Title: Subtyping relapsing-remitting multiple sclerosis using structural MRI

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Abstract

Background and purpose: Subtyping relapsing-remitting multiple sclerosis (RRMS) patients may help predict disease progression and triage patients for treatment. We aimed to subtype RRMS patients by structural MRI and investigate their clinical significances.

Methods: 155 relapse-remitting MS (RRMS) and 210 healthy controls (HC) were retrospectively enrolled with structural 3DT1, diffusion tensor imaging (DTI) and resting-state functional MRI. Z-scores of cortical and deep gray matter volumes (CGMV and DGMV) and white matter fractional anisotropy (WM-FA) in RRMS patients were calculated based on means and standard deviations of HC. We defined RRMS as "normal" (-2< z-scores of both GMV and WM-FA), DGM (z-scores of DGMV <-2), and DGM-plus types (z-scores of DGMV and [CGMV or WM-FA] <-2) according to combinations of z-scores compared to HC. Expanded disability status scale (EDSS), cognitive and functional MRI measurements, and conversion rate to secondary progressive MS (SPMS) at 5-year follow-up were compared between subtypes.

Results: 77 (49.7%) patients were "normal" type, 37 (23.9%) patients were DGM type and 34 (21.9%) patients were DGM-plus type. 7 (4.5%) patients who were not categorized into the above types were excluded. DGM-plus type had the highest EDSS. Both DGM and DGM-plus types had more severe cognitive impairment than "normal" type. Only DGM-plus type showed decreased functional MRI measures compared to HC. A higher conversion ratio to SPMS in DGM-plus type (55%) was identified compared to "normal" type (14%, $p<0.001$) and DGM type (20%, $p=0.005$).

Conclusion: Three MRI-subtypes of RRMS were identified with distinct clinical and imaging features and different prognosis.

Keywords: relapsing-remitting multiple sclerosis; magnetic resonance imaging; diffusion tensor imaging; gray matter volume; fractional anisotropy.

Introduction

Multiple sclerosis (MS) is recognized as a chronic inflammatory disease of the central nervous system. Disease mechanisms are heterogeneous in MS including demyelination, axonal loss and neurodegeneration[1]. Clinically, MS is typically classified as primary progressive MS, secondary progressive MS (SPMS), relapsing-remitting MS (RRMS) and clinically isolated syndrome (CIS), and could also be characterized into phenotypes (e.g., active or non-active, worsening or progression) by taking disease activity and disease progression into consideration [2-4]. RRMS is the most common phenotype with marked heterogeneity on MRI and variable treatment response and prognosis, reflecting the pathological heterogeneity of this phenotype[5]. Defining subtypes of RRMS based on MRI signatures might provide a more objective approach by reflecting the underlying pathological mechanisms. More accurate subtyping RRMS using MRI may benefit our understanding the pathological mechanisms, support prognostication and treatment planning.

Focal white matter (WM) demyelinating lesions, diffuse WM fiber integrity damage and gray matter (GM) atrophy are the most frequently reported MRI characteristics in MS patients[6-12]. WM lesions are an imaging hallmark of MS, but only account for less than 30% of the heterogeneity in clinical disability and progression[13, 14]. Beyond focal lesions, diffuse WM demyelination and neurodegeneration, represented by abnormal diffusion measures (e.g. reduced fractional anisotropy [FA])[9, 10] and loss of cortical and deep GM volume (CGMV and DGMV)[11, 12], are major determinants of physical disability and cognitive impairment. Structural MRI measures reflecting

demyelination and neurodegeneration are promising for objectively assessing the pathological heterogeneity and help subtyping patients[6, 9, 12, 15].

In the current study, we aimed to subtype RRMS patients using a combination of structural MRI measurements to understand clinical disease heterogeneity and predict disability progression. To achieve this aim, a retrospective multicenter study was carried out and we defined RRMS as subtypes by CGMV, DGMV, and WM integrity, and determined their distinct clinical, cognitive and functional MRI features.

Materials and methods

Subjects

From Jan 2009 to Sep 2019, we retrospectively enrolled 155 RRMS patients in the remitting phase and 210 healthy controls (HC) with multiparametric MRI (FLAIR, 3DT1, diffusion tensor imaging [DTI] and resting-state functional MRI [rs-fMRI]) from seven centers (center-1: Beijing Tiantan Hospital, Capital Medical University; center-2: China-Japan Union Hospital of Jilin University; center-3: Tianjin Medical University General Hospital; center-4: Xuanwu Hospital, Capital Medical University; center-5: Huashan Hospital Affiliated to Fudan University; center-6: The First Affiliated Hospital of Nanchang University; center-7: The First Hospital of Chongqing Medical University). The institutional review board approved this study, and written informed consent was obtained from each participant.

Clinical variables included gender, age, age-at-onset, disease duration, Expanded Disability Status Scale (EDSS) scores, relapse frequency. In a subset, cognitive testing was performed including Brief Visuospatial Memory Test (BVMT: 35 RRMS and 34 HC), California Verbal Learning Test (CVLT: 21 RRMS and 41 HC), and Paced Auditory Serial Addition Task (PASAT: 65 RRMS and 57 HC). In large subgroup (n=109), clinical follow-up over 5 years (median [interquartile range, IQR], 5 [5-7] years) included EDSS scores and assessments of conversion to SPMS. Disability progression was defined as an EDSS score increase $\geq$1.0, when baseline EDSS was $\leq$5.5 or an EDSS score increase $\geq$0.5, when baseline EDSS was >5.5. Conversion to SPMS was defined as disability progression occurring over a period of at least 6 months in the absence of a relapse and a resulting EDSS score of 4 or more[16].

MRI protocols

Imaging was performed at 3T MRI and included FLAIR, 3DT1, DTI and rs-fMRI. The main protocol parameters of FLAIR were as follows: 2D or 3D acquisition, Flip Angle (FA)=90°-150°, Repetition Time/Echo Time ([TR/TE] 4800-9000 ms)/(81-340 ms), Inversion Time (TI) =1650-2500 ms. 3DT1: FA=8°-12°, TR/TE=(7-8.3 ms)/(3-3.3 ms) for GE and Philips scanner and TR/TE=(1600-2300 ms)/(2.1-2.3 ms) for Siemens scanner, TI=400-1000 ms. For DTI, parameters were: FA=90°, TR/TE=(3700-15000 ms)/(70-100 ms), b values=0 and (1000-1500) s/mm^2, motion sensitive gradient direction number=30-62. For rs-fMRI, we used: FA=90°, TR/TE=(2000-2500 ms)/(22-45 ms), number of scans=180-240.

MR image processing

Lesions were manually segmented by a junior radiologist (C.G. with 5 years' experience in neuroradiology) and checked by a senior neuroradiologist (Y.D. with 12 years' experience in neuroradiology) based on FLAIR images. Lesions were filled on 3DT1 with surrounding normal appearing WM intensities before WM/GM segmentation. The lesion-filled 3DT1 images were then processed using Freesurfer software (*http://surfer.nmr.mgh.harvard.edu/*). CGMV and DGMV (including bilateral thalamus, caudate, putamen, pallidum, hippocampus, amygdala, accumbens area and ventral diencephalon) were obtained. DTI processing was carried out using FMRIB's Diffusion Toolbox (*FDT, http://www.fmrib.ox.ac.uk/fsl*). After skull removal and eddy current correction, FA was calculated based on the DTI tensor for each voxel. FA images were normalized to a pre-defined target FA template (FMRIB58_FA) by non-linear registration. Finally, the mean FA value within tracts defined by the JHU WM tractography atlas[17] (reflecting both WM lesion and the integrity of normal appearing WM) were calculated.

In order to investigate the functional patterns of RRMS subtypes, we determined the fractional amplitude of low frequency fluctuation (fALFF), regional homogeneity (ReHo) and degree centrality (DC) within GM based on rs-fMRI data.

Definition of MS subtypes by structural MRI

The RRMS patients were classified into subtypes according to the CGMV, DGMV, and WM-FA, which were adjusted for the multi-center effects, gender, age, the interaction of gender and age, and total intracranial volume (for CGMV and DGMV) by linear mixed models. Details were as follows (**Fig. 1**) and representative cases are shown in **Fig. 2**.

Step 1. For all structural MRI measurements, the mean and standard deviation (SD) of the corresponding measurements in HC (CGMV, 480.18 [45.77] ml; DGMV, 58.35 [4.83] ml and WM-FA, 0.49 [0.02]) were used to compute z-scores. The z-scores of MRI measurements in MS patients were defined as the following formula: z-score = (MRI measurement in MS - mean value of MRI measurement in HC)/SD of MRI measurement in HC.

Step 2. If z-scores of RRMS patients were not below -2 for any of the three measurements, the RRMS patients were designated as the “normal” type or Type1.

Step 3. There were seven potential combinations for the CGMV, DGMV and WM-FA with z-scores less than -2, which indicated significant CGM atrophy, DGM atrophy and WM integrity damage,

respectively. Three combinations (only WM integrity damage [n=5], only CGM atrophy [n=2], both CGM atrophy and WM integrity damage [n=0]) were excluded.

Step 4. The subtype with only DGM atrophy was designated as Type2: DGM type, while the one with both CGM and DGM atrophy as Type3a: DGM-CGM type, with both DGM atrophy and WM integrity damage as Type3b: DGM-WM type, and coexistence of CGM and DGM atrophy and WM integrity damage as Type3c: DGM-CGM-WM type. Given the small sample size and similar clinical features, the last three subtypes were considered as one group termed as Type3: DGM-plus type.

Statistical analyses

Statistical analyses were performed using IBM SPSS statistical software (Version 22.0, IBM Corp., Armonk, NY) and Matlab Statistics and Machine Learning Toolbox (Matlab 2019). Linear mixed models were firstly applied to structural and functional MRI measurements to correct for multi-center effects and adjust for gender, age, the interaction of gender and age, and total intracranial volume (for CGMV and DGMV). The mean returned residuals of the linear mixed regression models were adopted as the adjusted MRI measurements and used in the following analysis. For clinical and MRI measurements, categorical data were presented as proportions and analyzed using chi-square test. Continuous data and ranked data were presented with mean (SD) and median (IQR) respectively. They were analyzed using Mann-Whitney U or Kruskal-Wallis test followed by post-hoc multi-comparison with Tukey-Kramer tests. A statistical significance threshold of two-sided $p<0.05$ was adopted.

Validation strategies

We performed three validation analyses to validate our results of defining RRMS as three subtypes: (1) to exclude the effect of lesion volume and disease duration effect on the subtyping, we repeated the analysis with lesion volume and disease duration as covariate; (2) since the majority of the patients were females, we repeated the analyses in that subgroup only; (3) we repeated the analyses in the largest single center to exclude the potential of confounding by center.

Results

Demographic information and clinical variables

There were no differences in gender and age between RRMS and HC. RRMS patients had a relatively short disease duration (median=2 years) and mild disability (median EDSS=2). Their median lesion volume was 8.61 ml, with a large spread (IQR=2.92-20.67 ml) reflective of the typical heterogeneity in this phase of the disease. In the subgroup with cognitive testing, RRMS patients had lower CVLT scores (mean [SD], 45.50 [10.77]) and PASAT scores (39.84 [10.81]) than HC (53.17 [8.64]; p=0.009 and 47.67 [11.28]; p<0.001, respectively). In the subgroup with follow-up (n=109), the proportion of RRMS patients with disability progression was 34.9% (38/109) and conversion to SPMS conversion occurred in 26.6% (29/109).

Based on the Z-scores from HC, 148 (95.5%) RRMS patients could be categorized into the three proposed types (**Table 1**). Seventy-seven (46.7%) patients were classified as Type1: “normal” type, 37 (23.9%) patients were Type2: DGM type, and 34 (21.9%) patients were Type3: DGM-plus type. Within Type3, 12 (7.7%) patients were Type3a: DGM-CGM type, 11 (7.1%) patients were Type3b: DGM-WM type, while 11 (7.1%) patients were Type3c: DGM- CGM-WM type.

Clinical measurements of RRMS subtypes

A shown in **Table 1** and **Fig. 3**, no differences in the gender and age were found between different RRMS subtypes and HC. No difference in age-at-onset or baseline relapse frequency was found between different RRMS subtypes. Both the DGM type (median [IQR], 3 [1.23-8] years; p=0.002) and the DGM-plus type (4 [1-6] years; p=0.003) had longer disease duration than the “normal” type (1

[0.42-3] years). The DGM-plus type presented with higher baseline EDSS scores (3 [2-4.5]) than the "normal" type (2 [1-3]; p=0.008). The DGM-plus type (30.35 [8.22]) presented with lower PASAT scores than the "normal" type (44.19 [8.64]; p=0.003) and HC (47.67 [11.28]; p<0.001). No difference in BVMT and CVLT scores was found between RRMS subtypes and HC. As expected, due to the small sample sizes, no discernable differences in clinical measurements existed between Type3a, Type3b and Type3c (**Fig. S1**).

MRI measurements of RRMS subtypes

Both the DGM type (11.22 [6.26-19.79] ml; p<0.001) and the DGM-plus type (21.68 [15.02-35.98] ml; p<0.001) had larger lesion volumes than the "normal" type (4.30 [1.28-9.05] ml). The DGM-plus type had larger lesion volumes than the DGM type (p=0.023). The DGM-plus type (0.11 [0.04]) had lower mean (SD) fALFF values than the "normal" type (0.14 [0.04]; p=0.005) and HC (0.13 (0.03); p=0.012), and lower ReHo values (0.13 [0.04]) compared to HC (0.15 [0.05]; p=0.01). Additionally, the DGM-plus type (0.15 [0.04]) showed lower DC values compared to HC (0.19 [0.05]; p<0.001) and the "normal" type (0.18 [0.05]; p=0.01) (**Fig. 3**). No clear differences in MRI measurements were observed between Type3a, Type3b and Type3c (**Fig. S1**).

Follow-up clinical assessments of RRMS subtypes

The DGM-plus type (4.5 [3-6]) showed higher follow-up median (IQR) EDSS scores compared to the "normal" type (2 [1-3]; p<0.001) and the DGM type (2 [1-4]; p=0.009). The results remained significant when controlling for baseline EDSS as a covariate. A higher proportion of RRMS patients with the DGM-plus type (20/29, 69%) developed disability progression compared to the "normal" type

(11/50, 22%; p<0.001) and the DGM type (7/30, 23%; p<0.001) (**Fig. 3**). A higher conversion ratio to SPMS in DGM-plus type (16/29, 55%) was identified compared to the “normal” type (7/50, 14%; p<0.001) and DGM type (6/30, 20%; p=0.005). No trends in clinical outcomes were discernable between Type3a, Type3b and Type3c.

Validation results

When lesion volume and disease duration were included as covariates, the findings were largely remined (**Fig. S2, Fig. S3 and Fig. S4**). The validation results in different subsamples including (1) 213 female subjects (including 99 RRMS and 114 HC) and (2) 102 subjects (including 43 RRMS and 59 HC) from the largest single center showed distinct clinical and MRI characteristics between subtypes consistent with those in the whole group analysis (**Fig. S5 and Fig. S6**).

Discussion

In this study, we propose a novel MRI-based subtyping of RRMS patients to define “normal”, DGM and DGM-plus subtypes based on deviations from HC values of gray and white matter volume and integrity in a large multicenter dataset. These three subtypes have distinct clinical and other MRI features and carry a different prognosis. The “normal” type has the mildest physical disability, little cognitive impairment and best prognosis, with a small lesion load and preserved brain function, while assignment to the DGM-plus group conveyed the worst clinical status and prognosis, with a large lesion load and functional MRI abnormalities. The DGM type was in between these two subtypes, showing modest clinical disability and intermediate prognosis. These MRI subtypes were independent of demographic and clinical characteristics including gender, age, and disease duration. The substantially different patterns of structural alterations of different RRMS subtypes associated with distinct clinical and functional MRI features reflects the pathological heterogeneity and might provide a more objective stratification for treatment selection.

Type1 (“normal” type) was the most common subtype of RRMS in this study, and accounted for nearly half of the patients. This subtype is characterized by relatively intact GM and WM structure on structural MRI. Clinically, patients presented with subtle physical disability and limited cognitive impairment, and had the best prognosis (22% patients showed disability progression and 14% patients converted to SPMS) at 5 years follow-up. Although “normal” structure was at the core, WM lesions were present, implying a partial dissociation between lesions on the one hand and WM rarefaction and neurodegeneration on the other[13]. The underlying pathological alteration in this type therefore

appears to be localized demyelination with relatively intact GM and WM structures. One potential explanation for the “normal” type might be that the patients are in either in an early disease phase or tend to have non-destructive or non-strategic lesion[18]. The latter may be a more plausible interpretation; when disease duration was regressed out, the clinical outcomes remained favorable, indicating the “normal” type is a relatively benign phenotype independent of disease duration, rather than an early phase type of RRMS. No significant changes of fALFF (functional activity), ReHo (functional homogeneity) and DC (functional connectivity) in the “normal” type was identified, implying preserved brain function despite subtle structural damage including WM lesions, corroborating the mild disability, cognitive preservation and the good prognosis of this type[19, 20].

Type2 (DGM type) accounted for nearly one quarter of RRMS patients, and is characterized by DGM atrophy without significant cortical atrophy or diffuse WM damage. This type showed modest clinical disability and cognitive impairment, with 23% of patients presenting disability progression and 20% patients converting to SPMS at 5 years’ follow-up. DGM, especially the thalamus, comprises central hubs receiving and projecting signals between subcortical and cortical regions through the connecting fiber tracts (e.g. thalamocortical)[21]. DGM atrophy is well-established marker of RRMS and can occur in early phase of MS (e.g. CIS or even RIS phase), and drives disability worsening in MS[18, 22-24]. Fiber transection caused by WM lesion can lead to the neuronal loss in DGM as one of the potential underlying pathological mechanisms for DGM type[21, 25]. Other mechanisms include demyelination, primary neurodegeneration and iron deposition within the DGM[24, 26]. In this type, brain function still appears normal, possibly through functional adaption.

Type3 (DGM-plus type) is the severest type with most marked clinical disability, cognitive impairment and worst prognosis (nearly 70% patients showed disability progression and 55% patients converted to SPMS during 5 years of follow-up). It accounted for less than a quarter of RRMS patients. The hallmarks of DGM-plus group are significant DGM atrophy plus cortical atrophy or/and WM demyelination. All functional MRI measures (e.g. functional activity and functional connectivity) were significantly decreased in this type, indicating the brain functional reserve had been exhausted due to the severe structural damage[27]. This type can be furtherly divided into 3 subtypes depending on the predominant structural damage reflecting different pathological alterations (e.g. neuroinflammatory and/or neurodegeneration). While we did not have enough power to examine in detail, is seemed that Type3a and Typ3c (both with associated cortical atrophy) showed worse disability and more cognitive performance compared to Type3b (with associated WM fiber damage), implying that cortical atrophy rather than WM demyelination was the major driving force for permanent neurological disability[28, 29].

There was a very small proportion (4.5%) of patients that we could not classify into any of the above three types. Two patients had isolated CGM atrophy and five had isolated WM integrity damage, implying these may rarely occur independent of DGM atrophy[18]. No patient had both CGM atrophy and WM integrity damage without concomitant DGM atrophy, implying DGM is a key hub and connection between cortical degeneration and WM demyelination.

In terms of clinical significance of the proposed subtyping, stratification of patients with different subtypes of RRMS in clinical trials could potentially explain the variable responses typically observed

in such trials, and might offer more objective evidence for tailored treatment for individual patients[5, 30]. For example, early escalation to second-line therapy could be considered for Type2, while for Type3 addition of a neuroprotective treatment could be contemplated. Conversely, lower doses or treatment holidays could be considered for “normal” type, which has the best prognosis.

To exclude the possible confounding factors affecting our findings, we validated our results in several ways. The findings were replicated when repeating the analyses using lesion volume and disease duration as covariate, implying our subtyping is independent of lesion burden and disease duration. The subtypes of RRMS are not merely reflecting different disease stages, but classify RRMS as different independent groups with distinct pathological trajectories and clinical outcomes, indicating the intrinsic underlying feature in each type. Furthermore, the results were largely replicated in the female subsample and the single center subsample, supporting the robustness and generalizability of the results.

There are some limitations in this work. First, while we had a large number of HC to define normality, the number of RRMS patients was relatively small, especially in the DGM-plus group, precluding meaningful assessment of its subtypes. Further study in a larger sample and a longitudinal design is warranted to validate the current results, and investigate the features of Type3a, b and c. Second, cognitive assessment, treatment and follow-up information were incomplete due to the retrospective design of this study. Prospective studies with comprehensive clinical and cognitive assessment are needed and treatment responses of different RRMS subtypes should be investigated. Third, the RRMS subtypes were based on a slightly arbitrary cut-off (2SD) of the z-scores of structural parameters

compared to HC; further study is required to identify an optimal method to define the criteria or apply data-driven (e.g. event-based model, hierarchical and k-mean) methods[18, 24, 31, 32]. Lastly, the new subtypes were developed within the RRMS population; generalization to CIS and progressive MS, and those with and without disease activity needs further investigation and may require inclusion of additional MRI parameters (e.g. spinal cord) to better capture the pathological heterogeneity in progressive disease.

Conclusion

Based on structural MRI, we identified three subtypes of RRMS (Type1: “normal” type; Type2: DGM type and Type3: DGM-plus type) with distinct clinical, functional MRI features and different prognosis. The definition of new subtypes is promising step to disentangle disease heterogeneity in RRMS to facilitate prediction of disease progression and in the future hopefully to triage treatment in RRMS.

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Author contributions

Zhizheng Zhuo, Yongmei Li and Yunyun Duan made equal contributions to this work. Zhizheng Zhuo was responsible for data processing and statistical analyses, manuscript drafting and revision; Yongmei Li was responsible for the data processing, manuscript drafting and revision; Yunyun Duan was responsible for clinical and MRI data management, lesion segmentation and revision; Guanmei Cao was responsible for the lesion segmentation; Fenglian Zheng**,** Jinli Ding, Sven Hallar and Frederik Barkhof helped revising the manuscript; Jinhui Wang helped the MRI data preprocessing; Decai Tian, Xinli Wang, Xinghu Zhang, Kuncheng Li, Fuqing Zhou, Muhua Huang, Yuxin Li, Haiqing Li, Chun Zeng, Ningnannan Zhang, Jie Sun, Chunshui Yu, Xuemei Han, and Fudong Shi were responsible for patient recruitment in their centers; Yaou Liu was responsible for the study design, clinical and MRI data, manuscript revision and guarantor of the work.

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Declaration of conflicting interests

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Data sharing statements

The data can be made available upon reasonable request by a qualified researcher.

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Figure legends

Fig. 1. Flow chart of subtyping RRMS according to MRI structural characteristics.

RRMS, relapsing-remitting multiple sclerosis; HC, healthy controls; CGMV, cortical gray matter volume; DGMV, deep gray matter volume; WM-FA, whiter matter fractional anisotropy.

Fig. 2. **Representative cases for HC and RRMS subtypes.**

HC: a female healthy volunteer aged 34 years; "Normal" type (Type1): a female patient aged 46 years with disease duration of 28 months and EDSS of 1; DGM type (Type2): a female patient aged 37 years with disease duration of 36 months and EDSS of 3; DGM-CGM type (Type3a): a female patient aged 47 years and with disease duration of 41 months and EDSS of 4; DGM-WM type (Type3b): a male patient aged 57 years with disease duration of 13 months and EDSS of 2; DGM-CGM-WM type (Type3c): a male patient aged 44 years with disease duration of 16 months and EDSS of 6. A, anterior; L, left; RRMS, relapsing-remitting multiple sclerosis; HC, healthy controls; EDSS, expanded disability status scale; CGMV, cortical gray matter volume; DGMV, deep gray matter volume; WM-FA, whiter matter fractional anisotropy.

Fig. 3. The clinical and MRI measurements of HC and different RRMS subtypes.

Statistical analyses were performed based on Kruskal-Wallis followed by post-hoc multi-comparison with Tukey-Kramer tests. A significance threshold with two-sided testing of $p<0.05$ was adopted. HC, healthy controls; RRMS, relapsing-remitting MS; DGM, deep gray matter; EDSS, expanded disability status scale; PASAT, Paced Auditory Serial Addition Task; fALFF, fractional amplitude of low frequency fluctuation; ReHo, regional homogeneity; DC, degree centrality.

Table 1. Demographic information and clinical variables of the HC and RRMS subtypes

Characteristics	**HC (n=210)**	**RRMS (n=148)**	**"Normal" type (n=77)**	**DGM type (n=37)**	**DGM-plus type (n=34)**
Gender (Female/Male (Female ratio))	114/96 (54.3%)	94/54 (63.5%)	49/28 (64%)	26/11(70%)	19/15 (56%)
Age (mean (SD)) (years)	38.63 (12.25)	36.45 (11.39)	36.31 (11.92)	34.92 (10.20)	38.41 (11.43)
Age-at-onset (mean (SD)) (years)		32.61 (11.56)	33.65 (11.38)	29.82 (11.38)	33.28 (11.45)
Disease duration (mean (SD)) (years)		2 (0.67-6)	1 (0.42-3)	3 (1.23-8)[b]	4 (1-6)[b]
Relapse frequency (median (IQR))		3 (2-4)	3 (2-4)	4 (3-5)	2.5 (2-4)
EDSS (median (IQR))		2 (1-3.5)	2 (1-3)	2 (1-3.5)	3 (2-4.5)[b]
BVMT (mean (SD))	27.85 (5.17)	24.11 (8.84)	25.86 (9.31)	20.5 (7.81)	23.33 (6.66)
CVLT (mean (SD))	53.17 (8.64)	45.50 (10.77)[a]	47.07 (10.48)	46 (10.10)	33.5 (12.02)
PASAT (mean (SD))	47.67 (11.28)	39.84 (10.81)[a]	44.19 (8.64)	41.33 (11.32)	30.35 (8.22)[a,b]
EDSS (median (IQR)) at follow-up		3 (1.5-4.5)	2 (1-3)	2 (1-4)	4.5(3-6)[b,c]
Disability progressive ratio at follow-up		38/109 (34.9%)	11/50 (22%)	7/30 (23%)	20/29 (69%)[b,c]
Conversion ratio to SPMS at follow-up		29/109 (26.6%)	7/50 (14%)	6/30 (20%)	16/29 (55%)[b,c]
Lesion volume (mean (SD)) (ml)		8.61 (2.92-20.67)	4.30 (1.28-9.05)	11.22 (6.26-19.79)[b]	21.68 (15.02-35.98)[b,c]
TIV (mean (SD)) (ml)	1502.75 (130.42)	1468.19 (155.21)[a]	1449.32 (144.68)[a]	1475.87 (150.13)	1502.55 (180.03)
CGMV (mean (SD)) (ml)	480.18 (45.77)	452.81 (50.95)[a]	464.57 (49.62)	454.10 (52.01)[a]	424.79 (42.55)[a,b]
DGMV (mean	58.35	51.69 (6.96)[a]	55.26 (5.87)[a]	49.20 (5.33)[a,b]	46.32 (6.22)[a,b]

(SD)) (ml)	(4.83)				
WM-FA (mean (SD))	0.49 (0.02)	0.43 (0.04)[a]	0.45 (0.03)[a]	0.43 (0.03)[a]	0.39 (0.04)[a,b]
fALFF (z-score, mean (SD))	0.13 (0.03)	0·13 (0·05)	0.14 (0.04)	0.13 (0.05)	0.11 (0.04)[a,b]
ReHo (z-score, mean (SD))	0.15 (0.05)	0.14 (0·05)[a]	0.14 (0.05)	0.14 (0.06)	0.13 (0.04)[a]
DC (z-score, mean (SD))	0.19 (0.05)	0.17 (0·05)[a]	0.19 (0.05)	0.17(0.05)	0.15(0.04)[a,b]

Abbreviation: SD, standard deviation; IQR, interquartile range; HC, healthy controls; RRMS, relapsing-remitting MS; SPMS, secondary progressive MS; DGM, deep gray matter; EDSS, Expanded disability status scale; BVMT, Brief Visuospatial Memory Test; CVLT, California Verbal Learning Test; PASAT, Paced Auditory Serial Addition Task; TIV, total intracranial volume; CGMV, cortical gray matter volume; DGMV, deep gray matter volume; WM-FA, whiter matter fractional anisotropy; fALFF, fractional amplitude of low frequency fluctuation; ReHo, regional homogeneity; DC, degree centrality. BVMT, CVLT, PASAT, EDSS, disability progressive ratio and conversion ratio to SPMS at follow-up were only done in subgroups.

The categorical data were analyzed using chi-square test. The continuous data and ranked data were analyzed using Mann-Whitney U or Kruskal-Wallis test followed by post-hoc multi-comparison with Tukey-Kramer tests.

A statistical significance with two-sided $p<0.05$ was adopted;

[a] indicated $p<0.05$ compared to HC;

[b] indicated $p<0.05$ compared to “normal” type;

[c] indicated $p<0.05$ compared to DGM type.