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Early development of multiple sclerosis is associated with progressive grey matter atrophy in patients presenting with clinically isolated syndromes

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Summary

While brain atrophy occurs early in the clinical course of multiple sclerosis, exactly how early, which tissues are affected and the rate at which early atrophy occurs are unclear. Regional brain atrophy was investigated in 58 patients recruited within 3 months of onset of a clinically isolated syndrome (CIS) suggestive of multiple sclerosis, who were followed-up for 3 years. At 3 years, 31 subjects had developed multiple sclerosis as defined by the McDonald criteria, while 27 had not (13 had MRI-visible brain lesions and 14 did not). In those who developed multiple sclerosis, the mean decrease in grey matter fractional volume (GMF, as a fraction of total intracranial volume) was –0.017 (–3.3%) and was significantly larger than in the combined lesion-positive and lesion-negative CIS subjects [–0.005 (–1.1%), P = 0.001]. No decrease in white matter fractional volumes (WMF) was seen. Change in GMF correlated only modestly with the change in T2 lesion volume from baseline to year 3 (r = –0.428, P = 0.004). These results suggest that progressive grey matter, but not white matter, atrophy is seen in the earliest clinically observable stages of relapse onset multiple sclerosis, and this is only moderately related to lesion accumulation. Longer-term follow-up is required to determine whether early grey matter atrophy is associated with subsequent disability or cognitive impairment.

Keywords: multiple sclerosis; clinically isolated syndrome; grey matter; white matter; atrophy

Abbreviations: BPF = brain parenchymal fraction; CIS = clinically isolated syndrome; EDSS = Expanded Disability Status Score; GM = grey matter; GMF = grey matter fraction; NAWM = normal-appearing white matter; VV = ventricular volume; WM = white matter; WMF = white matter fraction

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Introduction

Multiple sclerosis initially presents as a clinically isolated syndrome (CIS) such as optic neuritis, isolated brainstem or spinal cord syndromes in 85–90% of patients. Sixty to eighty percent of patients presenting with CIS suggestive of multiple sclerosis who have MRI-visible brain lesions go on to develop clinically definite multiple sclerosis (Brex *et al*., 2002; Beck *et al*., 2003). However, while lesions are associated with the development of multiple sclerosis, there is only a limited relationship between lesion accrual and subsequent disability. This may reflect pathological heterogeneity within lesions, with variable effects on nerve conduction and clinical function. Axonal and neuronal damage have become the focus of significant attention (Trapp *et al*., 1998; Bjartmar and Trapp, 2001; Bjartmar *et al*., 2001; Cifelli *et al*., 2002) and may be more salient when considering long-term outcomes, with atrophy measures potentially acting as a more specific marker of such pathology than lesion volume measures (Miller *et al*., 2002). This is supported by the observation that in established multiple sclerosis, atrophy of the brain and spinal cord is

more noticeable with increasing disability (Losseff *et al*., 1996*a*, *b*).

Relatively little investigation of atrophy has been undertaken in patients in the earliest stages of multiple sclerosis. Studies at the early stage of disease have potential to identify prognostic markers and techniques for therapeutic monitoring and may provide insights into disease pathogenesis. In previous studies in patients with CIS, progressive ventricular enlargement was detected over 1 year in those with an abnormal MRI (Brex *et al*., 2000; Dalton *et al*., 2002*a*). In this study, we report global [brain parenchymal fraction (BPF)] and regional [grey matter (GM) fraction (GMF), white matter (WM) fraction (WMF)] measures of brain volume changes, as well as a measures of ventricular volume (VV) change in 58 subjects with CIS, scanned within 3 months of the onset of the CIS and again 3 years later, when 31 (53%) of patients had developed multiple sclerosis as defined by the McDonald criteria (McDonald *et al*., 2001). The clinical and MRI lesion findings of most subjects in the present cohort have been reported previously (Dalton *et al*., 2002*b*). The purposes of the present study were to determine: (i) the location of early brain atrophy; (ii) the relationship of early brain atrophy, both global and tissue specific, to the development of multiple sclerosis; and (iii) the relationship of early brain atrophy to focal lesion load measures.

Methods

Patients

Subjects aged between 17 and 50 years presenting at Moorfields Eye Hospital or the National Hospital for Neurology and Neurosurgery with CIS suggestive of multiple sclerosis were invited to participate in this study. Fifty-eight patients were recruited from 1995 to 1999, and followed-up for ~3 years. A CIS was defined as the first acute neurological event suggestive of CNS demyelination, e.g. unilateral optic neuritis, brainstem and partial spinal cord syndromes, where no alternative diagnosis was identified upon appropriate investigation. Overall, 40 patients presented with optic neuritis, 13 a brainstem syndrome, four a spinal cord syndrome (spinal MRI excluded an alternative structural cause in these cases) and one patient had a hemianopia attributable to an optic tract lesion. Patients were clinically assessed using the Kurtzke Expanded Disability Status Scale (EDSS) (Kurtzke, 1983) and MRI scans obtained upon entry to the study (within 3 months of symptom onset) and ~3 months, 1 and 3 years later. A diagnosis of multiple sclerosis was determined according to the McDonald criteria and included both MRI and clinical evidence for dissemination in space and time (McDonald *et al*., 2001). The study was undertaken with approval from the local medical ethics committees at Moorfields Eye Hospital, the National Hospital for Neurology and Neurosurgery and the Institute of Neurology, and written informed consent was obtained from all the study subjects.

Image acquisition

Brain MRI was performed using a 1.5 Tesla GE scanner (General Electric Medical Systems, Milwaukee, WI). At baseline, 3 months, 1 and 3 years, a proton density/T2-weighted fast spin echo sequence [repetition time (TR) 3200 ms, effective echo time (TE) 15/95 ms] and a T1-weighted spin echo sequence (TR 600 ms, TE 14 ms) were acquired in each patient after intravenous administration of 0.1 mmol/kg gadolinium (Gd) DTPA 5–7 min prior to the start of image acquisition. Forty-six, 3 mm contiguous axial slices were obtained with an in-plane resolution of 0.9 × 0.9 mm.

Lesion identification and measurements

The MRI scans were reviewed by an experienced neuroradiologist (K.A.M.), who was blinded to the patients' clinical status at follow-up. T2, Gd-enhancing and T1 hypointense lesions were identified at baseline, 3 months, 1 and 3 years of follow-up. Using a semi-automatic local thresholding technique, lesions were segmented on the proton density-weighted fast spin echo images and T1-weighted post-Gd-enhanced images, which provided T2 hyperintense and T1 hypointense lesion volumes, respectively (Plummer, 1992).

Brain tissue segmentation

The baseline and 3-year follow-up T2-weighted images were segmented, using a fully automated technique, into images representing the probability of any given voxel containing GM, WM, CSF and other tissues using SPM99 (Chard *et al*., 2002*a*). Lesions were identified and contoured using the same semi-automated lesion segmentation technique as noted above and used to allow for SPM99 misclassification of WM lesions as GM, CSF or other tissues. The lesion mask over-rode all SPM99 tissue classifications, otherwise a voxel was classified as GM, WM, CSF or other tissue, dependent on which mask had the greatest probability at that location. Results were assessed as fractions of total intracranial volume, determined by adding GM, WM, lesion and CSF volumes. BPF was calculated as GM, WM plus lesion volume divided by total intracranial volume. WMF was calculated as WM plus lesion volumes divided by total intracranial volume. GMF was calculated as the GM volume divided by the total intracranial volume.

The segmented brains were then visually checked for quality assurance to ensure GM, WM and CSF had been correctly segmented. In 13 scans, two measurements of the segmented brains were made at least 1 week apart in order to assess the reproducibility of the method. The mean coefficients of variation for GM, WM and CSF were 0.0002, 0.0003 and 0.0006%, respectively.

To investigate whether the higher lesion loads in the multiple sclerosis group when compared with the CIS group (see Table 4) might have influenced the segmented atrophy measures, simulated lesions were added to the 3 year scans of the CIS group such that the group had a median and range of lesion load increase similar to the multiple sclerosis group. The size and signal intensity of the simulated lesions were characteristic of multiple sclerosis lesions seen on T2-weighted scans. The signal intensity of lesions was set at a level half way between grey matter and CSF intensity. The lesions were placed at multiple locations in the cerebral white matter of the T2-weighted scans used for subsequent atrophy analysis. Measurements of the GMF and WMF of the CIS group with simulated lesions were obtained and compared with the measures made without such lesions.

Ventricle volume measurement

The ventricles were measured on baseline and 3-year follow-up T1-weighted scans using the MIDAS interactive image analysis package

by a single observer blinded to patient details in a previously described method (Freeborough *et al*., 1997; Brex *et al*., 2000; Dalton *et al*., 2002*a*). VV was estimated for the lateral ventricles and temporal horns but excluding the third and fourth ventricles; this approach has been applied previously in multiple sclerosis and demonstrated to have a good reproducibility and sensitivity to change (Fox *et al*., 2000; Dalton *et al*., 2002*a*).

Statistical methods

Patients were divided into one of two principal groups according to their 3-year follow-up status: (i) those with a diagnosis of multiple sclerosis by the McDonald criteria using both clinical and MRI evidence for dissemination in space and time (31 subjects); and (ii) those who still had a diagnosis of a CIS (27 subjects); for some analyses, this group was divided further into those who had MRI-visible brain lesions (13 subjects) and those without MRI-visible brain lesions (14 subjects) at any time during follow-up.

Mean fractional volume changes over time within groups were estimated by linear regression adjusting for the baseline value. Comparison of differences in mean changes in fractional volumes over time between groups was assessed by adding a group indicator to the regression with baseline covariate. Baseline adjustment did not alter the point estimates, but improved precision. Where there was evidence of non-normality, the validity of inference was checked using a non-parametric bias-corrected bootstrap with 1000 replicates (Carpenter *et al*., 2000), and, where the bootstrap estimates differed, these are reported. Non-parametric tests of change within group over time used the Wilcoxon signed rank test, and of differences in lesion volumes and changes between groups used the Mann–Whitney U test. Between-group comparison of lesion numbers used negative binomial regression. Correlations between lesion load and atrophy measures were assessed using Spearman rank correlation.

Results

Baseline clinical and MRI findings

The median time delay between the onset of symptoms and the baseline scan was 5 weeks, (range 1–12). Thirty-nine patients (67%) had an abnormal baseline T2-weighted MRI scan with one or more focal high signal lesions. Of those, 16 had Gd-enhancing and 23 had T1 hypointense lesions. The median EDSS at baseline in the group of patients with an abnormal baseline T2-weighted MRI scan was 2 (0–8), and 1 (0–3) in those with a normal scan.

Diagnosis at 3 years

The median time between the baseline and the 3-year follow-up scan was 37 months (range 31–72). At 3 years, 31 out of 58 (53%) had developed multiple sclerosis (13 males and 18 females), and 27 had not. Of the latter group, 13 had a diagnosis of CIS with brain lesions (six males and seven females) and 14 had a diagnosis of CIS without brain lesions (eight males and six females). The median EDSS at 3 years was 1 (0–8) in the multiple sclerosis group, 0 (0–1) in the CIS group with brain lesions and 0 (0–2) in the CIS group without brain lesions. The duration of follow-up was not significantly different between those developing multiple sclerosis and those with a remaining CIS (median 1125 versus 1111 days, $P = 0.47$).

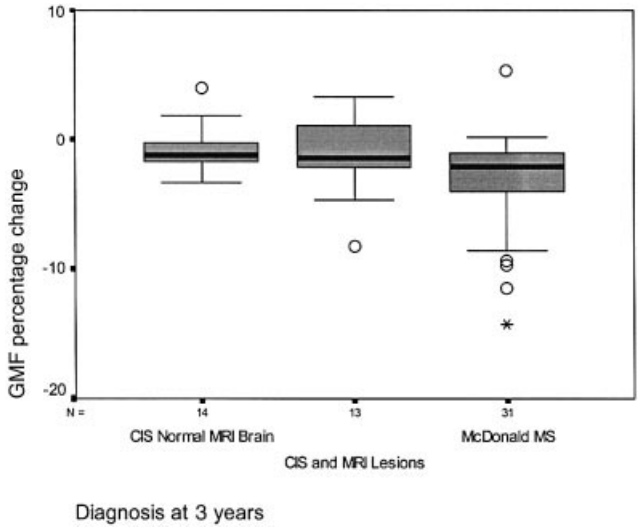

Fig. 1 Box plot showing the medians, interquartile ranges (box), highest and lowest values, excluding outliers (whiskers), outliers (circles) and extreme value (asterisk) for GMF percentage change in CIS patients with and without MRI lesions, and multiple sclerosis patients.

The relationship between atrophy measures and clinical outcome (Tables 1–3)

Table 1 shows global and regional brain and ventricular volume measures at baseline and 3 years for the multiple sclerosis and CIS subgroups. Table 2 shows absolute volume changes over 3 years in volume measures, and Table 3 shows median and mean percentage volume changes for the multiple sclerosis and CIS subgroups. No statistically significant differences were found in mean 3 year – baseline changes in BPF, GMF, WMF and VV between the CIS subgroups with and without lesions, and these two groups were combined for statistical comparisons.

A significant decrease in BPF over 3 years was seen in both the multiple sclerosis and combined CIS groups, but the decrease was significantly larger in the multiple sclerosis group (Tables 2 and 3). Figure 1 shows the median percentage change in GMF in the form of a box plot in CIS patients with and without MRI lesions and multiple sclerosis patients. There was a significant increase in VV between baseline and year 3 in those who developed multiple sclerosis but not in the combined CIS group; the difference between the groups was significant.

There was a significant decrease in GMF between baseline and year 3 in both the multiple sclerosis and combined CIS groups, but the decrease was significantly larger in the multiple sclerosis group (Tables 2 and 3). There was a weakly significant increase in mean WMF at 3 years compared with

Tables 1 *Brain and ventricular volume measures at baseline and 3 years [mean, median (range)]*

	Total cohort (n = 58)	McDonald multiple sclerosis (n = 31)	CIS		
			With lesions (n = 13)	Without lesions (n = 14)	CIS combined (n = 27)
BPF year 0	0.864, 0.860 (0.80–0.91)	0.863, 0.856 (0.80–0.91)	0.868, 0.860 (0.85–0.90)	0.862, 0.861 (0.83–0.91)	0.865, 0.860 (0.83–0.91)
BPF year 3	0.855, 0.853 (0.80–0.91)	0.851, 0.848 (0.80–0.91)	0.862, 0.856 (0.85–0.89)	0.858, 0.855 (0.83–0.91)	0.860, 0.855 (0.83–0.91)
GMF year 0	0.494, 0.490 (0.440–0.570)	0.490, 0.489 (0.44–0.55)	0.504, 0.500 (0.47–0.54)	0.494, 0.486 (0.47–0.57)	0.499, 0.491 (0.47–0.57)
GMF year 3	0.483, 0.479 (0.430–0.550)	0.473, 0.473 (0.43–0.54)	0.497, 0.497 (0.46–0.55)	0.490, 0.482 (0.46–0.55)	0.493, 0.493 (0.46–0.55)
WMF year 0	0.370, 0.369 (0.33–0.41)	0.373, 0.369 (0.35–0.41)	0.363, 0.363 (0.33–0.40)	0.368, 0.372 (0.33–0.40)	0.366, 0.365 (0.33–0.40)
WMF year 3	0.372, 0.371 (0.33–0.40)	0.377, 0.373 (0.33–0.40)	0.365, 0.363 (0.33–0.40)	0.368, 0.368 (0.34–0.39)	0.367, 0.365 (0.33–0.40)
VV (ml) year 0	8.605, 6.286 (0.610–26.86)	9.718, 8.569 (1.22–26.86)	6.844, 4.572 (1.68–19.88)	7.776, 6.563 (0.61–23.12)	7.327, 5.848 (0.61–23.12)
VV (ml) year 3	9.972, 7.499 (0.740–28.91)	12.128, 11.198 (1.42–28.91)	7.335, 5.247 (1.79–22.31)	7.647, 6.307 (0.74–3.49)	7.497, 5.701 (0.74–23.49)

Table 2 *Year 3 minus baseline changes in BPF, GMF, WMF and VV in the MS and CIS groups*

Atrophy	Multiple sclerosis		CIS (combined)		Baseline adjusted between-group difference	
	Mean change (CI)	P value	Mean change (CI)	P value	Mean multiple sclerosis – CIS difference (CI)	P value
BPF	–0.012 (–0.016 to –0.008)	0.001	–0.005 (–0.010 to –0.0003)	0.038	–0.008 (–0.014 to –0.001)	0.022
GMF	–0.017 (–0.022 to –0.011)	0.001	–0.005 (–0.011 to 0.004)	0.03*	–0.014 (–0.021 to –0.006)	0.001
WMF	0.005 (0.0007 to 0.009)	0.023	0.0005 (–0.004 to 0.005)	0.820	0.006 (0.0003 to 0.0123)	0.040
VV (ml)	2.410 (1.301 to 3.518)	0.001	0.170 (–1.018 to 1.358)	0.775	2.305 (0.651 to 3.958)	0.007

No statistical differences were noted in mean differences in BPF, GMF, WMF and VV changes from baseline to year 3 between the CIS groups with and without lesions so these groups were combined for statistical purposes.
CI = 95% confidence interval.
*Bootstrap-derived P value.

Table 3 *Median and mean percentage change in 3-year BPF, GMF, WMF and VV (relative to baseline values)*

MRI parameter	Multiple sclerosis (n = 31)		CIS					
			With MRI lesions (n = 13)		Without MRI lesions (n = 14)		CIS combined (n = 27)	
	Median	Mean	Median	Mean	Median	Mean	Median	Mean
BPF	–0.8	–1.4	–0.4	–0.7	–0.6	–0.5	–0.5	–0.6
GMF	–2.1	–3.3	–1.4	–1.4%	–1.2	–0.7	–1.4	–1.1
WMF	+1.2	+1.3	+1.1	+0.4	+0.2	+0.1	+0.8	+0.2
VV	+17.9	+38.9	+9.9	+7.9	+0.4	+3.1	+2.3	+5.4

baseline in the patients who developed multiple sclerosis (Table 2; P = 0.023); the median percentage change in these patients was +1.2% (Table 3; P = 0.046). However, there was no significant change in WMF in the combined CIS group.

Seven patients were treated with short courses of steroids (three prior to the baseline scan; four between the baseline and year 3 scan); two of these were also treated with β-interferon between baseline and year 3, and one patient received β-interferon but not steroids. Five of these eight were in the group who developed multiple sclerosis and three were in the CIS non-lesion group. Exclusion of these eight patients did not affect multiple sclerosis versus CIS comparisons, nor did exclusion of the patient with an EDSS of 8.

Table 4 *Comparison of lesion load measures and changes in lesion load measures (median plus ranges) in multiple sclerosis subjects versus CIS group with MRI lesions*

MRI parameter	Multiple sclerosis (n = 31)	CIS with MRI lesions (n = 13)	P value (Mann–Whitney U)
Baseline T2 lesion volume (ml)	1.1 (0.0 to 13.9)	0.2 (0.0 to 0.5)	<0.001
3 year T2 lesion volume (ml)	2.5 (0.3 to 58.5)	0.2 (0.03 to 1.4)	<0.001
T2 lesion volume change 0–3 (ml)	1.2 (–1.5 to 44.6)	0.1 (–0.3 to 1.0)	<0.001
Baseline T1 lesion volume (ml)	0.1 (0.0 to 4.5)	0.0 (0.0 to 0.3)	0.008
3 year T1 volume (ml)	0.1 (0.0 to 18.3)	0.0 (0.0 to 0.4)	0.006
T1 lesion volume change 0–3 (ml)	0.1 (–2.0 to 13.8)	0.0 (–0.3 to 0.2)	0.196
Baseline Gd lesion number	0 (0 to 21)	0 (0 to 3)	0.013*
3 year Gd lesion number	1 (0 to 6)	0 (0 to 1)	0.016*

*Negative binomial regression.

Lesion measures in multiple sclerosis group versus CIS group with abnormal MRI (Table 4)

The multiple sclerosis group had higher T2 and T1 hypointense lesion volumes and higher numbers of Gd-enhancing lesions at baseline and year 3. The multiple sclerosis group also displayed a greater increase in T2 volume between baseline and year 3, but no difference was observed between the two groups in change in T1 hypointense volume. Excluding patients treated with steroids or β-interferon did not alter comparisons except for numbers of Gd-enhancing lesions, which failed to reach significance.

Effect of simulated lesions on the segmented atrophy measures in the CIS group

When the 3 year CIS group segmented atrophy measures with and without simulated lesions were compared, the measures with simulated lesions displayed slightly higher GMFs and lower WMFs, but the differences were not significant (mean increase in GMF = +0.0032, 95% confidence intervals –0.00037 to +0.0067, P = 0.077, two-tailed paired t test; mean decrease in WMF = –0.00096, 95% confidence intervals –0.0022 to +0.0003, P = 0.135, two-tailed paired t test).

Associations between lesion and atrophy measures (Table 5)

Change in BPF (3 year – baseline) correlated negatively with the baseline number of Gd-enhancing lesions, and changes (3 year – baseline) in T1 hypointense and T2 lesion volume. Change in VV (3 year – baseline) correlated positively with baseline T1 hypointense and T2 lesion volumes and Gd-enhancing lesion number, and with change (3 year – baseline) in T1-hypointense and T2 lesion volumes. Change in GMF (3 year – baseline) was modestly correlated negatively with changes (3year – baseline) in T1 hypointense and T2 volume. There were no significant correlations between lesion volumes and WMF changes.

Associations between VV change and other tissue volume measurements

Three year – baseline changes in VV correlated with changes in GMF (r_s = –0.393, P = 0.002) and BPF (r_s = –0.453, P < 0.001), but not with changes in WMF (r_s = +0.027, P = 0.839).

Discussion

Previous cross-sectional studies have reported both total and neocortical GM atrophy, in excess of that seen in healthy controls, in early relapsing–remitting multiple sclerosis with disease durations of <3 (Chard *et al*., 2002*b*) and 5 (De Stefano *et al*., 2003) years respectively. The present study extends these observations in demonstrating that there is increasing GM atrophy in patients developing multiple sclerosis over 3 years following presentation with a CIS. The multiple sclerosis cohort also exhibited a significant increase in VV during the follow-up period. Although decreasing GMF was also seen in the 27 patients in the remaining CIS group, the decrease was significantly less than that observed in the multiple sclerosis group. While it is likely that some individuals in the CIS group will develop multiple sclerosis with longer follow-up, the present study shows that progressive GM atrophy is more prominent in those CIS patients who develop multiple sclerosis within 3 years using the McDonald criteria. Although the small sample size limits the sensitivity of the analysis, no significant atrophy was detected in the 14 CIS patients with normal imaging, this being a group that has a relatively low risk for developing multiple sclerosis (Brex *et al*., 2002). There was significantly greater ventricular enlargement seen in the multiple sclerosis versus the remaining CIS group, and the amount of increase in the multiple sclerosis group is also more than that reported from healthy young adult populations in the literature (Fox *et al*., 2000).

This study investigates tissue volume changes in multiple sclerosis at an early stage. This has been enabled by the recently developed McDonald criteria which allow a diagnosis based on MRI evidence for dissemination in space and

Table 5 *Spearman correlations between lesion load and atrophy measures*

Atrophy measures	Lesion load measures	r_s	*P*
VV change year 3 – 0	Volume of T2 lesions at baseline	0.528	0.0002
	Number of Gd-enhancing lesions at baseline	0.514	0.0004
	Volume of T1 hypo-intense lesions at baseline	0.529	0.0002
	T2 lesion volume change year 3 – 0	0.440	0.0028
	T1 lesion volume change year 3 – 0	0.369	0.0137
BPF change year 3 – 0	Volume of T2 lesions at baseline	–0.258	0.091
	Number of Gd-enhancing lesions at baseline	–0.440	0.0028
	Volume of T1 hypo-intense lesions at baseline	–0.167	0.2791
	T2 lesion volume change year 3 – 0	–0.410	0.0057
	T1 lesion volume change year 3 – 0	–0.306	0.0432
GMF change year 3 – 0	Volume of T2 lesions at baseline	–0.0003	0.9986
	Number of Gd-enhancing lesions at baseline	–0.1193	0.4405
	Volume of T1 hypo-intense lesions at baseline	–0.0049	0.9751
	T2 lesion volume change year 3 – 0	–0.4280	0.0037
	T1 lesion volume change year 3 – 0	–0.3071	0.0426

There were no significant correlations between the change in WMF and lesion load changes.

time in patients with single clinical episodes (McDonald *et al*., 2001). Studying multiple sclerosis at an early stage has potential to identify markers which predict the future clinical course, and to find useful techniques for treatment trial monitoring that can be applied prior to the emergence of persistent neurological deficits.

In contrast to the development of GM atrophy, there was no decrease in WM volume in the multiple sclerosis group over the 3 years, and indeed there was a suggestion of an increase in the amount of WM. The multiple sclerosis group had a large increase in WM lesion load during the study. Lesions in early multiple sclerosis often display inflammation, and increased glial cellularity is also reported in multiple sclerosis normal-appearing WM (NAWM) (Allen *et al*., 2001). Such processes might contribute to an increase in tissue volume. On the other hand, loss of axons and myelin within inflammatory lesions (Trapp *et al*., 1998), and axonal loss in NAWM would be expected to cause tissue loss. The observed result would be compatible with WM volume gain from inflammation compensating for loss due to axonal degeneration. Two previous magnetic resonance spectroscopy studies of NAWM in CIS have shown no significant reduction of *N*-acetyl-aspartate, an axonal marker, which suggests that substantial axonal loss is not prominent in WM at this early stage (Brex *et al*, 1999; Tourbah at al., 1999). Other techniques that assess intrinsic tissue characteristics may be of greater use than atrophy measures when evaluating longitudinal WM processes in early multiple sclerosis.

Because in the present study no healthy control group was available for comparison using an identical image acquisition sequence, we were unable to investigate directly whether the CIS cohort already had GM or WM atrophy at presentation. However, in a previous study of 40 CIS patients that used a different (5 mm thick) acquisition sequence for tissue volume analysis, we observed a significant decrease in WMF but not GMF compared with healthy controls, within 6 months of onset of the CIS (Traboulsee *et al*., 2002). Taking that observation together with the present study raises the possibility that there are differences in the temporal evolution of GM and WM atrophy in CIS and early relapse onset multiple sclerosis, with GM atrophy emerging later and then evolving more rapidly. Further studies of other patient and control cohorts are warranted to define the temporal evolution of GM and WM atrophy in early multiple sclerosis.

GM atrophy might reflect neuronal degeneration secondary to anterograde or retrograde degeneration from WM lesions in which there has been axonal transection. Alternatively, it could reflect the development of focal lesions within GM in which there is axonal loss. Current MRI methods are largely unable to detect focal GM lesions, although they are well recognized pathologically (Kidd *et al*., 1999; Peterson *et al*., 2001). GM lesions exhibit less inflammation than those seen in WM (Kidd *et al*., 1999). Volume changes in GM may thus provide a more direct measure of the neurodegenerative component of multiple sclerosis pathology, being relatively unaffected by fluctuations due to inflammation.

Perhaps surprisingly, the increase in VV was not accompanied by a decrease in WM volume. Possibly ventricular enlargement is more related to the focal effects of tissue loss in periventricular lesions rather than to global WM volumes (Kalkers *et al*., 2002). The robust correlation of ventricular enlargement with lesion volume measures indicates that there is a notable relationship between focal lesions and this particular measure of atrophy. Ventricular enlargement was also correlated with GMF changes, and a generalized remodelling of the brain secondary to GM atrophy may contribute to VV enlargement. In addition, the GMF includes deep periventricular structures such as thalamus and caudate nucleus, and atrophy in these regions may have contributed more directly to the ventricular enlargement.

VV, GMF and BPF all appear potentially useful outcome measures for monitoring disease-modifying therapies in early multiple sclerosis, as in this study they were sensitive to progressive tissue loss. The GMF change correlated only modestly with lesion volume changes (the strongest correlation, which was with T2 volume change, was $r = 0.428$;

Table 5), suggesting that it provides additional information in understanding disease evolution and monitoring treatment effects. This limited correlation between GMF and lesion load, accounting for <20% of variability in either measure ($r^2 = 0.17$), also suggested that segmentation bias associated with focal signal abnormalities (Chard *et al*., 2002*b*; Quarantelli *et al*., 2003) do not alone account for the observed tissue-specific volume changes. Furthermore, we found only minimal changes to the 3-year GMF and WMF measures in the CIS group when simulated lesion loads approximating the increases seen in the multiple sclerosis cohort were introduced; indeed, the small changes observed were to slightly increase GMF and decrease WMF, thus increasing the differences between the multiple sclerosis and CIS groups. Further studies are warranted to confirm the present findings using different scan acquisitions and segmentation methodologies. Future studies should investigate the potential for early VV and GM volume measures to predict disability or cognitive impairment, and thereby help to identify patients in whom early disease-modifying treatments are most needed. It will also be relevant to investigate the effect of current or future disease-modifying treatments on these atrophy measures.

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