On-line Appendix

MR Imaging Acquisition

Conventional MR imaging and diffusion tensor MR imaging scans of the brain were acquired from all subjects using identical 1.5T magnetic field strengths (Avanto; Siemens, Erlangen, Germany). The following MR imaging sequences of the brain were acquired from all subjects: a) axial dual-echo (DE) turbo spin-echo (TSE) (TR = 2650 ms, TE = 28–113 ms, echo-train length = 5, number of sections = 50, section thickness = 2.5 mm with no gap, matrix size = 512 × 512, FOV = 250 × 250-mm^2), b) axial pulsed-gradient spin-echo (PGSE) echo-planar diffusion MR imaging sequence (TR = 6400 ms, TE = 93 ms, number of sections = 40, section thickness = 2.5 mm with no gap, matrix size = 128 × 128, FOV = 240 × 240 mm^2), with diffusion-encoding gradients applied in 12 noncollinear directions (b factor = 1000 sec/mm^2, number of averages = 8), and c) sagittal 3D T1-weighted magnetization-prepared rapid acquisition gradient echo (MPRAGE) (TR = 2000 ms, TE = 3.93 ms, TI = 1100 ms, number of sections = 208, section thickness = 0.9 mm, matrix size = 256 × 224, FOV = 236 × 270 mm^2). All the scans were positioned following published guidelines.[1] Diffusion-weighted images were positioned with the same orientation as the DE scan, with the central section positioned to match the central section of the DE set.

In center B, using the same scanner, the following pulse sequences of the cervical cord were also acquired using a tailored cervical spine phased array coil for signal reception: a) sagittal T2-weighted TSE (TR = 4130 ms, TE = 104 ms, echo-train length = 25, number of sections = 12, section thickness = 3.0 mm, intersection gap = 0.3 mm, matrix = 448 × 336, FOV = 280 × 280 mm^2); b) sagittal 3D T1-weighted MPRAGE (TR = 1160 ms, TE = 4.24 ms, TI = 600 ms, number of sections = 88, section thickness = 0.9 mm, matrix size = 256 × 128, FOV = 115 × 230 mm^2); and c) axial 2D gradient-echo (TR = 640 ms, TE = 12 ms, number of averages = 2, number of sections = 20, section thickness = 5.0 mm, intersection gap = 0 mm, matrix size = 256 × 256, FOV = 250 × 250 mm^2) with and without an off-resonance radio-frequency saturation pulse (magnetic field strength = 1.5 kHz, flip angle = 20°).

MR Imaging Postprocessing

All images were assessed by consensus of 2 experienced observers blinded to patient identity. Brain T2 lesion loads (LL) were measured using a local thresholding segmentation technique (Jim; Xinapse System, Leicester, United Kingdom). On brain MPRAGE scans, normalized brain volume (NBV) was measured using the cross-sectional version of the fully automated Structural Imaging Evaluation of Normalized Atrophy (SIENAx) software.[2]

Using an in-house software, from diffusion-weighted images, the diffusion tensor was estimated for each voxel using linear regression,[3] and mean diffusivity (MD) and fractional anisotropy (FA) maps derived.[4] MD histograms of the normal-appearing white matter (NAWM) and gray matter (GM) and FA histograms of the NAWM were produced as previously described.[5] FA histograms were derived only for the NAWM, because no preferential direction of water molecular motion is expected to occur in the GM, due to the absence of a microstructural anisotropic organization of this tissue compartment. For each histogram, the average MD and FA were measured.

A FA atlas was created based on data from 24 healthy subjects (15 women and 9 men, mean age = 31.8 years, range = 21–40), with no previous history of neurologic dysfunction (reference group).[6–8] Then, using diffusion tensor MR imaging tractography, probability maps of the corpus callosum, corticospinal tract, thalamocortical connection (TCC), inferior fronto-occipital fasciculus (IFOF), uncinate fasciculus, cingulum, arcuate fasciculus, inferior longitudinal fasciculus (ILF), optic radiation (OR), superior cerebellar peduncle (SCP), and middle cerebellar peduncle (MCP) were produced, as described elsewhere.[6–8] MD and FA maps from controls, as well as MD, FA and lesion maps from patients were non-linearly transformed[9] to the FA atlas, using the FA maps to calculate the transformation. T2-visible lesions were removed from MD and FA maps, and WM fiber bundle probability maps were applied to patients' data to obtain average MD and FA of the tracts of interest. For white matter (WM) fiber bundles with a bilateral location in the brain, the averages of the MD and FA values measured in the right and left hemisphere entered the analysis. Volumes of T2-visible lesion in the different WM fiber bundles were derived by applying fiber bundle probability maps, obtained using diffusion tensor MR imaging tractography, and calculating the volume of lesions located inside each of them.[6] Figure 1 in the text shows an example of WM fiber bundle reconstruction in an individual subject prior to constructing the probability maps.

Cervical cord hyperintense lesions were identified on the sagittal T2-weighted scans. The original cervical cord MPRAGE data were reformatted and a set of 5 contiguous, 3-mm-thick axial sections were reconstructed using the center of the C2-C3 disk as the caudal landmark. Then a semiautomated technique was used to segment the cord tissue and to measure the cross-sectional cord area at the level of each section.[10] Values from the 5 sections were averaged to obtain a single value for each subject.

After coregistration of the 2 cord gradient-echo scans (ie, with and without the magnetization transfer > saturation pulse), MT ratio (MTR) images were derived pixel by pixel, using an automated technique based on pixel similarity measures. Extra cord tissue was then removed from the MTR maps using a local thresholding segmentation technique (Jim; Xinapse System) and the corresponding average MTR values obtained.[11]

Statistical Analysis

Between-group differences were assessed using the nonparametric Kruskall-Wallis test. Correlations between clinical and MR-derived quantities were estimated using the Spearman rank correlation coefficient.

Random forest analysis was performed to classify clinically impaired vs. unimpaired patients using a set of MR imaging covariates (including measures of global brain and cord damage as well as selective damage to brain WM fiber bundles). Because the number of observations for each value of the functional system and EDSS scales was small, potentially leading to unreliable results, we dichotomized the FS into impaired (FS

≥ 1) and nonimpaired (FS = 0) and the EDSS scale according to a cutoff of 4.0 (which identifies fully ambulatory patients). According to the random forest technique, 100 000 trees were built to classify compromized patients.[12] The training set used to grow each tree was a .632+ bootstrap resample of the observations.[13] Trees were allowed to grow to their full size without pruning; nodes with at least 1 event and minimum total size of 5 were used as stopping rules. The best split at each node was selected from a random subset of covariates. The left-out observations (ie, "out of bag" observations) were then predicted to obtain the classification error rate of the considered tree. Predictive ability of the classifier was assessed aggregating the single tree error rates. Furthermore, the random forest framework allowed us to estimate the importance of a variable by looking at how much the classification error increases when "out of bag" data for that variable are permuted while all others are left unchanged. We followed Strobl et al[14] to avoid possible bias in variable selection: individual classification trees were built using subsampling without replacement and adopting a conditional permutation scheme.[15]

The goodness of the fit of the classifier is reported as an "error rate," which is the rate of misclassified patients. When the random forest shows a large error rate, this may be due to at least 2 reasons: 1) the covariates do not explain well the patient's status, 2) the sample design is very unbalanced (eg, 95% compromised and 5% non-compromised patients). An output of the random forest corresponds to variable importance reported as a ranking: each covariate gets a score according to its ability to classify correctly the patient according to the decrease of classification accuracy. When the random forest achieved an error rate close to 100% in 1 of the outcome classes, the derived variable importance was not considered reliable.

Because cervical cord MR imaging was acquired in 1 center only, subgroup analysis was also required. Therefore, all the random forest analyses were re-run, including data from center B only, in order to confirm and/or understand better the importance of cord MR-derived variables.

A P value $<.05$ was considered as significant. All analyses were performed using SAS Release 9.1. For the random forest analysis, we used the package 'randomForest' version 4.5 implemented in R software (A language and environment for statistical computing; version 2.12.0).

References

1. Miller DH, Barkhof F, Berry I, et al. **Magnetic resonance imaging in monitoring the treatment of multiple sclerosis: concerted action guidelines.** *J Neurol Neurosurg Psychiatry* 1991;54:683–88
2. Smith SM, De Stefano N, Jenkinson M, et al. **Normalized accurate measurement of longitudinal brain change.** *J Comput Assist Tomogr* 2001;25:466–75
3. Basser PJ, Mattiello J, LeBihan D. **MR diffusion tensor spectroscopy and imaging.** *Biophys J* 1994;66:259–67
4. Basser PJ, Pierpaoli C. **Microstructural and physiological features of tissues elucidated by quantitative-diffusion-tensor MRI.** *J Magn Reson B* 1996;111:209–19
5. Ceccarelli A, Rocca MA, Falini A, et al. **Normal-appearing white and grey matter damage in MS. A volumetric and diffusion tensor MRI study at 3.0 Tesla.** *J Neurol* 2007;254:513–18
6. Pagani E, Filippi M, Rocca MA, et al. **A method for obtaining tract-specific diffusion tensor MRI measurements in the presence of disease: application to patients with clinically isolated syndromes suggestive of multiple sclerosis.** *Neuroimage* 2005;26:258–65
7. Rocca MA, Pagani E, Absinta M, et al. **Altered functional and structural connectivities in patients with MS: a 3-T study.** *Neurology* 2007;69:2136–45
8. Rocca MA, Valsasina P, Ceccarelli A, et al. **Structural and functional MRI correlates of Stroop control in benign MS**. *Hum Brain Mapp* 2009;30:276–90
9. Rohde GK, Aldroubi A, Dawant BM. **The adaptive bases algorithm for intensity-based nonrigid image registration.** *IEEE Trans Med Imaging* 2003;22:1470–79
10. Losseff NA, Kingsley DP, McDonald WI, et al. **Clinical and magnetic resonance imaging predictors of disability in primary and secondary progressive multiple sclerosis.** *Mult Scler* 1996;1:218–22
11. Filippi M, Rocca MA. **Magnetization transfer magnetic resonance imaging of the brain, spinal cord, and optic nerve.** *Neurotherapeutics* 2007;4:401–13
12. Breiman L. **Random forests.** *Machine Learning* 2001;45:5–32
13. Efron B, Tibshirani R. **Improvements on cross-validation: The .632+ bootstrap method.** *Journal of the American Statistical Association* 1997;92:548–60
14. Strobl C, Boulesteix AL, Zeileis A, et al. **Bias in random forest variable importance measures: illustrations, sources and a solution.** *BMC Bioinformatics* 2007;8:25
15. Strobl C, Boulesteix AL, Kneib T, et al. **Conditional variable importance for random forests.** *BMC Bioinformatics* 2008;9:307

On-line Table 1: Main demographic and clinical findings from the cohorts of patients with MS studied

	CIS	RRMS	SPMS	BMS	PPMS
	(22)	(51)	(44)	(20)	(35)
Men	27.3%	25.5%	47.7%	20.0%	51.0%
Women	72.7%	74.5%	52.3%	80.0%	49.0%
Mean age (range) (yr)	29.5	41.4	49.5	45.0	46.7
	(19–43)	(22–68)	(28–67)	(31–59)	(26–70)
Mean disease duration (range) (yr)	0.06	10.3	17.8	23.3	9.7
	(0.01–0.25)	(1–30)	(4–38)	(15–40)	(1–29)
Median EDSS score (range)	2.0	2.5	6.5	2.0	5.5
	(0.0–4.0)	(0.0–4.0)	(3.5–8.5)	(0.0–3.0)	(2.0–7.5)

Note:—CIS indicates clinically isolated syndromes.

On-line Table 2: Number of patients with impairment in the different functional systems of the EDSS

Functional systems		CIS	RRMS	SPMS	BMS	PPMS
Pyramidal	0	5	5	0	3	0
	1	9	11	1	12	2
	2	2	7	2	5	4
	3	6	18	11	0	13
	4	0	10	23	0	13
	5	0	0	7	0	3
	6	0	0	0	0	0
Cerebellar	0	14	18	1	8	10
	1	3	6	3	6	2
	2	4	11	14	6	10
	3	1	12	24	0	11
	4	0	4	2	0	2
	5	0	0	0	0	0
Brain stem	0	15	18	5	15	9
	1	4	7	8	3	7
	2	3	18	17	2	14
	3	0	7	13	0	5
	4	0	1	1	0	0
	5	0	0	0	0	0
Sensory	0	7	12	6	11	7
	1	4	12	7	6	6
	2	10	17	14	3	14
	3	1	9	15	0	8
	4	0	1	2	0	0
	5	0	0	0	0	0
	6	0	0	0	0	0
Bowel and bladder	0	19	27	9	17	10
	1	3	11	14	2	12
	2	0	9	11	1	7
	3	0	2	7	0	4
	4	0	1	2	0	1
	5	0	1	1	0	1
	6	0	0	0	0	0
Visual	0	17	46	29	10	26
	1	3	2	7	9	5
	2	2	3	8	1	4
	3	0	0	0	0	0
	4	0	0	0	0	0
	5	0	0	0	0	0
	6	0	0	0	0	0
Cerebral (mental)	0	22	40	25	18	27
	1	0	5	5	2	2
	2	0	6	11	0	6
	3	0	0	3	0	0
	4	0	0	0	0	0
	5	0	0	0	0	0
	6	0	0	0	0	0
Ambulation Index	0	15	24	10	14	0
	1	2	12	10	4	8
	2	4	14	5	2	6
	3	1	1	3	0	10
	4	0	0	1	0	3
	5	0	0	2	0	1
	6	0	0	2	0	1
	7	0	0	4	0	1
	8	0	0	4	0	2
	9	0	0	3	0	3

Note:—CIS indicates clinically isolated syndromes.

On-line Table 3: Structural MR imaging findings from healthy controls and patients with MS studied[2]

		Healthy Controls	All Patients	CIS Patients	RRMS Patients	SPMS Patients	BMS Patients	PPMS Patients	P^b
T2 LL (ml) (SD)		–	25.3 (27.0)	4.1 (6.1)	19.2 (17.9)	43.8 (29.5)	30.9 (35.4)	21.5 (23.9)	<0.0001
NBV (ml) (SD)		1562.9 (22.9)	1499.7 (113.9)	1621.3 (96.6)	1519.4 (107.2)	1425.4 (89.7)	1480.2 (84.4)	1498.8 (104.3)	<0.0001
AL FA (SD)		–	0.31 (0.04)	0.33 (0.05)	0.31 (0.04)	0.28 (0.03)	0.34 (0.02)	0.32 (0.04)	<0.0001
AL MD (SD)		–		1.00 (0.1)	1.07 (0.1)	1.18 (0.1)	1.03 (0.1)	1.07 (0.1)	<0.0001
NAWM Average FA (SD)		0.40 (0.03)	0.37 (0.04)	0.41 (0.02)	0.37 (0.05)	0.34 (0.03)	0.38 (0.3)	0.37 (0.04)	<0.0001
NAWM Average MD (SD)		0.73 (0.02)	0.78 (0.05)	0.75 (0.02)	0.76 (0.05)	0.81 (0.06)	0.78 (0.05)	0.77 (0.03)	<0.0001
GM Average MD (SD)		0.89 (0.04)	0.96 (0.09)	0.89 (0.04)	0.95 (0.08)	1.02 (0.13)	0.96 (0.06)	0.96 (0.07)	<0.0001
Number of cervical cord lesions (range)		0	1.1 (0–6)	0.5 (0–3)	1.5 (0–6)	2 (0–5)	1 (0–4)	1 (0–5)	n.s.
Cervical cord area (ml) (SD)		68.1 (8.5)	62.9 (9.9)	69.2 (8.8)	68.6 (7.6)	56.7 (8.0)	64.2 (7.8)	56.7 (10.0)	<0.0001
Cervical cord MTR (%) (SD)		48.7 (1.5)	46.1 (2.2)	47.8 (1.2)	47.2 (1.4)	44.6 (2.2)	45.7 (2.0)	45.1 (2.3)	<0.0001
	T2 LL (ml) (SD)	–	0.82 (0.8)	0.14 (0.2)	0.66 (0.8)	1.50 (0.9)	0.74 (0.6)	0.68 (0.7)	<0.0001
	AL FA (SD)	–	0.45 (0.08)	0.55 (0.10)	0.45 (0.07)	0.40 (0.06)	0.47 (0.06)	0.47 (0.08)	<0.0001
CC	AL MD (SD)	–	0.99 (0.14)	0.86 (0.12)	0.98 (0.14)	1.10 (0.14)	0.96 (0.10)	0.98 (0.13)	<0.0001
	NAWM FA (SD)	0.63 (0.05)	0.58 (0.07)	0.63 (0.03)	0.59 (0.07)	0.52 (0.09)	0.60 (0.05)	0.60 (0.05)	<0.0001
	NAWM MD (SD)	0.74 (0.03)	0.85 (0.10)	0.77 (0.03)	0.83 (0.17)	0.95 (0.20)	0.83 (0.08)	0.82 (0.08)	<0.0001
	T2 LL (ml) (SD)	–	0.2 (0.20)	0.02 (0.04)	0.2 (0.20)	0.4 (0.30)	0.3 (0.03)	0.1 (0.20)	<0.0001
	AL FA (SD)	–	0.38 (0.09)	0.29 (0.13)	0.39 (0.09)	0.39 (0.07)	0.40 (0.07)	0.38 (0.11)	n.s.
Arcuate fasciculus	AL MD (SD)	–	0.84 (0.2)	0.58 (0.2)	0.82 (0.2)	0.95 (0.2)	0.89 (0.2)	0.80 (0.2)	<0.0001
	NAWM FA (SD)	0.50 (0.04)	0.47 (0.04)	0.50 (0.03)	0.48 (0.04)	0.45 (0.04)	0.48 (0.04)	0.48 (0.03)	<0.0001
	NAWM MD (SD)	0.71 (0.03)	0.77 (0.06)	0.74 (0.02)	0.75 (0.06)	0.80 (0.06)	0.78 (0.07)	0.76 (0.04)	<0.0001
	T2 LL (ml) (SD)	–	0.01 (0.10)	0.01 (0.07)	0.01 (0.06)	0.02 (0.30)	0.01 (0.07)	0.01 (0.06)	<0.0001
	AL FA (SD)	–	0.25 (0.07)	0.23 (0.05)	0.25 (0.07)	0.25 (0.07)	0.27 (0.10)	0.26 (0.07)	n.s.
Cingulum	AL MD (SD)	–	0.53 (0.20)	0.42 (0.02)	0.51 (0.20)	0.65 (0.30)	0.46 (0.20)	0.47 (0.09)	0.01
	NAWM FA (SD)	0.56 (0.05)	0.52 (0.06)	0.56 (0.03)	0.53 (0.06)	0.48 (0.07)	0.54 (0.05)	0.55 (0.05)	<0.0001
	NAWM MD (SD)	0.73 (0.03)	0.78 (0.07)	0.76 (0.03)	0.76 (0.05)	0.81 (0.10)	0.78 (0.05)	0.77 (0.04)	<0.0001
	T2 LL (ml) (SD)	–	0.09 (0.10)	0.01 (0.03)	0.08 (0.10)	0.2 (0.10)	0.09 (0.10)	0.07 (0.10)	<0.0001
	AL FA (SD)	–	0.38 (0.09)	0.38 (0.10)	0.38 (0.10)	0.38 (0.07)	0.36 (0.10)	0.37 (0.10)	n.s.
IFOF	AL MD (SD)	–	0.94 (0.3)	0.73 (0.3)	0.93 (0.3)	1.07 (0.2)	0.86 (0.3)	0.90 (0.3)	<0.0001
	NAWM FA (SD)	0.55 (0.05)	0.50 (0.05)	0.56 (0.04)	0.51 (0.06)	0.46 (0.05)	0.51 (0.04)	0.52 (0.04)	<0.0001
	NAWM MD (SD)	0.77 (0.03)	0.85 (0.07)	0.79 (0.04)	0.83 (0.07)	0.90 (0.07)	0.85 (0.07)	0.84 (0.05)	<0.0001
	T2 LL (ml) (SD)	–	0.8 (0.90)	0.01 (0.02)	0.06 (0.08)	0.14 (0.10)	0.08 (0.10)	0.06 (0.08)	<0.0001
	AL FA (SD)	–	0.34 (0.09)	0.33 (0.08)	0.30 (0.10)	0.35 (0.06)	0.39 (0.08)	0.34 (0.10)	n.s.
ILF	AL MD (SD)	–	0.92 (0.3)	0.76 (0.2)	0.81 (0.3)	1.07 (0.2)	0.97 (0.2)	0.87 (0.3)	0.0002
	NAWM FA (SD)	0.47 (0.04)	0.43 (0.05)	0.48 (0.04)	0.44 (0.04)	0.40 (0.06)	0.44 (0.04)	0.44 (0.04)	<0.0001
	NAWM MD (SD)	0.76 (0.04)	0.86 (0.08)	0.81 (0.05)	0.83 (0.08)	0.90 (0.08)	0.88 (0.09)	0.85 (0.07)	<0.0001
	T2 LL (ml) (SD)	–	0.03 (0.05)	0.01 (0.03)	0.03 (0.05)	0.05 (0.07)	0.02 (0.06)	0.03 (0.04)	<0.0001
	AL FA (SD)	–	0.34 (0.01)	0.32 (0.10)	0.34 (0.10)	0.39 (0.10)	0.32 (0.20)	0.32 (0.20)	n.s.
MCP	AL MD (SD)	–	0.59 (0.2)	0.54 (0.2)	0.55 (0.2)	0.68 (0.2)	0.51 (0.2)	0.57 (0.2)	n.s.
	NAWM FA (SD)	0.55 (0.04)	0.53 (0.04)	0.56 (0.02)	0.54 (0.03)	0.50 (0.04)	0.54 (0.03)	0.54 (0.03)	<0.0001
	NAWM MD (SD)	0.71 (0.02)	0.74 (0.05)	0.72 (0.02)	0.74 (0.05)	0.77 (0.05)	0.75 (0.05)	0.74 (0.04)	<0.0001
	T2 LL (ml) (SD)	–	0.01 (0.01)	0.01 (0.01)	0.01 (0.01)	0.01 (0.01)	0.01 (0.01)	0.01 (0.01)	<0.0001
	AL FA (SD)	–	0.30 (0.01)	0.35 (0.1)	0.26 (0.1)	0.31 (0.1)	0.26 (0.1)	0.31 (0.1)	n.s.
SCP	AL MD (SD)	–	0.6 (0.2)	0.62 (0.2)	0.58 (0.2)	0.76 (0.3)	0.57 (0.2)	0.62 (0.3)	n.s.
	NAWM FA (SD)	0.55 (0.04)	0.53 (0.04)	0.55 (0.03)	0.53 (0.04)	0.51 (0.05)	0.56 (0.05)	0.54 (0.03)	0.0004
	NAWM MD (SD)	0.87 (0.04)	0.93 (0.07)	0.90 (0.06)	0.91 (0.07)	0.97 (0.08)	0.93 (0.07)	0.93 (0.05)	<0.0001
	T2 LL (ml) (SD)	–	0.01 (0.001)	0.01 (0.007)	0.01 (0.02)	0.02 (0.02)	0.01 (0.008)	0.01 (0.006)	<0.0001
	AL FA (SD)	–	0.22 (0.08)	0.16 (0.05)	0.20 (0.08)	0.23 (0.07)	0.21 (0.10)	0.25 (0.08)	n.s.
Uncinate fasciculus	AL MD (SD)	–	0.74 (0.3)	0.61 (0.2)	0.70 (0.4)	0.82 (0.4)	0.66 (0.2)	0.71 (0.2)	n.s.
	NAWM FA (SD)	0.42 (0.04)	0.40 (0.04)	0.43 (0.03)	0.40 (0.04)	0.37 (0.04)	0.41 (0.03)	0.41 (0.03)	<0.0001
	NAWM MD (SD)	0.78 (0.03)	0.84 (0.07)	0.82 (0.04)	0.83 (0.07)	0.88 (0.08)	0.85 (0.09)	0.83 (0.05)	<0.0001
	T2 LL (ml) (SD)	–	0.06 (0.05)	0.01 (0.01)	0.06 (0.06)	0.1 (0.08)	0.07 (0.07)	0.04 (0.04)	<0.0001
	AL FA (SD)	–	0.44 (0.08)	0.44 (0.05)	0.44 (0.09)	0.41 (0.05)	0.48 (0.04)	0.45 (0.10)	0.05
CST	AL MD (SD)	–	0.87 (0.1)	0.76 (0.2)	0.88 (0.1)	0.91 (0.1)	0.85 (0.09)	0.83 (0.08)	0.001
	NAWM FA (SD)	0.63 (0.03)	0.62 (0.03)	0.62 (0.02)	0.61 (0.02)	0.61 (0.04)	0.62 (0.02)	0.61 (0.02)	n.s.
	NAWM MD (SD)	0.70 (0.03)	0.74 (0.05)	0.72 (0.02)	0.73 (0.05)	0.76 (0.05)	0.75 (0.05)	0.73 (0.03)	<0.0001
	T2 LL (ml) (SD)	–	0.02 (0.05)	0.001 (0.001)	0.03 (0.04)	0.05 (0.04)	0.03 (0.03)	0.02 (0.02)	<0.0001
	AL FA (SD)	–	0.46 (0.09)	0.47 (0.1)	0.43 (0.07)	0.43 (0.06)	0.48 (0.09)	0.48 (0.1)	0.03
TCC	AL MD (SD)	–	0.91 (0.1)	0.81 (0.06)	0.91 (0.1)	0.95 (0.1)	0.88 (0.1)	0.88 (0.1)	0.001
	NAWM FA (SD)	0.60 (0.04)	0.58 (0.03)	0.59 (0.02)	0.58 (0.02)	0.58 (0.04)	0.58 (0.03)	0.58 (0.03)	0.02
	NAWM MD (SD)	0.71 (0.03)	0.74 (0.05)	0.74 (0.02)	0.74 (0.06)	0.78 (0.05)	0.77 (0.05)	0.75 (0.03)	<0.0001
	T2 LL (ml) (SD)	–	0.05 (0.06)	0.01 (0.01)	0.05 (0.06)	0.10 (0.06)	0.06 (0.06)	0.05 (0.06)	<0.0001
	AL FA (SD)	–	0.40 (0.08)	0.42 (0.10)	0.39 (0.09)	0.38 (0.06)	0.43 (0.06)	0.40 (0.08)	n.s.
OR	AL MD (SD)	–	1.06 (0.2)	0.86 (0.3)	1.02 (0.3)	1.21 (0.2)	1.07 (0.2)	1.02 (0.3)	<0.0001
	NAWM FA (SD)	0.58 (0.05)	0.50 (0.07)	0.57 (0.04)	0.50 (0.07)	0.45 (0.07)	0.50 (0.06)	0.51 (0.06)	<0.0001
	NAWM MD (SD)	0.82 (0.04)	0.96 (0.10)	0.86 (0.05)	0.95 (0.10)	1.05 (0.20)	0.97 (0.10)	0.94 (0.07)	<0.0001

Note:—MS indicates multiple sclerosis; CIS, clinically isolated syndromes; B, benign form; PP, primary-progressive; RR, relapsing-remitting; SP, secondary-progressive; LL, lesion load; SD, standard deviation; NBV, normalized brain volume; MD, mean diffusivity; FA, fractional anisotropy; NAWM, normal-appearing white matter; GM, gray matter; MTR, magnetization transfer ratio; AL, average lesion; CST, corticospinal tract; TCC, thalamocortical connection; CC, corpus callosum; IFOF, inferior fronto-occipital fasciculus; ILF, inferior longitudinal fasciculus; MCP, middle cerebellar peduncle; SCP, superior cerebellar peduncle; OR, optic radiation; n.s., not significant.

[a] Average MD is expressed in units of $mm^2s^{-1} \times 10^{-3}$, FA is a dimensionless index.

[b] Non-parametric Kruskall-Wallis test.

On-line Table 4: Results of the random forest analysis[a,b]

Clinical Variable	MS	CIS	RRMS	SPMS	BMS	PPMS
EDSS	18.0 26.2 10.9 NAWM FA[c] CC NAWM FA[c] CC T2 LL[c] MCP NAWM FA[c]	13.6 5 100 Cingulum AL FA SCP NAWM FA NAWM MD NBV	14.2 100 0 Cingulum AL FA Cingulum AL MD MCP AL MD CC NAWM MD	39.2 25.0 63.2 SCP NAWM MD[c] CC T2 LL[c] Cingulum NAWM FA[c] SCP NAWM FA[c]	–	6.8 100 0 CC NAWM MD OR NAWM MD Uncinate NAWM MD CC NAWM FA
Ambulation Index	30.7 41.4 22.8 NAWM FA[c] Cord area MCP[c] NAWM FA[c] SCP NAWM MD[c]	35.0 23.1 57.1 CST AL FA[c] SCP NAWM FA[c] CST T2 LL[c] CST AL MD[c]	42.8 21.4 85.7 Cord MTR[c] MCP[c] NAWM MD[c] NBV[c] NAWM FA[c]	– – – –	50.0 28.6 100 NAWM FA NAWM MD CST NAWM MD MCP NAWM FA	– – – –
Brain stem FS	21.5 43.5 9.1 NAWM FA[c] MCP NAWM FA[c] NBV[c] MCP T2 LL[c]	36.4 26.7 57.1 CST T2 LL[c] MCP T2 LL[c] T2 LL[c] NAWM MD[c]	13.7 27.8 6.0 CST NAWM[c] FA[c] NBV[c] SCP NAWM MD[c] CST T2 LL[c]	13.6 100 2.5 SCP NAWM MD SCP NAWM FA OR NAWM FA OR NAWM MD	30.0 20.0 60.0 GM MD[c] NAWM FA[c] NAWM MD[c] SCP NAWM FA[c]	22.8 66.7 7.7 CST NAWM[c] FA[c] NBV[c] SCP NAWM MD[c] CST T2 LL[c]
Cerebellar FS	19.3 43.5 5.8 T2 LL[c] CST NAWM[c] MD[c] SCP NAWM FA[c] NAWM FA[c]	27.3 14.3 50.0 MCP NAWM[c] MD[c] CST NAWM FA[c] SCP NAWM MD[c] SCP NAWM FA[c]	11.7 16.7 9.1 NBV[c] CST NAWM[c] MD T2 LL[c] SCP NAWM MD[c]	– – – –	25.0 37.5 16.7 NAWM MD[c] SCP NAWM FA[c] MCP NAWM FA[c] SCP NAWM MD[c]	22.8 60.0 8.0 T2 LL[c] SCP NAWM MD[c] OR NAWM MD[c] MCP T2 LL[c]
Pyramidal FS	7.5 100 0 NBV CST T2 LL MCP NAWM FA CC NAWM FA	22.7 100 0 CST AL MD CC NAWM MD CC NAWM FA CST T2 LL	9.8 100 0 NBV MCP NAWM FA OR NAWM MD OR NAWM FA	– – – –	20.0 100.0 5.8 CST T2 LL CST NAWM FA CC NAWM FA NAWM FA	– – – –
Visual FS	27.3 5.5 91.0 CST NAWM[c] MD[c] CC NAWM MD[c] OR NAWM FA[c] OR NAWM MD[c]	22.7 0 100 NAWM MD CC NAWM MD GM MD NBV	9.8 0 100 OR T2 LL MCP NAWM FA SCP NAWM FA NAWM MD	50.0 31.0 86.7 SCP NAWM[c] MD[c] OR NAWM FA[c] OR AL MD[c] NAWM MD[c]	30.0 30.0 30.0 NAWM MD[c] CC NAWM MD[c] CST NAWM MD[c] CST NAWM FA[c]	28.6 7.7 88.9 SCP NAWM[c] MD[c] OR NAWM FA[c] NAWM MD[c] OR NAWM[c] MD[c]
Sensory FS	25.5 88.4 4.6 TCC AL FA[c] NAWM FA[c] TCC T2 LL[c] NBV[c]	9.0 0 13.0 TCC AL FA[c] TCC AL MD[c] NBV[c] TCC T2 LL[c]	17.6 33.3 12.8 Cord MTR[c] Cord area[c] TCC NAWM[c] FA[c] NBV[c]	18.2 100 5.2 NAWM FA SCP NAWM MD TCC AL MD NBV	70.0 63.6 66.6 MCP NAWM FA[c] Cord MTR[c] NBV[c] MCP NAWM MD[c]	25.7 100 7.1 NAWM FA NAWM MD MCP NAWM MD Arcuate NAWM MD
Bowel and bladder FS	22.1 21.9 22.2 Cord area[c] Cord MTR[c] CST AL FA[c] CC NAWM FA[c]	13.6 0 100 CST AL FA CST AL MD CST T2 LL MCP NAWM MD	15.6 14.8 16.6 Cord area[c] Cord MTR[c] IFOF T2 LL[c] NBV[c]	20.4 100 0 CST AL FA Cord area NAWM MD T2 LL	20.0 5.8 100 CST NAWM FA Uncinate NAWM FA Cingulum NAWM FA IFOF NAWM FA	25.7 80.0 8.0 Uncinate[c] NAWM FA[c] CST NAWM MD[c] Cingulate[c] NAWM MD[c] Uncinate T2 LL[c]
Cerebral FS	21.5 6.8 70.0 NAWM FA[c] Cingulum T2 LL[c] CC T2 LL[c] ILF T2 LL[c]	– – – –	27.4 7.5 100 Cord MTR Cord area T2 LL ILF NAWM FA	29.5 28.0 31.6 CST T2 LL[c] Cord area[c] NBV[c] IFOF T2 LL[c]	15.0 5.5 100 Cingulum T2 LL CC NAWM MD ILF NAWM FA IFOF NAWM FA	25.7 3.7 100 Cingulum T2 LL Arcuate NAWM MD NAWM FA CST NAWM MD

Note:—MTR indicates magnetization transfer ratio; IFOF, inferior fronto-occipital fasciculus; ILF, inferior longitudinal fasciculus; CIS, clinically isolated syndromes; FS, functional system; LL, lesion load; OR, optic radiation; AL, average lesion; TCC, thalamocortical connection; GE, global error; UE, unimpaired error; IE, impaired error.

[a] The divided cells represent the variable importance of GE (%), UE (%), and IE (%), respectively.

Empty cells are due to the absence of a group of patients (eg, 100% impaired and 0% unimpaired).

[c] Variables whose importance was considered reliable (the others were not considered reliable due to an error rate of 100% in 1 of the outcome classes).