ON-LINE APPENDIX: METHODS

MR Imaging Data Acquisition

All MR images were acquired using the same 3T scanner (Tim Trio; Siemens, Erlangen, Germany). Acquisition protocol included the following sequences—MPRAGE: TR = 2500 ms, TE = 2.8 ms, TI = 900 ms, flip angle = 9°, voxel size = 1 × 1 × 1 mm^3, 176 sagittal slices, used for volumetric analyses; 3D-FLAIR: TR = 6000 ms, TE = 396 ms, TI = 2200 ms, flip angle = 120°, voxel size = 1 × 1 × 1 mm^3, 160 sagittal slices, for the quantification of demyelinating lesion load volume; an echo-planar imaging sequence (TR = 2000 ms, TE = 40 ms, 50 volumes, voxel size = 1 × 1 × 5 mm^3, 17 axial slices) for the DSC-PWI analysis; and an unenhanced 3D double-echo FLASH sequence (TR = 28 ms, TE_1 = 7.63 ms, TE_2 = 22.14 ms, flip angle = 20°, voxel size = 0.65 × 0.65 × 1.3 mm^3, 128 axial slices) for the calculation of QSM images.

Furthermore, in a subgroup of 59 patients (38 with RRMS and 21 with PMS) and 38 HC, an echo-planar imaging sequence (TR = 5200 ms, TE = 82 ms, 64 directions uniformly distributed in 3D space, b factors = 0 and 1000 s/mm^2, 9 B_0 images equally spaced throughout the DTI acquisition, voxel size = 1.8 × 1.8 × 3.3 mm^3, 45 axial slices) for a DTI analysis was also acquired.

MR Imaging Data Processing

For all patients, hyperintense lesions on FLAIR images were identified and segmented in consensus by 3 observers with specific training in brain imaging using a semiautomatic approach (Jim 7; http://www.xinapse.com/home.php), and lesion load volume was obtained for all patients.

Lesion masks were coregistered via affine registration to the MPRAGE and were used to correct for the possible impact of white matter lesions using the in-painting procedure implemented in FSL, Version 5.0.10 (FMRIB Software Library; http://www.fmrib.ox.ac.uk/fsl).[1] Lesions were filled with mean signal intensity values similar to those available in the normal-appearing WM. On lesion-filled MPRAGE images, gray matter and WM volumes were obtained via SIENAX (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/SIENA),[2] and the normalized volumes (namely, normalized GM, normalized WM, and normalized brain volumes, calculated as the sum of GM and WM) were obtained by multiplying by the V-scaling factor. DGM segmentation was achieved using the FIRST routine (FMRIB Integrated Registration and Segmentation Tool) to automatically obtain masks for both the right and left caudate nuclei, globus pallidus, putamen, and thalami. Similar to the calculation of the above-mentioned normalized volumes, DGM volumes were also normalized by multiplying by the V-scaling factor.

From the DTI datasets, fractional anisotropy and mean diffusivity maps were calculated as follows: Briefly, images were preliminary corrected for head motion and eddy current distortions using the eddy_correct routine (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/eddy/UsersGuide),[3] while diffusion-sensitizing gradient directions were corrected according to the corresponding deformation vectors.[4] For each subject, a brain mask was obtained using the Brain Extraction Tool routine (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/BET)[5] from the B_0 images, and both FA and MD maps were then generated by fitting a diffusion tensor model at each voxel.

PWI data were analyzed using Olea Sphere, Version 2.3 software (Olea Medical) to generate rCBV and relative CBF. After noise reduction, an arterial input function was automatically produced by sampling multiple areas, with rCBV and relative CBF maps that were derived from the resulting mean arterial input function using a vascular deconvolution.

Finally, a complete description of all processing steps used to obtain the QSM maps starting from the FLASH sequences is available in Borrelli et al[6] and Palma et al.[7,8]

After the coregistration procedure, mean values were extracted from all the structures using the fslmeants command (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/Fslutils).

An experienced neuroradiologist ensured the quality of all the processing steps by visually inspecting, patient by patient, the results of segmentation and coregistration steps.

RESULTS

Results of the Partial Correlation Analyses between Advanced MR Imaging Metrics and DGM Volumes

A comprehensive representation of the correlations between each DGM metric and the respective normalized volume within the different groups is provided in On-line Table 2. Briefly, partial correlation analyses showed that in patients with MS, thalamic volume was significantly associated with MD ($r = -0.684$, $P < .001$) and χ ($r = 0.464$, $P < .001$) values. Similarly, basal ganglia volumes were mostly associated with measures of microstructural damage (ie, negative correlations with MD and/or FA values, all with $P \leq .002$). When we considered the different subgroups, in patients with RRMS, thalamic volume was correlated with MD ($r = -0.537$, $P = .001$) and χ ($r = 0.293$, $P = .05$, not significant after Bonferroni correction) values, while the caudate volume was significantly associated with MD values ($r = -0.536$, $P = .001$). On the other hand, in the PMS subgroup, the volumes of the thalamus, caudate, and putamen showed significant correlations with measures of microstructural damage (ie, negative associations with MD or FA values, all with $P \leq .001$), with additional correlations between thalamic volume and χ values ($r = 0.708$, $P < .001$) and between caudate volume and rCBV values ($r = -0.506$, $P = .02$, not significant after Bonferroni correction).

Results of the Partial Correlation Analyses between DGM Metrics and Clinical Disability

A complete list of the correlations between clinical disability and DGM metrics that proved to be different between HC and patients with MS is shown in On-line Table 3. Briefly, correlation analyses showed the presence of a significant association between DGM volumes and clinical disability with, in particular, thalamic, globus pallidus, and putamen volumes that proved to be correlated with the EDSS score ($r = -0.308$, $P = .008$; $r = -0.312$, $P = .007$; and $r = -0.303$, $P = .009$, respectively). Furthermore, thalamic susceptibility values were related to the EDSS score ($r = -0.319$, $P = .009$).

REFERENCES

1. Battaglini M, Jenkinson M, De Stefano N. **Evaluating and reducing the impact of white matter lesions on brain volume measurements.** *Hum Brain Mapp* 2012;33:2062–71 CrossRef Medline
2. Smith SM, Zhang Y, Jenkinson M, et al. **Accurate, robust, and automated longitudinal and cross-sectional brain change analysis.** *Neuroimage* 2002;17:479–89 CrossRef Medline
3. Smith SM, Jenkinson M, Woolrich MW, et al. **Advances in functional and structural MR image analysis and implementation as FSL.** *Neuroimage* 2004;23(Suppl 1):S208–19 CrossRef Medline
4. Leemans A, Jones DK. **The B-matrix must be rotated when correcting for subject motion in DTI data.** *Magn Reson Med* 2009;61:1336–49 CrossRef Medline
5. Smith SM. **Fast robust automated brain extraction.** *Hum Brain Mapp* 2002;17:143–55 CrossRef Medline
6. Borrelli P, Palma G, Tedeschi E, et al. **Improving signal-to-noise ratio in susceptibility weighted imaging: a novel multicomponent non-local approach.** *PLoS One* 2015;10:e0126835 CrossRef Medline
7. Palma G, Comerci M, Alfano B, et al. **3D non-local means denoising via multi-GPU.** In: *Proceedings of the 2013 Federated Conference on Computer Science and Information Systems*, Kraków, Poland; September 8–11, 2013:495–98
8. Palma G, Tedeschi E, Borrelli P, et al. **A novel multiparametric approach to 3D quantitative MRI of the brain.** *PLoS One* 2015;10:e0134963 CrossRef Medline

On-line Table 1: Distributions of the different DMTs within the groups

DMT	MS	RRMS	PMS
No therapy	6/77 (7.8%)	3/52 (5.8%)	3/25 (12.0%)
Interferon β-1a	15/77 (19.5%)	14/52 (26.9%)	1/25 (4.0%)
Interferon β-1b	9/77 (11.7%)	7/52 (13.5%)	2/25 (8.0%)
Glatiramer acetate	2/77 (2.6%)	0/52 (0.0%)	2/25 (8.0%)
Natalizumab	23/77 (29.9%)	15/52 (28.8%)	8/25 (32.0%)
Fingolimod	20/77 (26.0%)	13/52 (25.0%)	7/25 (28.0%)
Dimethyl fumarate	1/77 (1.3%)	0/52 (0.0%)	1/25 (4.0%)
Teriflunomide	1/77 (1.3%)	0/52 (0.0%)	1/25 (4.0%)

On-line Table 2: Correlations between each DGM metric and the respective normalized volume within the different MS groups[a]

	MS	RRMS	PMS
Thalamus			
MD	−0.684 (<.001)[b]	−0.537 (.001)[b]	−0.779 (<.001)[b]
FA	−0.141 (.30)	−0.044 (.80)	−0.199 (.43)
CBV	−0.036 (.76)	−0.081 (.58)	0.040 (.86)
CBF	0.077 (.51)	0.123 (.40)	0.074 (.75)
χ	0.464 (<.001)[b]	0.293 (.05)[c]	0.708 (<.001)[b]
Caudate			
MD	−0.474 (<.001)[b]	−0.536 (.001)[b]	−0.366 (.14)
FA	−0.427 (.001)[b]	0.204 (.24)	−0.862 (<.001)[b]
rCBV	−0.099 (.40)	−0.007 (.96)	−0.506 (.02)[c]
rCBF	0.048 (.69)	0.260 (.07)	−0.178 (.43)
χ	0.146 (.24)	−0.060 (.70)	0.304 (.19)
Putamen			
MD	0.029 (.83)	0.154 (.38)	−0.039 (.88)
FA	−0.530 (<.001)[b]	−0.245 (.16)	−0.711 (.001)[b]
rCBV	−0.156 (.18)	−0.173 (.24)	−0.192 (.39)
rCBF	0.003 (.98)	0.103 (.48)	−0.079 (.73)
χ	−0.076 (.54)	0.007 (.97)	−0.090 (.71)
Globus pallidus			
MD	0.109 (.43)	0.258 (.14)	−0.049 (.85)
FA	−0.078 (.57)	−0.001 (.99)	−0.073 (.77)
rCBV	−0.198 (.09)	−0.180 (.22)	−0.249 (.27)
rCBF	−0.168 (.15)	−0.058 (.69)	−0.176 (.43)
χ	−0.124 (.32)	−0.071 (.65)	−0.182 (.44)

Note:—rCBF indicates relative cerebral blood flow.

[a] Data are presented as Pearson correlation coefficients *r* (*P* value).

[b] Significant correlation.

[c] Not significant after Bonferroni correction.

On-line Table 3: Correlations between altered DGM metrics and EDSS[a]

MRI Metric	EDSS
Thalamus volume	−0.308 (.008)[b]
Caudate volume	−0.208 (.08)
Putamen volume	−0.303 (.009)[b]
Globus pallidus volume	−0.312 (.007)[b]
MD thalamus	0.284 (.03)[c]
MD caudate	0.173 (.20)
FA putamen	0.159 (.24)
rCBV caudate	−0.203 (.08)
χ thalamus	−0.319 (.009)[b]

[a] Data are presented as Pearson correlation coefficients *r* (*P* value).

[b] Significant correlation.

[c] Not significant after Bonferroni correction.

On-line Table 4: Results of the hierarchic multiple linear regression analysis exploring the relationship between altered DGM metrics and clinical disability in patients with MS

	Model			Predictor		
	R^2 (ΔR^2)	F (ΔF)	*P* Value	Standardized β	*T*	*P* Value
EDSS	0.322 (0.056)	4.985 (5.247)	<.001			
Thalamus volume				−0.306	−2.291	.03