Exploring in vivo multiple sclerosis brain microstructural damage through T1w/T2w-ratio: a multicenter study

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Abstract

Objectives. To evaluate white matter and gray matter T1-weighted (w)/T2w-ratio (T1w/T2w-ratio) in healthy controls and multiple sclerosis patients, and its association with clinical disability.

Methods. In this cross-sectional study, 270 healthy controls and 434 multiple sclerosis patients were retrospectively selected from 7 European sites. T1w/T2w-ratio was obtained from brain T2w and T1w scans after intensity calibration using eyes and temporal muscle.

Results. In healthy controls, T1w/T2w-ratio increased until 50-60 years both in white and gray matter. Compared to healthy controls, T1w/T2w-ratio was significantly lower in white matter lesions of all multiple sclerosis phenotypes, and in normal-appearing white matter and cortex of relapsing-remitting and secondary-progressive multiple sclerosis patients ($p \leq 0.026$), but it was significantly higher in the striatum and pallidum of relapsing-remitting, secondary progressive and primary progressive multiple sclerosis patients ($p \leq 0.042$). In relapse-onset multiple sclerosis, T1w/T2w-ratio was significantly lower in white matter lesions and normal-appearing white matter already at Expanded Disability Status Scale [EDSS]<3.0 and in the cortex only for EDSS≥3.0 ($p \leq 0.023$). Conversely, T1w/T2w-ratio was significantly higher in the striatum and pallidum for EDSS≥4.0 ($p \leq 0.005$). In primary progressive multiple sclerosis, striatum and pallidum showed significantly higher T1w/T2w-ratio beyond EDSS=6.0 ($p \leq 0.001$). In multiple sclerosis, longer disease duration, higher EDSS, higher brain lesional volume, and lower normalized brain volume were associated with lower lesional and cortical T1w/T2w-ratio and a higher T1w/T2w-ratio in the striatum and pallidum (β from -1.168 to 0.286, $p \leq 0.040$).

Conclusions. T1w/T2w-ratio may represent a clinically relevant marker sensitive to demyelination, neurodegeneration, and iron accumulation occurring at the different multiple sclerosis phases.

What is already known on this topic

- T1w/T2w-ratio has been proposed as a proxy for myelin quantification; however, its specificity for myelin was not confirmed by several studies performed in multiple sclerosis, nor in other neurodegenerative diseases such as Alzheimer and Parkinson diseases.
- A few studies have investigated *in vivo* the differences of T1w/T2w-ratio between multiple sclerosis patients and healthy controls with conflicting results.

What this study adds

- We explored T1w/T2w-ratio in different brain compartments in a large cohort of multiple sclerosis patients with different ages and disease clinical phenotypes, and we evaluated its feasibility and clinical relevance in a multicenter setting.
- In healthy controls we demonstrated an increase of T1w/T2w-ratio with age in all the investigated brain compartments; conversely, in multiple sclerosis patients we found heterogeneous T1w/T2w-ratio abnormalities in the different brain regions according to the clinical phenotypes and levels of disability.
- Altogether, our data suggest that T1w/T2w-ratio results from several pathological substrates, including inflammation, demyelination, neurodegeneration and iron accumulation occurring at different phases of the disease.

How this study might affect research, practice or policy

- T1w/T2w-ratio may represent a clinically relevant tool that can be obtained from MRI sequences extensively used in clinical practice.

INTRODUCTION

Multiple sclerosis (MS) is an autoimmune disorder of the central nervous system (CNS) characterized by heterogeneous pathological processes, including inflammation, demyelination and neurodegeneration.[1] Although the hallmark of MS is the accumulation of focal white matter (WM) lesions, pathological studies have consistently shown the presence of abnormalities affecting the normal-appearing (NA) WM, deep gray matter (GM) and cortex.[1, 2]

The calculation of the ratio between conventional images with T1- and T2-weighted (w) contrast (i.e., T1w/T2w-ratio) may represent a clinically relevant tool easily obtained from MRI sequences, which are extensively used in clinical practice. This method has been proposed as a proxy for myelin, since some evidence suggested that T1- and T2-w signals are, respectively, positively and negatively associated with myelin content and may be specific to this pathological substrate.[3]

In line with this, previous studies found a good overlap between cortical T1w/T2w-ratio maps and histological cortical myeloarchitecture.[3] Moreover, MRI studies found moderate correlations between T1w/T2w-ratio and magnetization transfer ratio, a measure that is considered specific to quantify myelin.[4, 5] However, the specificity of T1w/T2w-ratio for myelin was not confirmed by other studies in MS,[6-8] nor in other neurodegenerative diseases such as Alzheimer and Parkinson diseases.[9, 10] These discrepancies suggest that T1w/T2w-ratio may be sensitive to different pathological processes.

At present, a few studies have investigated *in vivo* T1w/T2w-ratio in MS patients with conflicting results.[6, 11-18] T1w/T2w-ratio values were found significantly lower in the NAWM of early relapsing-remitting (RR) MS compared to healthy controls (HC).[12, 14, 18] However, this was not confirmed in other studies where no difference was detected in MS patients compared to HC.[11, 13, 16] Some studies found a significantly lower cortical T1w/T2w-ratio in RRMS and progressive MS patients,[6, 17] but not in the early phases of the disease.[16] So far, no study directly evaluated T1w/T2w-ratio in deep GM nuclei.

The clinical relevance of T1w/T2w-ratio still needs to be fully explored. A few studies in patients with mild disability and short disease duration showed mild correlations between Expanded Disability Status Scale (EDSS) and T1w/T2w-ratio in the NAWM and cortex.[6, 12]

Several factors could contribute to these conflicting results. Methodological heterogeneities in the T1w/T2w-ratio quantification must be taken into account.[3, 19] Moreover, some studies evaluated small cohorts of MS patients, not spanning the whole spectrum of disease severity and with limited age-range.

Therefore, the aims of this study were to characterize T1w/T2w-ratio in the different brain tissues in a large multicenter dataset of HC and MS patients across the lifespan and spanning the main clinical phenotypes and to assess the relationship between T1w/T2w-ratio and clinical disability.

METHODS

Ethics committee approval

Approval was received from the local ethical standards committees at each participating center; written informed consent was obtained from all study participants prior to enrollment. A MAGNIMS data-sharing agreement was signed among the participating centers.

Study population

Participants were retrospectively identified at 7 European centers (http://www.magnims.eu/): (1) the Amsterdam MS Center (the Netherlands); (2) the CEM-Cat, Hospital Vall d'Hebron, Barcelona (Spain); (3) Institute of Neurology, UCL, London (UK); (4) the Neuroimaging Research Unit, IRCCS San Raffaele Scientific Institute, Milan (Italy); (5) the MRI Center "SUN-FISM," University of Campania "Luigi Vanvitelli," Naples (Italy); (6) the Nuffield Department of Clinical Neurosciences, Oxford (UK); and (7) the Clinic of Neurology, Faculty of Medicine, University of Belgrade, Belgrade (Serbia). HC were mainly recruited among the spouses of

patients and by word of mouth. To be included, MS patients had to have a diagnosis of MS according to the 2010 revised McDonald criteria, have stable treatment during the last 6 months prior to imaging and received no corticosteroids during the last month. Patients with a clinically isolated syndrome (CIS) suggestive of MS had to have a first episode suggestive of CNS demyelination and a clinical assessment within 3 months from clinical symptoms onset. A diagnosis of neuromyelitis optica or of other conditions mimicking MS were carefully excluded from the study.

Exclusion criteria for both HC and MS were major comorbidities (i.e., cerebrovascular disease, metabolic disorders, brain tumors, psychiatric disorders); history of drug/alcohol abuse and contraindications to undergo MRI (i.e., claustrophobia, metal implants, pacemakers, pregnancy or breastfeeding).

Clinical assessment

Within 48 hours from MRI acquisition, MS patients underwent a complete neurologic evaluation by experienced neurologists, with definition of the clinical phenotype, rating of the EDSS score and recording of disease-modifying treatments (DMTs).

MRI acquisition

Using 3T or 1.5T scanners (without software/hardware upgrades during the study), the following brain sequences were acquired: (1) brain 3-dimensional (3D) T1-weighted scan; (2) axial brain 2-dimensional (2D) dual-echo fast spin-echo or brain 3D T2-weighted scan; (3) sagittal 3D fluid attenuation inversion recovery (FLAIR). See online supplementary methods and supplementary table 1 for additional details regarding scan geometry.

Conventional MRI analysis

Brain T2-hyperintense lesion volume (LV) was measured on the dual-echo or FLAIR scans, using, respectively, a local thresholding segmentation technique (n=562) (Jim 8, Xinapse Systems) or a fully automated deep-learning approach (n=142). Normalized brain volume (NBV), white matter volume (NWMV), gray matter volume (NGMV) and cortical GM volume were measured on lesion-filled 3D T1-weighted scans using the SIENAx2 software.[20] Deep GM nuclei (i.e., thalamus, caudate, putamen, pallidum) were segmented from the lesion-filled brain 3D T1-weighted images using FSL FIRST.[21] Masks of the aforementioned brain regions were then derived. GM maps were thresholded at probability value of 0.5 to limit partial volume effects from WM and CSF.

The results of segmentation were all visually checked. The caudate and putamen together comprised the striatum.

As performed previously,[22] volume of deep GM nuclei was calculated and normalized using FSL SIENAx scaling factor.

T1w/T2w-ratio image reconstruction

To obtain T1w/T2w-ratio maps, 3D T1-weighted and T2-weighted images were preprocessed and combined using an in-house dedicated pipeline adapted from Ganzetti et al.[19] This included intensity bias correction and calibration of each of the two input MRI sequences and the subsequent calculation of their ratio. In detail, first, 3D T1-weighted and T2-weighted images underwent intensity N4 bias field correction.[23] Then, 3D T1-weighted and T2-weighted images were further processed to normalize their signal intensity histograms using a linear scaling procedure described in Ganzetti et al. (implemented in Matlab® v2012).[19] Specifically, the intensity histograms were anchored using the lowest and the highest intensity peaks derived from the ocular and temporal muscle masks that were defined manually in Montreal Neurological Institute (MNI) space to improve the overlap with anatomy. The signal intensity distributions were extracted from both T1- and T2-weighted images after non-linear registrations of the tissue masks

into the subject space (http://stnava.github.io/ANTs/). The co-registrations of the masks in the subject's space were checked by visual inspection. The intensity bias-corrected and calibrated T2-weighted image was then co-registered to the 3D T1-weighted image space through a rigid-body transformation using FLIRT tool (FSL Library). The ratio between these images was calculated in native space.

T1w/T2w-ratio values within the cortical and deep GM were derived by imposing the previously obtained tissue segmentation masks on the T1w/T2w-ratio image. T1w/T2w-ratio values of cortical GM above the 99th percentile were excluded from the analysis.

To extract T1w/T2w-ratio values from WM lesions, the transformation estimated by co-registering T1- and T2-weighted images was applied to the WM lesion masks. T1w/T2w-ratio values within the NAWM were also derived after removing lesions from the WM mask.

Statistical analysis

Demographic and clinical variables were compared between groups using Chi-squared, Kruskal-Wallis and Mann-Whitney tests, or linear models, as appropriate. Age- and sex-adjusted linear mixed models for clustered data (participants within sites) were performed to assess differences in MRI features between HC and MS patients, as well as between clinical phenotypes, with the following a priori contrasts: RRMS *vs* CIS, secondary progressive (SP) MS *vs* RRMS, and SPMS *vs* primary progressive (PP) MS. Brain T2-hyperintense LV was log-transformed.

To estimate T1w/T2w-ratio expected lifespan trajectories in different brain compartments, we fitted linear mixed models, accounting for clustering (participants within sites), to HC data. Sex, age and age squared were included as fixed effects according to Akaike information criterion (AIC). We dealt with heterogeneity in residual variances, due to different T2-weighted sequences geometry (i.e., higher residual variability detected with 3D *vs* 2D sequences), by allowing heteroscedastic errors. First-order derivative (estimated slope) of each model was evaluated across ages (steps of 5 years) to assess the rates of T1w/T2w-ratio changes.

Estimated parameters from the described models were then used to convert T1w/T2w-ratio values measured in MS patients to z-scores, which are relative to healthy sex- and age-corrected values from HC. In particular, they represent a standardized measure of deviation from the sex-, age- and site-specific expected values in the HC population. We assessed (testing the nullity, i.e., healthy population reference value, of the estimated means) and compared z-scores in MS patients as a whole and in clinical phenotypes, by linear models. Obtained results suggested stratifying MS patients according to the relapse-onset continuum (i.e., CIS, RRMS, SPMS) or progressive-onset (i,e., PPMS) course. Therefore, we studied by linear regression models z-scores trends along increasing disability levels. To this aim, we chose the following EDSS scores, considered relevant milestones of disease severity: (1) EDSS=3.0; (2) EDSS=4.0; and (3) EDSS=6.0. Lastly, we ran linear regression models to investigate the association of T1w/T2w-ratio in different brain compartments with disease duration and EDSS. Differences of associations between relapse-onset and progressive-onset MS patients were tested by interaction terms. A random intercept for site was added to assess the association with brain T2-hyperintense LV and NBV.

All the analyses were repeated including the presence of DMT as a binary variable in our models. The significance of specific interaction terms was also tested.

Benjamini-Hochberg false discovery rate (FDR) correction was carried out to account for the overall number of tests performed, for each analysis separately.

SAS release 9.4 (SAS Institute, Cary, NC) was used for computations. P-values<0.05 were deemed statistically significant.

RESULTS

Demographic, clinical and conventional MRI findings

Among the 275 HC and 444 MS patients initially evaluated for the study, 5 HC and 10 MS patients were excluded due to the incomplete MRI protocol (n=5) or inadequate MRI scan quality

(poor positioning, movement artefacts, n=10). Ultimately, data from 270 HC and 434 MS patients were available for analysis, including 57 CIS, 196 RRMS, 106 SPMS and 75 PPMS (Table 1).

Table 1. Main demographic, clinical, and conventional MRI characteristics of healthy controls and multiple sclerosis patients as a whole, and according to their disease clinical phenotype.

Variable		HC (n=270)	All MS (n=434)	p (FDR p)	CIS (n=57)	RRMS (n=196)	SPMS (n=106)	PPMS (n=75)	p[a]
Sex									
Male (%)		121 (45)	177 (41)	0.293	18 (32)	78 (40)	42 (40)	39 (52)	0.131
Female (%)		149 (55)	257 (59)		39 (68)	118 (60)	64 (60)	36 (48)	
Mean age (SD) [years]		41.9 (15.4)	44.8 (12.2)	**0.009**	32.4[b] (8.8)	42.7[c] (11.3)	53.1[d] (8.6)	48.1[b,e] (11.7)	**<0.001**
Median disease duration (IQR) [years]			12.0 (2.6;20.2)	-	0.4 (0.3;1.0)	12.7[c] (4.5;19.3)	20.8[d] (15.0;27.0)	6.0[e] (2.0;16.0)	**<0.001**
Median EDSS (IQR)			3.5 (1.5;5.5)	-	1.5 (1.0;2.0)	2.0[c] (1.5;3.0)	6.0[d] (5.0;6.5)	5.5 (4.0;6.5)	**<0.001**
Patients receiving DMTs (%)			221 (51)	-	5 (9)	172[c] (88)	29[d] (27)	15 (20)	**<0.001**
Median brain T2-hyperintense LV (IQR) [mL]		0.1 (0.0;0.2)	5.0 (2.2;12.5)	-	3.1 (2.5;3.6)	3.7[c] (3.4;4.0)	4.1[d] (3.7;4.4)	3.7[e] (3.4;4.1)	**<0.001**
Estimated mean	**NBV (SE) [mL]**	1569 (12)	1523 (12)	**<0.001 (<0.001)**	1551[b] (12)	1528[c] (11)	1493 (12)	1522[e] (13)	**<0.001**
	NWMV (SE) [mL]	707 (18)	690 (18)	**<0.001 (<0.001)**	701 (18)	693 (18)	677[d] (18)	691[e] (18)	**<0.001**
	NGMV (SE) [mL]	862 (16)	833 (16)	**<0.001 (<0.001)**	848[b] (16)	836[c] (15)	818[d] (16)	832[e] (16)	**<0.001**
	Normalized cortical volume (SE) [mL]	650 (11)	625 (11)	**<0.001 (<0.001)**	638[b] (11)	626[c] (10)	612[d] (11)	623 (11)	**<0.001**

Normalized thalamic volume (SE) [mL]	22.1 (0.4)	20.3 (0.4)	**<0.001 (<0.001)**	21.7 (0.4)	20.4[c] (0.4)	19.0[d] (0.4)	20.3[e] (0.5)	**<0.001**
Normalized striatum volume (SE) [mL]	23.6 (0.3)	21.9 (0.3)	**<0.001 (<0.001)**	23.5 (0.4)	21.8[c] (0.3)	21.0[d] (0.4)	21.8[e] (0.4)	**<0.001**
Normalized pallidum volume (SE) [mL]	4.9 (0.1)	4.7 (0.1)	**<0.001 (<0.001)**	5.0 (0.1)	4.7[c] (0.1)	4.4[d] (0.1)	4.7 (0.1)	**<0.001**

Comparisons performed by Chi-squared (sex), Kruskal-Wallis and Mann-Whitney (disease duration and EDSS) tests, and linear models (age). FDR correction (Benjamini-Hochberg procedure) was applied to account for the overall number of tests. Age- and sex-adjusted linear mixed models, for clustered data (participants within sites), were performed for MRI features. Bold values denote statistical significance ($p < 0.05$).

p[a]Heterogeneity among MS phenotypes.
[b]Significant post hoc comparisons vs HC.
[c]Significant post hoc comparisons vs CIS.
[d]Significant post hoc comparisons vs RRMS.
[e]Significant post hoc comparisons vs SPMS.

Abbreviations: CIS = clinically isolated syndrome; DMT = disease-modifying therapy; EDSS = Expanded Disability Status Scale; FDR = false discovery rate; LV = lesion volume; MRI = magnetic resonance imaging; NBV = normalized brain volume; NGMV = normalized gray matter volume; NWMV = normalized white matter volume; PPMS = primary progressive multiple sclerosis; RRMS = relapsing-remitting multiple sclerosis; SPMS = secondary progressive multiple sclerosis.

Compared to HC, MS patients were significantly older (p=0.009), and as expected had significant higher brain T2-hyperintense LV, and lower NBV, NGMV, normalized cortical GMV, NWMV, as well as lower normalized thalamic, striatum and pallidum volumes (FDR p<0.001 for all).

Compared to CIS, RRMS patients had a higher EDSS score (p=0.001), longer disease duration (p<0.001), higher brain T2-hyperintense LV (FDR p<0.001), and lower NBV, NGMV, normalized cortical GM volume, normalized thalamic, pallidum and striatum volumes (FDR p ranging from <0.001 to 0.028).

Compared to RRMS, SPMS patients had a higher EDSS, longer disease duration, higher brain T2-hyperintense LV (p<0.001 for all), and lower NBV, NWMV, NGMV, normalized cortical GM volume, normalized thalamic, pallidum and striatum volumes (FDR p ranging from <0.001 to 0.011).

Compared to SPMS, PPMS patients had shorter disease duration (p<0.001). Compared to PPMS, SPMS patients had a higher brain T2-hyperintense LV (FDR p=0.024) and lower NBV, NGMV, NWMV, normalized thalamic, pallidum and striatum volumes (FDR p ranging from <0.001 to 0.042).

T1w/T2w-ratio trajectories in healthy controls

The T1w/T2w-ratio significantly increased until the age of 45 years in the thalamus, until 50 years in the WM and pallidum and until 60 years in the cortex and striatum (FDR p ranging from <0.001 to 0.022) (Figure 1, Supplementary Table 2). Interestingly, a steep slope was detected in the first decades especially in the pallidum and striatum. Higher T1w/T2w-ratio values were observed in females compared to males, consistently in all the investigated brain compartments (ß ranging from 0.023 to 0.065, all p≤0.022). The inclusion of an age × sex interaction term, which was not significant in all the models, did not lead to an improvement in AIC-based models fit.

T1w/T2w-ratio in multiple sclerosis clinical phenotypes

Compared to HC WM, T1w/T2w-ratio was significantly lower in T2-hyperintense WM lesions of MS patients, including all the main clinical phenotypes (FDR $p<0.001$ for all) (Table 2, Figure 2).

Table 2. Mean estimated T1w/T2w-ratio z-scores in multiple sclerosis patients as a whole and according to their clinical phenotype in different brain compartments.

	MS		**CIS**		**RRMS**		**SPMS**		**PPMS**		
	Mean (SD)	**p (FDR p)**	**Mean (SD)**	**p (FDR p)**	**Mean (SD)**	**p (FDR p)**	**Mean (SD)**	**p (FDR p)**	**Mean (SD)**	**p (FDR p)**	**p[a]**
WM lesions	-4.531 (1.426)	**<0.001 (<0.001)**	-4.251 (1.768)	**<0.001 (<0.001)**	-4.406 (1.317)	**<0.001 (<0.001)**	-4.897 (1.257)[b]	**<0.001 (<0.001)**	-4.536 (1.582)	**<0.001 (<0.001)**	**0.010**
NAWM	-0.266 (1.220)	**0.001 (0.001)**	-0.152 (1.224)	0.351 (0.508)	-0.366 (1.148)	**0.001 (0.001)**	-0.314 (1.245)	**0.011 (0.026)**	-0.021 (1.343)	0.890 (0.920)	0.213
Thalamus	-0.001 (1.163)	0.989 (0.989)	-0.089 (1.094)	0.541 (0.701)	-0.041 (1.080)	0.598 (0.718)	-0.023 (1.237)	0.849 (0.906)	0.202 (1.308)	0.185 (0.355)	0.491
Striatum	0.349 (1.328)	**0.001 (0.001)**	0.040 (1.145)	0.792 (0.864)	0.201 (1.227)	**0.023 (0.042)**	0.555 (1.338)[c]	**0.001 (0.001)**	0.681 (1.588)	**0.001 (0.001)**	**0.008**
Pallidum	0.536 (1.367)	**<0.001 (<0.001)**	0.186 (1.240)	0.262 (0.393)	0.351 (1.318)	**0.001 (0.001)**	0.913 (1.333)[d]	**0.001 (0.001)**	0.754 (1.492)	**0.001 (0.001)**	**<0.001**
Cortex	-0.270 (1.246)	**0.001 (0.001)**	-0.173 (1.090)	0.237 (0.392)	-0.246 (1.158)	**0.003 (0.008)**	-0.430 (1.364)	**0.001 (0.004)**	-0.183 (1.401)	0.262 (0.393)	0.523

p values of the test for the nullity (i.e., healthy population expected value) of the mean estimated z-scores in each group and of significant between-group comparisons are reported (linear models). FDR correction (Benjamini-Hochberg procedure) was applied to account for the overall number of tests. Bold values denote statistical significance ($p < 0.05$).

p[a]Heterogeneity among MS phenotypes.
[b,c,d]Significant SPMS vs RRMS post hoc comparisons: b) p (FDR p) = 0.002 (0.004); c) p (FDR p) = 0.025 (0.045); d) p (FDR p) = 0.001 (0.002).

Abbreviations: CIS = clinically isolated syndrome; FDR = false discovery rate; MS = multiple sclerosis; NAWM = normal-appearing white matter; PPMS = primary progressive multiple sclerosis; RRMS = relapsing-remitting multiple sclerosis; SPMS = secondary progressive multiple sclerosis; WM = white matter.

Similarly, T1w/T2w-ratio of NAWM and cortex were significantly lower in MS patients compared to HC (FDR p=0.001 for both), although this was significant only in RRMS and SPMS patients when the clinical phenotypes were evaluated separately (FDR p ranging from 0.001 to 0.026).

Conversely, T1w/T2w-ratio of the pallidum and striatum were significantly higher in MS patients compared to HC (FDR p≤0.001). In the analysis according to disease clinical phenotype, such a difference was detected only in RRMS, SPMS and PPMS patients (FDR p ranging from 0.001 to 0.042).

Thalamic T1w/T2w-ratio did not differ between MS patients and HC, also according to disease clinical phenotypes (FDR p≥0.355).

T2-hyperintense WM lesion T1w/T2w-ratio was significantly lower in SPMS *vs* RRMS (FDR p=0.004), whereas T1w/T2w-ratio of the striatum and pallidum were significantly higher in SPMS *vs* RRMS (FDR p=0.045 and 0.002, respectively).

T1w/T2w-ratio did not differ between RRMS *vs* CIS or between PPMS *vs* SPMS (FDR p ≥0.392) in any GM or WM region.

Compared to HC, larger increases in T1w/T2w-ratio were observed in males than in females MS patients in the pallidum (mean [SD] z-score males: 0.737 [1.302], FDR p<0.001; females: 0.398 [1.395], FDR p<0.001; males *vs* females: FDR p=0.022) and striatum (males: 0.542 [1.299], FDR p<0.001; females: 0.217 [1.335], FDR p=0.020; males *vs* females: FDR p=0.022).

Results did not change when adjusting for treatment. No influence of DMT on T1w/T2w-ratio within-group estimates (p≥0.071) and between-phenotypes comparisons (p≥0.222) was detected.

T1w/T2w-ratio according to EDSS milestones

In relapse-onset MS, compared to HC, T1w/T2w-ratio was significantly lower in WM lesions (all FDR p<0.001) and NAWM (FDR p ranging from 0.008 to 0.023) starting from mild disability

(EDSS<3.0) and in the cortex starting from EDSS≥3.0 (FDR p ranging from 0.007 to 0.023) (Table 3, Figure 3). Conversely, T1w/T2w-ratio was significantly higher in the striatum and pallidum only from EDSS≥4.0 onward (FDR p ranging from 0.001 to 0.005).

Table 3. Mean estimated T1w/T2w-ratio z-scores in relapse- and progressive-onset multiple sclerosis patients according to EDSS milestones in different brain compartments.

	Relapse-onset MS									**Progressive-onset MS**				
	EDSS < 3 (n=178)		**3 ≤ EDSS < 4 (n=45)**		**4 ≤ EDSS < 6 (n=68)**		**EDSS ≥ 6 (n=68)**			**EDSS < 6 (n=39)**		**EDSS ≥ 6 (n=36)**		
	Mean (SD)	**p (FDR p)**	**Mean (SD)**	**p (FDR p)**	**Mean (SD)**	**p (FDR p)**	**Mean (SD)**	**p (FDR p)**	**p[a]**	**Mean (SD)**	**p (FDR p)**	**Mean (SD)**	**p (FDR p)**	**p[a]**
WM lesions	-4.183 (1.514)	**<0.001 (<0.001)**	-4.766[b] (1.072)	**<0.001 (<0.001)**	-4.811[b] (1.127)	**<0.001 (<0.001)**	-4.949[b] (1.306)	**<0.001 (<0.001)**	**<0.001**	-5.096 (1.367)	**<0.001 (<0.001)**	-3.929[e] (1.592)	**<0.001 (<0.001)**	**0.001**
NAWM	-0.239 (1.185)	**0.008 (0.023)**	-0.473 (1.083)	**0.005 (0.019)**	-0.480 (1.224)	**0.002 (0.008)**	-0.379 (1.082)	**0.006 (0.020)**	0.408	-0.229 (1.366)	0.302 (0.422)	0.203 (1.298)	0.354 (0.425)	0.165
Thalamus	0.039 (1.095)	0.637 (0.800)	-0.357[b] (1.022)	**0.024 (0.049)**	-0.120 (1.123)	0.382 (0.551)	0.036 (1.266)	0.817 (0.942)	0.130	0.053 1.356)	0.809 (0.809)	0.364 (1.252)	0.090 (0.202)	0.305
Striatum	0.125 (1.131)	0.147 (0.253)	0.068 (0.964)	0.643 (0.800)	0.587[b,c] (1.222)	**<0.001 (0.002)**	0.610[b,c] (1.331)	**<0.001 (0.002)**	**0.005**	0.210 (1.363)	0.348 (0.425)	1.091[e] (1.634)	**<0.001 (0.001)**	**0.014**
Pallidum	0.177 (1.143)	0.044 (0.082)	0.202 (1.273)	0.293 (0.451)	0.586 (1.405)	**0.001 (0.005)**	1.188[b,c,d] (1.240)	**<0.001 (<0.001)**	**<0.001**	0.243 (1.323)	0.264 (0.422)	1.167[e] (1.34)	**<0.001 (<0.001)**	**0.004**
Cortex	-0.028 (1.024)	0.723 (0.866)	-0.479[b] (1.166)	**0.008 (0.023)**	-0.499[b] (1.234)	**0.001 (0.007)**	-0.509[b] (1.269)	**0.002 (0.008)**	**0.003**	-0.272 (1.432)	0.244 (0.422)	-0.087 (1.379)	0.709 (0.750)	0.570

p values of the test for the nullity (i.e., healthy population expected value) of the mean estimated z-scores in each group and of significant between-group comparisons are reported (linear models). FDR correction (Benjamini-Hochberg procedure) was applied to account for the overall number of tests. Bold values denote statistical significance (p <0.05).

p[a]Heterogeneity among multiple sclerosis patients' groups, classified according to EDSS milestones.
[b]Significant post hoc comparisons vs EDSS < 3 (FDR p range: <0.001-0.021; FDR p range: <0.001-0.045).
[c]Significant post hoc comparisons vs 3 ≤ EDSS < 4 (FDR p range: <0.001-0.015; FDR p range: <0.001-0.034).
[d]Significant post hoc comparisons vs 4 ≤ EDSS < 6 (FDR p=0.009; FDR p=0.023).
[e]Significant post hoc comparisons vs EDSS < 6 (FDR p range: 0.001-0.014; FDR p range: 0.004-0.037).

Abbreviations: CIS = clinically isolated syndrome; EDSS = Expanded Disability Status Scale; FDR = false discovery rate; MS = multiple sclerosis; NAWM = normal-appearing white matter; WM = white matter.

In PPMS patients, T1w/T2w-ratio was significantly lower in WM lesions already at EDSS<6.0 (FDR p<0.001) and significantly higher in the striatum and pallidum only after EDSS≥6.0 (FDR p=0.001 and <0.001, respectively).

Between-group comparisons confirmed the described T1w/T2w-ratio behavior along increasing disability levels (Table 3, Figure 3).

Results did not change when adjusting for treatment. No influence of DMT on T1w/T2w-ratio within-group estimates (p≥0.069) and between-group comparisons (p≥0.107) was detected.

Analysis of associations

A longer disease duration, higher EDSS score, higher brain T2-hyperintense WM LV, and a lower NBV were significantly associated with a lower T1w/T2w-ratio in T2-hyperintense WM lesions (ß ranging from -1.168 to 0.005, FDR p ranging from <0.001 to 0.011) and with a higher T1w/T2w-ratio in the pallidum and striatum (ß ranging from to -0.005 to 0.286, FDR p ranging from <0.001 to 0.040) (Table 4). A longer disease duration was also significantly associated with a lower cortical T1w/T2w-ratio (ß=-0.014, FDR p=0.036), whereas a higher brain T2-hyperintense WM LV was associated with a lower T1w/T2w-ratio both in the cortex and NAWM (ß=-0.313, FDR p=0.010 and ß =-0.442, FDR p<0.001).

Results did not change when adjusting for treatment. No significant interaction effects on the associations due to DMT were detected (p≥0.102), with no effect on the differences of association between relapse-onset and progressive-onset disease course (p>0.118).

Table 4. Associations between disease duration, Expanded Disability Status Scale (EDSS) score, brain T2 lesion volume (LV) and NBV (normalized brain volume) with T1w/T2w-ratio z-scores in multiple sclerosis patients as a whole, and classified in relapse- and progressive-onset patients, in different brain compartments.

		MS		**Relapse-onset MS**		**Progressive-onset MS**		**Progressive- vs relapse-onset MS**
		β (SE)	**p (FDR p)**	**β (SE)**	**p (FDR p)**	**β (SE)**	**p (FDR p)**	**p (FDR p)**
WM lesions T1w/T2w-ratio	Disease duration	-0.027 (0.007)	**<0.001 (<0.001)**	-0.036 (0.007)	**<0.001 (<0.001)**	0.020 (0.019)	0.287 (0.412)	**0.005 (0.016)**
	EDSS	-0.095 (0.032)	**0.003 (0.011)**	-0.152 (0.035)	**<0.001 (<0.001)**	0.278 (0.120)	0.021 (0.050)	**<0.001 (0.003)**
	Brain T2 LV	-1.168 (0.108)	**<0.001 (<0.001)**	-1.072 (0.115)	**<0.001 (<0.001)**	-1.772 (0.275)	**<0.001 (<0.001)**	0.020 (0.050)
	NBV	0.005 (0.001)	**<0.001 (<0.001)**	0.005 (0.001)	**<0.001 (<0.001)**	0.006 (0.003)	0.038 (0.081)	0.767 (0.817)
NAWM T1w/T2w-ratio	Disease duration	-0.005 (0.006)	0.377 (0.490)	-0.008 (0.006)	0.167 (0.272)	0.022 (0.016)	0.175 (0.275)	0.080 (0.151)
	EDSS	0.022 (0.028)	0.431 (0.531)	-0.02 (0.0300)	0.504 (0.598)	0.178 (0.103)	0.086 (0.159)	0.066 (0.130)
	Brain T2 LV	-0.442 (0.105)	**<0.001 (<0.001)**	-0.422 (0.111)	**<0.001 (<0.001)**	-0.741 (0.290)	**0.013 (0.036)**	0.304 (0.420)
	NBV	0.001 (0.001)	0.559 (0.632)	0.001 (0.001)	0.265 (0.391)	-0.001 (0.002)	0.624 (0.678)	0.395 (0.500)
Thalamic T1w/T2w-ratio	Disease duration	-0.004 (0.005)	0.405 (0.505)	-0.006 (0.006)	0.304 (0.420)	0.010 (0.016)	0.518 (0.606)	0.337 (0.456)
	EDSS	0.036 (0.026)	0.172 (0.275)	0.006 (0.029)	0.839 (0.852)	0.181 (0.100)	0.072 (0.139)	0.095 (0.168)
	Brain T2 LV	-0.115 (0.103)	0.269 (0.391)	-0.093 (0.109)	0.396 (0.500)	-0.370 (0.290)	0.206 (0.315)	0.372 (0.490)
	NBV	-0.001 (0.001)	0.239 (0.358)	-0.001 (0.001)	0.306 (0.420)	0.001 (0.002)	0.843 (0.852)	0.871 (0.871)
Striatum T1w/T2w-ratio	Disease duration	0.017 (0.006)	**0.005 (0.014)**	0.012 (0.006)	0.045 (0.092)	0.053 (0.018)	**0.003 (0.010)**	0.031 (0.068)
	EDSS	0.149 (0.028)	**<0.001 (<0.001)**	0.122 (0.030)	**<0.001 (<0.001)**	0.377 (0.114)	**0.001 (0.004)**	0.031 (0.068)
	Brain T2 LV	0.259 (0.112)	0.022 (0.051)	0.261 (0.115)	0.024 (0.055)	0.083 (0.350)	0.814 (0.845)	0.628 (0.678)
	NBV	-0.004 (0.001)	**<0.001 (<0.001)**	-0.004 (0.001)	**<0.001 (<0.001)**	-0.006 (0.003)	0.043 (0.091)	0.555 (0.632)

Pallidum T1w/T2w-ratio	Disease duration	0.026 (0.006)	**<0.001 (<0.001)**	0.024 (0.006)	**<0.001 (0.001)**	0.048 (0.016)	**0.003 (0.010)**	0.165 (0.272)
	EDSS	0.199 (0.028)	**<0.001 (<0.001)**	0.196 (0.032)	**<0.001 (<0.001)**	0.361 (0.102)	**<0.001 (0.002)**	0.123 (0.207)
	Brain T2 LV	0.286 (0.117)	**0.015 (0.040)**	0.303 (0.125)	**0.016 (0.042)**	0.073 (0.316)	0.819 (0.845)	0.498 (0.598)
	NBV	-0.005 (0.001)	**<0.001 (<0.001)**	-0.005 (0.001)	**<0.001 (<0.001)**	-0.003 (0.003)	0.190 (0.294)	0.593 (0.655)
Cortical T1w/T2w-ratio	Disease duration	-0.014 (0.006)	**0.013 (0.036)**	-0.018 (0.006)	**0.002 (0.007)**	0.012 (0.017)	0.489 (0.594)	0.092 (0.166)
	EDSS	-0.053 (0.028)	0.054 (0.108)	-0.087 (0.03)	**0.004 (0.012)**	0.097 (0.109)	0.377 (0.490)	0.105 (0.183)
	Brain T2 LV	-0.313 (0.104)	**0.003 (0.010)**	-0.298 (0.110)	**0.007 (0.022)**	-0.479 (0.295)	0.109 (0.187)	0.566 (0.632)
	NBV	0.002 (0.001)	0.020 (0.050)	0.002 (0.001)	**0.014 (0.040)**	0.001 (0.002)	0.564 (0.632)	0.775 (0.817)

Beta coefficients (β) and p values from linear models are reported. FDR correction (Benjamini-Hochberg procedure) was applied to account for the overall number of tests. Bold values denote statistical significance ($p < 0.05$).

Abbreviations: EDSS = Expanded Disability Status Scale; FDR = false discovery rate; LV = lesion volume; MS = multiple sclerosis; NAWM = normal-appearing white matter; NBV = normalized brain volume; SE = standard error; WM = white matter.

DISCUSSION

By evaluating T1w/T2w-ratio in different brain compartments in a large multicenter cohort of HC and MS patients including the main disease clinical phenotypes, this study provided relevant information regarding the trajectories of T1w/T2w-ratio occurring with aging and how clinical phenotypes, severity of disability and structural brain damage are associated with brain T1w/T2w-ratio abnormalities in MS.

Consistently with previous studies,[24, 25] the analysis on HC showed that T1w/T2w-ratio varied across the adult lifespan in the different brain compartments. In particular, T1w/T2w-ratio gradually increased until mid-age, followed by a plateau in all the investigated structures. Of note, in the deep GM nuclei, especially in the pallidum and striatum, a steeper increase occurred in the first decades. Moreover, sex showed a marginal effect on T1w/T2w-ratio trajectories, with higher T1w/T2w-ratio values observed in females compared to males in all the investigated brain compartments. Different physiological processes may explain our findings. T1w/T2w-ratio has been suggested to be associated with myelin density.[3] Accordingly, the process of brain myelination may contribute to the increase of T1w/T2w-ratio at least in the first decades of life[26] and could explain the sex-differences observed.[27] Since iron influences both signal on T1- and T2-weighted images[4, 28] and it co-localizes with myelin in the cortex and WM,[28, 29] it may also contribute, together with myelin macromolecules, to T1w/T2w-ratio values.[4] In T2 spin echo sequences, the effect of iron in reducing T2 relaxation time is related to water diffusivity. Specifically, it is evident when the diffusion distance and the size of the cells (where iron is presumably compartmentalized) are comparable in terms of diameter.[30] Considering the relatively low myelin content within the deep GM nuclei (except for the thalamus and globus pallidus), the observed trajectories support the contribution of iron and neurodegeneration in determining the increase of T1w/T2w-ratio with healthy aging.[31] When myelination is accomplished, there is no further iron accumulation in oligodendrocytes.[28] However, microglia and astrocytes continue to accumulate iron during adulthood and senescence,[32] possibly explaining the high iron levels found in the basal ganglia in

post-mortem studies,[28, 33] and the age-related increase of susceptibility on quantitative susceptibility mapping studies.[34, 35]

The most relevant results are those we obtained from the evaluation of T1w/T2w-ratio in the different brain compartments of MS patients according to their disease severity.

In line with a previous study,[16] we found a significantly lower T1w/T2w-ratio in T2-hyperintense WM lesions in all the clinical phenotypes and already from mild disability. According to histopathological studies,[36, 37] demyelination, axonal damage and loss of iron may explain our findings (Figure 4). Interestingly, more severe damage was found in SPMS patients and more severe disability. Our findings suggest that, although the pathological processes occurring in focal WM lesions are similar across the MS spectrum, a more severe disease is characterized by more relevant lesional microstructural abnormalities.

In the NAWM, compared to HC, T1w/T2w-ratio was significantly lower already from mild disability levels and in RRMS and SPMS patients, but not in those with CIS and PPMS. Although in contrast with some previous reports,[11, 16] our results in RRMS patients are consistent with other studies,[12-14, 18] showing no significant differences in the early phases of the disease but only in patients with a longer disease duration. Discrepancies among studies may be explained by heterogeneities in the cohorts of MS patients investigated, often with small sample sizes and short disease duration, and in the methods applied to quantify T1w/T2w-ratio. Despite this, our results further confirm that NAWM is characterized by the occurrence of microstructural abnormalities, with inflammation, demyelination, gliosis and axonal damage (Figure 4),[2] already from the early phases of the disease.[1, 2]

Consistently with previous findings,[6, 17] compared to HC, cortical T1w/T2w-ratio was significantly lower from moderate levels of disability and in RRMS and SPMS patients, but not in those with CIS and PPMS. Although we did not evaluate cortical lesions, our findings suggested that microstructural tissue abnormalities, including demyelination, iron loss and axonal damage,

occur in the cortex of MS patients[38-40] and further support cortical damage in determining clinical disability.

Unexpectedly, we found no differences in NAWM and cortical T1w/T2w-ratio between PPMS patients and HC nor a significant decline in SPMS compared to RRMS patients.

MS patients with a progressive disease course and more severe disability are characterized by a progressive reduction of myelin and oligodendrocytes, typically rich of iron, thus promoting a further decline of T1w/T2w-ratio in the NAWM and cortex. However, age-related physiological iron increase in oligodendrocytes and myelin may act in the opposite direction (Figure 4).[41] Moreover, a more substantial microglia activation and astrogliosis with a patchy iron redistribution, especially in progressive MS patients, may also contribute to explain our findings.[2, 33]

Interestingly, compared to HC, T1w/T2w-ratio in the pallidum and striatum was significantly higher from moderate levels of disability and in all disease clinical phenotypes, except for patients with CIS. Of note, the highest T1w/T2w-ratio values were found in SPMS and PPMS patients. Ferritin iron has a strong effect on decreasing T2w signal, but only a weak effect on increasing T1w signal,[4, 28] leading to an increase in T1w/T2w-ratio. This could explain the higher T1w/T2w-ratio values in MS patients compared to HC, as the pallidum and striatum have been shown to accumulate iron in MS patients.[42, 43] Our findings are consistent with previous studies that evaluated T1w/T2w-ratio in other neurodegenerative diseases.[9, 10, 44, 45] Patients with Parkinson disease showed higher T1w/T2w-ratio in the substantia nigra.[10] In Alzheimer disease,[9] Huntington disease[44] and multiple system atrophy,[45] cortical and WM T1w/T2w-ratio, respectively, were significantly higher compared to HC. Moreover, our analysis showed sex-differences in T1w/T2w-ratio values in the striatum and pallidum, confirming the evidence that males have worse neurodegeneration than females in MS.[46] All these findings suggest that T1w/T2w-ratio may reflect ongoing neurodegenerative processes (Figure 4).

Interestingly and differently from the other deep GM nuclei, we found no significant alterations of T1w/T2w-ratio values in the thalamus in MS patients, independently from the clinical phenotype and clinical disability. Heterogeneous pathological processes including demyelination, microglia activation, neuronal loss and iron accumulation may affect the thalamus in MS and may influence T1w/T2w-ratio in opposite ways.[43] Of note, discordant evidence exists regarding thalamic iron concentration in this condition, with the majority of pathological and MRI studies suggesting no differences in thalamic iron content compared to HC,[34, 43] not confirmed by others showing decreased iron.[42] The peculiar morphological architecture of the thalamus, characterized by more abundant WM fibers and iron-rich oligodendrocytes compared with other structures (i.e., pallidum, striatum) likely contribute to explain our findings in this nucleus.[33]

Regarding the clinical relevance of this metric, differently from previous studies,[6, 12] the significant associations found between both EDSS score and disease duration with T1w/T2w ratio in T2-hyperintense WM lesion, cortex, striatum, and pallidum, suggest a gradual and progressive accumulation of clinically relevant microstructural tissue abnormalities in the different brain compartments.

The significant associations between higher brain T2-hyperintense WM LV and lower T1w/T2w-ratio in the NAWM and cortex and higher T1w/T2w-ratio in the pallidum and striatum suggest that diffuse abnormalities may be, at least partially, secondary to retrograde degeneration due to the accumulation of focal demyelinating T2-hyperintense lesions in the WM. Moreover, the associations with brain volume also suggested the relevance of T1w/T2w-ratio values as a surrogate measure of irreversible tissue loss.

This study has some limitations. First, we evaluated T1w/T2w-ratio as a global measure in all the investigated brain compartments with a cross-sectional approach. Since different regions are characterized by specific cyto-architectural features and MS may affect the different regions with spatial and temporal heterogeneities, future studies are needed to better evaluate MS pathology at a regional level and in a longitudinal setting to explore the dynamic patterns of

T1w/T2w-ratio and their associations with disease progression. Second, we did not evaluate cortical lesions, thus the results found for cortical T1w/T2w-ratio may be influenced, at least partially, by focal cortical demyelination. Third, we did not evaluate calcification in the pallidum, thus the results found could be partially influenced by the presence of other diamagnetic tissue components (i.e., calcium), known to potentially accumulate in deep GM structures.

Fourth, the observed T1w/T2w-ratio values showed a relatively high variability, possibly related also to the multicenter design. Indeed, even though the proposed method uses a calibration to reduce the intensity variability, different scans and sequences were used. However, appropriate adjustments for site were included in the statistical models to provide site-specific T1w/T2w-ratio z-scores that were used in subsequent statistical analyses. In addition, we allowed heteroscedastic errors to deal with the higher residual variability detected with 3D *vs* 2D T2-weighted sequences. This combined approach allowed to model the variability of our data, contributing to provide robustly estimated z-scores and strengthening the generalizability of our results.

In conclusion, in HC we demonstrated an increase of T1w/T2w-ratio with age in all the investigated brain compartments. Conversely, heterogeneous T1w/T2w-ratio abnormalities were found in specific brain compartments according to different clinical phenotypes and levels of disability in MS patients. Altogether, our data suggest that T1w/T2w-ratio results from several pathological substrates, including inflammation, demyelination, neurodegeneration and iron accumulation. Given its broad availability, the T1w/T2w-ratio may represent a clinically relevant method to investigate in vivo the heterogeneous processes affecting the brain of MS patients.

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Data sharing

The dataset used and analyzed during the current study are available from the corresponding author on reasonable request.

Copyright statement

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FIGURE LEGENDS

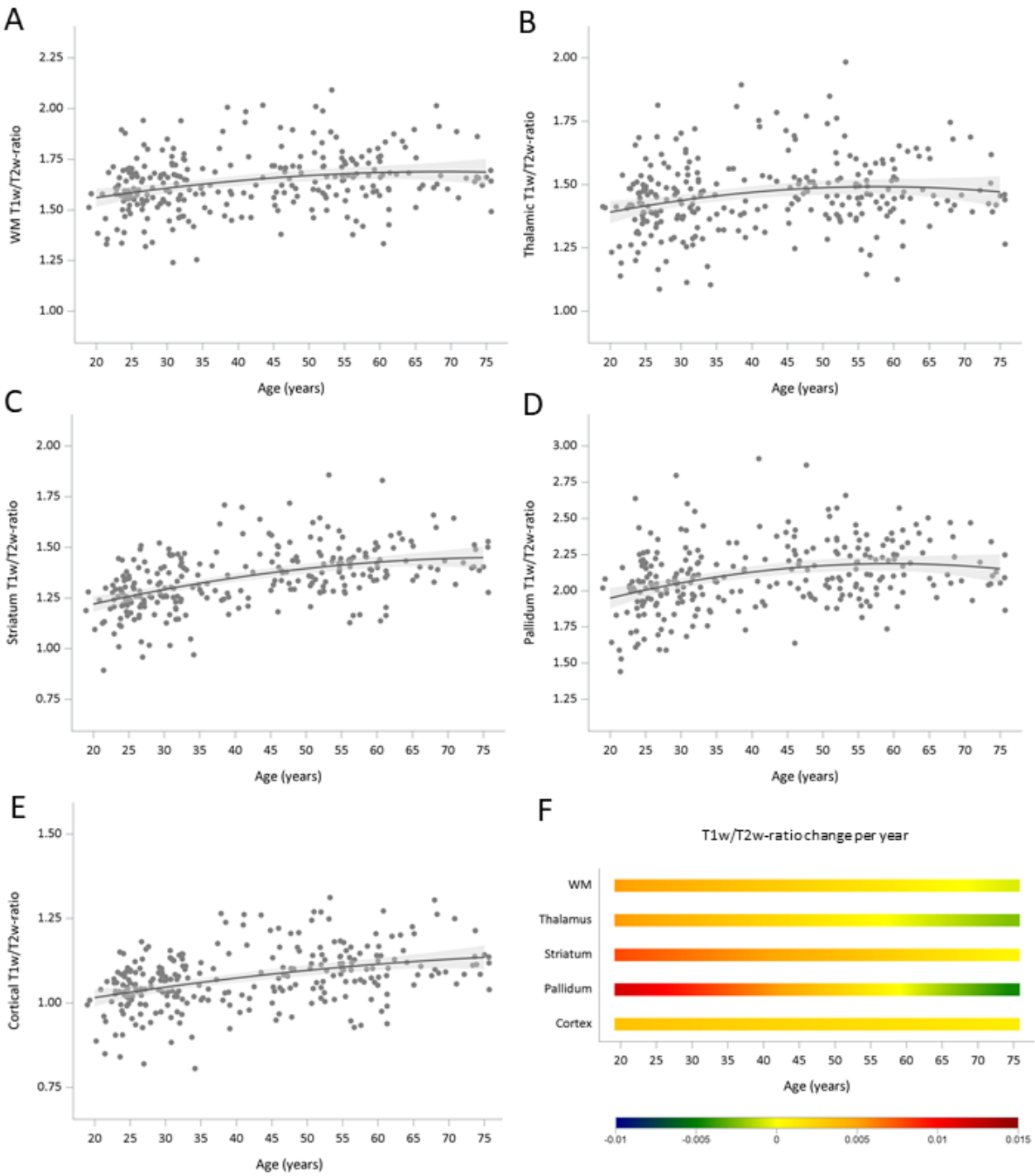

Figure 1. Estimated sex- and site-adjusted T1w/T2w-ratio lifespan trajectories in different brain compartments of healthy controls. The figure shows T1w/T2w-ratio mean estimated sex- and site-adjusted values (solid lines with 95% shaded confidence intervals) in (A) white matter, (B) thalamus, (C) striatum, (D) pallidum and (E) cortex, across ages in healthy controls (linear mixed models). (F) Rates of T1w/T2w-ratio change (trajectories estimated slopes) across ages in the different brain compartments studied in healthy controls.
Abbreviations: T1w/T2w-ratio = T1weighted/T2weighted ratio; WM = white matter.

Figure 2. T1w/T2w-ratio z-scores distribution in multiple sclerosis patients according to their clinical phenotype in different brain compartments. Violin plots show the distribution of T1w/T2w-ratio z-scores in (A) white matter lesions, (B) normal-appearing white matter, (C) thalamus, (D) striatum, (E) pallidum and (F) cortex, in multiple sclerosis patients. Healthy controls are only shown as a reference group for illustrative purposes. The symbol (*) indicates phenotypes with a significantly non-zero (i.e., healthy population expected value) mean estimated z-score, as well as significant between-group comparisons (linear models). FDR correction (Benjamini-Hochberg procedure) was applied. See main text and Table 2 for further details.
Abbreviations: CIS = clinically isolated syndrome; HC = healthy controls; NAWM = normal-appearing white matter; PPMS = primary progressive multiple sclerosis; RRMS = relapsing-remitting multiple sclerosis; SPMS = secondary progressive multiple sclerosis; T1w/T2w-ratio = T1weighted/T2weighted ratio; WM = white matter.

Figure 3. Mean estimated T1w/T2w-ratio *z*-scores in relapse- and progressive-onset multiple sclerosis patients according to EDSS milestones in different brain compartments.
T1w/T2w-ratio mean estimated *z*-scores with 95% confidence intervals in white matter lesions, normal-appearing white matter, thalamus, striatum, pallidum and cortex, according to increasing

disability levels**,** in (A) relapse-onset and (B) progressive-onset multiple sclerosis patients (linear models). See main text and Table 3 for further details.

Abbreviations: EDSS = Expanded disability status scale; NAWM = normal-appearing white matter; T1w/T2w-ratio = T1weighted/T2weighted ratio; WM = white matter.

Figure 4. Overview of the different pathological substrates of multiple sclerosis and their possible effects on T1w/T2w-ratio.

The pathological mechanisms involved in multiple sclerosis may influence T1w/T2w-ratio with opposite behaviors and heterogeneously in the different brain compartments investigated in the study. Demyelination/inflammation (yellow) and axonal damage (pink) are likely to reduce T1w/T2w-ratio; conversely, iron accumulation (red), microglia activation (violet) and astrogliosis (green) may increase T1w/T2w-ratio. In T2-hyperintense white matter lesions, normal-appearing white matter and cortex, the effects of demyelination/inflammation and axonal damage on T1w/T2w-ratio are likely to be more prominent than those of iron accumulation (red), microglia activation (violet) and astrogliosis (green), thus promoting an overall reduction of T1w/T2w-ratio. On the other hand, in the striatum and pallidum, the effects of microglia activation (violet) and astrogliosis (green), and, above all, iron accumulation (red) are likely to be more prominent, thus resulting in an overall increase of T1w/T2w-ratio. In the thalamus, the effects of the different pathological processes counteract each other, thus nullifying differences in T1w/T2w-ratio. Created with biorender.com.

Abbreviations: T1w/T2w-ratio = T1weighted/T2weighted ratio.