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Keywords: multiple sclerosis, sodium MRI, benign, neuronal degeneration, disability

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Abstract

Objective: Quantification of brain injury in patients with variable disability despite similar disease duration may be relevant to identify the mechanisms underlying disability in MS. We aimed to compare grey-matter sodium abnormalities (GMSA), a parameter reflecting neuronal and astrocyte dysfunction, in MS patients with benign MS (BMS) and non-benign MS (NBMS).

Methods: We identified never-treated BMS patients in our local MS database of 1352 patients. A group with NBMS was identified with same disease duration. All participants underwent ^{23}Na MRI. The existence of GMSA was detected by statistical analysis.

Results: In total, 102 individuals were included (21 BMS, 25 NBMS and 56 controls). GMSA was detected in 10 BMS and 19 NBMS (11/16 RRMS and 8/9 SPMS) patients (p=0.05). On logistic regression including the presence or absence of GMSA, thalamic volume, cortical grey matter volume and T2-weighted lesion load, thalamic volume was independently associated with BMS status (OR=0.64 for each unit). Nonetheless, the absence of GMSA was independently associated when excluding patients with significant cognitive alteration (n=7) from the BMS group (OR=4.6).

Conclusion: Detection of GMSA in individuals and thalamic volume are promising to differentiate BMS from NBMS as compared with cortical or whole grey-matter atrophy and T2-weighted lesions.

Introduction

Irreversible tissue loss driven by early inflammatory processes mainly underlies long-term disability in multiple sclerosis (MS). Atrophy measurement using MRI has been demonstrated as a relevant marker of irreversible tissue injury [1],[2] but MRI markers sensitive to ongoing and potentially reversible pathological processes are missed. Such markers are mandatory for developing therapies to limit the progression of irreversible tissue injury leading to disability.

^{23}Na MRI has been recently used in MS [3]–[10] and constitutes a promising tool to image neuronal dysfunction occurring from the first stages of MS [5],[10] and leading to neuronal loss. These studies have revealed that abnormal sodium concentration increase mainly occurs in the grey matter (GM) [3],[5],[7]–[9], especially in patients with long disease duration [5],[9]. Some associations between sodium increase and disability have been found. Thus, a recent study conducted by Brownlee and colleagues showed in a group of 78 MS patients followed 15 years that cortical grey matter (CGM) sodium concentration was independently associated with physical and cognitive disability. Nonetheless, as all previous studies considered groups of patients in their design, the relevance of the MRI technique to quantify at the patient-level the extent of brain injury underlying disability is largely unknown. Moreover, no definitive conclusion is possible because several confounding factors were not controlled for in these studies. Particularly, the potential confounding effect of disease-modifying therapies (DMT) has not been removed, nor has the effect of age and disease duration. Indeed, among the eight previous studies assessing ^{23}Na MRI in brain compartments, only two included a large proportion of followed MS patients without DMT, respectively 70 and 74% [6],[9] but none of them was originally designed to control potential DMT effects nor included specifically long-term and treatment-naïve patients.

The natural course of MS is highly variable among patients. Some patients are particularly notable for a relative good recovery after relapse and subsequent non-significant long-term

disability (so-called benign MS [BMS]) [11]. Nevertheless, the absence of long-term disability is not fully explained by T2-weighted lesion load seen on MRI, because high T2-weighted lesion load can also be found in patients with long disease duration (> 15 years) and no significant disability (Expanded Disability Status Scale [EDSS] score ≤ 3) [12]. The relative "favourable" evolution in such patients is probably related to less neurodegenerative process as compared with other MS patients. Particularly, previous MR studies reported lower GM damage in patients with BMS, including fewer GM lesions [13], GM atrophy [14] and GM destructuration [15]. However, severe GM damage has been described in BMS patients presenting cognitive impairment [16]. Sodium concentration, a parameter sensitive to cellular dysfunction, might be less altered in the GM of patients with a favourable evolution. If this assumption is validated, it would provide an important argument for the clinical relevancy of ^{23}Na MRI to image cellular dysfunction underlying disability in patients with MS.

It is worth noting that providing information that is distinct from other assessments is a first step reached by ^{23}Na MRI in the way of being a usable biomarker of brain injury in clinical trials of neuroprotective therapies. Passing the gap of group-level to individual-level analysis is an unavoidable second step that the present study aimed to surmount.

In the database of the tertiary MS centre of our university hospital (n = 1352 patients), we identified patients with long disease duration (15 years) who did not receive any disease-modifying drugs from the onset of MS and who did not have significant disability (EDSS score < 3). ^{23}Na MRI was used to assess GM sodium abnormalities (GMSA) in these patients and a group of patients with the same disease duration but a "typical" level of disability. To test the relevancy of ^{23}Na MRI for quantifying the extent of brain injury underlying disability at the individual level, we used a novel approach designed for the individual patient.

Material and Methods

Participants

In total, 485 MS patients with disease duration ≥ 15 years and EDSS score < 3.0 were identified in the database of the tertiary MS centre of our university hospital (n = 1352 patients); 62 of these 485 patients were treatment-naïve from the onset of their disease and 21 agreed to participate in the study. These patients were called the benign MS (BMS) group. A group of MS patients with the same disease duration but who did not qualify for the BMS definition were also included. The inclusion to the study was proposed to patients who met the criteria successively to their visit to the MS-clinic. We stopped the inclusion when we recruited enough patients to match the BMS group. These patients were called non-benign MS (NBMS). Considering the frequency of MS patients fulfilling the last criteria, we prospectively recruited a group of 25 age- and sex-matched NBMS patients in our outpatient clinic. All included patients fulfilled the 2010 McDonald revised criteria for MS. Non-inclusion criteria were: 1) any other central nervous diseases or any psychiatric diseases, 2) any drug abuse (including alcohol), 3) treatment with oral or intravenous steroids in the last 3 months, 4) relapse in the last 3 months, and 5) any history of disease-modifying drug in the BMS group. A control group of 56 age- and sex-matched healthy controls (HCs) was also included.

Standard Protocol Approvals, Registrations, and Patient Consents

Approval from the local ethics committee was obtained and all participants provided written consent before starting the study.

Clinical and Neuropsychological tests

Patient disability was assessed by the Kurtzke EDSS and Multiple Sclerosis Functional Composite (MSFC) on the day of the MRI exam. BMS patients received the Brief Repeatable

Battery (BRB), which included the Selective Reminding Test (SRTL: long-term storage, SRTC: consistent long-term retrieval, SRTD: delayed recall); Spatial Recall Test (SPARTi: immediate recall, SPARTD: delayed recall); Symbol Digit Modalities Test (SDMT); Paced Auditory Serial Addition Test (3') (PASAT); and Word List Generation (WLG) with semantic and phonemic fluencies. Patients were classified as cognitively impaired if, for at least two tests, they obtained a score below at least two standard deviations (SDs) of the mean score for healthy age-, sex- and educational level matched controls [17].

MRI Data Acquisition and Signal Processing

MRI acquisition

^{23}Na MRI was performed with a 3T Verio system (Siemens) using a volume ^{23}Na–^{1}H head coil (Rapid Biomedical) and a 3D density-adapted radial projection reconstruction pulse sequence (TE/TR=0.2/120 ms, 17,000 projections, isotropic resolution of 3.6 mm, 34 min acquisition time) [18]. The 3D ^{23}Na images were reconstructed by using a homemade Matlab procedure (R2014a, MathWorks). To measure ^{23}Na concentration within the brain, two phantoms with known ^{23}Na concentration (50 mmol/l) and 2% agar gel were placed close to the participant's head [5]. Moreover, rician noise was removed by using a non-local means algorithm (see [7]). As result of these steps, quantitative total sodium concentration (TSC) maps were obtained [5]. ^{1}H MRI involved the use of a 32-channel head coil (Siemens). A sagittal 3D high-resolution MPRAGE (TE/TR/TI=3/2,300/900 ms, 160 slices, isotropic resolution of 1 mm), and combined axial proton density-weighted and T2-weighted sequences (TE1/TE2/TR=15/85/8,500 ms, 49 contiguous sections with 3 mm thickness, spatial in-plane resolution of 1 x 1 mm) were acquired.

Determination of thalamic and cortical GM volumes

To measure thalamic and cortical GM volumes, 3D MPRAGE images were first processed with N4 to reduce low frequency intensity non-uniformity [19]. Later, FSL-FIRST and the Unified Segmentation approach in SPM12 were used to segment and measure the volume of two regions of interest: the thalamus and the cortical GM matter with a lesion filling method to avoid segmentation errors due to the WM T2 lesions [20],[21].

Individual statistical mapping analysis of GMSA and statistical threshold

To obtain the mask of WM, T2-weighted lesions and measure of T2-weighted lesion volume, the same experienced medical doctor (A.M.) marked out all T2-weighted lesions of patients by using an interactive semi-automated thresholding method [7]. T2-weighted and sodium images were co-registered onto the MPRAGE images (SPM8 software, http://www.fil.ion.ucl.ac.uk/spm/). VBM8 toolbox was used to segment the MPRAGE images. Because intensity levels of WM lesions and GM compartment are very close, the segmentation algorithms of SPM have the tendency to attribute WM lesions to the GM mask [22]. To minimize T2-weighted lesion misclassification into normal-appearing brain tissues, for each participant we subtracted the co-registered T2-weighted lesion mask from the GM probability map (80% threshold). The results of this step were checked by the same experienced medical doctor (A.M.) who marked out all T2-weighted lesions. Finally, for each participant, we applied the GM mask to the patient's co-registered TSC map. For HCs, we performed the same pipeline except the T2-weighted lesion mask subtraction.

A voxel-based statistical mapping analysis (SPM8) was used for the normalized (Montreal Neurological Institute template) and Gaussian smoothed GM quantitative TSC map (full width at the half maximum = 8 mm) for each patient compared to the control population to depict individual abnormality. The statistical threshold of significant GMSA was determined as the maximum p-value for which no significant cluster survived when comparing each control to

the whole control population, as proposed by Crespy et al. [23]. This conservative criterion allows for minimizing the false-positive cluster potentially observed in patients. Finally, we determined for each patient the extent of GMSA defined as the ratio of the volume of GMSA to the volume of total GM.

Statistical Analysis

Demographic comparisons

Between-group comparisons for age, sex, disease duration, EDSS, MSFC, T2LL, GM volumes and cognitive impairment were assessed by using non-parametric Wilcoxon test for continuous variables with multiple comparison correction performed with the Steel-Dwass procedure and non-parametric Fisher test for categorical variables.

Voxel wise analyses and GMSA ratio computation

Voxel-based statistical analysis was performed between each patient and the group of controls, corrected for age as covariable and corrected for multiple comparison using the false discovery rate method at the cluster level. The statistical threshold was defined as the maximum p value enabling to show no significant cluster when comparing any individual control with the whole population of controls, corrected for age as covariable.

Regression models

The potential MRI parameters (T2-weighted lesion load, thalamic and cortical grey matter volume, presence/absence of GMSA) associated with BMS status were assessed with a forward stepwise regression which was used to identify possible predictors of BMS. At each step, variables were chosen based on p-values and the p-value threshold of 0.1 was used to set a limit on the total number of variables included in a final logistic model, estimating odds ratios (ORs) and 95% confidence intervals (CIs).

Data Availability Statement

Generated datasets are available on reasonable request from the corresponding author.

Results

Demographic and MRI findings

Clinical and demographic characteristics of participants are in Table 1. In total, 102 individuals were included (21 BMS, 25 NBMS and 56 controls). The mean (± standard deviation) disease duration and EDSS score was 19.2±5.6 years and 1.33±0.67 for BMS patients, and 18.3±5.7 years and 4.45±1.78 ($p<0.001$) for NBMS patients (noticeably, two patients had an EDSS = 2 and were continuously treated with DMT introduced the first year of their disease). Among NBMS patients, 9 were classified as having secondary progressive MS and 16 as relapsing-remitting MS. T2-weighted lesion load did not significantly differ between BMS and NBMS patients (5.2±4 vs 8.6±11 ml; $p=0.52$). Scores for 7 of 21 BMS patients for at least two cognitive tasks were < 2 SDs of the mean for controls.

Statistical Threshold

Individual analyses comparing each healthy control to the whole group of controls allow for determining a maximum p value with no significant cluster when comparing any control to the whole population of controls, $p=0.001$, false discovery rate corrected.

GM Sodium Abnormalities

The GMSA for the BMS and NBMS patients are showed in Figures 1 and 2. Ten of 21 BMS (48%) and 19 of 25 NBMS (76%) patients showed GMSA ($p=0.05$), affecting respectively 4.4 ±9.6% of GM volume vs 5.4 ±9.3% ($p=0.13$). Among the 19 NBMS patients with GMSA, 11 were RRMS and 8 were SPMS. When present, GMSA usually affected < 25% of total GM volume in BMS and NBMS patients.

Among the 21 BMS patients, 7 patients had cognitive impairment and among them 5 showed GMSA (71%). Thus, if we applied a more conservative definition of BMS as no physical

disability but also no cognitive disability, 14 patients remained 'truly' BMS. On the other hand, the non-truly BMS group would thus account for the initial 25 NBMS plus the 7 BMS patients with cognitive impairment, corresponding to 32 'non-truly' BMS patients.

Regarding GMSA, among the 14 'truly' BMS, 5 patients showed GMSA (36%) while among the 32 'non-truly' BMS, 24 patients showed GMSA (75%), (p=0.01).

No linear correlation has been found between GMSA extent and EDSS (Spearman rho 0.13; p=0.41).

On stepwise logistic regression analysis including the presence of GMSA, thalamic volume, cortical grey matter volume and T2-weighted lesion load, only the thalamic volume was independently associated with BMS status (OR = 0.64 for each unit of volume, table 3). Moreover, performing the same analysis but excluding patients with significant cognitive alteration (n=7) from the BMS group and including them in the NBMS group, the two parameters that survived after the stepwise were, significantly, the absence of GMSA (OR = 4.6, p=0.03) and with a tendency, the thalamic volume (OR = 1.01, p=0.07, table 3).

Discussion

Individual detection of GMSA with ^{23}Na MRI revealed that patients with a benign clinical MS evolution (BMS) were less prone to present GMSA than those with non-benign evolution (NBMS). Less than half of BMS patients showed GMSA, but three-quarters with a common evolution (NBMS) and the same disease duration showed GMSA. This result is particularly observed in BMS patients when cognition is assessed and include in the definition. It is noteworthy that among NBMS, SPMS patients are more prone to show GMSA (almost all the patients). Moreover, statistical modelling revealed that deep grey matter atrophy is a robust MRI parameter to separate the two groups. Nonetheless, when the definition of BMS is more stringent, individual sodium mapping appears to be a better discriminator, with the added value

that it could be an early biomarker compared to atrophy [8],[10]. Furthermore, grey matter volume failed to be a robust biomarker associated to BMS status. All these findings demonstrate that ^{23}Na MRI can offer a sensitive marker of the pathological processes underlying disability in MS**.**

It is noteworthy that patients are sparse from the presence of GMSA when they are both sparse form physical and cognitive disability, but that the extent of GM involved by sodium abnormalities is not different. This result suggests that it is the presence of GMSA, regardless of its extension, that is associated with clinical disability and possibly on-going neurodegenerative processes [24]. Sodium imaging does not provide a direct structural information of the brain but provides a functional information of the dynamic ionic changes that may occur (in both ways, increase and decrease) [25]. The dynamic changes could explain that the extent is not higher in patients with progressive forms compared to patients with relapsing-remitting form. Indeed, it could be hypothesised that observing a GMSA extended over 30%, at a given time, but which would be at least partially reversible due to intrinsic repair processes, would be less unfavourable for the patient than an extension of 6% but which would be irreversible and lead to definitive neuro-axonal loss and subsequent atrophy. Assuming this hypothesis, it is rather the presence of GMSA, especially if observed in recurrent MRI scans of a given patient, that could be a relevant biomarker of homeostatic and pathological sodium variations, which are associated with ongoing neurodegenerative processes.

Many findings are converging toward the existence of disturbed sodium homeostasis in MS leading to intracellular sodium concentration increase [26],[27]. First, in demyelinating neurons, redistribution of sodium channels along the axons probably limits the impact of myelin loss on conduction velocity. However, this reorganization has high energy cost related to the Na/K ATPase involved in the sodium efflux. Meanwhile several products of inflammation such as nitric oxide and reactive oxygen species have a negative effect on

mitochondrial ATP production [27]–[29]. This discrepancy between increased energy demand and decreased energy production leads to intra-neuronal sodium concentration increase. Second, several studies have suggested that microglial cells may contribute to increase of intracellular brain sodium concentration [30],[31]. Particularly, upregulation of sodium channels has been described during microglia activation occurring in inflammation [31]. A magnetic resonance spectroscopy (MRS) study revealed the association of myoinositol level, a marker of microglia activation, with worsening disability [32], assuming that microglial activation strongly participates in the occurrence of irreversible disability in MS.

It makes sense that lower increase of sodium concentration, reflecting less cellular dysfunction, occurs in patients with a favourable clinical course of MS. In such patients, demyelination may be less pronounced, as suggested by studies that revealed reduced T2-weighted lesions in BMS patients [33] or by studies that revealed increased T2-weighted lesion load in BMS patients who will exhibit disability progression in the short term [34],[35]. We found lower T2-weighted lesion load in BMS than NBMS patients, although not significant. MRS studies argued also for less pronounced demyelination in BMS lesions, showing higher N-acetyl aspartate rates in BMS patients than those with progressive disease [36],[37]. Furthermore, several studies using quantitative MRI techniques such as magnetization transfer imaging or diffusion tensor imaging also strongly suggested that tissue damage within and, more importantly, outside T2-weighted lesions was less severe in BMS than relapsing-remitting MS or secondary progressive MS. Although the damage might be less severe in NAWM of BMS, its location in specific WM tracts is a determinant driver of under-recognized symptoms such as fatigue and cognitive impairment in BMS [38]. Thus, magnetization transfer ratio was higher within and outside of T2-weighted lesions in BMS than those with other disease types, at the same levels as patients with clinically isolated syndrome or healthy controls [39],[40]. In line with these findings, BMS patients were previously found to have higher T2-weighted lesion

volume, higher normalized brain volume, and lower average GM median diffusivity than patients with secondary progressive MS, except for the subgroup of BMS patients with cognitive impairment (12 of 62 included BMS patients) [16]. The sparseness of demyelination, neuronal suffering and microglial activation in benign MS may explain the lower prevalence of sodium abnormalities in this form of the disease. Furthermore, the presence of cortical lesions, particularly at later stage of the disease, is largely known [41] and may contribute to the sodium concentration increase depicted in the present study. The present study was performed at 3T, consequently robust in vivo visualisation of cortical lesions, which requires ultra-high field MRI (≥7T) [42], was not possible. It constitutes a limitation in the interpretation of the present results.

Results have converged for the existence of intracellular increased sodium concentration in MS [4], but we cannot rule out that the extracellular compartment may also substantially contribute to sodium abnormalities measured by ^{23}Na MRI. In regions showing atrophy, cerebrospinal fluid expansion leads to total sodium concentration increase. In these regions, no definitive interpretation is possible, and probably both intra- and extracellular increase of sodium concentration occur. Nonetheless, observed results in the present study did not support the hypothesis that atrophy plays a major role in MRI measured sodium concentration, as if sodium accrual reflects atrophy, higher GMSA extent in progressive patients should be expected, that was not the case. One hypothesis is the ionic and functional rather than structural information brings by sodium MRI. Nevertheless, previous MRI studies combining maps of atrophy and sodium abnormalities have clearly demonstrated that increase of sodium concentration is mainly present in regions spared by atrophy, which demonstrates that extracellular increase of sodium concentration is not the main determinant of total sodium abnormalities [7]–[10]. Especially, Collorone and collaborators evidenced at the first stage of the disease, abnormal sodium concentration in cortical regions spared from atrophy [10].

Emerging techniques such as triple quantum filtering and multi-echo ^{23}Na MRI applied at ultra-high field (at least 7 tesla) could help differentiate intra- and extracellular sodium signals [4],[43]. In addition, a long-term serial study exploring large cohorts of truly BMS and NBMS patients at the beginning of the disease could bring evidence on the progression of GMSA.

The present study used an individual analysis of ^{23}Na brain maps. This approach involved use of a method developed by our group and first applied to magnetization transfer ratio maps [23]. It consists of comparing each individual map to maps obtained for a large group of HCs by using a voxel-based approach. To prevent false-discovery findings, we used a statistical threshold determined in the group of controls as the minimum threshold whereby no individual control showed any differences compared to the whole group of controls. This approach provides a map of sodium abnormalities for each patient and allows for obtaining an individual measure of sodium abnormalities for each individual. We show that individual assessment of brain sodium abnormalities could clearly separate two groups of patients with the same disease duration but a definitive different level of disability independent of disease-modifying therapies. We demonstrate the sensitivity of this approach to the main mechanisms underlying irreversible disability in MS.

One limitation of this study is the small size of the groups studied. However, because of low frequency of patients, obtaining a sizeable BMS sample naïve of disease-modifying therapies from disease onset is difficult. Assessing GMSA in a group of BMS patients receiving disease-modifying therapies could provide evidence of the protective effect of these treatments on disease progression. Another limitation is that even if there is an association between GMSA and disability, the association is not linear. One may assume that this complex relationship is at least partly influenced by the topography of GMSA. Another factor is the crucial role of spinal cord injury in patients' disability, that is to date very challenging to explore with ^{23}Na MR imaging due to technical issues.

In conclusion, this paper opens new perspectives for the use of ^{23}Na MRI in clinical practice, especially for future therapeutic trials. ^{23}Na MRI can provide an individual assessment of sodium abnormalities in MS patients, reflecting neuronal dysfunction and allows for assessing in a restricted sample the potential effect of new but also current therapeutics for neuronal protection.

Acknowledgements: n/a

Statistical Analysis conducted by Pr. Jean-Philippe Ranjeva, PhD, Aix Marseille University

Disclosures: No conflict of interest for this study.

JP received an unconditional grant from Novartis for this study.
AM, BA, AR, SG, PV, MG, JPR and WZ have no relevant financial interest or relationship to disclose.

Funding Statement: This study was supported by ARSEP Fondation, Association SEP Pays d'Aix, and unconditional grant from Novartis France.

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Table 1. Demographic and clinical characteristics of participants with benign multiple sclerosis (BMS) and non-benign MS (NBMS) and healthy controls.

	BMS (n=21)	NBMS (n=25)	Healthy controls (n=56)	p-value BMSvsNBMS	p-value BMSvsHC	p-value NBMSvsHC
Age (years)	47.5 ±8.1	46.7 ±9.3	41.9 ±12.9	*0.86*	*0.22*	*0.21*
Sex (F/M)	16/5	18/7	33/23	*1*	*0.19*	*0.32*
Clinical phenotype (Lublin and Reingold 1996)	21 BMS	16 RRMS 9 SPMS	n/a	*n/a*	*n/a*	*n/a*
Disease duration (years)	19.2 ±5.6	18.3 ±5.7	n/a	*0.69*	*n/a*	*n/a*
DMT	0	25	n/a	*n/a*	*n/a*	*n/a*
EDSS	1.33 ±0.67	4.45 ±1.78	n/a	***<0.001***	*n/a*	*n/a*
MSFC	0.15 ±0.43	-0.44 ±0.83	n/a	***0.005***	*n/a*	*n/a*
T2LL (cm^3)	5.2 ±4	8.6 ±11	n/a	*0.52*	*n/a*	*n/a*
Cortical GM volume (cm^3)	589 ±36	566 ±58	575 ±57	*0.36*	*0.67*	*0.76*
Thalamic volume (cm^3)	14.2 ±1.4	13.0 ±2.0	16.4 ±1.5	*0.10*	***<0.001***	***<0.001***

Data are expressed as mean ±SD.
BMS: benign MS; NBMS: non-benign MS; DMT: Disease Modifying Treatments; EDSS: Expanded Disability Status Scale; MSFC: Multiple Sclerosis Functional Composite; T2LL: T2-weighted lesion load; n/a: not applicable. Statistical test: Wilcoxon with Stell-Dwass procedure correction or Fisher exact test.

Table 2. Demographic and clinical characteristics of participants with BMS by the presence or not of abnormal sodium concentration.

	With GMSA (n=10)	**Without GMSA (n=11)**	**p ***
Age (years)	46.2 ±10.6	48.8 ±5.0	*0.75*
Sex (F/M)	8/2	8/3	*1*
Cognitive impairment (Y/N)	5/5	2/9	*0.14*
Disease duration (years)	20.5 ±7.5	18.1 ±3.0	*0.89*
EDSS	1.6 ±0.8	1.1 ±0.5	***0.04***
MSFC	-0.09 ±0.46	0.40 ±0.23	***0.01***
T2LL (cm^3)	7.0 ±2.8	3.6 ±3.9	***0.01***
Cortical GM volume (cm^3)	579 ±45	598 ±26	*0.17*
Thalamic volume (cm^3)	13.6 ±1.7	14.8 ±0.9	***0.02***

Data are expressed as mean ±SD.
BMS: benign MS; NBMS: non-benign MS; EDSS: Expanded Disability Status Scale; GMSA: grey matter sodium abnormalities, MSFC: Multiple Sclerosis Functional Composite; T2LL: T2-weighted lesion load. *Statistical test: Wilcoxon, comparing with and without abnormal sodium concentration or Fisher exact test

Table 3. Results of logistic regressions to explore BMS associated MRI parameters.

a)	*Odds Ratio*	*Lower 95%*	*Upper 95%*	*p-value*
Model				0.01
Thalamic volume	0.64	0.44	0.95	0.03

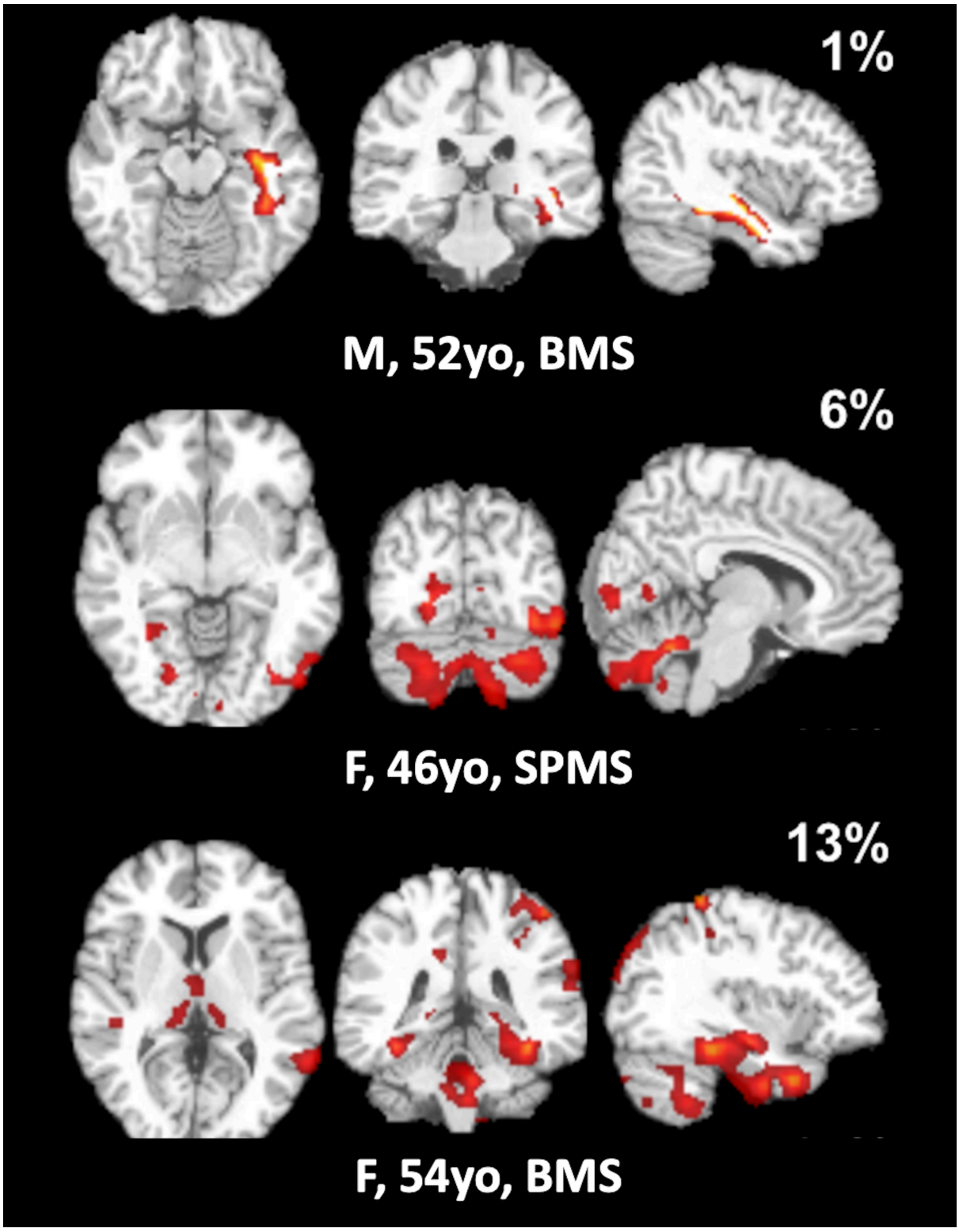
b)	*Odds ratio*	*Lower 95%*	*Upper 95%*	*P-value*
Model				0.008
GMSA (No)	4.6	1.14	18.8	0.03
Thalamic volume	1.01	0.99	1.03	0.07

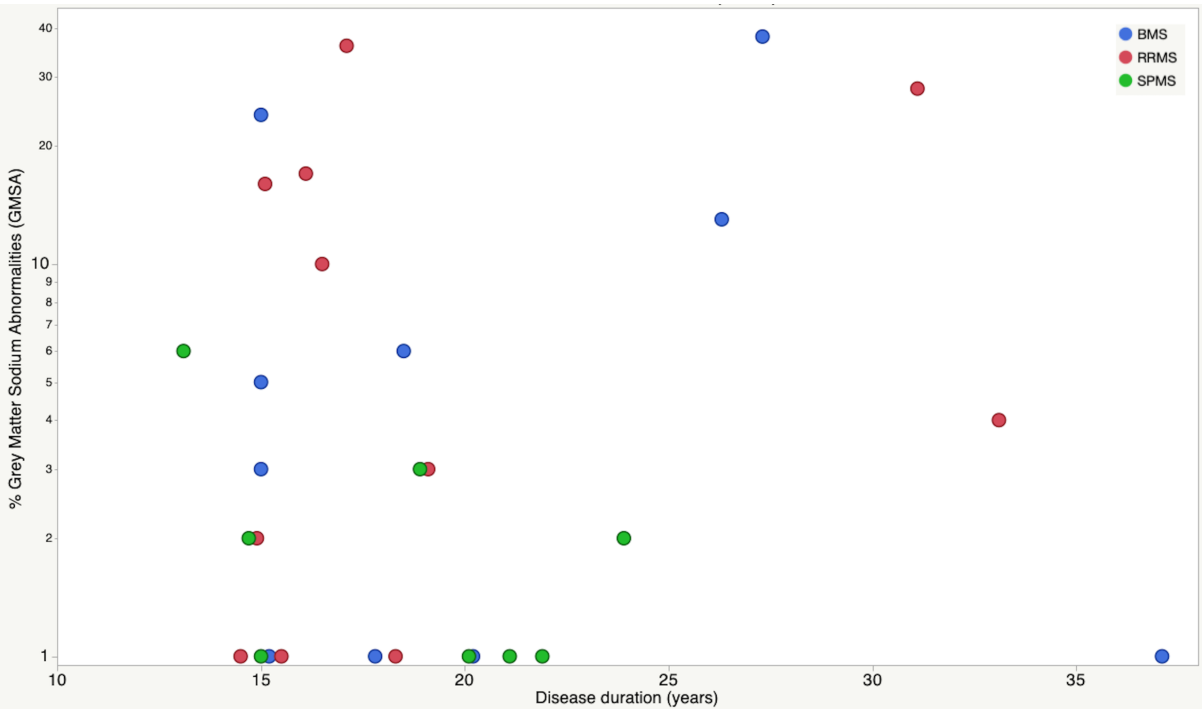
a. Parameters associated with BMS status, after stepwise regression. b. parameters associated with 'truly' BMS status, after stepwise regression.

Figures legend

Figure 1. Examples of extent of grey-matter sodium abnormalities in three patients.

Figure 2. Extension of grey-matter sodium abnormalities in the 29 patients which had GMSA, according to the disease duration.


1%
M, 52yo, BMS
6%
F, 46yo, SPMS
13%
F, 54yo, BMS


BMS
RRMS
SPMS
% Grey Matter Sodium Abnormalities (GMSA)
40
30
20
10
9
8
7
6
5
4
3
2
1
10
15
20
25
30
35
Disease duration (years)