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Quantification of Brain Gray Matter Damage in Different MS Phenotypes by Use of Diffusion Tensor MR Imaging

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BACKGROUND AND PURPOSE: **Increasing evidence exists that cerebral gray matter (GM) from patients with multiple sclerosis (MS) is not spared. This study was performed to quantify in vivo the extent of cerebral GM pathologic abnormality in patients with relapsing-remitting (RR), secondary progressive (SP), and primary progressive MS, by using diffusion tensor (DT) MR imaging.**

METHODS: **Dual-echo and DT MR imaging of the brain were performed in 102 patients with MS and 30 healthy volunteers. After GM segmentation using a technique based on diffusion anisotropy thresholding, average diffusivity ($\bar{D}$) histograms of the cerebral GM were produced for all participants.**

RESULTS: **All $\bar{D}$ histogram-derived metrics of the GM were significantly different between control volunteers and the whole MS population. No significant difference was found for any of the $\bar{D}$ histogram-derived metrics between control volunteers and patients with RRMS, whereas significant differences were found for $\bar{D}$ and $\bar{D}$ histogram peak location between control volunteers and patients with PPMS. All the $\bar{D}$ histogram-derived metrics differed significantly between patients with RRMS and patients with SPMS. Patients with SPMS also had significantly lower $\bar{D}$ than did patients with PPMS. All $\bar{D}$ histogram-derived metrics of the GM were strongly correlated with the T2 lesion volume.**

CONCLUSIONS: **This study confirms the presence of brain GM changes in patients with MS. It also shows that the extent of such changes is greater during the progressive forms of the disease.**

Although CNS white matter is the preferential site of the pathologic abnormalities associated with multiple sclerosis, postmortem studies (1–4) have shown that brain gray matter (GM) of patients with MS is also not spared. A considerable proportion of discrete MS lesions are located in the cerebral GM (1–4). Retrograde degeneration of GM neurons secondary to white matter damage is also likely to contribute to GM pathologic abnormalities in cases of MS. Because of their sizes and relaxation characteristics and because of partial volume effects with CSF, GM lesions are usually missed on T2-weighted images (3, 5). Although the sensitivity of fast fluid-attenuated inversion recovery (6, 7) images for GM lesion detection is higher than that of T2-weighted images, the ability of conventional MR imaging to detect and quantify MS-related GM pathologic abnormalities is still limited.

To overcome these limitations, we recently developed an automated technique, based on fractional anisotropy thresholding, to segment large portions of brain GM (5). Using this approach, we quantified the extent of GM pathologic abnormalities shown on magnetization transfer and diffusion tensor (DT) MR maps of patients with MS. Patients with MS had significantly decreased magnetization transfer ratios and significantly increased water diffusivity ($\bar{D}$) of GM than did matched healthy volunteers (5). The present study was based on a much larger sample of patients and was performed to confirm previous preliminary observations. The large sample size also allowed investigation of whether GM damage is present in all the main clinical phenotypes of MS, whether its severity varies according to the disease phenotype, and whether its severity is correlated with the extent of lesions visible on T2-weighted MR images.

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TABLE 1: Demographic, clinical, and MR imaging characteristics of patients with different multiple sclerosis phenotypes

	RRMS	SPMS	PPMS
Mean age in years (SD)	34.8 (7.5)	47.8 (9.1)	50.8 (7.5)
Mean disease duration in years (range)	6.5 (2–17)	15.5 (3–34)	10.0 (2–20)
Median EDSS score (range)	1.5 (1.0–4.5)	6.0 (3.5–7.5)	6.0 (3.0–8.5)
Median T2 lesion load in milliliters (range)	12.2 (0.4–38.2)	31.2 (6.1–87.7)	15.1 (1.7–65.0)
Mean brain volume in milliliters (SD)	1137 (98)	1074 (91)	1116 (108)

Note.—RRMS indicates relapsing-remitting multiple sclerosis; SPMS, secondary progressive multiple sclerosis; PPMS, primary progressive multiple sclerosis; EDSS, Expanded Disability Status Scale.

TABLE 2: Average diffusivity (SD) metrics in gray matter of control volunteers and of different multiple sclerosis phenotypes studied

	Controls	All MS	RRMS	SPMS	PPMS
Average $\bar{D}$	1.02 (0.04)	1.13 (0.09)	1.05 (0.05)	1.20 (0.07)	1.13 (0.06)
$\bar{D}$ peak height	73.0 (11.5)	55.0 (12.7)	67.0 (11.2)	45.8 (7.6)	52.3 (8.1)
$\bar{D}$ peak location	0.86 (0.04)	0.92 (0.08)	0.89 (0.06)	0.95 (0.09)	0.90 (0.07)

Note.—Average diffusivity and the peak location of the average diffusivity histogram are expressed in units of $mm^2s^{-1} \times 10^{-3}$. RRMS indicates relapsing-remitting multiple sclerosis; SPMS, secondary progressive multiple sclerosis; PPMS, primary progressive multiple sclerosis; $\bar{D}$ = mean diffusivity. For further details and statistical analysis, see text.

Methods

We studied 102 patients (60 women and 42 men; mean age, 44.5 years; age range, 21–63 years) with MS. The median disease duration was 10 years (range, 2–34 years), and the median Expanded Disability Status Scale score (8) was 4.5 (range, 1.0–8.5). According to the criteria presented by Lublin and Reingold (9), 35 patients were classified as having relapsing-remitting (RR) MS, 36 as having secondary progressive (SP) MS, and 31 as having primary progressive (PP) MS. None of these patients participated in our previous study assessing GM abnormalities associated with MS (5). At the time of MR imaging examination, all patients with RRMS and SPMS had been free from relapses and steroid treatments for at least 3 months. The demographic and clinical characteristics of the three groups of patients are summarized in Table 1. Thirty healthy volunteers (18 women and 12 men; mean age, 42.4 years; age range, 22–62 years) served as control participants. Local ethics committee approval and written informed consent from all participants were obtained before study initiation.

On a single occasion and using a 1.5-T magnet, the following pulse sequences were performed in all participants: dual-echo turbo spin-echo (3300/16, 98 [TR/first TE, second TE] with an echo train length of 5); T1-weighted conventional spin-echo (768/14); pulsed-gradient spin-echo echo-planar pulse sequence (inter-echo spacing, 0.8; TE, 123), with diffusion gradients applied in eight non-collinear directions, chosen to cover 3D space uniformly (10). The duration and maximum amplitude of the diffusion gradients were 25 ms and 21 mTm^{-1}, respectively, yielding a maximum b factor in each direction of 1044 s mm^{-2}. To optimize the measurement of diffusion, only two b factors were used (11) ($b_1 \approx 0$, $b_2 = 1044$ s mm^{-2}). Fat saturation was performed using a 4-RF binomial pulse train to avoid chemical shift artifact. A birdcage head coil of approximately 300 mm in diameter was used for signal transmission and for reception. For the dual-echo and T1-weighted images, 24 contiguous interleaved axial sections were acquired with 5-mm section thickness, a 256 × 256 matrix, and a 250 × 250 mm field of view. The sections were positioned to run parallel to a line that joins the most inferoanterior and inferoposterior parts of the corpus callosum (12). For the echo-planar images, 10 5-mm-thick sections were acquired, with the same orientation of the other image sets, positioning the penultimate caudal section to match exactly the central sections of the other image set. This brain portion was chosen because these central sections are less affected by the distortions caused by B_0 field inhomogeneity, which can affect image co-registration. A 128 × 128 matrix and 240 × 240 mm field of view were used. The manufacturer's phase correction and regridding algorithm were used before Fourier transformation and interpolation to a 256 × 256 image matrix.

All MR image analysis and postprocessing were performed by a single observer, unaware of to whom the images belonged. After lesion identification on dual-echo images, lesion volumes were measured using a segmentation technique based on local thresholding (13). The volume of the whole brain was measured using a seed-growing technique for brain parenchyma segmentation (14). The technical aspects of this method, which are characterized by very high intra-observer reproducibility, are reported elsewhere (14). We produced $\bar{D}$ histograms of cerebral GM, as previously described (5, 15). To correct for the between-participant differences in GM volume, each histogram was normalized by dividing the height of each bin by the total number of pixels contributing to the histogram. For each histogram, the following measures were derived: the relative peak height (ie, the proportion of pixels at the most common $\bar{D}$ value), the peak location (ie, the most common $\bar{D}$ value), and the average $\bar{D}$.

The following five comparisons were decided a priori to assess the differences in $\bar{D}$ histograms of the GM from the different clinical phenotypes studied: control volunteers versus all MS cohort, control volunteers versus patients with RRMS, control volunteers versus patients with PPMS, patients with RRMS versus patients with SPMS, and patients with PPMS versus patients with SPMS. The nature of the contrasts was decided based on clinical considerations (ie, MS onset is either RRMS or PPMS, RRMS can evolve to SPMS, patients with SPMS and PPMS experience progressive accumulation of disability). This analysis was conducted using an analysis of variance model with age and brain volume as covariates. The Bonferroni correction was also applied to correct for multiple comparisons. As a consequence, only P values $\leq .003$ were considered significant. Univariate correlations were evaluated using the Pearson Correlation Coefficient.

Results

No abnormalities were found on the images of the control volunteers. Their mean brain volume was 1156 mL (SD, 100 mL). Conventional MR findings for the three MS phenotypes are presented in Table 1. Diffusivity histogram-derived metrics of the GM of all patients with MS, each of the three MS clinical

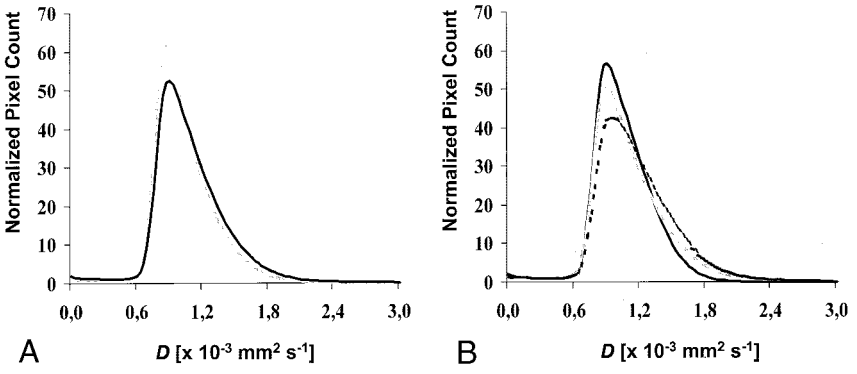

FIG 1. $\bar{D}$ histograms of the whole patient population, of each clinical subgroup, and of the control volunteers.

A, GM from normal control volunteers (*gray line*) and whole patient population (*black line*).

B, GM from patients with RRMS (*black line*), patients with PPMS (*gray line*), and patients with SPMS (*dotted line*).

TABLE 3: Results of the age-adjusted, brain volume-adjusted, and Bonferroni-corrected comparisons among control volunteers and different multiple sclerosis clinical phenotypes

	Average $\bar{D}$	$\bar{D}$ Histogram Peak Height	$\bar{D}$ Histogram Peak Position
Control volunteers vs. all patients with MS	<0.00001	<0.00001	0.003
Control volunteers vs. RRMS	0.93	0.08	0.98
Control volunteers vs. PPMS	0.00001	0.00001	0.14
RRMS vs. SPMS	<0.00001	<0.00001	0.0009
PPMS vs. SPMS	0.0002	0.07	0.12

Note.—MS indicates multiple sclerosis; RRMS, relapsing-remitting multiple sclerosis; SPMS, secondary progressive multiple sclerosis; PPMS, primary progressive multiple sclerosis. For further details and statistical analysis, see text.

phenotypes, and control volunteers are reported in Table 2. Figure 1 shows the $\bar{D}$ histograms of the whole patient population, of each clinical subgroup, and of the control volunteers. The age-adjusted, brain volume–adjusted, and Bonferroni-corrected *P* values of the five a priori contrasts for the GM $\bar{D}$ histogram metrics are reported in Table 3. All $\bar{D}$ histogram-derived metrics of the GM were significantly different between control volunteers and the whole MS population. No significant difference was found for any of the $\bar{D}$ histogram-derived metrics between control volunteers and patients with RRMS obtained in isolation. This was not the case for the comparisons between control volunteers and patients with PPMS, for whom significant differences were found for $\bar{D}$ and $\bar{D}$ histogram peak location. All the $\bar{D}$ histogram-derived metrics differed significantly between patients with RRMS and patients with SPMS. Patients with SPMS had significantly lower $\bar{D}$ obtained from GM histograms than did patients with PPMS.

Average $\bar{D}$ obtained from GM histograms (r, 0.70; $P < .0001$), $\bar{D}$ histogram peak height (r, 0.57; $P < .0001$), and $\bar{D}$ histogram peak position (r, 0.58; $P < .0001$) were strongly correlated with the T2 lesion volume.

Discussion

The traditional description of MS emphasizes the notion that this disease is an inflammatory and demyelinating condition of the white matter of the CNS. Consistent with this, a large number of MR imaging studies have been devoted to the detection, description, and quantification of white matter abnormalities of the brain and spinal cord of patients with MS (16). The increased use of MR imaging in the assessment of MS has, however, disclosed that the extent of white matter abnormalities measured using conventional MR imaging contributes only partially to the clinical manifestations of the disease, including the accumulation of irreversible disability. One of the main reasons for this discrepancy is likely to be the inability of conventional MR imaging to detect subtle structural changes (including axonal loss) known to occur in the so-called normal appearing tissue of the brain of patients with MS (1–4, 17, 18). In this context, several studies have shown that quantitative MR imaging technology is sensitive for the detection of subtle tissue changes in the white matter of patients with MS, which appear normal on conventional MR images (19).

This study shows that brain GM is also not spared by the MS pathologic process. This is in agreement with the results of preliminary studies (5, 20–22) based on smaller samples of patients and using different MR imaging technology. Although correlative studies with histopathology are needed to confirm the nature of GM pathologic abnormalities in cases of MS, retrograde degeneration of neurons secondary to the damage of fibers transversing MS white matter lesions and occult GM lesions (which previous postmortem studies [1–4] have shown to be relatively frequent in the cerebral GM of patients with MS) are both likely to contribute to increased water diffusivity in the cerebral GM of patients with MS. Considering that it has been reported that fractional anisotropy of normal appearing white matter of patients with MS is significantly reduced (23, 24) and considering that our segmentation technique (based on fractional anisotropy thresholds) might have classified pixels actually belonging to diseased subcortical white matter as GM pixels in patients with MS, we cannot exclude a con-

tribution of subcortical white matter damage to the observed $\bar{D}$ histogram changes.

The number of patients we studied also enabled us to investigate whether GM changes are present in all three major clinical phenotypes of the disease. After adjusting for differences attributable to age and brain volume, we found that none of the $\bar{D}$ histogram-derived metrics differed significantly between normal control volunteers and patients with RRMS, whose $\bar{D}$ histogram-derived metrics were significantly different from the corresponding quantities of patients with SPMS. Although this was not a longitudinal study (and, as a consequence, caution must be used when drawing any conclusion about MS evolution), the finding of more extensive cerebral GM pathologic abnormalities in patients with SPMS than in those with RRMS is consistent with the more profound cognitive impairment found in patients with SPMS (25). This supports the concept that GM pathologic abnormalities associated with MS are, at least in part, secondary to lesion accumulation in the white matter. We also found (again, after adjusting for differences attributable to age and brain volume) that patients with PPMS had significantly increased water diffusivity of GM than did normal volunteers. Interestingly, we also found that patients with PPMS had significantly lower $\bar{D}$ obtained from GM histograms than did patients with SPMS but reduced (albeit not significantly) $\bar{D}$ histogram peak height. Because $\bar{D}$ histogram peak height is considered to be a measure of the residual amount of truly normal tissue (17, 26), these findings suggest that GM pathologic abnormalities differ between the two major progressive forms of the disease, being more severe in SPMS but perhaps more diffuse in PPMS.

Conclusion

This study confirms that cerebral GM is not spared by the MS pathologic process. The large sample we studied also enabled us to investigate whether GM changes are present in all major clinical phenotypes of the disease. Interestingly, we found that GM pathologic abnormalities seem to be more pronounced in patients with the progressive forms of the disease than in those with RRMS. This suggests that increased GM pathologic abnormality might be an additional factor contributing to the presence and severity of cognitive impairment in patients with progressive MS (25, 27).

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