White and gray matter damage in primary progressive MS

The chicken or the egg?

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ABSTRACT

Objective: The temporal relationship between white matter (WM) and gray matter (GM) damage in vivo in early primary progressive multiple sclerosis (PPMS) was investigated testing 2 hypotheses: (1) WM tract abnormalities predict subsequent changes in the connected cortex ("primary WM damage model"); and (2) cortical abnormalities predict later changes in connected WM tracts ("primary GM damage model").

Methods: Forty-seven patients with early PPMS and 18 healthy controls had conventional and magnetization transfer imaging at baseline; a subgroup of 35 patients repeated the protocol after 2 years. Masks of the corticospinal tracts, genu of the corpus callosum and optic radiations, and of connected cortical regions, were used for extracting the mean magnetization transfer ratio (MTR). Multiple regressions within each of 5 tract-cortex pairs were performed, adjusting for the dependent variable's baseline MTR; tract lesion load and MTR, spinal cord area, age, and sex were examined for potential confounding.

Results: The baseline MTR of most regions was lower in patients than in healthy controls. The tract-cortex pair relationships in the primary WM damage model were significant for the bilateral motor pair and right visual pair, while those in the primary GM damage model were only significant for the right motor pair. Lower lesion MTR at baseline was associated with lower MTR in the same tract normal-appearing WM at 2 years in 3 tracts.

Conclusion: These results are consistent with the hypothesis that in early PPMS, cortical damage is for the most part a sequela of normal-appearing WM pathology, which, in turn, is predicted by abnormalities within WM lesions. *Neurology*® 2016;86:170-176

GLOSSARY

CST = corticospinal tract; **EDSS** = Expanded Disability Status Scale; **GM** = gray matter; **MS** = multiple sclerosis; **MTR** = magnetization transfer ratio; **NAWM** = normal-appearing white matter; **OR** = optic radiation; **PD** = proton density; **PPMS** = primary progressive multiple sclerosis; **WM** = white matter.

Little is known about the pathologic relationship linking white matter (WM) and gray matter (GM) damage in multiple sclerosis (MS). Postmortem studies provide a snapshot of pathology in WM and GM and have demonstrated demyelination and neuroaxonal damage in both tissues.¹⁻⁶ However, a key question is to what extent pathologic abnormalities in the GM are related to contiguous WM damage, either as a cause or consequence, or are the result of independent disease mechanisms. Longitudinal MRI studies represent a valuable approach to explore the dynamic associations between WM and GM pathology. Patients with early primary progressive multiple sclerosis (PPMS) should be an informative group in which to explore this question, as brain MRI lesion load is smaller compared with relapse-onset MS.

We have previously investigated in early PPMS the spatial relationship between pathology in WM tracts and connected GM areas using cross-sectional MRI data.⁷ We found that in some brain regions, pathology in WM tracts is correlated with that in the adjacent GM regions.⁷

Supplemental data
at *Neurology.org*

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In this study, we sought to determine whether (1) pathology in WM tracts is associated with (i.e., “predicts”) subsequent changes in connected cortical GM (“primary WM damage model”), or (2) pathology in cortical GM is associated with (i.e., “predicts”) subsequent changes in the connected normal-appearing WM (NAWM) tracts (“primary GM damage model”) in early PPMS, recognizing that both may occur simultaneously perhaps with one dominating. We also investigated the relationship between tract-specific lesions at baseline and NAWM/GM abnormality changes over time. Microstructural changes were assessed using magnetization transfer ratio (MTR), as lower MTR has been shown to reflect demyelination and neuroaxonal loss in MS.^{8,9}

METHODS Subjects and study design. MRI and clinical data, including the Expanded Disability Status Scale (EDSS) scores¹⁰ from 47 people with definite or probable PPMS,¹¹ no other known neurologic condition, and a history of clinical progression of less than 5 years, were analyzed in this study (table 1). All patients had MRI at baseline, while a subgroup of 35 patients repeated the imaging protocol 24 months later (mean time interval 24.5 months, SD 1.5). The MRI scans of 4 of these 35 patients were unusable because of movement artifacts. A group of 18 healthy subjects (included if in good general health, no known history of medical conditions known to affect the brain, and without contraindications to MRI scanning) underwent the same imaging protocol (table 1).

Standard protocol approvals, registrations, and patient consents. This work was approved by the Joint Medical Ethics Committee of the National Hospital for Neurology and Neurosurgery and the UCL Institute of Neurology, London, and written informed consent was obtained from all participants.

Image acquisition. Imaging was performed using a 1.5T GE Signa scanner (General Electric, Milwaukee, WI). At baseline and 24 months, all participants had a 3-dimensional inversion-prepared fast spoiled gradient recall T1-weighted sequence of the brain, and a fast spin echo scan (i.e., proton density

[PD]-weighted and T2-weighted scans), and a magnetization transfer dual echo interleaved spin echo sequence (details on MRI sequences are given in the supplementary material on the *Neurology*[®] Web site at Neurology.org). In the PPMS groups, a fast spoiled gradient echo of the spinal cord was performed; a series of 5 contiguous 3-mm axial slices (perpendicular to the spinal cord) were reformatted using the center of the C2-3 disk as the caudal landmark.

To generate a set of tracts and associated cortical GM regions, spin echo diffusion-weighted echo planar imaging scans were obtained from a separate group of 23 healthy controls (12 women; mean age 35.1 years, SD 7.9).

Image analysis. Using the method described by Tozer et al.,¹² templates for the following WM tracts and their associated cortical GM areas were derived (figure 1A):

1. The left and right motor pair (composed of corticospinal tract [CST] and connected GM in the pre- and postcentral cortex)
2. The callosal pair (consisting of the genu of the corpus callosum and its connected GM region in the frontal lobe)
3. The left and right visual pair (composed of the optic radiation [OR] and its connected GM area in the visual cortex)

Using the baseline and 24-month MRI data from the PPMS and control groups, native space MTR maps were calculated.¹³ Tract-cortex pair templates were transformed into native space¹² (figure 1B) and visually checked for registration errors, allowing WM tract and associated cortical GM MTR to be determined. In the PPMS group, WM lesions were delineated on the PD images, and lesion masks were binarized. The PD/T2-weighted scans were coregistered to the MTR maps and associated lesion masks moved into native MTR space. The WM lesion masks were subtracted from the WM component of each tract-cortex pair at each time-point, thus leaving NAWM. In the PPMS group, the total volume of each WM tract (including lesions and NAWM) and associated cortical GM regions were calculated computing the number of voxels for each region, and tract-cortex-specific NAWM and GM mean MTR values were determined. Except for lesion volumes, the same measures were derived from the healthy control data.

Since spinal cord damage is thought to have an important role in the pathogenesis of PPMS,¹⁴ in the patient group, the cord cross-sectional area at the C2-3 level was calculated as previously described.¹⁵

Statistics. Changes in EDSS score between baseline and 2 years were assessed using the sign test.

Differences in mean MTR and volume between patients and controls at baseline and 24 months in WM tracts and cortical GM regions were assessed using multiple regressions, with age and sex as covariates. Where regression residuals showed deviations from normality and homoscedasticity (all relatively minor), a non-parametric bias-corrected and accelerated bootstrap¹⁶ was performed (1,000 replicates). Where a potentially influential data point was identified, bootstrapped regression was repeated omitting it. In the PPMS group, changes in mean MTR of the WM lesions, the NAWM, and the connected GM area from baseline to 24 months, were tested for using 1-sample *t* tests. Univariable (pair-wise) associations between MTR values in each tract-cortex pair were assessed with Pearson correlation, and the effect of omitting potentially influential data points was explored. To assess cross-sectional associations between WM and GM pathology in the PPMS group, multiple regression was used between each tract-cortex pair's NAWM mean MTR and the corresponding GM region mean MTR; age, sex, disease duration, NAWM and GM

Table 1 Demographic, clinical, and radiologic characteristics of patients and healthy controls at study entry

Characteristic	Patients	Healthy controls
No.	47	18
Age, y, mean (SD)	43.9 (11.2)	35.2 (6.02)
Sex, female/male	18/29	11/7
Disease duration, y, mean (SD)	3.4 (0.9)	—
EDSS score, median (range)	4.5 (1.5–7)	—
T2 lesion load, mL, mean (SD)	15.4 (17.3)	—
Spinal cord area, mm ² , mean (SD)	70.3 (9.4)	—

Abbreviation: EDSS = Expanded Disability Status Scale.

Figure 1 Reconstruction of the 5 tract-cortex pairs

(A) The left and right motor pair (composed of CST and connected GM in the pre- and post-central cortex, voxels in blue), the callosal pair (voxels in yellow, consisting of the genu of the corpus callosum and its connected GM region in the frontal lobe), and the left and right visual pair (voxels in red, composed of optic radiation and its connected GM area in the visual cortex), reconstructed from the diffusion tensor imaging control group in standard space. (B) Left and right CST (voxels in red) and associated voxels in the precentral/postcentral cortex (voxels in blue) in a single patient, overlaid onto the patient's T1-weighted image in native space. CST = corticospinal tract; GM = gray matter.

volumes, tract-specific lesion MTR and volume, and spinal cord area were separately included (because of the relatively small number of patients) as potentially confounding covariates.

To assess the temporal relationship between tract-specific NAWM and GM values, we tested 2 models in each tract-cortex pair: (1) the primary WM damage model, to examine whether early NAWM MTR predicts late GM MTR; and (2) the primary GM damage model, to examine whether early GM MTR predicts late NAWM MTR. To enable joint testing (to reduce the number of tests), and to permit direct testing of the primary WM vs GM models, these models were implemented with multivariate regressions as follows. For model 1, the primary WM damage model, 5 simultaneous regressions (for each of the 5 tract-cortex pairs) regressed the 24-month GM MTR outcome on the tract-specific baseline NAWM MTR predictor; the corresponding tract-specific baseline GM MTR was a covariate for each regression, to ensure that any baseline NAWM vs 24-month GM association was not explained by cross-sectional baseline NAWM vs GM association, which could induce the longitudinal association without prior WM damage. The null hypothesis, that baseline was not associated with 24-month MTR in any of the tract-cortex pairs, was jointly tested as a single hypothesis that all 5 baseline MTR coefficients (one in each regression) were zero. For model 2, the primary GM damage model, the simultaneous regressions used 24-month NAWM MTR with tract-specific baseline GM MTR predictors, adjusting

for corresponding baseline NAWM MTR. To examine whether age, sex, disease duration, NAWM or GM global volumes, tract-specific lesion MTR or volume, or spinal cord area explained the associations, these were included singly as covariates in each tract-cortex pair regression. The role of early lesions in contributing to later NAWM or GM damage was assessed when tract-specific baseline lesion volume was included in the 2 multivariate models above.

Analyses were performed in Stata 13 (StataCorp, College Station, TX); the multivariate regressions were performed using the Stata structural equation modeling command, using, as estimation method, maximum likelihood with missing values; this requires the assumptions of multivariate normality, and that the mechanism for missing data is either completely at random or associated with variables in the model. Results are reported as significant at $p < 0.05$.

RESULTS Clinical assessment. Patients clinically deteriorated over the follow-up period (baseline: median EDSS score 4.5, range 1.5–7; 2 years: median EDSS score 6, range 1.5–8; $p = 0.017$).

Baseline difference in MTR and volume between patients and healthy controls and MTR evolution over the follow-up. At baseline, patients showed reduced MTR in the NAWM of the bilateral CST compared with controls, but did not differ significantly in the GM MTR of the connected motor cortex (table 2, figure e-1). Patients showed significantly lower MTR than controls at baseline in the callosal tract and connected cortex, and in the right OR and bilateral visual cortex (table 2, figure e-1). Regional differences in NAWM and GM volume between patients and controls at baseline are reported in table e-1.

In patients, a statistically significant decrease in mean MTR over 24 months was seen in the GM of the left visual cortex (percentage of change in MTR over time = -1.27% , $p = 0.045$), while a trend toward a significant decrease over the follow-up was found in the GM of the right visual cortex (percentage of change in MTR over the follow-up = -0.89% , $p = 0.051$) (table e-2).

Correlation between WM and GM MTR at each time-point. In the patient group at baseline, a lower mean MTR of each tract's NAWM was significantly associated with a lower mean MTR of the corresponding GM target in the left motor pair ($r = 0.36$, $p = 0.014$), in the callosal pair ($r = 0.56$, $p < 0.001$), and in the left and right visual pair (both $r = 0.53$, $p < 0.001$), but not in the right motor pair ($r = 0.17$, $p = 0.260$). When adjusting for potential confounders, these associations remained significant for the callosal and the visual pairs.

At 24 months, a lower mean MTR of each tract was associated with a lower mean MTR of the corresponding GM region in all tract-cortex pairs independently of all the other covariates, except for the left motor pair when adjusting for baseline whole WM MTR (figure e-2).

The primary WM damage model. The joint test for the primary WM damage model gave $p = 0.006$,

Table 2 Mean MTR values at baseline in GM and WM regions in patients and healthy controls, with *p* values of the comparison between the 2 groups adjusting for age and sex

	NAWM, patients	WM, controls	<i>p</i> Values, patients vs controls	GM, patients	GM, controls	<i>p</i> Values, patients vs controls
Motor pair						
Left	36.2 (1.4)	37.2 (0.59)	0.002	35.1 (2.4)	35.7 (1.2)	0.392
Right	36.1 (1.3)	37.0 (0.66)	0.006	33.9 (2.5)	35.3 (1.8)	0.235
Callosal pair	38.1 (1.7)	39.5 (0.46)	<0.001	32.4 (1.3)	33.6 (0.97)	0.004
Optic pair						
Left	34.1 (2.0)	35.5 (1.4)	0.080 ^b	31.2 (1.7)	32.6 (1.4)	0.011 ^{a,b}
Right	34.4 (1.9)	36.1 (0.85)	0.002	32.2 (1.4)	33.6 (0.94)	0.001

Abbreviations: GM = gray matter; MTR = magnetization transfer ratio; NAWM = normal-appearing white matter; WM = white matter.

Data represent mean (SD) MTR.

^aThis comparison lost significance when an influential data point was omitted.

^bUnder a crude Bonferroni correction, multiplying all *p* values by the number of tests, these 2 *p* values are no longer significant at the 5% error rate.

rejecting the hypothesis of no association in any of the tracts: specifically, a lower baseline MTR of tract NAWM was associated with lower GM MTR of the connected cortex at 24 months in the bilateral motor pair and in the right visual pair, adjusting for tract-specific baseline GM MTR (table 3A, figures 2 and e-2). The inclusion of age, sex, disease duration, baseline NAWM and GM volumes, baseline lesion

MTR and lesion volume, and baseline spinal cord area in the model did not materially alter the results.

The primary GM damage model. In the primary GM damage model, although the joint test was again significant ($p = 0.007$), this was driven by a single significant association in the right motor cortex, where a lower baseline GM MTR predicted higher right CST

Table 3 Standardized regression coefficients for the primary WM damage (A) and primary GM damage (B) multivariate models

				Partial correlation coefficients	
Tract-cortex pairs	Baseline WM MTR standardized regression coefficient ^a	95% CI	p Value	WM	GM
A: Standardized regression coefficients for the primary WM damage multivariate model ^b					
Left motor pair	0.32	0.03, 0.60	0.031	0.40	0.53
Right motor pair	0.47	0.21, 0.72	<0.001	0.45	0.36
Callosal pair	−0.25	−0.60, 0.10	0.161	−0.12	0.67
Left visual pair	0.08	−0.15, 0.31	0.476	0.22	0.82
Right visual pair	0.22	0.01, 0.43	0.036	0.33	0.83
Baseline GM MTR standardized regression coefficient ^c					
B: Standardized regression coefficients for the primary GM damage multivariate model ^d					
Left motor pair	−0.08	−0.20, 0.04	0.218	0.77	−0.56
Right motor pair	−0.28	−0.44, −0.12	0.001	0.71	−0.62
Callosal pair	−0.03	−0.22, 0.17	0.800	0.82	−0.30
Left visual pair	0.08	−0.06, 0.23	0.237	0.89	0.16
Right visual pair	0.17	−0.07, 0.41	0.172	0.83	0.31

Abbreviations: CI = confidence interval; GM = gray matter; MTR = magnetization transfer ratio; NAWM = normal-appearing white matter; WM = white matter.

^aThe standard regression coefficient reported is the number of SDs by which 24-month GM MTR is estimated to increase per 1-SD increase in baseline WM MTR.

^bFor each of the 5 pairs, 24-month GM MTR is regressed on the tract-specific baseline NAWM MTR predictor, adjusting for the corresponding tract-specific baseline GM MTR.

^cThe standard regression coefficient reported is the number of SDs by which 24-month WM MTR is estimated to increase per 1-SD increase in baseline GM MTR.

^dFor each of the 5 pairs, 24-month WM MTR is regressed on the tract-specific baseline GM MTR predictor, adjusting for the corresponding tract-specific baseline NAWM MTR.

Figure 2 Tract-specific associations between baseline and 24-month MTR values

In these panels are reported the 6 scatterplots between the 4 MTR measures (baseline NAWM and GM, 24-month NAWM and GM) for the right motor tract-cortex pair. The 24-month GM against baseline NAWM and 24-month NAWM against baseline GM are the unadjusted pairwise versions of the primary WM damage and primary GM damage models for this tract-cortex pair. Pearson correlation coefficients and *p* values are reported. GM = gray matter; MTR = magnetization transfer ratio; NAWM = normal-appearing white matter; WM = white matter.

MTR at 24 months after adjusting for baseline NAWM tract MTR (table 3B, figures 2 and e-2). When including age, sex, disease duration, baseline NAWM and GM volumes, baseline lesion MTR and lesion volume, and baseline spinal cord area in the model, results did not change materially.

Correlation between lesional metrics at baseline and tissue damage at 2 years. In all tract-cortex pairs, no significant association was found between tract-specific lesion MTR and volume at baseline, and the corresponding cortical region's GM MTR at 24 months. Lower tract-specific lesion MTR at baseline was associated with lower MTR in the same tract NAWM at 24 months in all tracts, except the visual tracts bilaterally.

DISCUSSION Our results are consistent with an evolving relationship between anatomically linked GM and WM pathology in early PPMS. WM changes appear to predict subsequent GM abnormalities, more so than GM abnormalities predict subsequent WM damage. Over the 2 years of observation, abnormalities in NAWM rather than the WM lesions appeared to have a greater association with later GM damage. Over the same period, baseline WM lesion measures predicted subsequent changes in NAWM. Overall, this is consistent with a sequence of events, unfolding over

2 or more years, arising from WM lesions, through NAWM change, and leading to subsequent GM abnormalities.

At baseline, MTR in NAWM tracts was significantly lower in patients than in healthy controls (i.e., CST, genu of the corpus callosum, and OR) and in the corresponding GM targets, with the exception of the left OR and the motor cortex bilaterally, consistent with previous findings in this same cohort.^{7,17–19} The relative sparing of motor cortex is in line with previous MTR studies on patients with relapsing-remitting MS.^{20,21}

Cross-sectional correlations between NAWM and GM damage at each time-point were significant in all tract-cortex pairs, except in the right motor tract-cortex pair, confirming our previous results,⁷ which indicate that pathologic processes affecting the 2 compartments are correlated. Allowing for these baseline tract-cortex associations, statistical tests of the primary WM damage model were more consistently and robustly significant than those testing the primary GM damage model. Of note, baseline T2-weighted lesion load did not materially alter the models, i.e., that over the 2 years of observation, GM changes were mostly related to NAWM abnormalities rather than lesions. However, baseline T2-weighted lesion load was associated with subsequent NAWM abnormalities, and so the ultimate consequence of lesion formation

may include cortical changes, albeit taking more than 2 years to become manifest.

Axons are the cellular component linking WM lesions, WM tracts, and cortical GM, and axonal degeneration in WM lesions²² and NAWM²³ could well be the initiating element leading to subsequent cortical neuronal pathology.^{3,24} Several mechanisms within WM may contribute toward axonal pathology including glutamatergic excitotoxicity, disrupted intra-axonal transport, and mitochondrial dysfunction.³ It has also been suggested that the pathogenesis of PPMS, when compared with relapse-onset disease, is primarily one of cellular degeneration, and that this may begin years before the first onset of symptoms.²⁵ This would provide a plausible mechanism connecting a primary WM axonal pathology with a subsequent neuronal loss in the connected GM areas.

When the primary GM damage model was tested, the only significant finding was the association in the right motor pair between lower baseline MTR in the right motor cortex and the higher MTR in the right CST at 24 months; this association is not consistent with a primary GM damage hypothesis. In any case, it is possible that this correlation was induced by an outlying data point. It should be noted that this study specifically recruited people with clinically early PPMS, and our results may not be applicable to people with long-standing progressive MS. There is increasing evidence from histopathology and imaging studies suggesting that a substantial proportion of GM pathology, in particular subpial demyelination and cortical neurodegeneration, develops independently of WM damage.^{26–28} This component of GM damage is thought to be influenced by meningeal inflammation, which is particularly prominent in long-standing progressive MS.^{29,30} As such, it is possible that a primary GM damage model may have a more substantial role later on in PPMS.

While we have interpreted our results as being consistent with tract-mediated processes, they could also represent coincident but independent development of regional pathology, with pathologic changes in NAWM and GM progressing at similar rates. However, in this case, it would then be expected to see baseline GM MTR predicting subsequent NAWM MTR in the same tract-cortex pairs in which the primary WM damage model was significant, but only in 1 of the 3 significant pairs did we find this. Our results do not exclude the possibility of both dependent and independent components working in parallel, with one prevailing over the other at different stages of disease or in different brain regions. Further results from longer longitudinal studies, investigating the dynamics of WM and GM changes in a larger number of brain regions, are needed to confirm and generalize our conclusions, which should be interpreted with caution also

in light of the number of missing values (appendix e-1) at 24 months. To reduce the effect of missing MTR values, we relied on multivariate normality and “missing-at-random” assumptions, which allowed all available data points to be used. While there was no evidence to suggest violation of these assumptions, they are inherently difficult to assess.

Given the challenges of gathering large clinical and MRI datasets, we used previously acquired data for this work. Inaccuracies in tract alignment with cortical targets (and thus associated partial volume effects) could have limited our sensitivity to tract-cortex associations. Sequences tuned to detect GM lesions were not available when these data were collected, and unseen GM lesions are likely to increase the variability of GM MTR across subjects, and therefore further decrease our sensitivity to finding associations in the primary WM damage model. Because this was a retrospective study, we were limited in our scope to select healthy control scan data obtained over a comparable time period, giving rise to relatively poor matching with older patients with MS. Although there was very little evidence of an association between age or sex and MTR values, the patient vs healthy control adjusted differences may still have been influenced in part by this age distribution mismatch. Moreover, some MTR values lying in the tails of the data influenced the strength and significance of some correlations. As such, the results from this dataset should be interpreted with due caution, and further studies, informed by our methods, are required to clarify the actual strength of associations in the tract-cortex pairs.

In conclusion, our results in patients with early PPMS suggest a temporal evolution of pathology from WM to GM, and more specifically, from lesions to NAWM and from NAWM to GM.

AUTHOR CONTRIBUTIONS

Benedetta Bodini, Declan Chard, and Olga Ciccarelli designed and carried out the study and the writing of the manuscript. Claudia Wheeler-Kingshott, Daniel Tozer, and Daniel Altmann were involved in the imaging and statistical analysis of data. David Miller and Alan Thompson critically revised the manuscript for intellectual content.

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