subjects. PSIR has not previously been used to define lesional and extra-lesional GM, but should yield a substantially larger sample of cortical GM lesions, and reduce contamination of MRI-defined extra-lesional GM by unseen lesions, when compared with studies using either PD/T2-weighted or DIR scans to detect GM lesions.

In this study we investigated lesional and extra-lesional cortical GM abnormalities in RRMS and SPMS, and their relationship with disability. To achieve this, we used a combination of MRI techniques: PSIR to detect cortical lesions,[15] high-resolution MTR (sensitive to GM demyelination),[16] and DTI to assess tissue structural integrity. To avoid contamination of GM lesion measures by WM, we confined our analysis to intracortical GM lesions. [17] We addressed two questions:

1. Is lesional and extra-lesional cortical GM more abnormal in SPMS compared with RRMS?

2. Is neurological and cognitive disability in MS more closely related to abnormalities in lesional or extra-lesional cortical GM?

METHODS

Participants were between 18 and 65 years old, and had a diagnosis of clinically definite MS according to McDonald criteria.[18] The control group had no known neurological disease. MS subtypes were classified using the Lublin-Reingold criteria.[19] All participants gave written informed consent. This study was approved by our local institutional ethics committee.

Participants

We included 72 people with MS and 36 healthy control subjects. Demographic details are given in Table 1.

Clinical assessments

Expanded Disability Status Scale (EDSS),[20] MS Functional Composite (MSFC),[21] and Symbol Digit Modalities Test (SDMT)[22] scores were determined. MSFC and SDMT scores were also obtained from controls.

Image acquisition

Using a 3T Philips Achieva system (Philips Healthcare, Best, The Netherlands) with a 32-channel head coil and multi-transmit technology, the following sequences were acquired: T1-weighted volumetric, PSIR, PD/T2-weighted, fluid attenuated inversion recovery (FLAIR), DTI, and MTR. Acquisition parameters are given in supplemental eTable 1.

MRI analysis

Lesion identification

Cortical lesions confined to GM (intracortical lesions) were outlined on PSIR images using JIM (Version 6.0, Xinapse Systems, Northants) and their volume calculated (Figure 1). As in previous work, PD/T2-weighted and FLAIR-scans were used for reference.[8] White matter (WM) lesions were identified using the PD/T2-weighted scans. Marking of all scans was carried out blinded to clinical data by OY and VS, under the guidance of TY, an expert neuroradiologist.

Generation of the cortex and white matter masks

Registrations were carried out using NitfyReg.[23,24] PSIR images with their corresponding lesion masks were affine registered to the T1-weighted volumetric images. After lesion filling [25], the T1-weighted volume images were segmented using SPM8 (Wellcome Trust Centre for Neuroimaging, London, UK) and voxels with a ≥95% probability of being GM were included in the GM masks used in subsequent analyses. The Montreal Neurological Institute (MNI)-152 T1-template was segmented using SPM8, and the supratentorial cortex was manually extracted from the GM map using FSL (Functional magnetic resonance imaging of the brain Software Library, Oxford, UK). The MNI-152 template was affine registered to each subject's T1-weighted volume image, and a cortical GM mask prepared by extracting only GM voxels that fell within both the thresholded SPM8 segmentation of the T1-weighted image and the MNI-derived cortical

mask. A mask of extra-lesional cortical GM was obtained subtracting the PSIR-derived cortical lesion mask from the cortical GM mask. A NAWM mask was prepared by thresholding the SPM8 WM probability maps at 95%, and subtracting WM lesions detected on the PD/T2-weighted scan. Using the SPM8 segmentations of the lesion-filled T1-weighted volume images, brain parenchymal fraction (BPF) was calculated by dividing the sum of GM and WM volume by the total intracranial volume.[38]

Diffusion tensor imaging processing

Diffusion-weighted images were registered to the non-diffusion-weighted (b0) images and eddy current corrected using FSL. For each subject a mean b0-image was created by averaging the seven b0-images acquired. Fractional anisotropy (FA) and mean diffusivity (MD) maps were generated using Camino.[26] For each subject, the DTI-scans were registered to the T1-weighted volumetric images via a pseudo (p)T1-weighted image generated from the PD/T2-weighted scan.[27] The mean b0-image was registered to the pT1-image using first affine and then non-linear transformations. The pT1-image was registered to the T1-weighted volumetric scan using a rigid-body registration. The b0-to-pT1 and pT1-to-T1 transformations were combined, bringing the b0-images (and the associated MD and FA maps) into alignment with the T1-weighted volumetric scans.

Magnetisation transfer imaging processing

MT(on) and MT(off) images for each subject were affine registered to their T1-weighted volume scan. MT ratio (R) was calculated for each voxel as S(0)-S(RF)/S(0), where S(0) and S(RF) are the signal intensities without and with the application of the off-resonance pulse.

Lesional and extra-lesional cortical grey matter quantitative MRI measures
With the MTR and DTI maps, and lesion and tissue masks aligned with the T1-weighted volumetric scans, FA, MD and MTR measures were extracted from each of the masked regions.

Quality assessment
The MT and diffusion images were reviewed for artefacts, for example associated with subject motion, by OY. Every registration step for every image was reviewed by OY for accuracy. The segmented T1-weighted images were also reviewed for quality by OY. The registration steps and images of every tenth patient were re-reviewed by DTC for artefacts, registration and segmentation quality.

Statistical analysis
Continuous data are presented as mean±standard deviation. EDSS scores are presented as median (range). There was evidence of non-normality in FA, MD and MTR in lesional GM, FA and MD in extra-lesional GM, and cortical lesion volume measures (all $p<0.05$, Shapiro-Wilk test). Associations between age, disease duration and quantitative MRI-

measures were investigated using Spearman correlation. The differences in cortical lesion count and volume between RRMS and SPMS were investigated using Mann-Whitney tests. General linear model was used to determine if MTR and DTI measures differed between subject groups, with age and disease duration as covariates. All group comparisons were confirmed by bootstrap analysis (case resampling, n=1000). The Wilcoxon Signed-rank test was used to compare MTR and DTI measures in lesional and extra-lesional GM in the subgroups.

Associations between clinical (EDSS, MSFC and SDMT scores) and MRI measures (lesional and extra-lesional GM MTR and DTI, PD/T2-weighted lesion volumes and BPF) were investigated using Spearman correlation, and further explored using a stepwise linear regression model including all significant MRI variables from the univariate analysis as covariates confirmed by bootstrap analysis (case resampling, n=1000). Adjusted R-square values are given for each significant covariate. MSFC scores were transformed into z-scores with reference to the control group. Given the problems associated with formally correcting for multiple comparisons[28] we present results flagged using the conventional (p<0.05) significance threshold. We used SPSS (Version 21, Chicago, IL, USA) for the statistical analysis.

RESULTS

Age and gender effects

We did not find any association between sex and MTR and DTI measures in cortical GM in controls, or lesional or extra-lesional GM in people with MS. Age correlated with lesional GM MTR and BPF in MS, and with BPF in controls (all p<0.05, Spearman correlations).

Cortical grey matter lesions

Compared with people with RRMS those with SPMS had higher median intracortical lesion counts and volumes (Table 2).

MRI abnormalities in extra-lesional cortical grey matter

In extra-lesional cortical GM, mean MTR and FA were lower in MS compared with the controls, while mean MD was higher in the MS group than controls (all p<0.001, general linear model). Mean MTR was lower in the SPMS than RRMS groups (p<0.05), and remained so after adjusting for age, disease duration and extra-lesional cortical GM mask volume (p<0.05). There was a trend to higher MD in SPMS than RRMS, but FA did not differ significantly (Table 2). Disease duration correlated with FA, MD and MTR in extra-lesional cortical GM (all p<0.05, Spearman correlation) but this was not significant in a multivariate linear regression model when MS subtype was also included. MTR results are illustrated in Figure 2. For completeness, mean MTR, FA and MD values in the whole cortex, leucocortical lesions (those including both GM and WM), PD/T2 WM lesions and NAWM are given in supplemental eTable 2.

MRI abnormalities in cortical grey matter lesions

In the combined MS group, FA was higher and MD lower in cortical lesions when compared with extra-lesional cortical GM (both p<0.001, Wilcoxon Signed-rank test). The greatest difference in FA and MD was seen in people with SPMS. In SPMS, cortical lesion MTR was lower than in extra-lesional cortical GM (p=0.03, Wilcoxon Signed-rank test). In people with RRMS, lesional and extra-lesional cortical GM MTR did not differ significantly (Figure 2).

In SPMS compared with RRMS mean MTR was lower and mean MD higher in cortical GM lesions (both p<0.01, Table 2), and this remained significant after adjusting for age, disease duration, extra-lesional cortical GM mask volume and cortical GM lesion volume (all p<0.05). In contrast, mean FA in cortical GM lesions did not differ significantly between RRMS and SPMS. Disease duration correlated with MD and MTR in cortical GM lesions (both p<0.05, Spearman correlation) but this association was not significant in a multivariable linear regression model when disease subtype was also included in the model.

Associations of MRI measures with disability scores

In the combined MS-group the following associations were observed (all p<0.05, supplemental eTable 3):

(1) EDSS scores correlated with MD and MTR in lesional and extra-lesional cortical GM, and with BPF

(2) MSFC scores correlated with extra-lesional GM MD and MTR, lesional GM MTR, PD/T2-lesion volume and BPF

(3) SDMT scores correlated with FA, MD and MTR in extra-lesional cortical GM, MTR in GM lesions, PD/T2-lesion volume and BPF.

In a multivariable linear regression model (including all the variables found to significantly correlate with EDSS, MSFC or SDMT), *extra-lesional cortical GM MTR* was most consistently associated with clinical outcome measures (Table 3). The same was found for mean MTR in the whole cortex (supplemental eTable 4).

DISCUSSION

In this study we found that MTR and MD were both more abnormal in cortical GM lesions in SPMS than RRMS, and MTR was also more abnormal in extra-lesional cortical GM in SPMS compared with RRMS. These differences were independent of disease duration, suggesting that SPMS *per se* is characterised by more marked cortical GM pathology. Of the MRI measures included in this work, MTR in extra-lesional cortical GM emerged as being most consistently correlated with clinical outcome measures.

To the best of our knowledge, there has only been one previous *in vivo* study on MTR in cortical GM lesions in a mixed group of people with RRMS and SPMS, using DIR scans obtained with a 1.5T MRI system to identify lesions.[29] This demonstrated a relative reduction in MTR in lesional compared with extra-lesional GM. Our study, using PSIR obtained at 3T to identify and classify cortical lesions (and so, when compared with DIR, less likely to inadvertently include WM lesions[9]), confirms that MTR is reduced in GM lesions when compared with extra-lesional GM, and builds on this by demonstrating that a reduction in MTR is more apparent in SPMS than RRMS (Figure 2). However, it should be recalled that while the majority of cortical lesions are subpial[1], these are very rarely detected using DIR[30] or PSIR[8], and so it cannot be assumed that the results of the present study are representative of subpial GM lesions.

Previous *in vivo* work using DIR at 1.5T to identify cortical lesions has shown lower MD and higher FA values relative to extra-lesional cortical GM.[14] A relatively higher FA is contrary to changes seen in WM

lesions (in which FA is decreased[14]). In recent work we found that nearly a third of intracortical lesions (lesions confined to GM alone) identified on DIR appeared to involve WM when viewed on a PSIR scan[9]. This raises the possibility that previous GM lesion findings may in part have been due to WM contamination (which has a higher FA than GM[14]). However, the present results, obtained using PSIR, confirm previous observations. This suggests that partial volume alone does not explain the high FA values in cortical GM lesions. The reason for higher FA and lower MD in cortical lesions relative to extra-lesional GM is not known. One possible explanation comes from a study on the preterm human cortex which found that FA values are high at 26 weeks of gestational age, when the pyramidal cells have prominent apical dendrites radially oriented perpendicular to the pial surface, and in the subsequent weeks FA declines, which coincides with the development of basal dendrites.[32] In cortical GM lesions the dendritic arbor is damaged[6], which may result in a reversal of FA changes associated with normal cortical development, i.e. an increase in FA.

We found that cortical lesion MTR was significantly lower in SPMS than RRMS. While disease duration correlated with cortical lesion MTR, this association was lost once the MS phenotype was included in the statistical model, suggesting that the greater reduction in MTR seen in SPMS is not simply the result of a having had MS longer, but that SPMS is itself associated with greater abnormalities. This is consistent with previous work on WM lesions, which are known to be more intensely

abnormal in SPMS compared with RRMS: Filippi *et al.* demonstrated that the ratio of T1-hypointense to PD/T2-weighted lesions varied markedly between MS subtypes, being higher in SPMS (0.24) than RRMS (0.12).[33]

Our extra-lesional cortical GM findings also agree with previous work investigating MTR and diffusion MRI features.[13] As with cortical GM lesions, we found that MTR in extra-lesional GM was reduced in the MS group when compared with controls, and this reduction was greater in SPMS compared with RRMS. In extra-lesional GM we found a lower FA and higher MD in the MS group compared with controls, which is consistent with most,[14,34–36] but not all previous studies on cortical GM diffusion.[13] While there was a trend to a higher MD in extra-lesional GM in SPMS compared with RRMS, this did not reach statistical significance. Recalling that even using PSIR it is likely that only a minority of cortical lesions will be seen, these results may partly reflect the effects of unseen lesions. However, the discrepant FA abnormalities in cortical lesions (increased) and extra-lesional cortical GM (decreased) suggest that not all changes in extra-lesional GM can be explained by undetected GM lesions. This is also supported by histopathological studies which have shown reductions in oligodendrocyte and neuronal densities in extra-lesional GM, both of which may lead to alterations in MTR and diffusion MRI measures.[6,37] Pathological interpretation of MTR and DTI abnormalities in cortical GM is difficult as there have only been two combined MRI and histopathological studies of cortical MTR in MS, demonstrating associations between

reduced MTR and cortical demyelination, and no studies assessing the pathological substrates of diffusion abnormalities.[16,17]

Physical and cognitive disability, as measured using EDSS, SDMT and MSFC, were associated both with abnormalities in lesional and extra-lesional cortical GM albeit with relatively modest strengths. However, in the regression models only changes in extra-lesional GM consistently associated with EDSS, SDMT and MSFC. This is perhaps not surprising considering that the majority of GM is extra-lesional, and so likely to contribute more of the overall pathological burden of MS than GM lesions. In addition, a substantial proportion of GM lesions (and virtually all subpial lesions) will go undetected using DIR or PSIR, and so be included in MRI-based extra-lesional GM measures.

In addition to those noted above, there are a few other study limitations worth mentioning. The cortex is only 3-5 mm thick, and convoluted, and so partial volume may affect the results. Given that the MTR data was acquired at $1x1x1mm^3$ and the diffusion data at $2x2x2mm^3$, partial volume effects are more likely to influence DTI rather than the MTR results, although we used a conservative segmentation method to limit this, and included the cortical GM volume as a factor in the statistical model as an extra precaution. We assessed intracortical rather than leucocortical lesions to avoid contamination from WM, however a combined analysis of the intracortical lesions and the GM part of leucocortical lesions yielded similar results (see supplemental Table e2).

In this multimodal MRI study, it has been necessary to register each

subject's MTR, DTI, PSIR and PD/T2-weighted scans to their T1-weighted volumetric scans. These registrations may still leave images very slightly out of alignment (each set of scans were checked for visible misalignment) and re-sampling the images will also add a small amount of noise. This noise will be random and, as the same methods were used in all subjects, should not lead to spurious differences between the groups or associations with clinical outcome measures being introduced. However, additional random noise may have reduced sensitivity to group differences and the apparent strength of correlations with clinical outcomes. When comparing the results of the present work with previous studies, it should be recalled that the MRI measures in this study produce method specific rather than method independent values. As such we cannot directly compare the absolute values of the MRI measures from this study with those produced using a different scanner or scan protocol, although relative differences between tissue types should be consistent. It should also be noted that the SPMS group was smaller than the RRMS group. This will have resulted in there being less statistical power when analysing the SPMS group data, and so relative to results from the RRMS group, we may have underestimated the significance of differences between the SPMS group and either the control or RRMS groups, and associations of MRI measures with clinical outcomes. Lastly, while we have found statistically significant differences in MTR and diffusion measures between MS groups, there is considerable overlap between them; on a person-by-person basis, reliable separation into RRMS and SPMS groups could not be achieved

using these methods.

In conclusion, we have found that MTR and DTI measures in lesional and extra-lesional cortical GM differ between RRMS and SPMS. This suggests that not only are cortical lesions more extensive in people with SPMS compared with RRMS, but also that the intensity of pathological changes in lesional and extra-lesional GM may be greater. The intensity of pathology (as measured using MTR) was not accounted for by disease duration alone, suggesting that a SPMS compared with RRMS course is associated with greater GM abnormalities. Disability scores correlated more consistently with extra-lesional (and whole cortical) GM measures than cortical lesion measures, and so in clinical studies of RRMS and SPMS assessing MRI features of the whole cortex rather lesions may be more useful.

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Acknowledgments

Conflicts of interest

Participant's consent

Informed consent form approved by local ethics committee (London Queen Square R.E.C) was used for this project and signed by every participant.

Ethics approval

London Queen Square REC.

Authors contributions:

Dr Özgür Yaldizli had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Dr Özgür Yaldizli, Dr Declan T Chard, Dr Matteo Pardini, Dr Varun Sethi, Dr Zheng Liu, Dr Claudia Wheeler-Kingshott, Prof David H Miller.

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Table 1: Subject demographics and clinical parameters.

	Controls	Patients		
		RRMS	SPMS	Sig. (RRMS vs. SPMS)
N	36	46	26	
Age (years)	41.5±12.85	41.7±9.9	51.3±7.2	<0.001
Females (%)	19(52.8)	30(65.2)	18(69.2)	ns
Disease duration (years)		11±7.0	19.9±9.2	<0.001
Median EDSS (range)		2(0-7)	6.5(4-8.5)	<0.001
MSFC	0.7±0.27	-0.27±1.26	-2.3 ±2.5	<0.001
- PASAT	0.61±0.63	-0.13±1.0	-0.44±1.0	ns
- TWT	0.51±0.10	-0.58±3.0	-5.7±6.5	<0.001
- 9HPT	0.98±0.5	-0.09±0.6	-0.8±1.05	0.001
SDMT	61.8±9.5	49.7±11.6	42.5±10	0.01
Currently taking DMT(%)		28(60.9)	7(26.9)	<0.001

Legend: Continuous variables are presented as mean ± standard deviation. Components of the MS Functional Composite (MSFC, expressed as z-scores) were determined, calculated using own controls as reference population.
Abbreviations: 9HPT: nine hole peg test; DMT: disease modifying treatment; EDSS: Expanded Disability Status Scale; PASAT: Paced Auditory Serial Addition Test; RRMS: relapsing–remitting multiple sclerosis; SPMS: secondary progressive multiple sclerosis; TWT: Timed 25-Foot walk test, SDMT Symbol Digit Modalities Test.

Table 2: Cortical magnetic resonance imaging measures in relapsing-remitting and secondary-progressive multiple sclerosis compared with healthy control subjects

MRI parameters	Tissue	Controls	Patients		
			RRMS	SPMS	Sig. (RRMS vs. SPMS)
FA	Extra-lesional cortical GM	0.14 (0.01)	0.14 (0.02)	0.13 (0.01)	ns
	Intracortical lesions	-	0.20 (0.03)	0.21 (0.04)	ns
MD	Extra-lesional cortical GM	0.85 (0.03)	0.92 (0.07)	0.95 (0.07)	ns
	Intracortical lesions	-	0.84 (0.08)	0.90 (0.10)	<0.01
MTR	Extra-lesional cortical GM	32.6 (0.8)	31.4 (1.4)	30.5 (1.2)	<0.05
	Intracortical lesions	-	31.1 (2.15)	29.6 (1.79)	<0.05
Counts, median (range)	Intracortical lesions	0	24.5 (1-108)	32.0 (4-105)	<0.05
Volume	Intracortical lesions	0	0.47 (0.43)	0.70 (0.56)	<0.05
BPF		0.82 (0.02)	0.80 (0.02)	0.79 (0.02)	ns

Legend: Variables are given in mean (standard deviation) unless indicated differently. Abbreviations: RR relapsing-remitting, SP secondary progressive, MS multiple sclerosis, FA fractional anisotropy, MD mean diffusivity, MTR magnetisation transfer ratio, GM grey matter, BPF brain parenchymal fraction

Average MD is expressed in units of $mm^2 \, s^{-1} x10^{-3}$, FA is a dimensionless quantity, MTR a percentage, lesion volume in mL and lesion counts in median and range

Table 3: Stepwise linear regression analyses with EDSS, SDMT, and MSFC scores as outcome parameters and all significant variables from the univariate analysis as covariates.

Clinical outcome parameter	Significant covariates	Standardised Beta coefficient	Sig.	Adjusted R-square
SDMT	MTR in extra-lesional cortical GM	0.424	<0.001	0.23
	PD/T2 WM lesion volume	-0.269	<0.05	0.13
MSFC	MTR in extra-lesional cortical GM	0.407	<0.001	0.15
EDSS	MTR in extra-lesional cortical GM	-0.280	<0.05	0.16
	MTR intracortical lesions	-0.344	<0.05	0.20

Legend: Covariates included FA, MD, and MTR in extra-lesional cortical GM, MTR in intracortical lesions, PD/T2 WM lesion volume and brain parenchymal fraction.
Results including their individual adjusted R square are demonstrated for significant variables only.
Abbreviations: MSFC Multiple sclerosis functional composite, EDSS Expanded Disability Status Scale, SDMT Symbol Digit Modalities Test, FA fractional anisotropy, MD mean diffusivity, MTR magnetisation transfer ratio, PD proton density, GM grey matter, WM white matter, Sig. significance level
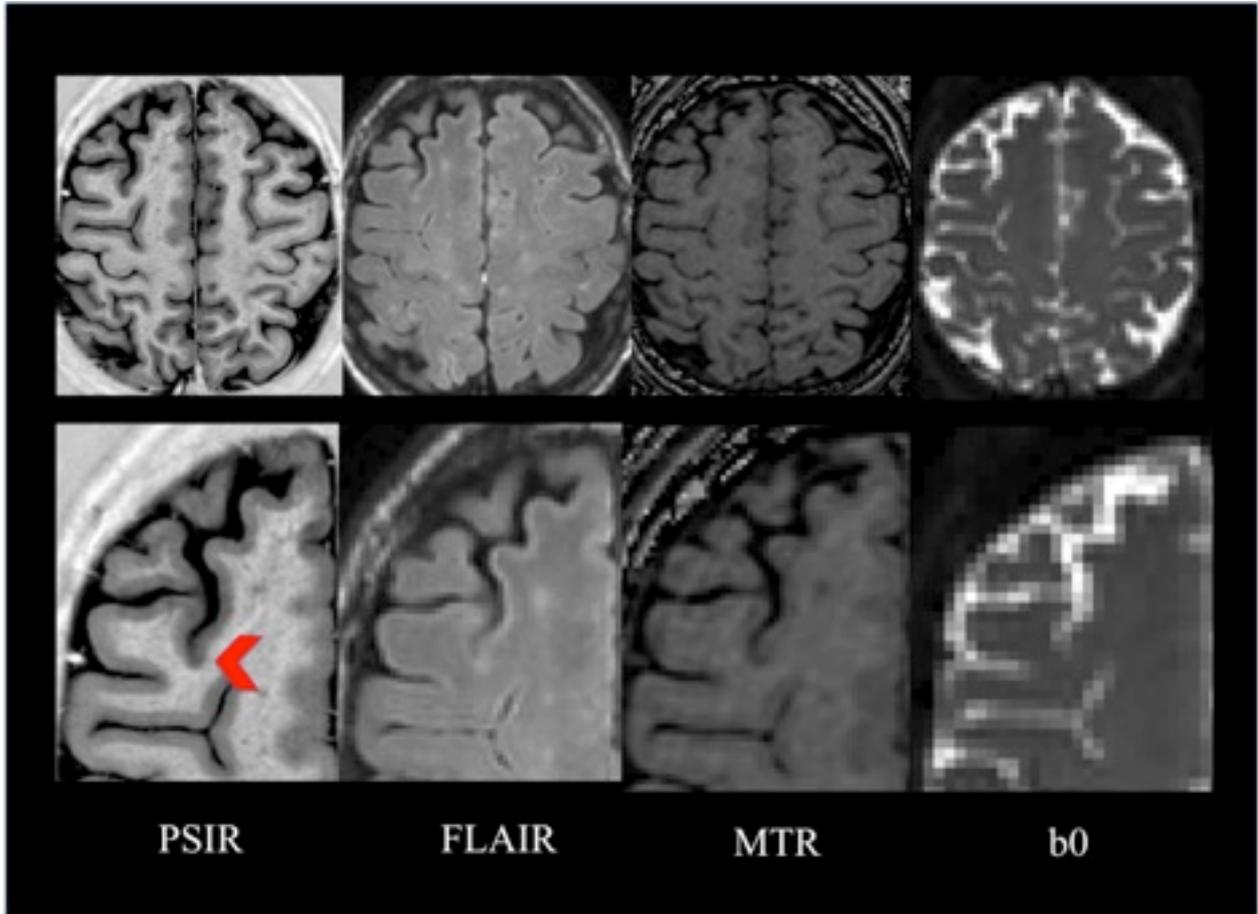
PSIR   FLAIR   MTR   b0

Figure 1: Example of an intracortical lesion (chevron) on PSIR and corresponding FLAIR, MTR and DTI maps.

Abbreviations: PSIR phase sensitive inversion recovery, FLAIR fluid attenuated inversion recovery, MTR magnetisation transfer ratio, b0 diffusion tensor imaging
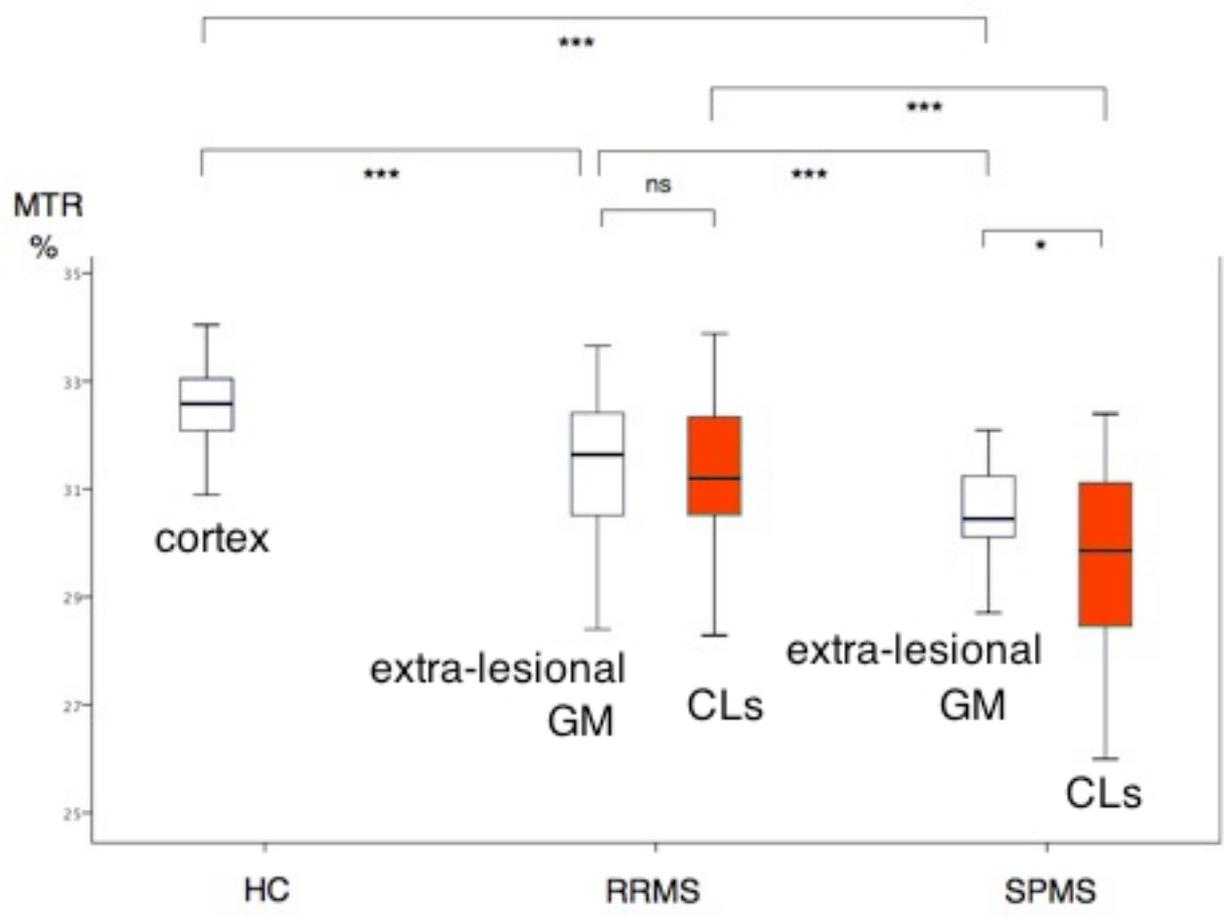
Figure 2: Magnetisation transfer ratio (MTR) in intracortical lesions (ICLs) and extra-lesional cortical grey matter in relapsing-remitting (RR) and secondary-progressive (SP) MS compared with healthy control (HC) subjects.

Significances *p<0.05, ***p<0.001.

Supplement eTable 1:

MRI acquisition parameter, all images have been aligned to the anterior posterior commissure line

|	Dimension	Acquisition Plane	Resolution (mm)	FOV (mm)	Matrix	TR (ms)	TE (ms)	TI (ms)	Slices	SENSE	Time (mins:sec)
T1	3D	Sagittal	1x1x1	256x256	256x256	6.9	3.1	824	180	2	6:32
PSIR	2D	Axial-oblique	0.5x0.5x2	240x180	480x360	7301	13	400	75	-	11:26
PD/T2	2D	Axial-oblique	1x1x3	240x180	240x180	3500	19/85	-	50	1.7	4:01
FLAIR	2D	Axial-oblique	1x1x3	240x180	240x180	8000	125	2400	50	1.3	3:44
DTI*	2D	Axial-oblique	2x2x2	192x224	96x112	~24000	68	-	72	3.1	~34:00
MTR**	3D	Sagittal	1x1x1	256x256	256x256	6.4	2.7/4.3	-	180	-	26:00

NOTE

* High angular resolution diffusion imaging consisting of a cardiac-gated spin-echo echo-planar imaging, 60 isotropically distributed diffusion-weighted directions with b=1200 s/mm^2, 7 non diffusion-weighted [b=0] volumes, TR depending on cardiac rate.

** 3D slab-selective fast field echo sequence with two echoes was used. A turbo field echo readout was used, with an echo train length of four and turbo field echo shot interval of 32.5 ms giving a total time between successive MT pulses of 51.9 ms, and scan time of approximately 26 minutes. The two echoes were averaged (thereby increasing the signal to noise ratio) for both the MT(on) and MT(off) data used to calculate the MTR. Sinc-Gaussian shaped MT pulses with flip angle of 360° and 16 ms duration but in total, the time between MT pulses was 51.9 ms (including the MT pulse duration and gradients).

Legend: D dimensional FFE fast field echo, TR repetition time, TE echo time, TI inversion time, SENSE sensitivity encoding factor, FOV Field of view FLAIR fluid attenuated inversion recovery, DTI diffusion tensor imaging, MTR magnetisation transfer ratio

Supplement eTable 2: Additional quantitative MRI measures in whole cortex, cortical lesions, leucocortical lesions, PD/T2 white matter lesions and normal-appearing white matter.

MRI measure	Tissue	HCs	MS	Sig. (HCs vs. MS)	RRMS	SPMS	Sig. (RRMS vs. SPMS)
FA	Whole cortex	0.16 (0.01)	0.15 (0.01)	**<0.001**	0.15 (0.01)	0.15 (0.01)	0.16
	Cortical lesions (purely intracortical plus GM part of leucocortical lesions)	-	0.19 (0.04)	-	0.19 (0.04)	0.19 (0.04)	0.87
	Leucocortical lesions	-	0.22 (0.05)	-	0.22 (0.05)	0.22 (0.03)	0.54
	PD/T2 WM lesions	0.31 (0.04)	0.30 (0.04)	0.36	0.30 (0.04)	0.29 (0.03)	0.50
	NAWM	0.38 (0.01)	0.36 (0.03)	**<0.001**	0.36 (0.03)	0.35 (0.02)	0.06
MD	Whole cortex	0.85 (0.03)	0.95 (0.06)	**<0.001**	0.94 (0.07)	0.96 (0.06)	0.12
	Cortical lesions (purely intracortical plus GM part of leucocortical lesions)	-	0.89 (0.09)	-	0.87 (0.08)	0.92 (0.10)	**0.035**
	Leucocortical lesions	-	0.91 (0.11)	-	0.89 (0.10)	0.94 (0.12)	0.064
	PD/T2 WM lesions	0.85 (0.10)	1.03 (0.11)	**0.03**	1.01 (0.12)	1.07 (0.10)	**0.03**
	NAWM	0.72 (0.03)	0.75 (0.05)	**<0.001**	0.75 (0.05)	0.75 (0.06)	0.43
MTR	Whole cortex	31.0 (0.9)	29.9 (1.5)	**<0.001**	29.8 (1.4)	28.7 (1.1)	**0.001**
	Cortical lesions (purely intracortical plus GM part of leucocortical lesions)	-	30.3 (1.7)	-	30.6 (1.7)	29.8 (1.6)	**0.04**
	Leucocortical lesions	-	28.7 (3.2)	-	29.8 (1.4)	28.7 (1.1)	0.054
	PD/T2 WM lesions	37.3 (2.5)	28.9 (4.3)	**<0.001**	29.0 (3.5)	26.3 (3.3)	**0.02**
	NAWM	39.3 (1.1)	38.4 (1.3)	**<0.001**	38.4 (1.2)	37.5 (1.4)	**0.006**

Legend: Abbreviations NAWM normal-appearing white matter, FA fractional anisotropy, MD mean diffusivity, MTR magnetisation transfer ratio, RRMS relapsing-remitting multiple sclerosis, SPMS secondary-progressive multiple sclerosis.

Supplement eTable 3: Correlation between whole cortex and leucocortical lesion measures and clinical outcome parameter.

	EDSS	MSFC	SDMT
Whole cortex			
- FA	ns	0.21*	0.27*
- MD	ns	-0.34**	-0,43**
- MTR	-0.40**	0.51*	0.55**
Leucocortical lesions			
- volume	ns	ns	-0.34*
- FA	ns	-0.22*	ns
- MD	ns	ns	ns
- MTR	-0.27*	-0.31*	ns

Legend: Values represent Spearman rho coefficients, significances are given as * (p<0.05), ** (p<0.01), *** (p<0.001).

Abbreviations: EDSS Expanded Disability Status Scale score, MSFC Multiple sclerosis function composite score, SDMT Symbol Digit Modalities Test score, FA fractional anisotropy MD mean diffusivity MTR magnetization transfer ratio

Supplement eTable 4: Multivariate linear regression model with measures of the whole cortex as predictors of clinical outcome measures

Clinical outcome parameter	Significant covariates	Standardised Beta coefficient	P-value	Adjusted R-square
SDMT	**MTR whole cortex**	0.546	<0.001	0.292
	PD/T2 WM lesion volume	-0.463	<0.01	0.208
MSFC	**MTR whole cortex**	0,419	<0.001	0.166
EDSS	**MTR whole cortex**	-0.404	<0.01	0.154
	MTR intracortical lesions	-0.349	<0.05	0.112

Legend: Covariates included FA, MD, and MTR in whole cortex, MTR in intracortical lesions, PD/T2 WM lesion volume and BPF.

Results including their individual adjusted R square are demonstrated for significant variables only.

Abbreviations: MSFC Multiple sclerosis functional composite, EDSS Expanded Disability Status Scale, SDMT Symbol Digit Modalities Test, FA fractional anisotropy, MD mean diffusivity, MTR magnetization transfer ratio, PD proton density, WM white matter, BPF brain parenchymal fraction