

RESEARCH ARTICLE

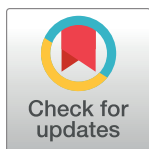
Patterns of brain atrophy in recently-diagnosed relapsing-remitting multiple sclerosis

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Abstract

Recurrent neuroinflammation in relapsing-remitting MS (RRMS) is thought to lead to neurodegeneration, resulting in progressive disability. Repeated magnetic resonance imaging (MRI) of the brain provides non-invasive measures of atrophy over time, a key marker of neurodegeneration. This study investigates regional neurodegeneration of the brain in recently-diagnosed RRMS using volumetry and voxel-based morphometry (VBM). RRMS patients (N = 354) underwent 3T structural MRI <6 months after diagnosis and 1-year follow-up, as part of the Scottish multicentre ‘FutureMS’ study. MRI data were processed using FreeSurfer to derive volumetrics, and FSL for VBM (grey matter (GM) only), to establish regional patterns of change in GM and normal-appearing white matter (NAWM) over time throughout the brain. Volumetric analyses showed a decrease over time ($q < 0.05$) in bilateral cortical GM and NAWM, cerebellar GM, brainstem, amygdala, basal ganglia, hippocampus, accumbens, thalamus and ventral diencephalon. Additionally, NAWM and GM volume decreased respectively in the following cortical regions, frontal: 14 out of 26 regions and 16/26; temporal: 18/18 and 15/18; parietal: 14/14 and 11/14; occipital: 7/8 and 8/8. Left GM and NAWM asymmetry was observed in the frontal lobe. GM VBM analysis showed three major clusters of decrease over time: 1) temporal and subcortical areas, 2) cerebellum, 3) anterior cingulum and supplementary motor cortex; and four smaller clusters within the occipital lobe. Widespread GM and NAWM atrophy was observed in this large recently-diagnosed RRMS cohort, particularly in the brainstem, cerebellar GM, and subcortical and occipital-temporal regions; indicative of neurodegeneration across tissue types, and in accord with limited previous studies in early disease. Volumetric and VBM results emphasise different features of longitudinal lobar and loco-regional change, however identify consistent atrophy patterns across individuals. Atrophy measures targeted to specific brain

Aberdeen, as well as the Grampian, Tayside, Lothian and Greater Glasgow Health boards, states that personal data cannot be released or made available to any third party unless the FutureMS steering committee have expressly permitted the data sharing, or personal data has been anonymized sufficiently. The magnetic resonance imaging data used in the current paper are not defaced and hence not sufficiently anonymized for open data sharing. Unrestricted access to these images in current form would present a confidentiality/privacy risk for participants. Additionally, the informed consent, approved by the South East Scotland Research Ethics Committee 02 under reference 15/SS/0233, states that by consenting to the FutureMS study, participants agree to sharing their data with the wider study team. The authors have not obtained explicit consent for deidentified data sharing beyond the wider study team. As such, they cannot make deidentified data openly available. The authors do, however, highly encourage collaboration with other research teams, and any researchers may request access to anonymized patient data from FutureMS-1 by contacting future-ms@ed.ac.uk. Proposals will be reviewed by the FutureMS steering committee and if approved, a signed data sharing agreement will be issued.

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regions may provide improved markers of neurodegeneration, and potential future imaging stratifiers and endpoints for clinical decision making and therapeutic trials.

1. Introduction

Multiple sclerosis (MS) is a neuroinflammatory and neurodegenerative disease affecting two million people worldwide [1–3]. Disease progression and severity varies between individuals, and symptoms are diverse, including mobility and vision problems, pain, depression, fatigue and cognitive impairment [4]. Several MS subtypes have been identified, of which relapsing-remitting disease (RRMS) is the most common [5,6]. There is currently no cure for MS. More accurate biomarkers of disease progression and improved understanding of disease mechanisms, particularly in terms of neurodegeneration, are required for more suitable treatment of MS. Magnetic resonance imaging (MRI) allows for studying neurodegeneration *in vivo*, through measurement of tissue volume change (atrophy) over time, and may thus provide such valuable biomarkers of MS severity and progression.

White matter lesions (WMLs) on MRI, reflecting underlying inflammatory demyelinating lesions, are considered an imaging hallmark of RRMS and are required for RRMS diagnosis [7]. Modulating neuroinflammation is also the primary target for currently available disease modifying treatment (DMT) for RRMS. However, these appear to have only a limited effect on reducing associated neurodegenerative processes [8,9]. Previous studies have shown that neurodegeneration also plays a prominent role in the disease evolution of RRMS and is importantly already present in the early stages of the disease [10–15]. Early-stage grey matter (GM) atrophy has been observed in specific brain areas, including the cingulate gyrus, precuneus, thalamus, basal ganglia, brainstem and cerebellum [11,16–18]. Less is known about regional patterns of WM atrophy in RRMS, but WM atrophy also occurs in early disease and has been suggested to occur independently of WML development [10,19,20]. Furthermore, previous studies have shown microstructural changes reflective of neurodegeneration within the normal-appearing WM (NAWM) [21]. Importantly, atrophy appears to be a better predictor of clinical disability and deterioration than WMLs [13,17,22–32]. This suggests it could provide a useful surrogate for neurodegeneration, to help stratify patients and evaluate the efficacy of disease-slowing treatments. Existing studies investigating the effect of current DMTs on atrophy suggest a slowing of neurodegeneration after DMT use [33–38]. Further research is required to investigate the effect of current DMTs and possible future neuroprotective therapies on brain atrophy. Such studies would benefit from detailed knowledge of the location and extent of early-stage GM and WM atrophy in RRMS [39].

Current literature does not provide detailed insights into regional WM volume loss in recently-diagnosed RRMS, and only a limited number of studies have examined specific regional GM volume in early stages of the disease [11,16–18]. The aim of this study was to investigate neurodegenerative changes reflected in loco-regional atrophy in recently-diagnosed people with RRMS. Additionally, two analysis approaches were used to understand disparate results in the literature and the degree to which they result from different measurement methodology. Baseline and 1-year follow-up MR imaging data was used from the FutureMS study [40,41], a large multicentre cohort of RRMS patients within six months after diagnosis. This allows for identification of brain areas affected by early atrophy in RRMS, which may provide possible biomarkers of disease progression for DMT trials.

design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: The authors have declared that no competing interests exist.

2. Methods

2.1 Participants

Participants with a recent diagnosis of RRMS according to the 2017 McDonald criteria (< 6 months) [7], were recruited (2016–2019) across five neurology sites in Scotland: Aberdeen, Dundee, Edinburgh, Inverness and Glasgow, as part of the FutureMS study [40,41]. Participants were 18 years or older and had the capacity to provide informed consent. Exclusion criteria were participation in a clinical trial prior to baseline assessment, contraindications for MRI, and intake of DMTs prescribed prior to baseline assessment. The latter criteria was selected because DMT intake in the Scottish population is very low compared with other countries, creating a unique opportunity to develop natural history predictive tools. Study visits took place at baseline (wave 0 [w0]) and 1-year follow-up (w1) and participants underwent brain MRI and expanded-disability status scale (EDSS) assessment at both time points. Full details of the FutureMS study have been previously described in Kearns et al. 2021 [40].

N = 431 participants underwent MR imaging at w0 and N = 382 participants underwent MR imaging at w1. The main reasons for not returning for w1 were not being able to reach participants at the provided contact details or participants having moved away from Scotland and not wanting to travel back. Additionally, the COVID-19 pandemic prematurely terminated w1 visits, which prevented several participants returning for their second visit. Additionally, 77/431 participants were excluded for various reasons (see Fig 1), resulting in N = 354 available MRI datasets for analysis.

All participants provided written informed consent before study entry. The study received ethical approval from the South East Scotland Research Ethics Committee 02 under reference 15/SS/0233 and was conducted in accordance with the Declaration of Helsinki and ICH guidelines on good clinical practice. Authors did not have access to information that could identify individual participants during or after data collection. All data were anonymised with unique study identifiers.

2.2 MR image acquisition

MR image acquisition was performed across four sites in Scotland using comparable 3T MRI systems (Siemens in Glasgow, Dundee and Edinburgh; Philips in Aberdeen). Protocol harmonisation was implemented during the course of the study to increase between-site comparability and facilitate image analysis. Importantly, each participant underwent both MRI scans at the same centre using the same protocol. All participants underwent T1-weighted, T2-weighted, and 2D FLAIR imaging. Full details have been previously described in Meijboom et al. (2022) [41] (see S1 Table for an overview of all MR parameters).

2.3 MR image processing

Image processing methods have been previously described in full in Meijboom et al. (2022) [41] and are briefly summarised in the sections below.

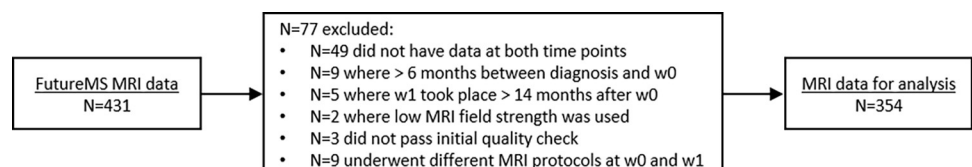


Fig 1. Overview of data exclusions.

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2.3.1 Registration and ICV. All images were registered to the T1W image with a rigid body transformation (degrees of freedom = 6) using FSL FLIRT (FSL v6.0.1) [42,43] separately for each time point. Brain tissue was isolated using FSL BET2 (FSL v6.0.1) [44], followed by manual editing (i.e. removal of any non-intracranial tissue) of the resulting w0 intracranial image using ITK-SNAP v3.8.0 [45]. For each participant, the edited w0 intracranial image was registered to the w1 T1W image, to avoid within-subject variability between time points. Intracranial volume (ICV) was extracted using fslstats (FSL6.0.1).

2.3.2 WML segmentation. Hyperintense voxels on 2D FLAIR were identified by thresholding intensity values to 1.69 SDs > mean, using an adjusted method from Zhan et al. (2014) [46]. Resulting hyperintense areas unlikely to reflect pathology were removed using a pre-defined lesion distribution template [47], followed by Gaussian smoothing. Resulting WML masks were manually edited using ITK-SNAP [45]. WML volumes were extracted using fslstats and expressed as a ratio of ICV (r-ICV) to correct for head size.

2.3.3 Tissue segmentation. Tissue segmentation was performed on T1W images using the longitudinal processing stream [48] in FreeSurfer v6.0 (<http://surfer.nmr.mgh.harvard.edu/>) with default settings and the edited intracranial image as brain mask. Tissue segmentations were based on the Desikan-Kiliany atlas [49] and pial surfaces were improved using additional contrast from T2W images. FreeSurfer output was manually edited by a trained neuroscientist where necessary using FreeView v2.0 (FreeSurfer v6.0) and following FreeSurfer editing guidelines (<https://surfer.nmr.mgh.harvard.edu/fswiki/Edits>). WML masks were then subtracted from the resulting tissue segmentations using fslmaths (FSL6.0.1), to create final tissue masks. Global and regional tissue volumes (see Table 1) were calculated from the final tissue masks using fslstats, and subsequently expressed as r-ICV to correct for head size.

2.3.4 Voxel-based morphometry. Longitudinal voxel-based morphometry (VBM), applying a voxel-wise comparison of local GM concentration between w0 and w1, was performed using the FSL VBM pipeline (FSL6.0.1; <http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FSLVBM>) [50–53].

Table 1. Global and regional GM/WM and subcortical areas.

Global WM and GM	Brainstem Cortical GM Cerebellar GM Cerebral WM Cerebellar WM		Subcortical regions	Amygdala Basal ganglia Hippocampus Nucleus accumbens Thalamus Ventral diencephalon	
Regional WM and GM	Frontal	Parietal	Temporal	Occipital	
	Superior frontal	Superior parietal	Superior temporal	Lateral Occipital	Insula
	Caudal middle frontal	Inferior parietal	Middle temporal	Lingual	
	Rostral middle frontal	Supramarginal	Inferior temporal	Cuneus	
	Pars opercularis	Postcentral	Superior temporal sulcus banks	Pericalcarine	
	Pars triangularis	Precuneus	Fusiform		
	Pars orbitalis	Posterior cingulate	Transverse temporal		
	Lateral orbitofrontal	Isthmus cingulate	Entorhinal		
	Medial orbitofrontal		Temporal Pole		
	Precentral		Parahippocampal		
	Paracentral				
	Frontal pole				
	Caudal anterior cingulate				
	Rostral anterior cingulate				

Left and right hemisphere were separately included for all areas, excluding the brainstem. WM = white matter, GM = grey matter.

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It was performed for GM only, because GM VBM is a more commonly used and accepted method (e.g. a WM VBM pipeline is not implemented in FSL). The first step of the VBM pipeline (i.e. brain extraction) was omitted, as we were able to use the previously created intracranial brain images, as described above. In step two, GM, WM and CSF were segmented from T1W images and the study-specific GM template was created. Specifically, all subject GM images at both time points were affine-registered to the GM ICBM-152 template, and then concatenated and averaged to create a study-specific GM template. The subject GM images were then non-linearly registered to this study-specific template, and concatenated and averaged again to then create a final study-specific GM template in standard space ($2 \times 2 \times 2 \text{ mm}^3$ resolution). In the last step, subject GM at both time points was then non-linearly registered to this study-specific template, after which a Jacobian modulation was applied, resulting in modulated GM images for each time point per subject.

As an additional step, to allow for longitudinal VBM analysis, we subtracted the modulated GM images for w1 from those at w0 using `fslmaths`, resulting in a GM difference file for each subject. Modulated GM difference files were then smoothed with a Gaussian size 2 kernel using `fslmaths`, and concatenated using `fslmerge` (FSL6.0.1) to form a final 4D group GM difference image for statistical analysis.

2.4 Statistical analyses

2.4.1 Volumetrics. Statistical analysis for tissue volumes was performed using R v4.0.2 [54] and package `lmerTest` [55], with further packages `ggplot2` [56] and `lattice` [57] used to create figures. A linear mixed-effects model was applied for each tissue volume (Table 1) with time point (w0, w1) as regressor of interest and age, sex, imaging site, WML change (w1 – w0) and DMT status at w1 as covariates. WML volume change was added as a covariate to correct for GM and WM volume changes induced by enlarging and/or receding WMLs. DMT status was included as a covariate to correct for the difference in effect of undergoing or not undergoing DMT (between baseline and follow-up) on GM and WM volume change. Additionally, to correct for any differences in volume change over time due to older and younger participants, the interaction between time and age was added. The interaction was removed from the model if not significant. For each outcome variable, extreme outliers (>3 SD) as well as participants with missing data at either time point were excluded. In case of missing DMT data, the mode of the group was used instead, i.e. yes to DMT use at w1. Subsequently, continuous variables were scaled before being entered into the linear model. False discovery rate (FDR) correction was performed to adjust p-values for multiple comparisons, with corrected p-values (q-values) considered significant at $q < 0.05$.

2.4.2 Voxel-based morphometry. FSL Randomise (FSL v6.0.1) [58] was used to perform nonparametric permutation analysis on the VBM output. A general linear model (GLM) design matrix was created with GM difference, age, sex, imaging site, DMT status at w1 and subject-specific WML mask as explanatory variables (EV). In case of missing DMT data, the mode of the group was used instead, i.e. yes to DMT use at w1. The interaction of age and time was not added here. Given the interaction was not significant in 98% of volumetric regions studied, it is unlikely it will have a significant effect on the overall VBM results. EVs were recentered to zero where appropriate.

WMLs were included as EVs to ensure their exclusion from the GM images. Subject-specific WML EVs were created by a) registering all subject-specific WML masks at w0 and w1 to the subject's modulated GM segmentation image at respectively w0 and w1, with the VBM transformation matrix that was created for registration of the native T1W to the group-specific template (`fslmaths`), and b) using FSL tool 'setup_masks' (FSL6.0.1; <https://fsl.fmrib.ox.ac.uk/>

[fsl/fslwiki/Randomise/UserGuide](https://fsl.fmrib.ox.ac.uk/fslwiki/Randomise/UserGuide)) to generate a concatenated 4D subject-specific WML image from the step a output images, as well as add a WML EV for each subject separately to the design matrix.

One t-contrast was defined, which assessed GM change over time, corrected for the remaining EV's. The 'randomise' function was run using 5000 permutations, and with threshold-free cluster enhancing (TFCE) [59] to perform a voxel-wise analysis with a specific focus on voxel clusters. Cluster (FSL6.0.1) was used to extract resulting clusters and atlasquery (FSL v6.0.1) was used to obtain labels for the included anatomical regions. Results were family-wise error (FWR) corrected for multiple comparisons ($p < 0.001$).

3. Results

3.1 Demographics

Data from $N = 315$ participants were used for tissue volume analysis and data from $N = 351$ were used for VBM analysis. Specifically, for tissue volume analysis, 39/354 cases were excluded due to segmentations of insufficient quality and for VBM analysis, 3/354 datasets were excluded for data processing purposes. In addition, for the tissue volume dataset, for participants where segmentation failed for one or a small number of areas only (usually subcortical), the appropriate areas were excluded from analysis, but the remaining data of the participant was included in analysis. See Table 2 for participant demographics for each analysis type.

Participants were DMT-naïve at w0, but most started receiving DMTs between w0 and w1 (Table 2). Post-hoc regression analyses showed tissue volume change was not significantly different between participants positive or negative for DMTs at w1 (S2 Table). The majority of DMTs administered to this cohort consisted of dimethyl fumarate (97 volumetrics sample; 104 VBM sample), but glatirimer acetate (31; 37), beta interferon (16; 20) and alemtuzumab (22; 24) were also used. A few participants received natalizumab (8 volumetrics sample; 9 VBM sample), fingolimod (7; 7), teriflunomide (5; 9), azathioprine (1; 1), a combination of the

Table 2. Participant demographics.

	Tissue volumes	VBM
N (female)	315 (237)	351 (264)
Mean age at w0 (SD)	38.18 (10.21)	38.22 (10.38)
Median EDSS at w0 (IQR)	2 (1.5)	2 (1.5)
Median EDSS at w1 (IQR)	2.5 (1)	2.5 (1)
Mean diagnosis to w0 in days (SD)	63.77 (39.12)	64.02 (39.04)
DMT use	w0: none w1: 224 (8 unknown)	w0: none w1: 252 (unknown 13)
MRI sites	ABN = 15, ED1 = 74, ED2 = 71, DUN = 34, GLA = 121	ABN = 17, ED1 = 84, ED2 = 77, DUN = 38, GLA = 135
MRI protocol	protocol A = 134, protocol B = 181	protocol A = 149, protocol B = 202
Mean WML volume %ICV at w0 (SD)	0.82 (0.67)	0.91 (0.89)
Mean WML volume %ICV at w1 (SD)	1.00 (0.66)	1.09 (0.84)

N = sample size, SD = standard deviation, IQR = interquartile range, EDSS = expanded disability status scale, VBM = voxel-based morphometry, w0 = baseline, w1 = 1-year follow-up, MRI = magnetic resonance imaging, WML = white matter lesion, ICV = intracranial volume, ABN = Aberdeen, ED1 = Edinburgh site 1, ED2 = Edinburgh site 2, DUN = Dundee, GLA = Glasgow.

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aforementioned DMTs (16; 18) or a DMT not further specified (21; 24). Additionally, 9 participants received a short course of oral or intravenous steroids in keeping with UK guidelines for managing MS relapse [60], within six weeks prior to the MRI scan (range 3 to 31 days; 7/9 prior to w0 and 2/9 prior to w1).

3.2 Global GM/WM, subcortical and whole-brain volumes

Whole-brain, brainstem and left and right cerebral GM and WM, right cerebellar GM, brainstem, amygdala, basal ganglia, hippocampus, nucleus accumbens, thalamus and ventral diencephalon volumes significantly ($q < 0.05$) decreased over time, whereas left and right cerebellar WM did *not* significantly ($q > 0.05$) change over time (Table 3, Fig 2). Additionally, atrophy rates for the left cerebellar GM were significantly different across age (See S3 Table for interaction effects). Covariate significance ($p < 0.05$) was variable across regions. Age and sex were significant in nearly all regions, except the bilateral cerebral NAWM (age and sex), brainstem (sex), right cerebellar GM (sex), and bilateral cerebellar NAWM (age). Site was significant for the bilateral amygdala, left accumbens, right cerebellar GM, bilateral cerebral GM, bilateral hippocampus, bilateral cerebellar NAWM, left cerebral NAWM, left thalamus (site), and

Table 3. Global GM/NAWM, subcortical and whole-brain volumes results for change over time.

	B _{standardised}	SE	df	t	P _{uncorrected}	CI 2.5.	CI 97.5.	Mean w0 (%ICV)	SD w0 (%ICV)	Mean w1 (%ICV)	SD w1 (%ICV)	Mean 1-year % change
Accumbens L	-0.0546	0.0210	313	-2.6078	0.0096	-0.0958	-0.0135	0.0371	0.0070	0.0367	0.0071	-1.0328
Accumbens R	-0.0787	0.0229	288	-3.4413	0.0007	-0.1236	-0.0338	0.0407	0.0063	0.0403	0.0064	-0.8147
Amygdala L	-0.0963	0.0222	312	-4.3368	<0.0001	-0.1398	-0.0527	0.1018	0.0119	0.1007	0.0120	-1.1297
Amygdala R	-0.1458	0.0217	313	-6.7159	<0.0001	-0.1885	-0.1032	0.1156	0.0117	0.1139	0.0116	-1.4730
Basal Ganglia L	-0.1510	0.0192	313	-7.8818	<0.0001	-0.1887	-0.1134	0.6456	0.0826	0.6359	0.0832	-1.5068
Basal Ganglia R	-0.1646	0.0200	313	-8.2182	<0.0001	-0.2039	-0.1253	0.6477	0.0779	0.6362	0.0761	-1.7785
Brainstem	-0.0671	0.0136	312	-4.9164	<0.0001	-0.0938	-0.0403	1.3243	0.1193	1.3165	0.1196	-0.5904
GM cerebellar L ^a	-0.0520	0.0115	313	-4.5135	<0.0001	-0.0745	-0.0294	3.4160	0.3488	3.3981	0.3459	-0.5247
GM cerebellar R	-0.0615	0.0127	313	-4.8253	<0.0001	-0.0865	-0.0365	3.4578	0.3922	3.4367	0.3888	-0.6097
GM cortical L	-0.0861	0.0165	313	-5.2088	<0.0001	-0.1186	-0.0537	15.5401	1.0006	15.4544	0.9889	-0.5513
GM cortical R	-0.0608	0.0169	313	-3.5949	0.0004	-0.0941	-0.0276	15.5598	0.9803	15.5002	0.9790	-0.3829
Hippocampus L	-0.1005	0.0177	313	-5.6859	<0.0001	-0.1352	-0.0658	0.2669	0.0254	0.2645	0.0255	-0.8973
Hippocampus R	-0.0951	0.0163	313	-5.8384	<0.0001	-0.1271	-0.0632	0.2784	0.0673	0.2762	0.0696	-0.8018
NAWM cerebellar L	-0.0215	0.0168	313	-1.2787	0.2019	-0.0546	0.0115	0.9693	0.5568	0.9658	0.5386	-0.3551
NAWM cerebellar R	0.0096	0.0162	311	0.5956	0.5519	-0.0221	0.0414	0.9165	0.1113	0.9169	0.1098	0.0444
NAWM cerebral L	-0.1378	0.0121	311	-11.3639	<0.0001	-0.1617	-0.1140	14.0731	1.1201	13.9223	1.1618	-1.0716
NAWM cerebral R	-0.1263	0.0120	313	-10.5151	<0.0001	-0.1498	-0.1027	14.0646	1.0971	13.9226	1.1497	-1.0100
Thalamus L	-0.1163	0.0116	313	-10.0684	<0.0001	-0.1390	-0.0937	0.4972	0.7444	0.4908	0.7296	-1.2765
Thalamus R	-0.1055	0.0107	311	-9.8826	<0.0001	-0.1265	-0.0846	0.4456	0.0441	0.4409	0.0450	-1.0525
Ventral DC L	-0.0763	0.0173	311	-4.4036	<0.0001	-0.1103	-0.0423	0.2568	0.0230	0.2550	0.0231	-0.6912
Ventral DC R	-0.1306	0.0186	289	-7.0225	<0.0001	-0.1671	-0.0941	0.2587	0.0246	0.2560	0.0245	-1.0304
Whole-brain	-0.1001	0.0146	313	-6.8463	<0.0001	-0.1288	-0.0714	74.1152	3.7355	73.7433	3.7808	-0.5018

Global GM/NAWM, subcortical and whole-brain volumes results for change over time (w1-w0) as assessed with a linear mixed-effects model, corrected for age, sex, imaging site, DMT status at w1 and WML change. Regression coefficient shown are standardised. Mean and SD are shown for volumes as % of intracranial volume (% ICV), without covariate correction. B = standardised beta value, SE = standard error, df = degrees of freedom, CI = confidence interval for beta value, SD = standard deviation, w0 = baseline, w1 = 1-year follow-up, L = left, R = right, GM = grey matter, NAWM = normal-appearing white matter, WML = white matter lesion, DC = diencephalon, ICV = intracranial volume. *p*-values surviving FDR correction ($q < 0.05$) are highlighted in bold.

^aThe model for GM cerebellar L included the significant interaction term for time*age. See S3 Table.

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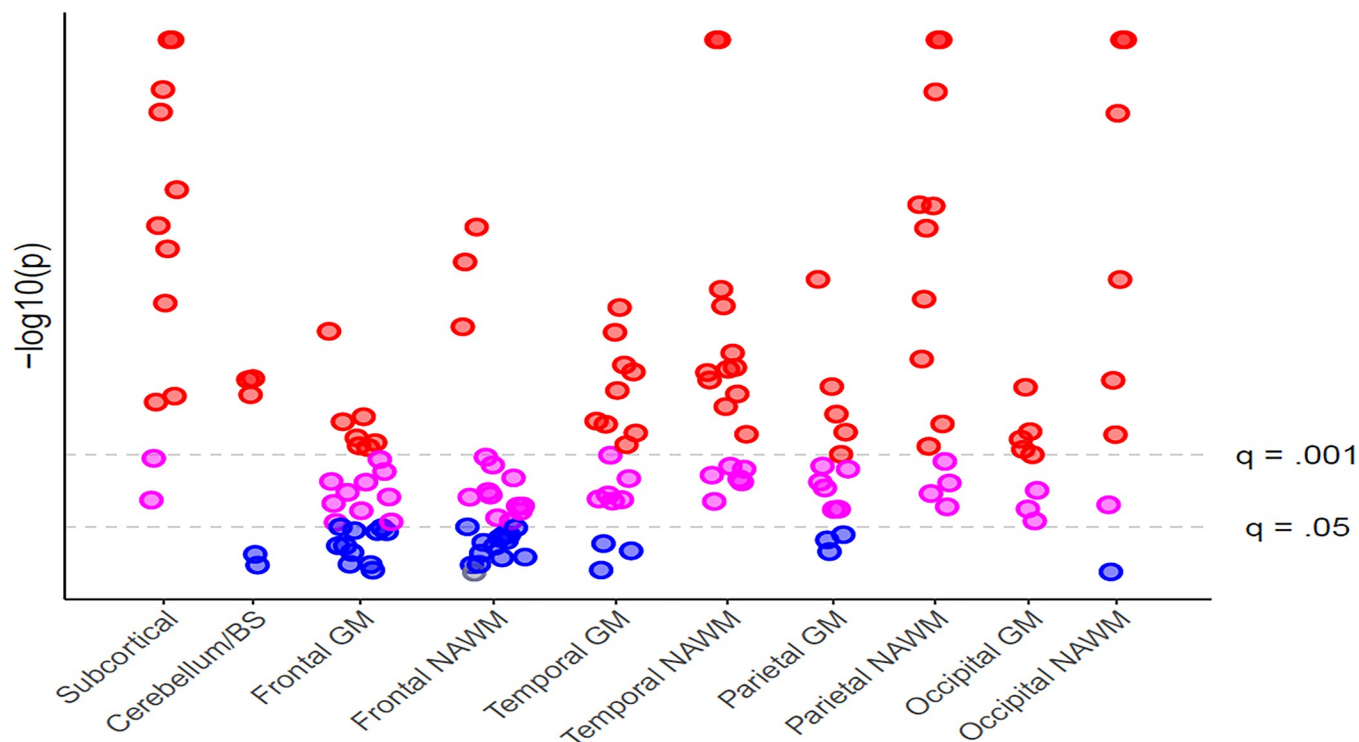


Fig 2. Manhattan plot of longitudinal brain volume change. Each point represents a tissue region within the given brain area category (x-axis). P-values were inverse log transformed (y-axis). Insignificant p-values ($p > 0.05$) are shown in blue. Significant ($p < 0.05$) false discovery rate (FDR) corrected p-values at $q = 0.05$ are shown in pink, and in red for $q = 0.001$. Regardless of significance, the direction of all effects – but one frontal NAWM area (in grey)—showed a longitudinal volume decrease. BS = brainstem, GM = grey matter, NAWM = normal-appearing white matter.

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whole-brain (site). WML change was significant for the bilateral amygdala and right thalamus only. DMT status at w1 did not reach significance for any brain region. See [S3 Table](#) for an overview of all significant covariates and interaction effects.

3.3 Regional cortical GM and cerebral WM

Regional GM and NAWM volume decrease ($q < 0.05$) was observed in most regions and predominantly in the temporal, parietal and occipital lobe. GM volume decreased over time in 15/18 temporal regions, 16/26 frontal regions, 11/14 parietal regions, 8/8 occipital regions and the bilateral insula. NAWM volume decreased over time in 18/18 temporal regions, 14/26 frontal regions, 14/14 parietal regions, 7/8 occipital regions and the insula (L). More GM and NAWM regions showed volume loss in the left than in the right hemisphere, particularly in the frontal lobe. See [Figs 2 and 3](#), and [S4 Table](#). Covariate significance was variable across regions, but age was most consistently significant and sex as well as site to a lesser extent. In contrast, WML change and DMT status at w1 only reached significance for very few brain regions. Additionally, 2/14 frontal NAWM regions, i.e. the left and right superior frontal NAWM, showed differential atrophy rates across age. See [S3 Table](#) for an overview of all significant covariates and interactions.

3.4 Voxel-based GM change

Twelve clusters of various sizes ([Table 4](#)) showed a change in local concentration of GM over time. The largest clusters were centred within 1) the temporal lobe and subcortical areas, 2)

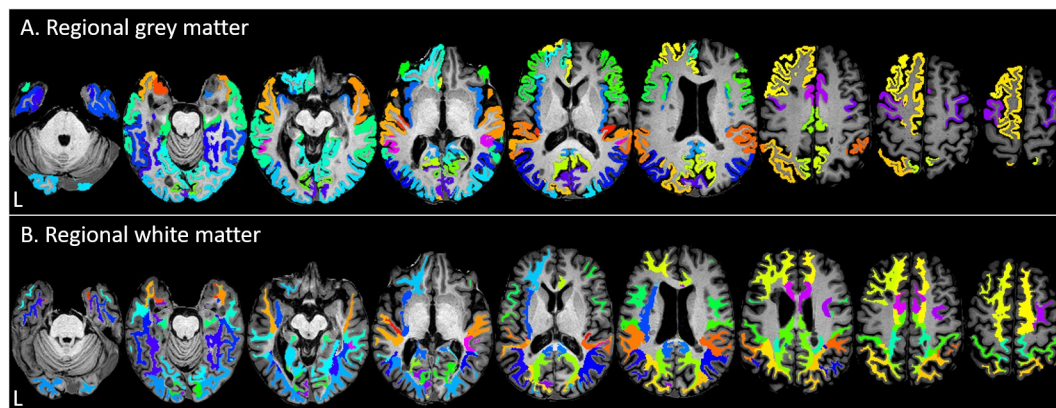


Fig 3. Volumetric results. For illustration purposes, regional grey matter (GM) (A) and normal-appearing white matter (NAWM) (B) areas with significant volume decrease ($w1-w0$; $q < 0.05$) are shown on an example subject's axial T1-weighted image. Colours were chosen to emphasise borders between regions and have no further meaning in terms of results. This figure was created using MRIcron (<https://www.nitrc.org/projects/mricron>).

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cerebellum, 3) anterior cingulum and supplementary motor cortex. Additional smaller clusters were also observed in the temporal lobe and cerebellum. Furthermore, four smaller clusters were observed in the occipital lobe, as well as one cluster in the posterior frontal lobe and one in the parietal supramarginal and angular gyrus. See Fig 4 for VBM results. See Fig 5 for a visual comparison of volumetric (regional GM, cerebellar GM and subcortical areas) and VBM results.

Table 4. Voxel-based morphometry results.

Cluster Index	Voxels	MAX X (mm)	MAX Y (mm)	MAX Z (mm)	COG X (mm)	COG Y (mm)	COG Z (mm)	MAX anatomical location	COG anatomical location
1	7415	34	4	-44	3.03	-1.47	-4.59	Temporal Pole, Anterior Temporal Fusiform Gyrus, Anterior Inferior Temporal Gyrus	Right thalamus
2	2882	20	-64	-52	1.76	-65.2	-32.1	Cerebellum	Cerebellum
3	794	0	22	20	-0.111	-1.98	45.5	Anterior Cingulate Gyrus	Anterior Cingulate Gyrus, Supplementary Motor Cortex
4	83	42	12	26	42.1	9.71	30.8	Inferior/Middle Frontal Gyrus, Precentral Gyrus	Inferior/Middle Frontal Gyrus, Precentral Gyrus
5	74	-24	-76	-12	-22.8	-73.9	-11.4	Occipital Fusiform Gyrus, Lateral Occipital Gyrus, Lingual Gyrus	Occipital Fusiform Gyrus, Lingual Gyrus
6	62	34	-96	-10	27.1	-97.8	-5.35	Occipital Pole, lateral occipital cortex	Occipital Pole
7	39	14	-98	18	10.7	-95.4	22.9	Occipital Pole	Occipital Pole
8	38	60	-48	18	55.7	-47.2	20.9	Angular Gyrus, Posterior Supramarginal Gyrus	Angular Gyrus, Posterior Supramarginal Gyrus
9	37	-44	-20	-36	-45.9	-19.1	-31.5	Posterior Inferior Temporal Gyrus, Posterior Temporal Fusiform Gyrus	Posterior Inferior Temporal Gyrus, Posterior Temporal Fusiform Gyrus
10	32	20	-102	4	16.8	-101	7.44	Occipital Pole	Occipital Pole
11	27	-62	-24	4	-63.3	-23.6	8.96	Posterior Superior Temporal Gyrus, Planum Temporale	Posterior Superior Temporal Gyrus, Planum Temporale
12	26	-32	-44	-42	-31.6	-40.5	-40.9	Cerebellum	Cerebellum

VBM cluster results (cluster-size > 20 , $p_{\text{corrected}} < 0.001$) for GM change over time ($w1-w0$) in RRMS ($N = 351$). VBM = voxel-based morphometry, GM = grey matter, RRMS = relapsing-remitting multiple sclerosis, MAX X/Y/Z = maximum cluster coordinates, mm = millimetres, COG X/Y/Z = centre of gravity cluster coordinates.

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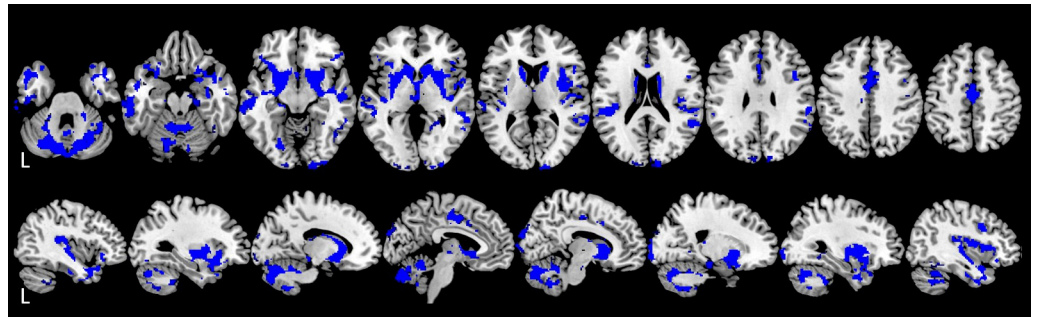


Fig 4. Voxel-based morphometry (VBM) results. VBM results for significant grey matter (GM) change over time ($w1-w0$; $p_{corrected} < 0.001$; $N = 351$) in RRMS are shown in blue on a template axial (top row) and sagittal (bottom row) T1-weighted image. This figure was created using MRIcron (<https://www.nitrc.org/projects/mricron>) and the implemented 'ch2' template (<http://www.bic.mni.mcgill.ca/ServicesAtlases/Colin27>).

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4. Discussion

The current study used imaging data from the FutureMS cohort, a large longitudinal multicentre cohort of people with recently-diagnosed RRMS in Scotland. The aim of our study was to establish a profile of regional NAWM and GM atrophy in recently-diagnosed RRMS. In addition, the effects of different imaging analysis approaches on observed patterns of atrophy were compared.

Widespread NAWM and GM atrophy was observed using both approaches. With the volumetric approach, people with recently-diagnosed RRMS showed brain volume loss in the brainstem, all subcortical regions and the cerebellar GM but not NAWM, over one year. Furthermore, cerebral NAWM and GM volume loss over one year was also evident. Specifically, GM and NAWM volume decreased in nearly all temporal, parietal and occipital regions and in about half of frontal regions. Slightly more NAWM regions showed volume decrease in the temporal-parietal lobes and slightly more GM regions showed a volume decrease in the frontal lobe. Additionally, more *left* frontal GM and NAWM regions showed volume decrease than *right* frontal regions. With the VBM approach (GM only), most of these subcortical and cortical GM findings in recently-diagnosed RRMS were replicated. Large areas of change in GM concentration were observed in temporal and subcortical regions, as well as in the cerebellum and the anterior cingulum, with some smaller areas in the occipital lobe. However, overall,

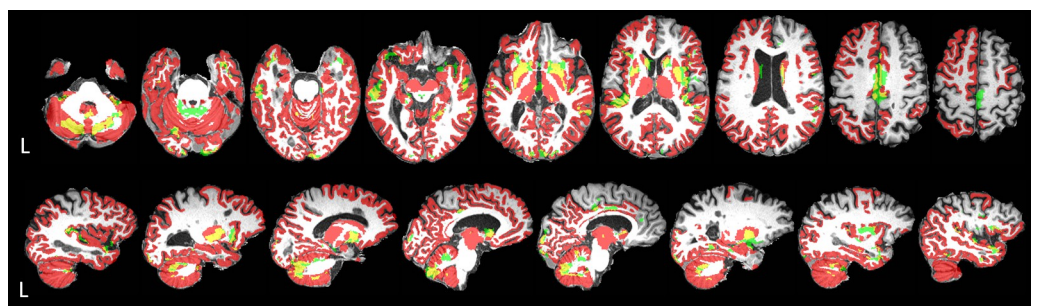


Fig 5. Comparison of voxel-based morphometry (VBM) and volumetric results in relapsing-remitting multiple sclerosis (RRMS). For illustration purposes, VBM results for significant grey matter (GM) change over time ($w1-w0$; $p_{corrected} < 0.001$) (green) are overlaid on volumetric results (red) for regional GM, cerebellar GM and subcortical areas with significant volume decrease ($w1-w0$; $q < 0.05$) (red). Areas where VBM and volumetry overlap are shown in yellow. Overlays are shown on an example subject's axial (top row) and sagittal (bottom row) T1-weighted image. This figure was created using MRIcron (<https://www.nitrc.org/projects/mricron>).

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fewer regions of GM change were observed than with the volumetric approach, particularly in the parietal and frontal lobes, without prominent hemispheric differences.

4.1 Subcortical atrophy

The importance of subcortical neurodegeneration in MS has been recently summarised by Ontaneda et al. (2021), emphasising that atrophy of deep GM structures is common in MS and may be a suitable target for DMT treatment [61]. In this study, we observed prominent subcortical volume changes as well as a large cluster of GM concentration changes within subcortical regions in early RRMS. These results are in line with previous studies reporting early-stage subcortical atrophy, specifically in the thalamus and basal ganglia [11,16,22]. The thalamus has been suggested to play an important role in MS disease symptomatology, with lower volumes being predictive of clinical worsening [62], even in the early stages [63]. Basal ganglia changes have also frequently been observed and related to clinical change [64,65]. Additionally, our study showed that other subcortical regions, such as the amygdala, hippocampus, ventral diencephalon and nucleus accumbens, also show early-stage atrophy. This is in line with previous studies reporting similar subcortical volume changes as well as associations with clinical changes in clinical isolated syndrome (CIS) or later stages of RRMS [65–72].

4.2 Cerebellar and brainstem atrophy

The observed GM volume changes are in line with previous reports of early-stage cerebellar atrophy [16] and they are unsurprising as the cerebellum has been shown to play an essential role in both sensorimotor and cognitive dysfunction in MS [73–76]. VBM analysis also indicated a large cluster of GM changes within the cerebellum. Cerebellar NAWM change was not observed, which is in line with previous studies reporting absence of cerebellar NAWM volume change in CIS and RRMS [77,78]. In contrast, some studies do report cerebellar NAWM atrophy in MS [79,80], however this may be explained by different disease stages being studied as well as study samples lacking a distinction between MS subtypes. Moreover, studies using diffusion tensor imaging (DTI) to explore cerebellar changes in RRMS have observed early-stage NAWM microstructural changes in absence of macrostructural NAWM volume change [81]. This suggests that microstructural cerebellar damage is already present in early-stage RRMS, which may go on to develop into macrostructural cerebellar NAWM atrophy in later stages.

The brainstem is also known to be involved in MS, showing both evidence of WMLs as well as volume loss, which are associated with clinical symptomatology [82–84]. Here we observed brainstem volume loss in recently-diagnosed RRMS, which is corroborated by findings from Eshaghi et al. (2018) [11]. Overall, this may suggest that atrophy of both the brainstem and cerebellar GM plays an important role in early-stage neurodegeneration in RRMS.

4.3 Cerebral regional NAWM and GM atrophy

This study suggests that both regional NAWM and GM are evident in recently-diagnosed RRMS in all brain lobes in variable degrees. Using volumetrics, cerebral GM volume loss was observed more prominently in the temporal, parietal and occipital lobes than in the frontal lobe. Similarly, with VBM, prominent GM changes were observed in the temporal, occipital and posterior frontal lobes, but less so in the anterior frontal lobe and parietal lobe. This discrepancy may be explained by the changes within the latter regions being less localised which may have left them undetected by VBM. The early GM changes observed here are partly in line with previous studies showing prominent early changes in the occipital [16], temporal [17,72,85], parietal (posterior cingulum) [11] and frontal lobe [85]. However, these

observations differ from the current results in that they do not report on GM changes in all lobes simultaneously. This is likely explained by differences in methodology [86] (e.g. processing methods, inclusion of healthy controls, design), which is also supported by the discrepancy shown between VBM and volumetric results within this study.

Cerebral NAWM loss has been observed in previous studies [10,19,20], but to our knowledge, regional NAWM atrophy has not been investigated before. Similar to regional GM change, we observed widespread cerebral NAWM loss, independent of lesion accumulation, which was most prominent in the temporal, occipital and parietal regions. This suggests that NAWM neurodegeneration is also evident in recently-diagnosed RRMS. In comparison with cerebral GM volume loss, cerebral NAWM volume loss was observed in slightly more regions in temporal and parietal lobes, but not in the frontal lobe where more GM regions showed volume loss. This may support the notion that pathological processes underlying GM and NAWM change are at least partly dissociated [87].

More frontal NAWM and GM regions were affected in the left than in the right hemisphere - using the volumetric approach - whereas the temporal, parietal and occipital lobes were symmetrically affected. A left-hemispheric predilection for atrophy in all brain lobes has been observed previously in MS, although only in a small number of studies [88–90]. Moreover, conflicting results have been reported in studies investigating hemispheric asymmetry in damage accumulation, in both ageing and neurological disease [88]. Overall, it remains unclear whether asymmetry of neurodegeneration is a feature of MS, and whether the degree of differentially-affected cognitive functions is associated with the left and right frontal hemispheres; further research is required to elucidate this.

Surprisingly, we did not detect volumetric changes within areas in the frontal lobe associated with sensorimotor functioning, typically impaired in MS, such as in the precentral GM/NAWM and paracentral GM. However, paracentral NAWM volumetric change was indeed observed and the VBM results were also indicative of a small cluster of GM change within the supplemental motor area and precentral gyrus. Previous literature has shown GM volume change in the precentral gyrus in early RRMS compared with healthy controls [91], as well as abnormal iron deposition in the precentral gyrus in later stage MS [92]. This suggests that motor cortex abnormalities are involved in MS as expected, however the lack of volumetric precentral GM changes observed in our study may indicate that widespread motor cortex change over time may not be as fast as in other frontal regions in early RRMS.

4.4 Volumetry and VBM

The findings of this study emphasise that different analysis approaches can lead to significantly different results from the same data. Although GM change was observed in overlapping areas, there were key differences between the VBM and volumetric output. A volumetric approach like FreeSurfer segments brain regions, allowing calculation of the total number voxels within these regions. VBM on the other hand does not require regions to be pre-defined and looks for changes voxel-by-voxel, allowing for detection of local and possibly smaller changes [53]. Furthermore, FSL VBM also uses TFCE, which enhances clusters of voxels that show change increasing focus on spatially-localised changes, even when these changes are small in overall magnitude [59]. Conversely volumetry may not detect such subtle localised change, but it is more sensitive to distributed change across a region, which would be likely missed by VBM.

Further differences in processing steps and statistical methods are also likely to cause outcome differences. For example, VBM treats all data as one group and registers all participants to a common template, which may lead to some individual change being lost. FreeSurfer avoids this by processing participant data separately in their own native space. However,

between-subject cortical variability can affect accuracy of atlas-based approaches and may thus bias the volumetric results [93]. Another example is that FSL VBM uses FWE correction for multiple comparisons, whereas we have applied FDR for the volumetric analysis. As FWE is considered to be more stringent than FDR, it is possible that VBM underestimated and FDR overestimated actual change, leading to a difference in results [94]. Additionally, a larger number of data failed quality checks with FreeSurfer than with VBM, leading to a difference in sample size between the two methods. Although this may suggest that VBM is a more robust method in case of lower data quality, alternatively, this may also indicate that FreeSurfer has a better and stricter quality-check procedure in place.

Specifically, for our RRMS cohort, the difference in results may indicate that the frontal and parietal changes observed with the volumetric approach are more widespread and more variable across participants. On the other hand, the overlapping temporal, occipital and subcortical results, may be indicative of prominent changes within these regions. Overall, both VBM and volumetric approaches have advantages and important limitations that should be taken into consideration when interpreting results.

4.5 Limitations

This study has some limitations. First, FutureMS is a multi-centre study involving five different MR systems and two different (although very similar) MR protocols, which may have influenced the results. The parameters for the T1W acquisition, upon which the current analyses were mainly based are, however, nearly identical between protocols. Additionally, all participants underwent their MR assessment with the same system and protocol at both time points. Pooling of data across centres is important for clinical studies, as it allows for drawing conclusions based on larger datasets. Second, we have not studied the relationship between atrophy and clinical features in this study. The reason for this is that divergence in clinical disease course is limited over one year in early MS and may be confounded by recovery from acute inflammatory episodes that led to initial diagnosis. We are therefore initially concentrating on patterns of atrophy between the point of diagnosis and one year and will focus our future research on clinical correlates of atrophy as they develop over a longer timescale. Third, pseudoatrophy may have been caused by spontaneous recovery from inflammation and associated swelling. This cannot be corrected for, and should be taken into consideration when interpreting results. Pseudoatrophy may also have been caused by DMTs, which we have carefully corrected for by including adding DMT as a covariate. Results showed that DMTs did not have a significant effect on whole-brain, cerebral, cerebellar, brainstem or subcortical atrophy, and only in 6 out of 105 WM/GM regions that showed significant atrophy, which was corrected for accordingly. Finally, matched healthy control data were not available for comparison, which may also be considered a limitation. It is worth noting, however, that overall whole-brain volume decrease observed in this study was 0.5%; this exceeds brain atrophy expected to occur in healthy individuals within the same age range (30–40 years) [95], as well as the atrophy threshold (0.4%) proposed in the 2020 MAGNIMS guidelines [39]. Importantly, the longitudinal study design mitigates significant variations in brain volume between individuals, and allows regional and global atrophy to be mapped in MS patients at an early disease stage and compared between disease phenotypes as they evolve with disease progression.

4.6 Conclusion

Widespread WM and GM atrophy is present in recently-diagnosed RRMS, suggesting neurodegeneration across tissue types. This is observed particularly in the brainstem, cerebellar GM, subcortical regions and temporal-occipital GM and NAWM, which accords with limited

previously published data in other MS cohorts. Analyses based on volumetry and VBM demonstrate different patterns of atrophy, albeit with some regional overlap. Atrophy measures targeted to these specific brain regions may provide improved markers of neurodegeneration, and potential future imaging stratifiers of disease progression and endpoints for therapeutic trials. Our future aims are directed at mapping neurodegenerative patterns across a ten-year time-period in the FutureMS cohort, as well as correlating regional atrophy with evolving clinical disability and other imaging and liquid biomarkers of neurodegeneration available in FutureMS.

Supporting information

S1 Table. Future MS MRI parameters for protocol A and B.

(DOCX)

S2 Table. Statistical parameters for linear mixed-effects models evaluating the effect of DMT use at w1 (yes/no) on brain tissue volume change over time.

(DOCX)

S3 Table. Detailed statistical parameters for linear mixed-effect models evaluating the effect of time on brain volumes.

(DOCX)

S4 Table. Regional GM and NAWM volume results for change over time (w1-w0).

(DOCX)

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References

1. Lassmann H. Multiple Sclerosis Pathology. *Cold Spring Harb Perspect Med*. 2018; 8: a028936. <https://doi.org/10.1101/cshperspect.a028936> PMID: 29358320
2. Reich DS, Lucchinetti CF, Calabresi PA. Multiple Sclerosis. Longo DL, editor. *N Engl J Med*. 2018; 378: 169–180. <https://doi.org/10.1056/NEJMra1401483> PMID: 29320652
3. Wallin MT, Culpepper WJ, Nichols E, Bhutta ZA, Gebrehiwot TT, Hay SI, et al. Global, regional, and national burden of multiple sclerosis 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet Neurol*. 2019; 18: 269–285. [https://doi.org/10.1016/S1474-4422\(18\)30443-5](https://doi.org/10.1016/S1474-4422(18)30443-5) PMID: 30679040
4. Kister I, Bacon TE, Chamot E, Salter AR, Cutter GR, Kalina JT, et al. Natural history of multiple sclerosis symptoms. *Int J MS Care*. 2013; 15: 146–58. <https://doi.org/10.7224/1537-2073.2012-053> PMID: 24453777
5. Lublin FD, Reingold SC, Cohen JA, Cutter GR, Sorensen PS, Thompson AJ, et al. Defining the clinical course of multiple sclerosis: The 2013 revisions. *Neurology*. 2014; 83: 278–286. <https://doi.org/10.1212/WNL.0000000000000560> PMID: 24871874
6. MS Trust. Types of MS. <https://www.ms-trust.org.uk/about-ms/what-ms/types-ms>. 2018.
7. Thompson AJ, Banwell BL, Barkhof F, Carroll WM, Coetzee T, Comi G, et al. Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. *Lancet Neurol*. 2018; 17: 162–173. [https://doi.org/10.1016/S1474-4422\(17\)30470-2](https://doi.org/10.1016/S1474-4422(17)30470-2) PMID: 29275977
8. McGinley MP, Goldschmidt CH, Rae-Grant AD. Diagnosis and Treatment of Multiple Sclerosis. *JAMA*. 2021; 325: 765. <https://doi.org/10.1001/jama.2020.26858> PMID: 33620411
9. Preziosa P, Rocca MA, Riccitelli GC, Moiola L, Storelli L, Rodegher M, et al. Effects of Natalizumab and Fingolimod on Clinical, Cognitive, and Magnetic Resonance Imaging Measures in Multiple Sclerosis. *Neurotherapeutics*. 2020; 17: 208–217. <https://doi.org/10.1007/s13311-019-00781-w> PMID: 31452082
10. Chard DT, Griffin CM, Parker GJM, Kapoor R, Thompson AJ, Miller DH. Brain atrophy in clinically early relapsing–remitting multiple sclerosis. *Brain*. 2002; 125: 327–337. <https://doi.org/10.1093/brain/awf025> PMID: 11844733
11. Eshaghi A, Marinescu R V, Young AL, Firth NC, Prados F, Jorge Cardoso M, et al. Progression of regional grey matter atrophy in multiple sclerosis. *Brain*. 2018; 141: 1665–1677. <https://doi.org/10.1093/brain/awy088> PMID: 29741648
12. Nygaard GO, Walhovd KB, Sowa P, Chepkoech J-L, Bjørnerud A, Due-Tønnessen P, et al. Cortical thickness and surface area relate to specific symptoms in early relapsing–remitting multiple sclerosis. *Mult Scler J*. 2015; 21: 402–414. <https://doi.org/10.1177/1352458514543811> PMID: 25139946
13. Calabrese M, Atzori M, Bernardi V, Morra A, Romualdi C, Rinaldi L, et al. Cortical atrophy is relevant in multiple sclerosis at clinical onset. *J Neurol*. 2007; 254: 1212–1220. <https://doi.org/10.1007/s00415-006-0503-6> PMID: 17361339
14. Zivadinov R. A longitudinal study of brain atrophy and cognitive disturbances in the early phase of relapsing–remitting multiple sclerosis. *J Neurol Neurosurg Psychiatry*. 2001; 70: 773–780. <https://doi.org/10.1136/jnnp.70.6.773> PMID: 11385012

15. Andravizou A, Dardiotis E, Artemiadis A, Sokratous M, Siokas V, Tsouris Z, et al. Brain atrophy in multiple sclerosis: mechanisms, clinical relevance and treatment options. *Autoimmun Highlights*. 2019; 10: 7. <https://doi.org/10.1186/s13317-019-0117-5> PMID: 32257063
16. Dekker I, Schoonheim MM, Venkatraghavan V, Eijlers AJC, Brouwer I, Bron EE, et al. The sequence of structural, functional and cognitive changes in multiple sclerosis. *NeuroImage Clin*. 2021; 29: 102550. <https://doi.org/10.1016/j.nicl.2020.102550> PMID: 33418173
17. Radetz A, Koirala N, Krämer J, Johnen A, Fleischer V, Gonzalez-Escamilla G, et al. Gray matter integrity predicts white matter network reorganization in multiple sclerosis. *Hum Brain Mapp*. 2020; 41: 917–927. <https://doi.org/10.1002/hbm.24849> PMID: 32026599
18. Bergsland N, Horakova D, Dwyer MG, Uher T, Vaneckova M, Tyblova M, et al. Gray matter atrophy patterns in multiple sclerosis: A 10-year source-based morphometry study. *NeuroImage Clin*. 2018; 17: 444–451. <https://doi.org/10.1016/j.nicl.2017.11.002> PMID: 29159057
19. Deppe M, Marinell J, Krämer J, Duning T, Ruck T, Simon OJ, et al. Increased cortical curvature reflects white matter atrophy in individual patients with early multiple sclerosis. *NeuroImage Clin*. 2014; 6: 475–487. <https://doi.org/10.1016/j.nicl.2014.02.012> PMID: 25610761
20. Turner B, Lin X, Calmon G, Roberts N, Blumhardt LD. Cerebral atrophy and disability in relapsing-remitting and secondary progressive multiple sclerosis over four years. *Mult Scler J*. 2003; 9: 21–27. <https://doi.org/10.1191/1352458503ms868oa> PMID: 12617263
21. Bao J, Tu H, Li Y, Sun J, Hu Z, Zhang F, et al. Diffusion Tensor Imaging Revealed Microstructural Changes in Normal-Appearing White Matter Regions in Relapsing–Remitting Multiple Sclerosis. *Front Neurosci*. 2022;16. <https://doi.org/10.3389/fnins.2022.837452> PMID: 35310094
22. Deppe M, Krämer J, Tenberge J-G, Marinell J, Schwindt W, Deppe K, et al. Early silent microstructural degeneration and atrophy of the thalamocortical network in multiple sclerosis. *Hum Brain Mapp*. 2016; 37: 1866–1879. <https://doi.org/10.1002/hbm.23144> PMID: 26920497
23. Deloire MSA, Ruet A, Hamel D, Bonnet M, Dousset V, Brochet B. MRI predictors of cognitive outcome in early multiple sclerosis. *Neurology*. 2011; 76: 1161–1167. <https://doi.org/10.1212/WNL.0b013e318212a8be> PMID: 21444901
24. Horakova D, Dwyer MG, Havrdova E, Cox JL, Dolezal O, Bergsland N, et al. Gray matter atrophy and disability progression in patients with early relapsing–remitting multiple sclerosis. *J Neurol Sci*. 2009; 282: 112–119. <https://doi.org/10.1016/j.jns.2008.12.005> PMID: 19168190
25. Rudick RA, Lee J-C, Nakamura K, Fisher E. Gray matter atrophy correlates with MS disability progression measured with MSFC but not EDSS. *J Neurol Sci*. 2009; 282: 106–111. <https://doi.org/10.1016/j.jns.2008.11.018> PMID: 19100997
26. Ruggieri S, Petracca M, De Giglio L, De Luca F, Gianni C, Gurreri F, et al. A matter of atrophy: differential impact of brain and spine damage on disability worsening in multiple sclerosis. *J Neurol*. 2021. <https://doi.org/10.1007/s00415-021-10576-9> PMID: 33942160
27. Haider L, Prados F, Chung K, Goodkin O, Kanber B, Sudre C, et al. Cortical involvement determines impairment 30 years after a clinically isolated syndrome. *Brain*. 2021. <https://doi.org/10.1093/brain/awab033> PMID: 33880511
28. Marchesi O, Vizzino C, Meani A, Conti L, Riccitelli GC, Preziosa P, et al. Fatigue in multiple sclerosis patients with different clinical phenotypes: a clinical and magnetic resonance imaging study. *Eur J Neurol*. 2020; 27: 2549–2560. <https://doi.org/10.1111/ene.14471> PMID: 32780554
29. Tóth E, Faragó P, Király A, Szabó N, Veréb D, Kocsis K, et al. The Contribution of Various MRI Parameters to Clinical and Cognitive Disability in Multiple Sclerosis. *Front Neurol*. 2019; 9. <https://doi.org/10.3389/fneur.2018.01172> PMID: 30728801
30. Hänninen K, Viitala M, Paavilainen T, Karhu JO, Rinne J, Koikkalainen J, et al. Thalamic Atrophy Predicts 5-Year Disability Progression in Multiple Sclerosis. *Front Neurol*. 2020;11. <https://doi.org/10.3389/fneur.2020.00606> PMID: 32760339
31. Haider L, Chung K, Birch G, Eshaghi A, Mangesius S, Prados F, et al. Linear brain atrophy measures in multiple sclerosis and clinically isolated syndromes: a 30-year follow-up. *J Neurol Neurosurg Psychiatry*. 2021; jnnp-2020-325421. <https://doi.org/10.1136/jnnp-2020-325421> PMID: 33785581
32. Kadrozkova L, Vaneckova M, Sobisek L, Benova B, Kucerova K, Motyl J, et al. Combining clinical and magnetic resonance imaging markers enhances prediction of 12-year employment status in multiple sclerosis patients. *J Neurol Sci*. 2018; 388: 87–93. <https://doi.org/10.1016/j.jns.2018.02.045> PMID: 29627038
33. Khan O, Bao F, Shah M, Caon C, Tselis A, Bailey R, et al. Effect of disease-modifying therapies on brain volume in relapsing–remitting multiple sclerosis: Results of a five-year brain MRI study. *J Neurol Sci*. 2012; 312: 7–12. <https://doi.org/10.1016/j.jns.2011.08.034> PMID: 21920559

34. Sotirchos ES, Gonzalez-Caldito N, Dewey BE, Fitzgerald KC, Glaister J, Filippatou A, et al. Effect of disease-modifying therapies on subcortical gray matter atrophy in multiple sclerosis. *Mult Scler J*. 2020; 26: 312–321. <https://doi.org/10.1177/1352458519826364> PMID: 30741108
35. Zivadinov R, Medin J, Khan N, Korn JR, Bergsland N, Dwyer MG, et al. Fingolimod's Impact on MRI Brain Volume Measures in Multiple Sclerosis: Results from MS-MRIUS. *J Neuroimaging*. 2018; 28: 399–405. <https://doi.org/10.1111/jon.12518> PMID: 29749661
36. Yousuf F, Dupuy SL, Tauhid S, Chu R, Kim G, Tummala S, et al. A two-year study using cerebral gray matter volume to assess the response to fingolimod therapy in multiple sclerosis. *J Neurol Sci*. 2017; 383: 221–229. <https://doi.org/10.1016/j.jns.2017.10.019> PMID: 29146095
37. Zivadinov R, Bergsland N, Hagemeier J, Carl E, Kolb H, Hojnacki D, et al. Effect of teriflunomide on gray and white matter brain pathology in multiple sclerosis using volumetric and diffusion-tensor imaging MRI measures. *J Neurol Sci*. 2018; 388: 175–181. <https://doi.org/10.1016/j.jns.2018.03.028> PMID: 29627017
38. Masuda H, Mori M, Hirano S, Kojima K, Uzawa A, Uchida T, et al. Relapse numbers and earlier intervention by disease modifying drugs are related with progression of less brain atrophy in patients with multiple sclerosis. *J Neurol Sci*. 2019; 403: 78–84. <https://doi.org/10.1016/j.jns.2019.06.011> PMID: 31233973
39. Sastre-Garriga J, Pareto D, Battaglini M, Rocca MA, Ciccarelli O, Enzinger C, et al. MAGNIMS consensus recommendations on the use of brain and spinal cord atrophy measures in clinical practice. *Nat Rev Neurol*. 2020; 16: 171–182. <https://doi.org/10.1038/s41582-020-0314-x> PMID: 32094485
40. Kearns PKA, Martin SJ, Chang J, Meijboom R, York EN, Chen Y, et al. FutureMS Cohort Profile: A Scottish Multi-Centre Inception Cohort Study of Relapsing-Remitting Multiple Sclerosis. *medRxiv*. 2021; 2021.04.15.21253274. <https://doi.org/10.1101/2021.04.15.21253274>
41. Meijboom R, Wiseman SJ, York EN, Bastin ME, Valdés Hernández M del C, Thrippleton MJ, et al. Rationale and design of the brain magnetic resonance imaging protocol for FutureMS: a longitudinal multi-centre study of newly diagnosed patients with relapsing-remitting multiple sclerosis in Scotland. *Wellcome Open Res*. 2022; 7: 94. <https://doi.org/10.12688/wellcomeopenres.17731.1> PMID: 36865371
42. Jenkinson M, Smith S. A global optimisation method for robust affine registration of brain images. *Med Image Anal*. 2001; 5: 143–156. [https://doi.org/10.1016/s1361-8415\(01\)00036-6](https://doi.org/10.1016/s1361-8415(01)00036-6) PMID: 11516708
43. Jenkinson M. Improved Optimization for the Robust and Accurate Linear Registration and Motion Correction of Brain Images. *Neuroimage*. 2002; 17: 825–841. [https://doi.org/10.1016/s1053-8119\(02\)91132-8](https://doi.org/10.1016/s1053-8119(02)91132-8) PMID: 12377157
44. Smith SM. Fast robust automated brain extraction. *Hum Brain Mapp*. 2002; 17: 143–55. <https://doi.org/10.1002/hbm.10062> PMID: 12391568
45. Yushkevich PA, Piven J, Hazlett HC, Smith RG, Ho S, Gee JC, et al. User-guided 3D active contour segmentation of anatomical structures: Significantly improved efficiency and reliability. *Neuroimage*. 2006; 31: 1116–1128. <https://doi.org/10.1016/j.neuroimage.2006.01.015> PMID: 16545965
46. Zhan T, Zhan Y, Liu Z, Xiao L, Wei Z. Automatic method for white matter lesion segmentation based on T1-fluid-attenuated inversion recovery images. *IET Comput Vis*. 2015; 9: 447–455. <https://doi.org/10.1049/iet-cvi.2014.0121>
47. Chen L, Tong T, Ho CP, Patel R, Cohen D, Dawson AC, et al. Identification of Cerebral Small Vessel Disease Using Multiple Instance Learning. Navab N, Hornegger J, Wells W, Frangi A (eds) *Medical Image Computing and Computer-Assisted Intervention – MICCAI 2015* MICCAI 2015 Lecture Notes in Computer Science, vol 9349 Springer, Cham. 2015. https://doi.org/10.1007/978-3-319-24553-9_64
48. Reuter M, Schmansky NJ, Rosas HD, Fischl B. Within-subject template estimation for unbiased longitudinal image analysis. *Neuroimage*. 2012; 61: 1402–1418. <https://doi.org/10.1016/j.neuroimage.2012.02.084> PMID: 22430496
49. Desikan RS, Ségonne F, Fischl B, Quinn BT, Dickerson BC, Blacker D, et al. An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest. *Neuroimage*. 2006; 31: 968–980. <https://doi.org/10.1016/j.neuroimage.2006.01.021> PMID: 16530430
50. Douaud G, Smith S, Jenkinson M, Behrens T, Johansen-Berg H, Vickers J, et al. Anatomically related grey and white matter abnormalities in adolescent-onset schizophrenia. *Brain*. 2007; 130: 2375–2386. <https://doi.org/10.1093/brain/awm184> PMID: 17698497
51. Good CD, Johnsrude IS, Ashburner J, Henson RNA, Friston KJ, Frackowiak RSJ. A Voxel-Based Morphometric Study of Ageing in 465 Normal Adult Human Brains. *Neuroimage*. 2001; 14: 21–36. <https://doi.org/10.1006/nimg.2001.0786> PMID: 11525331
52. Smith SM, Jenkinson M, Woolrich MW, Beckmann CF, Behrens TEJ, Johansen-Berg H, et al. Advances in functional and structural MR image analysis and implementation as FSL. *Neuroimage*. 2004; 23 Suppl 1: S208–19. <https://doi.org/10.1016/j.neuroimage.2004.07.051> PMID: 15501092

53. Ashburner J, Friston KJ. Voxel-based morphometry—the methods. *Neuroimage*. 2000/06/22. 2000; 11: 805–821. <https://doi.org/10.1006/nimg.2000.0582> PMID: 10860804
54. R Core Team. R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing; Available: <https://www.r-project.org/>.
55. Kuznetsova A, Brockhoff PB, Christensen RHB. lmerTest Package: Tests in Linear Mixed Effects Models. *J Stat Softw*. 2017;82. <https://doi.org/10.18637/jss.v082.i13>
56. Wickham H. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York; 2016. Available: <https://ggplot2.tidyverse.org>.
57. Sarkar D. Lattice: Multivariate Data Visualization with R. Springer-Verlag New York; 2008. Available: <http://lmdvr.r-forge.r-project.org>.
58. Winkler AM, Ridgway GR, Webster MA, Smith SM, Nichols TE. Permutation inference for the general linear model. *Neuroimage*. 2014; 92: 381–97. <https://doi.org/10.1016/j.neuroimage.2014.01.060> PMID: 24530839
59. Smith SM, Nichols TE. Threshold-free cluster enhancement: addressing problems of smoothing, threshold dependence and localisation in cluster inference. *Neuroimage*. 2008/05/27. 2009; 44: 83–98. <https://doi.org/10.1016/j.neuroimage.2008.03.061> PMID: 18501637
60. National Institute for Health and Care Excellence. Multiple Sclerosis in adults: management. Available: <https://www.nice.org.uk/guidance/ng220/chapter/Recommendations#ms-symptom-management-and-rehabilitation>.
61. Ontaneda D, Raza PC, Mahajan KR, Arnold DL, Dwyer MG, Gauthier SA, et al. Deep grey matter injury in multiple sclerosis: A NAIMS consensus statement. *Brain*. 2021. <https://doi.org/10.1093/brain/awab132> PMID: 33757115
62. Minagar A, Barnett MH, Benedict RHB, Pelletier D, Pirko I, Sahraian MA, et al. The thalamus and multiple sclerosis: Modern views on pathologic, imaging, and clinical aspects. *Neurology*. 2013; 80: 210–219. <https://doi.org/10.1212/WNL.0b013e31827b910b> PMID: 23296131
63. Nourbakhsh B, Azevedo C, Maghzi A-H, Spain R, Pelletier D, Waubant E. Subcortical grey matter volumes predict subsequent walking function in early multiple sclerosis. *J Neurol Sci*. 2016; 366: 229–233. <https://doi.org/10.1016/j.jns.2016.04.054> PMID: 27288812
64. Matías-Guiu JA, Cortés-Martínez A, Montero P, Pytel V, Moreno-Ramos T, Jorquera M, et al. Identification of Cortical and Subcortical Correlates of Cognitive Performance in Multiple Sclerosis Using Voxel-Based Morphometry. *Front Neurol*. 2018;9. <https://doi.org/10.3389/fneur.2018.00920> PMID: 30420834
65. Batista S, Zivadinov R, Hoogs M, Bergsland N, Heininen-Brown M, Dwyer MG, et al. Basal ganglia, thalamus and neocortical atrophy predicting slowed cognitive processing in multiple sclerosis. *J Neurol*. 2012; 259: 139–146. <https://doi.org/10.1007/s00415-011-6147-1> PMID: 21720932
66. Morelli ME, Baldini S, Sartori A, D'Acunto L, Dinoto A, Bosco A, et al. Early putamen hypertrophy and ongoing hippocampus atrophy predict cognitive performance in the first ten years of relapsing-remitting multiple sclerosis. *Neurol Sci*. 2020; 41: 2893–2904. <https://doi.org/10.1007/s10072-020-04395-5> PMID: 32333180
67. Koubiyr I, Deloire M, Coupé P, Dulau C, Besson P, Moroso A, et al. Differential Gray Matter Vulnerability in the 1 Year Following a Clinically Isolated Syndrome. *Front Neurol*. 2018;9. <https://doi.org/10.3389/fneur.2018.00824> PMID: 30364223
68. Koenig KA, Rao SM, Lowe MJ, Lin J, Sakaie KE, Stone L, et al. The role of the thalamus and hippocampus in episodic memory performance in patients with multiple sclerosis. *Mult Scler J*. 2019; 25: 574–584. <https://doi.org/10.1177/1352458518760716> PMID: 29512427
69. Planche V, Koubiyr I, Romero JE, Manjon J V., Coupé P, Deloire M, et al. Regional hippocampal vulnerability in early multiple sclerosis: Dynamic pathological spreading from dentate gyrus to CA 1. *Hum Brain Mapp*. 2018; 39: 1814–1824. <https://doi.org/10.1002/hbm.23970> PMID: 29331060
70. Lorefice L, Fenu G, Mammoliti R, Carta E, Sechi V, Frau J, et al. Event-related potentials and deep grey matter atrophy in multiple sclerosis: Exploring the possible associations with cognition. *Mult Scler Relat Disord*. 2021; 49: 102785. <https://doi.org/10.1016/j.msard.2021.102785> PMID: 33508572
71. Batista S D'Almeida OC, Afonso A, Freitas S, Macário C, Sousa L, et al. Impairment of social cognition in multiple sclerosis: Amygdala atrophy is the main predictor. *Mult Scler J*. 2017; 23: 1358–1366. <https://doi.org/10.1177/1352458516680750> PMID: 28273767
72. Fujimori J, Fujihara K, Wattjes M, Nakashima I. Patterns of cortical grey matter thickness reduction in multiple sclerosis. *Brain Behav*. 2021;11. <https://doi.org/10.1002/brb3.2050> PMID: 33506628
73. Argento O, Spanò B, Pisani V, Incerti CC, Bozzali M, Foti C, et al. Dual-Task Performance in Multiple Sclerosis' Patients: Cerebellum Matters? *Arch Clin Neuropsychol*. 2020. <https://doi.org/10.1093/arclin/aaa089> PMID: 33067615

74. Odom AD, Richmond SB, Fling BW. White Matter Microstructure of the Cerebellar Peduncles Is Associated with Balance Performance during Sensory Re-Weighting in People with Multiple Sclerosis. *The Cerebellum*. 2021; 20: 92–100. <https://doi.org/10.1007/s12311-020-01190-y> PMID: 32970313
75. Ruggieri S, Bharti K, Prosperini L, Gianni C, Petsas N, Tommasin S, et al. A Comprehensive Approach to Disentangle the Effect of Cerebellar Damage on Physical Disability in Multiple Sclerosis. *Front Neurol*. 2020;11. <https://doi.org/10.3389/fneur.2020.00529> PMID: 32695059
76. Kalron A, Menascu S, Givon U, Dolev M, Achiron A. Is the walk ratio a window to the cerebellum in multiple sclerosis? A structural magnetic resonance imaging study. *Eur J Neurol*. 2020; 27: 454–460. <https://doi.org/10.1111/ene.14119> PMID: 31696586
77. Anderson V, Fisniku L, Altmann D, Thompson A, Miller D. MRI measures show significant cerebellar gray matter volume loss in multiple sclerosis and are associated with cerebellar dysfunction. *Mult Scler J*. 2009; 15: 811–817. <https://doi.org/10.1177/1352458508101934> PMID: 19465449
78. Kugler A V., Deppe M. Non-lesional cerebellar damage in patients with clinically isolated syndrome: DTI measures predict early conversion into clinically definite multiple sclerosis. *NeuroImage Clin*. 2018; 19: 633–639. <https://doi.org/10.1016/j.nicl.2018.04.028> PMID: 29984171
79. Preziosa P, Rocca MA, Pagani E, Storelli L, Rodegher M, Muiola L, et al. Two-year regional grey and white matter volume changes with natalizumab and fingolimod. *J Neurol Neurosurg Psychiatry*. 2020; 91: 493–502. <https://doi.org/10.1136/jnnp-2019-322439> PMID: 32111638
80. Ramasamy DP, Benedict RHB, Cox JL, Fritz D, Abdelrahman N, Hussein S, et al. Extent of cerebellum, subcortical and cortical atrophy in patients with MS. *J Neurol Sci*. 2009; 282: 47–54. <https://doi.org/10.1016/j.jns.2008.12.034> PMID: 19201003
81. Schneider R, Genç E, Ahlborn C, Gold R, Lukas C, Bellenberg B. Temporal Dynamics of Diffusion Metrics in Early Multiple Sclerosis and Clinically Isolated Syndrome: A 2-Year Follow-Up Tract-Based Spatial Statistics Study. *Front Neurol*. 2019;10. <https://doi.org/10.3389/fneur.2019.01165> PMID: 31749760
82. Kerbrat A, Gros C, Badji A, Bannier E, Galassi F, Combès B, et al. Multiple sclerosis lesions in motor tracts from brain to cervical cord: spatial distribution and correlation with disability. *Brain*. 2020; 143: 2089–2105. <https://doi.org/10.1093/brain/awaa162> PMID: 32572488
83. Fujimori J, Fujihara K, Ogawa R, Baba T, Wattjes M, Nakashima I. Patterns of regional brain volume loss in multiple sclerosis: a cluster analysis. *J Neurol*. 2020; 267: 395–405. <https://doi.org/10.1007/s00415-019-09595-4> PMID: 31654116
84. Bellenberg B, Schneider R, Weiler F, Suchan B, Haghighi A, Hoffjan S, et al. Cervical cord area is associated with infratentorial grey and white matter volume predominantly in relapsing–remitting multiple sclerosis: A study using semi-automated cord volumetry and voxel-based morphometry. *Mult Scler Relat Disord*. 2015; 4: 264–272. <https://doi.org/10.1016/j.msard.2015.04.003> PMID: 26008944
85. Sailer M. Focal thinning of the cerebral cortex in multiple sclerosis. *Brain*. 2003; 126: 1734–1744. <https://doi.org/10.1093/brain/awg175> PMID: 12805100
86. Singh S, Tench CR, Tanasescu R, Constantinescu CS. Localised Grey Matter Atrophy in Multiple Sclerosis and Clinically Isolated Syndrome—A Coordinate-Based Meta-Analysis, Meta-Analysis of Networks, and Meta-Regression of Voxel-Based Morphometry Studies. *Brain Sci*. 2020; 10: 798. <https://doi.org/10.3390/brainsci10110798> PMID: 33143012
87. Calabrese M, Favaretto A, Martini V, Gallo P. Grey matter lesions in MS. *Prion*. 2013; 7: 20–27. <https://doi.org/10.4161/pri.22580> PMID: 23093801
88. Preziosa P, Pagani E, Mesaros S, Riccitelli GC, Dackovic J, Drulovic J, et al. Progression of regional atrophy in the left hemisphere contributes to clinical and cognitive deterioration in multiple sclerosis: A 5-year study. *Hum Brain Mapp*. 2017; 38: 5648–5665. <https://doi.org/10.1002/hbm.23755> PMID: 28792103
89. Prinster A, Quarantelli M, Orefice G, Lanzillo R, Brunetti A, Mollica C, et al. Grey matter loss in relapsing–remitting multiple sclerosis: A voxel-based morphometry study. *Neuroimage*. 2006; 29: 859–867. <https://doi.org/10.1016/j.neuroimage.2005.08.034> PMID: 16203159
90. Achiron A, Chapman J, Tal S, Bercovich E, Gil H, Achiron A. Superior temporal gyrus thickness correlates with cognitive performance in multiple sclerosis. *Brain Struct Funct*. 2013; 218: 943–950. <https://doi.org/10.1007/s00429-012-0440-3> PMID: 22790785
91. Pietroboni AM, Colombi A, Carandini T, Contarino VE, Ghezzi L, Fumagalli GG, et al. Low CSF β -amyloid levels predict early regional grey matter atrophy in multiple sclerosis. *Mult Scler Relat Disord*. 2020; 39: 101899. <https://doi.org/10.1016/j.msard.2019.101899> PMID: 31884385
92. Rumzan R, Wang J, Zeng C, Chen X, Li Y, Luo T, et al. Iron deposition in the precentral grey matter in patients with multiple sclerosis: A quantitative study using susceptibility-weighted imaging. *Eur J Radiol*. 2013; 82: e95–e99. <https://doi.org/10.1016/j.ejrad.2012.09.006> PMID: 23079047

93. Mikhael S, Hoogendoorn C, Valdes-Hernandez M, Pernet C. A critical analysis of neuroanatomical software protocols reveals clinically relevant differences in parcellation schemes. *Neuroimage*. 2018; 170: 348–364. <https://doi.org/10.1016/j.neuroimage.2017.02.082> PMID: 28279814
94. Nichols K. False Discovery Rate procedures. *Statistical Parametric Mapping*. Elsevier; 2007. pp. 246–252. <https://doi.org/10.1016/B978-012372560-8/50020-6>
95. Battaglini M, Gentile G, Luchetti L, Giorgio A, Vrenken H, Barkhof F, et al. Lifespan normative data on rates of brain volume changes. *Neurobiol Aging*. 2019; 81: 30–37. <https://doi.org/10.1016/j.neurobiolaging.2019.05.010> PMID: 31207467