STRUCTURAL MRI CORRELATES OF COGNITIVE IMPAIRMENT IN PATIENTS WITH MULTIPLE SCLEROSIS: A MULTICENTER STUDY

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Abstract

In a multicenter setting, we applied voxel-based methods to different structural MR imaging modalities to define the relative contributions of focal lesions, normal-appearing white matter (NAWM) and gray matter (GM) damage and their regional distribution to cognitive deficits as well as impairment of specific cognitive domains in multiple sclerosis (MS) patients.

Approval of the institutional review boards was obtained, together with written informed consent from all participants. Standardized neuropsychological assessment and conventional, diffusion tensor and volumetric brain MRI sequences were collected from 61 relapsing-remitting MS patients and 61 healthy controls (HC) from seven centers. Patients with ≥2 abnormal tests were considered cognitively impaired (CI). The distribution of focal lesions, GM and WM atrophy and microstructural WM damage were assessed using voxel-wise approaches. A random forest analysis identified the best imaging predictors of global cognitive impairment and deficits of specific cognitive domains.

Twenty-three (38%) MS patients were CI. Compared to cognitively preserved (CP), CI MS patients had GM atrophy of the left thalamus, right hippocampus and parietal regions. They also showed atrophy of several WM tracts, mainly located in posterior brain regions and widespread WM diffusivity abnormalities. WM diffusivity abnormalities in cognitive-relevant WM tracts followed by atrophy of cognitive-relevant GM regions explained global cognitive impairment. Variable patterns of NAWM and GM damage were associated with deficits in selected cognitive domains.

Structural, multiparametric, voxel-wise MRI approaches are feasible in a multicenter setting. The combination of different images modalities is needed to assess and monitor cognitive impairment in MS.

Key words: multiple sclerosis, cognitive impairment, diffusion tensor MRI, atrophy, voxel-wise analysis, multicenter.

Introduction

The definition of the mechanisms responsible for the presence and severity of cognitive impairment in multiple sclerosis (MS) patients is of paramount importance, given the high frequency from of such an impairment in this condition, ranging from 40 to 70% (Chiaravalloti and DeLuca, 2008), and the dramatic impact cognitive dysfunction has on activities and quality of life of patients and their caregivers.

MRI is extremely sensitive in detecting MS related tissue abnormalities, and, during the past decades, several quantitative MRI techniques, capable to estimate different aspects of MS pathology have been developed and are currently being used. The application of these techniques to define the structural MRI correlates of cognitive impairment in different cohort of MS patients has shown that global and regional damage of brain white matter (WM) and gray matter (GM) in terms of focal lesions (Calabrese, et al., 2012; Roosendaal, et al., 2009; Rossi, et al., 2012), diffuse microstructural abnormalities (Dineen, et al., 2009; Hulst, et al., 2013; Mesaros, et al., 2012), and irreversible tissue loss (Amato, et al., 2007; Calabrese, et al., 2010b) play a significant role for the presence and severity of cognitive impairment.

Only a few studies have integrated measures of focal lesions, normal-appearing (NA) WM injury and GM involvement to assess the relative contribution of each of these factors to cognitive deficits in MS (Hulst, et al., 2013; Llufriu, et al., 2014). Interestingly, these studies showed that damage of WM tracts, quantified using diffusion tensor (DT) MRI, influences significantly cognitive performance in MS, whereas GM damage seems to add no (Hulst, et al., 2013) or only a small increment to the variance explained by WM damage (Llufriu, et al., 2014).

To identify objective outcome measures of cognitive impairment to be applied not only for patients monitoring, but also as a target for innovative treatment strategies, several unmet needs still remain. These include the confirmation of the previous findings in different cohorts of patients and their validation in a multicenter context.

Against this background, we applied voxel-based methods to different structural MRI modalities to define the relative contributions of focal lesions, NAWM and GM damage and their regional distribution to cognitive deficits as well as impairment of specific cognitive domains in MS patients in a multicenter setting.

Materials and methods

This study was approved by the Local Ethical Committes on human studies in each participant center and written informed consent from each subject was obtained prior to their enrolment.

Subjects. Subjects were recruited from January 2009 to May 2012 as part of a project on imaging correlates of cognitive impairment in MS at seven European centers (www.magnims.eu), which included: a) the Department of Radiology, VU University Medical Centre, Amsterdam (Netherlands) (9 healthy controls [HC] and 8 MS patients); b) the CEM-Cat, Hospital Vall d'Hebron, Barcelona (Spain) (3 HC and 8 MS patients); c) the Research Unit for Neuronal Repair and Plasticity at the Dept. of Neurology, Medical University of Graz, Graz (Austria) (12 HC and 7 MS patients); d) the Queen Square Imaging Centre, Institute of Neurology, University College London, London (UK) (9 HC and 7 MS patients); e) the Neuroimaging Research Unit, San Raffaele Scientific Institute, Milan (Italy) (10 HC and 10 MS patients); f) the MRI Center "SUNFISM", Second University of Naples, Naples (Italy) (8 HC and 11 MS patients); and g) the Department of Neurological and Behavioral Sciences, University of Siena, Siena, Italy (10 HC and 10 MS patients).

The inclusion criteria for this study required all subjects to be right-handed and aged between 20 and 65 years. In addition, patients had to have a diagnosis of relapsing remitting (RR) MS (Lublin, et al., 2014; Polman, et al., 2011), no relapse or corticosteroids treatment within the month prior to scanning and no history of psychiatric conditions, including major depression.

The final dataset used for this analysis included 61 RRMS patients (21/40 men/women; mean age = 39.7 years, standard deviation [SD] = 8.5 years; mean disease duration = 8.2 years, range = 2-33 years; median Expanded Disability Status Scale [EDSS] score = 1.5, range = 0.0-6.0) and 61 healthy controls (HC) (26/35 men/women, mean age = 36.0 years, SD = 9.5 years). Sex did not differ significantly between HCs and MS patients (p=0.3), whereas HCs were significantly younger than MS patients (p=0.02) and had more years of education ($p<0.0001$). As a consequence age was included as a covariate in all statistical models.

Clinical and neuropsychological assessment. Within 48 hours from the MRI acquisition, MS patients underwent a neurological evaluation with rating of the EDSS score and a neuropsychological assessment, performed at each participating site by an experienced neurologist and neuropsychologist, unaware of the MRI results, using validated translations of the neuropsychological tests. Cognitive performance was assessed using the Brief Repeatable Battery of Neuropsychological Tests (BRB-N) (Rao, 1991), only marginally influenced by language or cultural differences (Sepulcre, et al., 2006). BRB-N includes the Selective Reminding Test (SRT), to assess verbal memory; the 10/36 Spatial Recall Test (10/36 SRT), to assess visual memory; the Symbol Digit Modalities Test (SDMT) and the Paced Auditory Serial Addition Test (PASAT) 2" and 3", to assess attention and information processing speed; and the Word List Generation (WLG) test, to assess verbal fluency. As previously described (Rocca, et al., 2014; Sepulcre, et al., 2006), raw data were corrected according to normative values and Z-scores for each of the previous domains were calculated.

In addition, the Wisconsin Card Sorting Test (WCST) was administered to evaluate executive functions (Heaton, 1993). Performance at the WCST was evaluated by computing scores related to the total errors (WCSTte), the number of perseverative errors (WCSTpe), and the number of perseverative responses (WCSTpr). Patients with a score ≤ 2SD in at least one of these measures were considered impaired at the WCST (Mattioli, et al., 2010).

Patients were considered as cognitively impaired (CI) if they had at least two abnormal tests (defined as a score more than 2 SDs below the normative value provided by Boringa et al. (Boringa, et al., 2001) for the BRB-N and by Heaton et al. (Heaton, 1993) for the WCST).

MRI acquisition. Brain MRI scans were obtained using magnets operating at 3 Tesla at all sites (Amsterdam and Naples: GE Signa; Barcelona, Graz and London: Siemens Trio; Milan and Siena: Philips Intera). The following sequences were collected during a single session: a) dual-echo turbo-spin-echo (TSE): TR=ranging from 4000 to 5380 ms, TE_1=ranging from 10 to 23 ms, TE_2=ranging from 90 to 102 ms, echo train length (ETL)=ranging from 5 to 11, 44 contiguous, 3-mm thick axial slices, parallel to the AC-PC plane, with a matrix size=256x192 and a FOV= 240x180 mm^2 (recFOV=75%); b) 3D T1-weighted scan: TR=ranging from 5.5 to 8.3 ms (for GE/Philips scanners) and from 1900 to 2300 ms (for Siemens scanners); TE=ranging from 1.7 to 3.0 ms; flip angle ranging from 8° to 12°, 176 to 192 sagittal slices with thickness=1 mm and in-plane resolution=1x1 mm^2; c) pulsed gradient spin echo (PGSE) (Stejskal, 1965) single-shot echo planar imaging (SS-EPI) sequence with a double-refocused variant (Reese, et al., 2003) on all Siemens scanners to minimize eddy-current distortions, and single-echo EPI acquisition on all Philips scanners. The following target scan parameters were used: TR: 6000–12000 ms; TE: 70–100 ms, FOV: 320x240 mm^2; acquisition matrix: 128x96, 50 slices with an isotropic resolution (cubic voxels) of 2.5 mm. Thirty DW volumes (Jones, 2004) were acquired, each with a different diffusion encoding gradient vector direction, and with a constant b-factor of 900 s/mm^2. On scanners where it was not possible to use 30 directions because of pulse sequence limitations, the maximum number available was used, but with an increased number of repetitions such that the product of the number of DW directions and the number of repetitions was kept close to 30. Parallel acquisition with a reduction factor=2 was used (Pagani, et al., 2010).

MRI analysis. MRI data analysis was done centrally at the Neuroimaging Research Unit (Milan, Italy) by experienced observers blinded to subjects' identity.

Brain T2-hyperintense and T1-hypointense lesion volumes (LV) were measured on dual-echo TSE and 3D T1-weighted scans, respectively, using a local thresholding segmentation technique (Jim 6.0, Xinapse Systems, West Bergholt, UK). Normalized brain (NBV), WM (WMV) and GM (GMV) volumes were measured on 3D T1-weighted scans using the SIENAx software, after T1-hypointense lesion refilling (Chard, et al., 2010). In MS patients, to create maps of T2-hyperintense lesions, binarized masks from T2 lesions were obtained, coregistered to the 3D T1-weighted scans (using the rigid transformation calculated between the T2-weighted and the 3D T1-weighted image), normalized to the standard SPM space (using the DARTEL non-linear transformation calculated for the Voxel-Based Morphometry [VBM] analysis), and averaged to obtain T2 lesion probability maps (LPMs).

VBM analysis was performed using SPM8. First, 3D T1-weighted images were segmented into GM, WM and cerebrospinal fluid (CSF). Then, GM and WM segmented images of all subjects, in the closest possible rigid-body alignment with each other, were used to produce GM and WM templates and to drive the deformation to the templates. At each iteration, the deformations, calculated using the Diffeomorphic Anatomical Registration using Exponentiated Lie algebra (DARTEL) registration method, were applied to GM and WM, with an increasingly good alignment of subject morphology, to produce the templates. Spatially normalized images were then modulated to ensure that the overall amount of each tissue class was not altered by the spatial normalization procedure. To better align the final template with the Montreal Neurologic Institute (MNI) space, an affine registration between the customized GM template and the SPM GM template (in the MNI space) was also calculated and added to the header of each image as a new orientation, in order to have all the images in a standard space. The same transformation was applied to the WM customized template. The images were then smoothed with an 8 mm FWHM Gaussian kernel.

Diffusion-weighted images were first corrected for distortions caused by the eddy currents, for movements and transformed to MNI space (http://white.stanford.edu/mrdiff). Then, using the FMRIB's Diffusion Toolbox (FDT tool, FSL 4.1, http://www.fmrib.ox.ac.uk), the diffusion tensor

(DT) was estimated in each voxel using a linear regression (Basser, et al., 1994) and mean diffusivity (MD) and fractional anisotropy (FA) maps were derived. GM and WM probability maps previously segmented from 3DT1-weighted scans were transformed to DT space, concatenating the rigid transformation between 3D T1-weighted scans and T2-weighted scans and the non linear transformation between T2-weighted scans and b=0 images. This second transformation, useful to compensate off resonance distortions was obtained using FNIRT from FSL Library. Average FA and MD values in the NAWM were obtained, masking out T2 lesions. Average MD values in the GM were also calculated.

For voxel-wise analysis, FA maps were transformed using FNIRT on FMRIB58_FA atlas, smoothed and statistically analyzed with SPM8, with the application of a WM mask, in order to consider only NAWM. The same procedure was used for MD.

Statistical analysis. Patients' clinical and MRI characteristics were reported as medians and ranges or as frequencies and percentages for continuous and categorical variables, respectively. Between-group and between-center comparisons were performed using the Pearson chi-squared test and Mann-Whitney U-test or Kruskal-Wallis test for categorical and continuous variables, respectively.

Between-group (MS patients *vs* HC and CI *vs* cognitively preserved [CP] MS patients) comparison of T2 LPMs, GM atrophy, WM atrophy and diffusivity abnormalities was performed using SPM8 and a full factorial analysis of covariance, considering group and center as factors, and including age and sex as covariates. Analyses were repeated including also education as covariate. For the analysis of atrophy, the normalization factor derived from SIENAx (which can be considered as a measure of head size) was also included. Multiple linear regression models, adjusted for age, sex and center, were used to assess the correlation between regional brain damage and neuropsychological variables (z scores of individual cognitive domains and WCST). Analyses were repeated considering also education as covariate. For all analyses run with SPM8, results were tested both at $p < 0.001$, uncorrected, and at $p < 0.05$, family-wise error (FWE) corrected (cluster

extent of = 5 voxels). The localization of areas of WM and GM atrophy and of diffusivity abnormalities was defined using available atlases (Mori, 2005; Tzourio-Mazoyer, et al., 2002).

A random forest analysis (RF) was run to identify the best predictors, among all MRI variables explored, of global cognitive impairment as well as impairment of specific cognitive domains (Breiman, 2001; Mesaros, et al., 2012). An output of the RF corresponds to variable importance reported as a ranking: each covariate receives a score according to its ability to classify or to predict correctly the patient's outcome when data are permuted. For convenience of interpretation, variable importance was normalized with respect to the best predictor. For the RF analysis, MRI measures which were significantly different between CI and CP MS patients and HC and those which showed significant correlation with neuropsychological scores were considered. C-statistics and R^2 were also reported for dichotomous and continuous outcomes, respectively.

Results

Clinical, neuropsychological, and conventional MRI measures. Table 1 summarizes the main demographic, clinical and MRI characteristics of MS patients and HC. Twenty-three (38%) MS patients were classified as CI. The cognitive domains most frequently involved were attention and information processing speed (33% of the patients), executive functions (23%), verbal fluency (21%), verbal memory (18%) and visual memory (16%).

Compared to HC, MS patients had lower NBV ($p<0.0001$), GMV ($p<0.0001$), WMV ($p<0.0001$), WM FA ($p=0.0001$) as well as higher WM MD ($p=0.03$) and GM MD ($p=0.02$). A significant center effect was found for age ($p=0.005$), EDSS ($p=0.01$), NBV ($p<0.0001$), GMV ($p=0.0001$), WMV ($p=0.04$), WM MD ($p<0.0001$) and WM FA ($p<0.0001$); whereas no effect was found for sex ($p=0.21$), education ($p=0.06$), disease duration ($p=0.18$), T2 LV ($p=0.17$), and T1 LV ($p=0.21$).

Compared to HC, both CP and CI MS patients had fewer years of education (p values=0.0001 for CP MS patients and 0.001 for CI MS patients), lower NBV, GMV and WMV (p

values ranging from <0.0001 to 0.005), and lower WM FA (p values=0.005 and 0.0002). Compared to HC, CI MS patients were also older (p=0.001). Compared to CP, CI MS patients were older (p=0.01), had higher EDSS (p=0.04), T2 LV (p=0.01) and T1 LV (p=0.008), as well as lower NBV (p=0.02) and GMV (p=0.01).

LPMs. In MS patients, T2-hyperintense lesions were mostly located in the periventricular WM and corona radiate, bilaterally. Compared to CP, CI MS patients showed a significantly higher T2 lesion frequency in periventricular regions and several WM tracts, including the inferior fronto-occipital fasciculus (IFOF), corona radiata, cingulum, corpus callosum (CC), inferior (ILF) and superior (SLF) longitudinal fasciculus, forceps major and minor and anterior thalamic radiation (ATR), bilaterally (Figures 1 and 2) (p<0.001 uncorrected).

VBM. Table 2 and Figure 1 summarize the results of GM and WM atrophy distribution among the different study groups. Compared to HC, both CP and CI MS patients showed diffuse GM atrophy involving deep GM nuclei, several fronto-parieto-temporal-occipital regions, the cingulate cortex, and cerebellum bilaterally. They also showed diffuse WM atrophy, involving the majority of brain WM tracts (p<0.001).

Compared to CP, CI MS patients showed significant GM atrophy of the bilateral postcentral gyrus, left (L) thalamus, L supramarginal gyrus and R hippocampus. They also showed significant atrophy of several clusters located in different WM tracts, including the ILF, IFOF, SLF, ATR bilaterally, L cingulum, R splenium of the CC, fornix and R uncinate fasciculus (p<0.001). Such volume differences were only partially overlapping with T2 LPM differences (Table 2 and Figure 1). Similar results were obtained when including education as a covariate (data not shown).

Voxel-wise analysis of DT MRI abnormalities. Table 3 and Figure 2 summarize the results of MD and FA abnormalities distribution among the different study groups (p<0.05 FWE corrected). Compared to HC, both CP and CI MS patients showed an increased MD in several WM tracts, including the CC, cingulum, corona radiata, ILF, IFOF, and SLF bilaterally. They also showed decreased FA in the majority of WM tracts. Compared to CP, CI MS patients showed an

increased MD of the majority of WM tracts, while FA abnormalities were more limited, with the involvement of bilateral IFOF, bilateral ILF, R forceps major, R cingulum and L SLF (Table 3 and Figure 2). Such diffusivity abnormalities were only partially overlapping with T2 LPM differences (Table 3 and Figure 2). Similar results were obtained when including education as a covariate (data not shown).

Analysis of correlations. In MS patients, GM and WM atrophy of several brain regions were significantly correlated with performances in different cognitive domains (r values ranging from 0.69 to 0.48; $p<0.001$ uncorrected) (Figure 3, Table 4). No correlation was found between regional GM atrophy and verbal memory performance as well as between regional WM atrophy and verbal memory, fluency and WCST performances. Similar results were obtained when including education as a covariate (data not shown).

In MS patients, MD and FA abnormalities of several brain WM tracts were significantly correlated with performances in different cognitive domains (r values ranging from -0.66 to 0.71; $p<0.001$ uncorrected) (Figure 4, Table 5). No correlation was found between diffusivity measures and performance at verbal memory and fluency.

Multimodal analysis. Figure 5 summarizes the results of RF analysis performed to identify the MRI variables significantly associated with the presence of cognitive impairment as well as with performance at specific cognitive domains. For each cognitive variable, the five most important MRI predictors are listed. The best predictors of global cognitive impairment were L SLF FA (C-statistic=0.89), R cingulum FA (C-statistic=0.85), L posterior corona radiata MD (C-statistic=0.82), L postcentral gyrus atrophy (C-statistic=0.86) and R hippocampal atrophy (C-statistic=0.82) (in this order of ranking).

Considering performance at specific cognitive domains, impairment of attention/information processing speed was predicted by R ILF FA (R^2=0.30), followed by R inferior frontal gyrus (IFG) atrophy (R^2=0.27) and left thalamus atrophy (R^2=0.29); visual memory impairment was associated with atrophy of the splenium of the CC (R^2=0.24) and L ILF (R^2=0.22); verbal fluency impairment

was predicted by R insula (R^2=0.08) and L postcentral gyrus atrophy (R^2=0.07); finally executive function deficits were associated with R IFG atrophy (R^2=0.14) and L IFOF MD (R^2=0.13).

The analysis was not performed for verbal memory, since no cluster resulted significantly correlated with their z scores.

Discussion

By combining voxel-wise analysis methods and a multiparametric structural MRI approach, which included both measures of irreversible tissue loss and microstructural tissue damage, this study proves the applicability of these techniques in a multicenter setting and shows that CI in this condition is the result of a complex interplay between NAWM and GM damage. Our results also support the notion that different substrates are likely to contribute to global CI and impairment of specific cognitive domains in these patients. Indeed, when considering global CI, WM diffusivity abnormalities in cognitive-relevant WM tracts followed by atrophy of critical GM regions had the highest relevance to explain cognitive performance, possibly as a consequence of a disconnection syndrome occurring between GM regions following WM damage. Conversely, impairment of selected cognitive domains was associated with variable patterns of NAWM and GM damage, reflecting structural damage to regions with a critical role for the studied function. All of this suggests that the use of a single MR modality is not sufficient to properly explain cognitive impairment in MS and to monitor its progression and the effects of therapeutic interventions, but a multimodal imaging approach is needed.

In agreement with the results from previous studies (Dineen, et al., 2009; Hulst, et al., 2013; Mesaros, et al., 2012; Roosendaal, et al., 2009) and with that of a recent meta-analysis (Welton, et al., 2015) which after including data from 12 MS studies (495 MS patients and 253 HC) has suggested that brain WM damage is more functionally relevant for cognitive dysfunction than for physical disability, we found that, compared to HC and CP patients, CI MS patients had increased MD of the majority of WM tracts as well as reduced FA of the L IFOF, R ILF, L SLF and R

anterior cingulum. Moreover, diffusivity abnormalities of several clusters in cognitive-relevant WM tracts significantly correlated with performance at different cognitive domains, except for verbal memory and fluency. The relevance of WM diffusivity abnormalities in explaining cognitive impairment is further supported by the RF analysis, which showed that diffusivity abnormalities of WM tracts enabled the best differentiation between CI and CP MS patients and also contributed to explain deficits of attention and executive functions.

WM tract damage in MS is partially driven by secondary degenerative phenomena due to the presence of focal T2 hyperintense lesions at this level (Henry, et al., 2009; Preziosa, et al., 2012; Rocca, et al., 2013), which are another contributor to cognitive dysfunction in these patients (Mesaros, et al., 2012). To define the role of focal WM lesions on our findings, we investigated the volume and distribution of T2-hyperintense lesions in MS patients based on the presence or not of CI. In agreement with previous studies (Rocca, et al., 2015a; Rossi, et al., 2012; Sepulcre, et al., 2009), CI MS patients had higher T2 LV as well as a higher probability of harboring T2 lesions in WM tracts involved in cognitive functions. Despite this, we found only a minimal overlap between diffusivity abnormalities and T2 LPMs, thus suggesting that the accumulation of NAWM damage, independently from the presence of focal lesions, is relevant for cognitive performances.

The third important substrate of cognitive impairment in MS patients is atrophy, with a more prominent role played by tissue loss in the GM rather than the WM (Rocca, et al., 2015b) and a distinct pattern of GM atrophy distribution in CI *vs* CP patients, involving several cortical regions in the frontal, parietal and temporal lobes as well as the thalami and caudate nuclei (Morgen, et al., 2006; Riccitelli, et al., 2011). This is also substantiated by our results, including the RF analysis, which identified atrophy of the left postcentral gyrus and right hippocampus among the best MRI variables discriminating CI from CP MS patients. The notion that damage to the GM is more clinically relevant for cognition than that of the WM is also supported by the analysis of correlation with performance at individual cognitive domains, which demonstrated that atrophy of selected GM regions contributed to explain deficit at attention, visual memory, fluency and executive functions,

whereas it did not explain verbal memory impairment. Conversely, regional WM atrophy was associated with deficits of attention and visual memory only, with atrophy of the posterior part of the CC and left ILF identified as the best predictors of impairment of visual memory by the RF analysis. The latter finding is in agreement with the results of a recent investigation, which showed that damage to the WM, measured using DT MRI, is more clinically relevant that that of the GM to explain visual memory deficits (Llufriu, et al., 2014).

At present, only a few studies have attempted to weight the relative contribution of damage to the GM and WM on cognitive impairment in MS patients, providing conflicting results (Hulst, et al., 2013; Llufriu, et al., 2014; Sanfilipo, et al., 2006). One study that limited the analysis to global WM and GM atrophy (without assessing the regional distribution of such an atrophy) found that WM atrophy was the best predictor of mental processing speed and working memory deficits, whereas GM atrophy predicted impairment of verbal memory, euphoria, and disinhibition (Sanfilipo, et al., 2006). By combining VBM, tract-based spatial statistical analysis and T2 LPMs, a recent study showed that CI MS patients diverged from CP ones only for DTI measures of WM integrity in areas that are highly relevant for cognition, whereas GM atrophy and T2 lesion distribution did not differ between the two groups (Hulst, et al., 2013). By using voxel-wise methods to analyze the relative contribution of GM and WM integrity on performance at single cognitive tests, another study found that GM damage assessed by DT MRI adds only a small increment (less than 5%) to the variance of single neuropsychological tests score attributable to WM damage (Llufriu, et al., 2014). Several factors may contribute to explain discrepancies between available studies, including differences in the clinical characteristics of the patients enrolled, methods used for neuropsychological evaluation, type of imaging modality and analysis methods.

Our study is not without limitations. First, we did not assess the contribution of cortical lesions, which have been associated with the presence and severity of cognitive impairment in MS patients (Calabrese, et al., 2010a). However, currently a standardization of this sequence in a multicenter setting is lacking. Second, we selected *a priori* a set of imaging modalities (3D T1

weighted and DT MRI scans) which have been proven to be sensitive to some of the pathological substrates of MS. As a consequence, we cannot rule out that the use of other imaging modalities (e.g., magnetization transfer imaging, MR spectroscopy) would have provided different results.

Third, the analysis of correspondence between lesional, DTI and atrophy abnormalities was based on a visual inspection. A larger sample size and a more specific assessment of the heterogeneity of microstructural tissue damage within T2-hyperintense lesions might allow a better characterization of the role of focal lesions on cognitive impairment. Fourth, even though patients with major depression were excluded, we cannot rule out that other factors, such as mild depression, anxiety and fatigue, might have partially influenced our results.

Future studies should use more sophisticated approaches (such as DT tractography) to assess numerical correlations and their reciprocal influence. Longitudinal studies, including patients with different clinical phenotypes of the disease, are now warranted to examine the temporal evolution of WM and GM damage and their relationship with cognitive impairment and to design rehabilitative trials based on multimodal MRI measures.

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Table 1. Main demographic, clinical and conventional MRI characteristics of healthy controls and patients with multiple sclerosis (MS) according to the presence or not of cognitive impairment enrolled at seven European centers.

Group	HC	MS	MS *vs* HC p	CP	CI	CI *vs* CP p
M / F	26 / 35	21 / 40	n.s.*	14 / 24	7 / 16	n.s.*
Mean age (SD) [years]	36.0 (9.5)	39.7 (8.5)	0.02°	37.5 (7.9)	43.3 (8.4)	0.01°
Mean education (SD) [years]	16.5 (2.9)	13.7 (3.2)	<0.0001°	13.7 (3.1)	13.6 (3.4)	n.s.°
Median EDSS (range)	-	1.5 (0.0-6.0)	-	1.5 (0.0-4.0)	2.0 (1.0-6.0)	0.04°
Mean disease duration (SD) [years]	-	8.2 (6.4)	-	7.1 (4.9)	9.9 (8.2)	n.s.°
Mean T2 LV (SD) [ml]	-	10.8 (14.0)	-	7.3 (9.7)	16.6 (17.9)	0.01°
Mean T1 LV (SD) [ml]	-	5.6 (5.9)	-	3.7 (3.5)	8.7 (7.6)	0.008°
Mean NBV (SD) [ml]	1531 (80)	1435 (108)	<0.0001°	1460 (99)	1395 (114)	0.02°
Mean GMV (SD) [ml]	826 (57)	776 (68)	<0.0001°	793 (68)	748 (58)	0.01°
Mean WMV (SD) [ml]	705 (40)	659 (69)	<0.0001°	666 (55)	648 (88)	n.s.°
Mean WM FA (SD)	0.47 (0.03)	0.45 (0.03)	0.0001°	0.45 (0.02)	0.45 (0.03)	n.s.°
Mean WM MD (SD)	0.76 (0.03)	0.77 (0.04)	0.03°	0.77 (0.03)	0.78 (0.05)	n.s.°
Mean GM MD (SD)	0.91 (0.22)	0.97 (0.22)	0.02°	0.94 (0.24)	1.02 (0.20)	n.s.°

HC=healthy controls; CP=cognitively preserved; CI=cognitively impaired; M=male; F=female; SD=standard deviation; EDSS=Expanded Disability Status Scale; LV=lesion volume; NBV=normalized brain volume; GMV=gray matter volume; WMV=white matter volume; WM=white matter; GM=gray matter; FA=fractional anisotropy; MD=mean diffusivity; n..s.=not significant.

Mean MD is expressed in units of $mm^2/s \times 10^{-3}$, FA is dimensionless index.

*Chi-Square Test; °Mann-Whitney Test.

Table 2. Brain regions with gray matter (GM) and white matter (WM) atrophy and T2-hyperintense lesion differences among the different study groups (p<0.001 uncorrected, ke=5 voxels).

				MNI coordinates			
Comparison	Contrast	Anatomical region	Side	x	y	z	t value
CP MS *vs* HC	GM atrophy	Thalamus	R	16	-27	1	6.93*
			L	-18	-30	-2	6.67*
		MTG	R	65	-35	1	5.33*
		Posterior cingulate cortex	L	-2	-30	29	4.62
		STG	R	63	-44	10	4.52*
		Parahippocampal gyrus	R	18	-19	-24	4.16
		Insula	L	-44	-11	4	4.00
		Caudate nucleus	R	21	17	1	3.90
		Caudate nucleus	L	-10	10	15	3.74
		Calcarine cortex	R	6	-63	15	4.30
		MTG	L	-66	-36	5	4.21
		SFG	R	24	57	8	4.02
		Lingual gyrus	R	18	-54	-11	4.02
		Cerebellum (lobule 6)	R	16	-64	-27	3.41
		IPL	R	56	-47	42	4.00
		Precentral gyrus	L	-24	-10	64	3.98
		MFG	L	-26	46	11	3.95
		IFG	R	37	20	-15	3.90
		IPL	L	-58	-53	42	3.79
		Cerebellum (lobule 6)	L	-23	-61	-32	3.79

		Precentral gyrus	R	29	-4	52	3.68
		SPL	R	27	-61	50	3.56
		SFG	L	-1	56	22	3.52
		Hippocampus	L	-23	-13	-24	3.51
		SPL	L	-77	45	3.29	-24
	WM atrophy	Fornix	L/R	0	-5	13	5.89*
		ATR	R	26	-33	1	5.66*
		ATR	L	-29	-30	-6	5.18*
		Body of the CC	L	-5	-16	24	4.98*
		Body of the CC	R	6	-17	24	4.97*
		ILF	R	45	-7	-13	4.90*
		Posterior cingulum	L	-10	-19	36	4.86*
		SLF	R	55	-38	21	4.65*
		IFOF	R	45	-24	-11	4.63*
		Forceps minor	L	-19	46	22	3.84
		SLF	L	-52	-55	38	3.58
		ILF	L	-28	-81	-4	3.36
CI MS *vs* HC	GM atrophy	Thalamus	L	-16	-29	-1	8.12*
		Thalamus	R	18	-29	-1	7.92*
		Posterior cingulate cortex	L	-0	-33	27	5.91*
		Precuneus	R	3	-62	17	5.41*
		Posterior cingulate cortex	R	2	-36	28	5.26*
		Putamen	R	32	-2	1	5.01*
		Putamen	L	-19	9	3	4.98*
		Caudate nucleus	R	13	3	20	4.82*

		IPL	L	-40	-37	45	4.76*
		Amygdala	R	31	3	-18	4.71
		Caudate nucleus	L	-10	8	15	4.63
		Insula	L	-53	-9	8	4.63
		Amygdala	L	-10	-8	-15	4.46
		ITG	L	-58	-10	-15	4.45
		MTG	L	-54	-60	1	4.27
		Cerebellum (lobule 6)	R	21	-59	-9	4.17
		Insula	R	48	-27	8	4.09
		STG	R	61	-35	1	5.10*
		MOG	L	-29	-87	12	4.54
		MTG	R	47	-21	-25	4.13
		MFG	L	-24	-4	48	4.13
		Precentral gyrus	R	39	-11	47	3.98
		STG	L	-62	-38	5	3.96
		Postcentral gyrus	L	-45	-19	34	3.79
		Cerebellum (left crus I)	L	-45	-54	-25	3.70
		Postcentral gyrus	R	21	-42	61	3.51
		Precentral gyrus	L	-32	-17	45	3.50
		SPL	R	27	-60	49	3.48
		SMA	L	-2	24	57	3.45
		IFG	R	44	8	25	3.29
	WM atrophy	Fornix	L/R	0	0	3	8.08*
		ATR	R	19	-33	8	8.08*
		ATR	L	-32	-24	-6	7.55*

		Forceps major	L	-13	-38	15	6.38*
		Splenium of the CC	L	-10	-41	19	6.32*
		Body of the CC	L	-7	-19	24	6.27*
		SLF	L	-34	-46	20	6.27*
		Body of the CC	R	8	-19	24	6.26*
		ILF	L	-37	-60	1	6.25*
		SLF	R	44	-46	1	6.18*
		Posterior cingulum	L	-13	-47	22	6.17*
		Splenium of the CC	R	18	-47	22	6.05*
		ILF	R	40	-8	-17	6.02*
		Posterior corona radiata	L	-21	-28	36	5.86*
		IFOF	R	35	-55	14	5.60*
CI *vs* CP MS	T2 LPM	IFOF	L	-37	-44	-6	5.84*
		Forceps major	L	-24	-55	3	4.42
		ATR	L	-24	-31	22	4.27
		SLF	R	34	-31	36	4.69
		IFOF	R	35	-44	10	4.39
		Forceps major	R	22	-55	12	3.88
		Splenium of the CC	R	26	-52	8	3.59
		Body of the CC	R	3	14	20	4.45
		Forceps minor	L	-10	33	-13	4.16
		ATR	R	3	-19	-6	4.27
		Posterior cingulum	L	-20	-49	31	4.20
		ILF	R	21	-59	-4	4.19
		Middle cingulum	L	-11	-27	31	4.04

		Splenium of the CC	R	14	-43	29	3.99
		Anterior cingulum	R	8	24	27	3.85
		SCP	R	6	-48	-27	3.79
		Corona radiata	R	21	-8	34	3.53
		Genu of the CC	R	15	33	9	3.32
	GM atrophy	Postcentral gyrus	R	23	-42	56	3.51
			L	-45	-17	38	3.26
		Thalamus	L	-16	-27	1	3.48
		Supramarginal gyrus	L	-45	-38	36	3.41
		Hippocampus	R	29	-30	-2	3.32
	WM atrophy	ILF	L	-34	-60	8	4.22
		IFOF	L	-34	-32	3	4.14
		ATR	R	18	-32	8	4.07
		Posterior cingulum	L	-11	-43	22	3.86
		SLF	L	-31	-36	22	3.83
		Splenium of the CC	R	11	-39	17	3.61
		Fornix	L/R	0	0	3	3.87
		ILF	R	37	-55	12	3.46
		Uncinate fasciculus	R	36	10	-34	3.32
Conjunction analysis CI MS *vs* (HC and CP MS)	GM atrophy	Thalamus	L	-16	-27	1	3.48
		Supramarginal gyrus	L	-45	-38	36	3.41
		Hippocampus	R	29	-30	-2	3.32
		SPL	R	26	-42	54	3.28
		Postcentral gyrus	L	-45	-17	38	3.26
	WM	ILF	L	-34	-60	8	4.22

	atrophy	IFOF	L	-34	-32	3	4.14
		ATR	R	18	-32	8	4.07
		Posterior Cingulum	L	-11	-43	22	3.86
		SLF	L	-31	-36	22	3.83
		Splenium of the CC	R	11	-39	17	3.61
		Fornix	L/R	0	0	3	3.87
		ILF	R	37	-55	12	3.46
		Uncinate fasciculus	R	36	10	-34	3.32

HC=healthy controls; MS=multiple sclerosis;CP=cognitively preserved; CI=cognitively impaired; MNI=Montreal Neurological Institute; GM=gray matter; WM=white matter; LPM=lesion probability map; R=right, L=left; MTG=middle temporal gyrus; STG=superior temporal gyrus; SFG=superior frontal gyrus; IPL= inferior parietal lobule; MFG=middle frontal gyrus; IFG=inferior frontal gyrus; SPL=superior parietal lobule; ATR=anterior thalamic radiation; CC=corpus callosum; ILF=inferior longitudinal fasciculus; SLF=superior longitudinal fasciculus; IFOF=inferior fronto-occipital fasciculus; ITG=inferior temporal gyrus; MOG=middle occipital gyrus; SMA=supplementary motor area; SCP=superior cerebellar peduncle.

*$p<0.05$ FWE corrected for multiple comparisons.

Table 3. Normal appearing white matter regions with mean diffusivity and fractional anisotropy differences among the different study groups (p<0.05 FWE corrected for multiple comparisons, ke=5 voxels).

				MNI coordinates			
Comparison	Contrast	Anatomical region	Side	x	y	z	t value
CP MS *vs* HC	MD increase	ILF	R	45	-4	-16	6.11
		Corona radiata	L	-25	-9	36	5.39
		Splenium of the CC	R	9	-36	8	5.28
		Body of the CC	L	-7	0	29	5.26
		SLF	L	-54	-22	6	5.00
		Corona radiata	R	22	-23	37	4.95
		ILF	R	45	-36	-9	4.82
		Posterior cingulum	R	25	-32	-13	4.79
		ATR	L	-23	29	19	4.76
		SLF	R	49	-38	0	4.76
		Anterior cingulum	R	6	15	24	4.73
	FA decrease	IFOF	L	-32	-22	-6	6.51
		ILF	L	-35	-58	5	6.47
		Posterior corona radiata	R	24	-23	28	6.19
		IFOF	R	31	-36	22	5.32
		SLF	R	39	-13	25	5.16
		ILF	R	44	-32	-9	6.00
		Fornix	L	-1	1	4	5.60
		Posterior corona radiata	L	-21	-29	30	5.38
		SLF	L	-30	-15	27	4.88

		Posterior cingulum	L	-11	-17	38	5.36
		MCP	R	12	-41	-30	5.36
		MCP	L	-18	-37	-29	5.28
		Posterior cingulum	R	24	-28	-12	5.11
		Anterior cingulum	L	-12	18	28	5.02
CI MS *vs* HC	MD increase	Posterior cingulum	L	-7	-25	41	10.50
		Posterior corona radiata	L	-23	-48	26	10.48
		Posterior cingulum	R	10	-28	39	9.18
		SLF	L	-40	-2	43	8.99
		SLF	R	40	15	24	8.63
		IFOF	L	-33	-13	-7	8.45
		Forceps major	R	25	-54	19	8.27
		Splenium of the CC	R	13	-44	13	8.27
		SLF	R	31	-39	27	8.11
		Forceps major	L	-2	-35	14	7.95
		Splenium of the CC	L	-13	-45	13	7.86
		Body of the CC	R	7	-24	26	7.78
		Anterior corona radiata	R	24	-9	50	7.69
		MCP	L	-12	-30	-29	6.16
		MCP	R	17	-32	-35	6.11
		IFOF	R	17	-83	-2	5.72
		Forceps minor	L	-11	55	6	5.70
		Uncinate fasciculus	R	33	-1	-22	4.70
	FA decrease	IFOF	L	-34	-19	-8	9.30
		ILF	L	-36	-53	-4	8.92

		ILF	R	35	-54	9	8.55
		Posterior corona radiata	R	16	-25	67	8.54
		Posterior cingulum	R	11	-7	39	7.83
		Forceps major	R	25	-63	18	7.79
		Posterior cingulum	L	-23	-31	-16	7.75
		SLF	R	34	-15	22	7.60
		SLF	L	-49	16	10	7.51
		Body of the CC	R	2	18	17	7.21
		ATR	L	-14	-19	-4	7.21
		Forceps major	L	-22	-66	18	7.18
		Body of the CC	L	-5	-24	26	7.17
		MCP	R	8	-45	-33	6.65
		Uncinate fasciculus	L	-39	32	-7	5.93
		Forceps minor	R	12	51	-9	5.08
CI *vs* CP MS	MD increase	Posterior cingulum	L	-7	-25	41	8.30
		Posterior corona radiata	L	-24	-47	26	8.00
		Splenium of the CC	L	-2	-34	15	5.51
		SLF	L	-40	-3	43	7.15
		IFOF	L	-33	-13	-7	6.74
		Posterior cingulum	R	9	-27	39	6.73
		Splenium of the CC	R	12	-44	13	5.88
		IFOF	R	31	-38	25	5.29
		SLF	R	39	15	23	5.81
		Anterior cingulum	L	-5	19	31	5.72
		Forceps minor	R	12	41	10	5.62

		Anterior cingulum	R	9	31	37	5.45
		Uncinate fasciculus	R	29	16	-6	5.25
		ATR	L	-37	34	9	5.23
		Uncinate fasciculus	L	-30	18	3	4.87
	FA decrease	IFOF	L	-28	-45	24	6.19
		IFOF	R	30	-55	19	5.43
		SLF	L	-47	13	5	5.38
		Anterior cingulum	R	8	-3	39	5.12
Conjunction analysis CI MS *vs* (HC and CP MS)	MD increase	Posterior cingulum	L	-7	-25	41	8.30
		Posterior corona radiata	L	-19	-26	51	4.95
		Posterior corona radiata	L	-24	-47	26	8.00
		Splenium of the CC	L	-2	-34	15	5.51
		SLF	L	-40	-3	43	7.15
		IFOF	L	-33	-13	-7	6.74
		Posterior cingulum	R	9	-27	39	6.73
		Splenium of the CC	R	12	-44	13	5.88
		IFOF	R	31	-38	25	5.29
		SLF	R	39	15	23	5.81
		Anterior cingulum	L	-5	19	31	5.72
		Forceps minor	R	12	41	10	5.62
		Anterior cingulum	R	9	31	37	5.45
		Uncinate fasciculus	R	29	16	-6	5.25
		ATR	L	-37	34	9	5.23
		Uncinate fasciculus	L	-30	18	3	4.87
	FA	IFOF	L	-28	-45	24	6.19

	decrease	ILF	R	30	-55	19	5.43
		SLF	L	-47	13	5	5.38
		Anterior cingulum	R	8	-3	39	5.12

HC=healthy controls; MS=multiple sclerosis; CP=cognitively preserved; CI=cognitively impaired; MNI=Montreal Neurological Institute; MD=mean diffusivity; FA=fractional anisotropy; R=right, L=left; ILF=inferior longitudinal fasciculus; CC=corpus callosum; SLF=superior longitudinal fasciculus; IFOF=inferior fronto-occipital fasciculus; ATR=anterior thalamic radiation; MCP=middle cerebellar peduncle.

Table 4. Brain regions with gray matter and white matter atrophy correlated to performance at different cognitive domains (z scores) and number of errors at Wisconsin Card Sorting Test (WCST) ($p<0.001$ uncorrected, ke=5 voxels).

				MNI coordinates				
Cognitive domain	Contrast	Anatomical region	Side	x	y	z	t value	r value
Attention (z score)	GM atrophy	Putamen	L	-29	5	-4	5.53*	0.69
		Putamen	R	19	19	3	5.49*	0.69
		Caudate nucleus	R	11	21	4	5.45*	0.68
		Thalamus	L	-15	-25	-2	4.77	0.63
		Thalamus	R	6	-8	-3	4.71	0.63
		Hippocampus	L	-19	-18	-11	4.28	0.59
		Amygdala	L	-13	-10	-13	4.25	0.59
		Amygdala	R	21	3	-18	4.23	0.59
		Insula	L	-40	13	-1	4.07	0.57
		Caudate nucleus	L	-15	-8	24	4.03	0.57
		Posterior cingulate cortex	L	-3	-41	19	5.32*	0.68
		Precuneus	R	3	-62	14	4.07	0.57
		MOG	L	-41	-66	24	3.89	0.56
		Precentral gyrus	R	44	-8	36	3.84	0.55
		STG	R	55	-36	14	3.81	0.55
		MTG	R	53	-37	-4	3.78	0.54
		IFG	R	41	32	17	3.71	0.54
		Anterior cingulate cortex	R	13	-7	48	3.64	0.53

		STG	L	-52	-22	-6	3.43	0.51
		MFG	L	-34	46	2	3.43	0.51
	WM atrophy	Posterior cingulum	L	-5	-49	21	5.54*	0.69
		ATR	L	-7	-16	13	4.85*	0.64
		Body of the CC	R	5	-22	26	4.65	0.62
		IFOF	R	38	-8	-18	4.50	0.61
		ATR	R	5	20	-5	4.34	0.60
		IFOF	L	-26	28	2	4.25	0.59
		ILF	L	-39	-11	-24	4.22	0.59
		SLF	L	-36	8	13	4.08	0.57
		ILF	R	37	-27	-15	4.06	0.57
		Posterior corona radiata	L	-21	-28	54	4.13	0.58
		SLF	R	43	-51	5	3.57	0.52
Visual memory (z score)	GM atrophy	MFG	L	-37	47	4	4.39	0.57
		Postcentral gyrus	L	-31	-39	45	4.01	0.53
		ITG	R	53	-40	-16	3.99	0.53
		Precuneus	R	6	-58	70	3.98	0.53
		MTG	R	65	-24	-16	3.90	0.52
		IFG	R	49	35	4	3.88	0.52
		Precentral gyrus	R	50	3	24	3.67	0.50
		Posterior cingulate cortex	R	10	-38	5	3.63	0.50
		Precentral gyrus	L	-47	-6	31	3.57	0.49
		Amygdala	R	16	-7	-11	3.51	0.48
		MFG	L	-34	32	25	3.44	0.48
		Posterior cingulate cortex	L	-7	-28	38	3.40	0.48

	WM atrophy	SLF	R	47	-57	26	5.08*	0.62
		ILF	R	47	-37	-13	4.29	0.56
		Posterior cingulum	L	-5	-25	33	4.26	0.56
		Posterior corona radiata	L	-18	-42	52	4.23	0.55
		IFOF	R	37	-19	-2	4.19	0.55
		ILF	L	-19	-50	54	4.10	0.54
		Splenium of the CC	R	5	-36	24	3.82	0.52
		SLF	L	-34	-30	31	3.74	0.51
		Splenium of the CC	L	-10	-36	20	3.70	0.50
		Forceps major	L	-10	-41	6	3.44	0.48
Verbal memory (z score)	GM atrophy	-	-	-	-	-	-	-
	WM atrophy	-	-	-	-	-	-	-
Fluency (z score)	GM atrophy	Precentral gyrus	L	-5	-29	77	3.68	0.57
		Postcentral gyrus	L	-44	-26	43	3.64	0.56
		MTG	R	68	-10	-16	3.62	0.56
		Insula	R	39	6	-4	3.58	0.56
		Insula	L	-40	19	-8	3.41	0.54
	WM atrophy	-	-	-	-	-	-	-
Executive functions (WCST number	GM atrophy	IFG	R	44	33	-5	4.20	0.65
		Thalamus	L	-3	-9	15	4.20	0.65
		Thalamus	R	6	-24	15	3.48	0.58
		Anterior cingulate cortex	L	-6	16	41	4.11	0.65

of errors)		SFG	R	7	49	2	3.37	0.57
		Caudate nucleus	R	15	5	25	3.34	0.57
		Posterior cingulate cortex	R	3	-36	27	3.33	0.57
	WM atrophy	-	-	-	-	-	-	-

MNI=Montreal Neurological Institute; GM=gray matter; WM=white matter; R=right, L=left; MTG=middle temporal gyrus; MOG=middle occipital gyrus; STG=superior temporal gyrus; IFG=inferior frontal gyrus; MFG=middle frontal gyrus; ATR=anterior thalamic radiation; CC=corpus callosum; IFOF=inferior fronto-occipital fasciculus; ILF=inferior longitudinal fasciculus; SLF=superior longitudinal fasciculus; ITG=inferior temporal gyrus; SFG=superior frontal gyrus.

*$p<0.05$, FWE corrected for multiple comparisons.

Table 5. Normal-appearing white matter regions with mean diffusivity and fractional anisotropy values correlated to performance at different cognitive domains (z scores) and number of errors at Wisconsin Card Sorting Test (WCST) ($p<0.001$ uncorrected, ke=5 voxels).

				MNI coordinates				
Cognitive domain	Contrast	Anatomical region	Side	x	y	z	t value	r value
Attention (z score)	MD increase	Uncinate fasciculus	L	-28	21	12	5.22*	-0.66
		Anterior cingulum	R	13	17	38	5.13*	-0.66
		Uncinate fasciculus	R	26	19	-7	5.03*	-0.65
		Posterior cingulum	R	11	-15	39	4.93*	-0.64
		Posterior cingulum	L	-13	-24	36	4.72	-0.63
		IFOF	L	-25	23	-1	4.72	-0.63
		ATR	L	-11	2	5	4.65	-0.62
		Body of the CC	R	14	-13	33	4.54	-0.61
		SLF	L	-52	-41	-14	4.90	-0.64
		SLF	R	52	-54	2	4.56	-0.61
		IFOF	R	26	-84	-10	3.78	-0.54
	FA decrease	SLF	L	-49	-34	-13	5.90*	0.71
		ATR	L	-4	-12	7	5.80*	0.70
		Forceps minor	R	19	51	21	5.58*	0.69
		Body of the CC	R	3	-21	26	5.58*	0.69
		Posterior corona radiata	L	-21	-26	53	5.57*	0.69
		ILF	R	40	-2	-34	5.17*	0.66
		IFOF	L	-32	-25	4	5.15*	0.66

		SLF	R	41	9	16	4.97	0.65
		Forceps major	R	-16	-89	20	4.12	0.57
		Posterior corona radiata	R	17	-34	67	3.90	0.55
		ILF	L	-48	7	-17	3.63	0.53
Visual memory (z score)	MD increase	Posterior cingulum	L	-8	-27	30	4.55	-0.58
		Splenium of the CC	L	-16	-35	29	4.05	-0.53
		Posterior corona radiata	L	-25	-17	33	4.05	-0.53
		SLF	R	47	-46	34	3.94	-0.52
		Posterior cingulum	R	8	-30	30	3.89	-0.52
		Posterior corona radiata	R	25	-43	28	3.73	-0.50
		Forceps minor	R	17	38	12	3.72	-0.50
		SLF	L	-42	-34	6	3.64	-0.49
		ATR	L	-16	3	6	3.64	-0.49
		Forceps major	R	16	-81	4	3.51	-0.48
		Uncinate fasciculus	R	18	9	-10	3.50	-0.48
	FA decrease	IFOF	L	-24	-74	-3	5.36*	0.64
		ILF	L	-29	-87	9	4.55	0.58
		ATR	L	-18	4	7	4.53	0.57
		Posterior cingulum	L	-22	-48	-3	4.53	0.57
		Forceps major	L	-16	-82	4	4.33	0.56
		SLF	L	-58	-28	-14	4.07	0.53
		Forceps major	R	18	-81	9	5.13	0.62
		IFOF	R	27	-71	-2	4.18	0.54
		Anterior cingulum	R	14	27	31	4.80	0.60
		Body of the CC	R	1	-2	28	4.11	0.54

		Forceps minor	R	16	42	15	3.89	0.52
		Body of the CC	L	-4	-24	28	3.58	0.48
		Posterior corona radiata	R	33	-24	58	4.77	0.59
		SLF	R	37	-12	22	4.38	0.56
Verbal memory (z score)	MD increase	-	-	-	-	-	-	-
	FA decrease	-	-	-	-	-	-	-
Fluency (z score)	MD increase	-	-	-	-	-	-	-
	FA decrease	-	-	-	-	-	-	-
Executive functions (WCST number of errors)	MD increase	ILF	R	35	-63	-12	4.34	-0.66
		SLF	L	-38	-14	33	4.04	-0.63
		Forceps major	R	19	-88	19	4.03	-0.63
		Anterior cingulum	R	7	37	19	3.99	-0.63
		Splenium of the CC	R	10	-42	7	3.86	-0.62
		SLF	R	49	-45	35	3.77	-0.61
		IFOF	R	31	2	-5	3.71	-0.60
		Body of the CC	R	9	-20	24	3.66	-0.60
		IFOF	L	-30	-4	-3	3.64	-0.59
	FA decrease	IFOF	R	43	36	6	4.56	0.68
		SLF	R	54	-8	35	4.41	0.67
		Uncinate fasciculus	L	-39	34	12	3.87	0.62
		SLF	L	-50	-3	37	3.56	0.59

MNI=Montreal Neurological Institute; MD=mean diffusivity; FA=fractional anisotropy; R=right, L=left; IFOF=inferior fronto-occipital fasciculus; ATR=anterior thalamic radiation; CC=corpus callosum; ILF=inferior longitudinal fasciculus; SLF=superior longitudinal fasciculus.

*$p<0.05$, FWE corrected for multiple comparisons.

Figure legends

Figure 1. Statistical parametric mapping (SPM) analysis showing regions of gray matter (GM) (yellow coded) and white matter (WM) (blue coded) volume loss and T2 lesion probability maps (LPMs) differences (red-coded) superimposed on the customized GM template ($p<0.001$ uncorrected; cluster extent=5 voxels): a) GM and WM atrophy in cognitively preserved (CP) MS patients *vs* healthy controls (HC); b) GM and WM atrophy in cognitively impaired (CI) MS patients *vs* HC; c) GM and WM atrophy and regions with higher T2 occurrence in CI *vs* CP MS patients; d) GM and WM atrophy in CI MS patients *vs* both HC and CP MS patients. See text for further details. Images are in neurological convention (right side of the images is right side of the brain).

Figure 2. Statistical parametric mapping (SPM) analysis showing regions with increased mean diffusivity (MD) (blue-coded), reduced fractional anisotropy (FA) (green-coded) and T2 lesion probability maps (LPMs) differences (red-coded) superimposed on the customized FA template ($p<0.05$ family-wise error corrected for multiple comparisons; cluster extent=5 voxels): a) significantly increased MD and decreased FA in cognitively preserved (CP) MS patients *vs* healthy controls (HC); b) significantly increased MD and decreased FA in cognitively impaired (CI) MS patients *vs* HC; c) significantly increased MD and decreased FA and regions with higher T2 lesion occurrence in CI *vs* CP MS patients (the overlaps between diffusivity differences and T2 LPMs differences are yellow-coded); d) significantly increased MD and decreased FA in CI MS patients *vs* both HC and CP MS patients. See text for further details. Images are in neurological convention (right side of the images is right side of the brain).

Figure 3. Statistical parametric mapping (SPM) analysis showing regions with gray matter (GM) (yellow coded) and white matter (WM) (blue coded) atrophy in multiple sclerosis patients significantly correlated to performance at different cognitive domains ($p<0.001$ uncorrected; cluster

extent=5 voxels). Results are superimposed on the customized GM template. See text for further details. Images are in neurological convention (right side of the images is right side of the brain). WCST=Wisconsin Card Sorting Test.

Figure 4. Statistical parametric mapping (SPM) analysis showing regions with mean diffusivity (MD) (blue-coded) and fractional anisotropy (FA) (green-coded) being correlated to the performance at the different cognitive domains superimposed on the customized FA template in multiple sclerosis (MS) patients ($p<0.001$ uncorrected; cluster extent=5).

Figure 5. Results of the random forest analysis. Normalized variable importance, ranging from 0 (the less important) to 100 (the most important), of the five most important MRI variables in predicting global and specific cognitive scores. CI=cognitively impaired; CP=cognitively preserved; MS=multiple sclerosis; L=left; R=right; SLF=superior longitudinal fasciculus; FA=fractional anisotropy; MD=mean diffusivity; ILF=inferior longitudinal fasciculus; IFG=inferior frontal gyrus; CC=corpus callosum; MTG=middle temporal gyrus; IFOF=inferior fronto-occipital fasciculus.