Label-Fusion-Segmentation and Deformation-Based Shape Analysis of Deep Gray Matter in Multiple Sclerosis: The Impact of Thalamic Subnuclei on Disability

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Abstract: Deep gray matter (DGM) atrophy has been reported in patients with multiple sclerosis (MS) already at early stages of the disease and progresses throughout the disease course. We studied DGM volume and shape and their relation to disability in a large cohort of clinically well-described MS patients using new subcortical segmentation methods and shape analysis. Structural 3D magnetic resonance images were acquired at 1.5 T in 118 patients with relapsing remitting MS. Subcortical structures were segmented using a multitlas technique that relies on the generation of an automatically generated template library. To localize focal morphological changes, shape analysis was performed by estimating the vertex-wise displacements each subject must undergo to deform to a template. Multiple linear regression analysis showed that the volume of specific thalamic nuclei (the ventral nuclear complex) together with normalized gray matter volume explains a relatively large proportion of expanded disability status scale (EDSS) variability. The deformation-based displacement analysis confirmed the relation between thalamic shape and EDSS scores. Furthermore, white matter lesion volume was found to relate to the shape of all subcortical structures. This novel method for the analysis of subcortical vol-

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ume and shape allows depicting specific contributions of DGM abnormalities to neurological deficits in MS patients. The results stress the importance of ventral thalamic nuclei in this respect. *Hum Brain Mapp* 35:4193–4203, 2014. © 2014 Wiley Periodicals, Inc.

Key words: MRI; brain volume; thalamus; neuroimaging

INTRODUCTION

During the last decade, a growing body of evidence has suggested that deep gray matter (DGM) structures in the brains of multiple sclerosis (MS) patients are affected by inflammation, demyelination and neuronal loss already at early stages of the disease. Postmortem studies have shown neuronal loss and atrophy in the caudate and thalamus [Cifelli et al., 2002; Vercellino et al., 2009]. Moreover, DGM alterations have been observed in vivo using magnetic resonance imaging (MRI) such as iron accumulation [Khalil et al., 2011], reduced *N*-acetylaspartate levels [Geurts et al., 2006], reduced cerebral blood flow [Inglese et al., 2007; Papadaki et al., 2012], and atrophy [Ramasamy et al., 2009] in MS patients compared to healthy controls. Evidence suggests a relation between disease progression and the volume of subcortical gray matter (GM) structures and specifically, thalamic atrophy seems to be the most relevant measure related to cognitive deficits [Houtchens et al., 2007]. However, the relation between subcortical volume and the expanded disability status scale (EDSS) scores, the most frequently used global disability measure in MS, is still unclear [Bergsland et al., 2012; Houtchens et al., 2007].

It has been shown that DGM structures are organized topographically [Gerfen and Bolam, 2010; e.g., striatum] or into subnuclei [Hirai and Jones, 1989] with different functions (e.g. thalamic nuclei). Hence, the study of local subcortical abnormalities may be a way to improve the clinico-radiological correlation. Voxel-based morphometry (VBM) has been applied to investigate local subcortical changes [Ceccarelli et al., 2008], but the nature of abnormalities identified with VBM is still poorly understood. Thus, the relationship between local DGM alterations and MS-related disability is insufficiently understood so far. In this regard, the analysis of subcortical shape and the measurement of the volume of thalamic subnuclei may be ways forward to precisely locate local changes.

The aims of this study were: (1) to investigate the relationship between striatal, pallidal, and thalamic volume and disability in relapsing-remitting MS; (2) to investigate the relationship between the volume of thalamic nuclei and disability in relapsing-remitting MS; and (3) to investigate changes of the shape of subcortical structures induced by white matter lesions and the relation between such changes and the degree of disability. We addressed these questions by using cross-sectional high resolution MRI data of relapsing remitting MS patients. The MRI data

were analyzed with a novel fully automatic model-based analysis pipeline, which uses multiple automatically generated templates for the segmentation of the DGM brain structures.

PATIENTS AND METHODS

Subjects

Data of one hundred-eighteen patients with a diagnosis of relapsing-remitting MS [McDonald et al., 2001] taking part in an ongoing cohort study on the genotypic-phenotypic characterization of MS, recruited at a tertiary center, were retrospectively analyzed. All patients underwent a thorough medical and neurological examination with structured EDSS assessment (www.neurostatus.net), which included assessment of the EDSS functional system scores (FSS) and review of the past medical history (Table I). Written informed consent was obtained from each participant after a detailed explanation of all procedures. The study was approved by the local ethics committee and was conducted in concordance with the Declaration of Helsinki.

MRI Protocol

Morphological analyses were performed on high-resolution three-dimensional T1-weighted (T1w) MPRAGE images acquired in sagittal plane (TR/TI/TE = 2080/1100/3.0 ms; $\alpha = 15^\circ$, 160 slices, isotropic resolution of 1 mm³). Additionally, a double spin echo proton density (PDw)/T2-weighted (T2w) sequence was applied (TR/

TABLE I. Demographic and clinical data

	Mean $\pm$ SD	Range
Gender M/F	35/83	
Age (years)	44.86 $\pm$ 10.46	23–68
Disease duration (years)	15.24 $\pm$ 8.6	3–43
	Median	Range
EDSS	2.5	0–7
FSS pyramidal	1	0–4
FSS cerebellar	1	0–4
FSS sensory	1	0–4

Demographical features and clinical characteristics of relapsing-remitting MS patients. EDSS = Expanded Disability Status Scale; FSS = Functional System Scores.

Figure 1.

Exemplary subcortical segmentation of two of the study participants with different degrees of atrophy. (a) 41 year old woman, disease duration = 7 years, EDSS = 3, WMLV = 317 mm³; (b) 41 year old man, disease duration = 5, EDSS = 0, WMLV = 835 mm³. First column: axial view of the individual T1w MPRAGE data-

set of the patients in radiological convention. Second column: segmented DGM structures: striatum (red), globus pallidus (green), and thalamus (blue). Third column: segmented thalamic nuclei. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

TE1/TE2 = 3980/14/108 ms; flip angle 180, 40 slices 3 mm thick without gap with an in-plane resolution of 1 × 1 mm²). All MRI scans were performed on a 1.5 T Magnetom Avanto MR scanner (Siemens Medical Solutions, Erlangen, Germany).

MRI Segmentation and Shape Analysis

Subcortical structures were automatically identified using a newly developed segmentation method. This method applies multiple automatically generated templates from different brains [MAGeT Brain algorithm; Chakravarty et al., 2006, 2012] for the segmentation of individual brains.

Briefly, the MAGeT algorithm uses a single atlas derived from manually segmented serial histological data [Chakravarty et al., 2006]. The atlas contains definitions for 108 basal ganglia and thalamic structures based on Schaltenbrand et al. [1977], Hirai and Jones [1989], and Gloor [1997]. The first step of the MAGeT Brain algorithm is to customize the atlas to a subset of subjects that are distributed with respect to age, sex, and disease category to adequately capture neuroanatomical variability within the sample using a region-of-interest based nonlinear registration scheme [Chakravarty

et al., 2008, 2009b]. This newly segmented set of subjects now acts as a template library for the remainder of the dataset. This has the benefit of averaging different sources of random error prior to the estimation of the final segmentation. Once each subject is matched to each of these templates, there are numerous candidate segmentations that are fused using a voxel-wise majority vote (i.e., the label occurring most frequently at a specific location is retained; [Collins and Pruessner, 2010]). Two sets of segmentation are produced from the pipeline (Fig. 1). The first are the striatum, thalamus, and pallidum and the second are the thalamic subnuclei as per the Hirai and Jones [1989]. All DGM segmentations were visually inspected to confirm a correct segmentation. A representative sample (for age, gender, disease duration, and EDSS scores) of 31 patients was selected from the whole group to create the template library. The mean age of these subjects was 45.76 years ± 11.05 (range 23–67), the mean disease duration was 16.38 years ± 10.47 (range 4–38), and the median EDSS score was 3 (range 1.5–6).

Shape analysis was carried out using an extension of the surface-based methodology proposed by Lerch et al. [2008]. First, surface-based representations were generated for each structure. For each subject, the 31 nonlinear transformations

mapped to the original template were concatenated and then averaged to limit noise and error and to increase accuracy [Dorr et al., 2008; Frey et al., 2011]. The dot product between the nonlinear transformation and the surface model normals were used as indices of shape that were estimated to provide a local measure of inwards and outwards displacement along the normal. Inwards and outwards displacements were computed using the structures as defined in the atlas [Chakravarty et al., 2006] as a reference. Specifically, outwards displacements indicate that the segmented subcortical structure is smaller than the corresponding point in the template and local inwards displacements indicate that the segmented structure is larger than the template.

GM volume was computed for each patient using the high-resolution T1w images with the fully automated tool Structural Image Evaluation, using Normalization, of Atrophy for cross-sectional studies (SIENAX version 2.6; [Smith et al., 2004]) using lesion filling to correct for tissue misclassification due to MS lesions [Battaglini et al., 2012]. The SIENAX volume-correction factor was used for normalizing the volume of the DGM structures and GM volume regarding variations of head size. Specifically, native volumes were multiplied by the volume-correction factor. All analyses were performed on these corrected volumes.

All white matter lesions were segmented by trained expert observers according to the standard operating procedures used at our institution for the analysis of clinical phase II and phase III trials. Lesions are first marked by manually putting a cursor into the lesion and then semiautomatically segmented using intensity thresholding with Amira 3.1.1 (Mercury Computer System). Manual adjustments are performed when necessary. The lesions are marked on PDw images, but the according slices of T2w images are displayed at the same time to confirm the lesion site and extent. All raters undergo a training period with consecutive reliability testing before working on any study. Reliability is retested in all raters at fixed intervals (once a year). This ensures a consistently high quality of lesion marking and segmentation. After lesion marking and segmentation, there is a final quality control step with verification of all segmentations by a neuroradiologist.

The white matter lesions volume (WMLV) was computed for the whole brain and for each lobe. In this regard, the T1w images were nonlinearly registered to the MNI atlas and each brain lobe was anatomically labelled using the ANIMAL algorithm [Collins et al., 1995]. The estimated transformation matrices were then applied to the binary lesion mask.

Statistical Analysis

Volume analysis

Hierarchical multiple linear regression (MLR) analyses including three blocks was performed to investigate the relation between EDSS and DGM volumes. Age, gender, and disease duration were entered in the first block and

maintained to take them into account in all subsequent blocks. DGM volumes were entered in the second block and stepwise method (variable entry at $P < 0.05$, variable removal $P > 0.10$) was used. In the third block, stepwise method was used to investigate the relevance of WMLV and normalized GM volume (nGMV).

Moreover, multinomial logistic regression was used to investigate how the significant predictors of global EDSS observed in the hierarchical MLR relate to FSS (pyramidal, cerebellar, and sensory). In case of the cerebellar FSS, the analysis was restricted to a subgroup of 102 (79 women, mean age: 44.18 years $\pm$ 10, range 23–68) patients without severe motor deficits (pyramidal FSS less than or equal to 2).

For each regression model, the following assumptions were tested: linearity and homoscedasticity were tested by plotting the regression standardized predicted value. The assumption of normality was tested using the Kolmogorov-Smirnov (K-S) test and the probability-probability (P-P) plot of the standard residuals. Moreover, autocorrelations (independent errors) were tested by using Durbin-Watson (D-W) test. Collinearity was also tested using the Pearson correlation between independent variables and variance inflated factor (VIF) as indexes. In order to reduce the multicollinearity problem, continuous interdependent variables were centered with transformation of the raw values into z-scores [Kraemer and Blasey, 2004]. To meet the normality assumption of standardized residuals, a base-10 logarithmic transformation was applied to the EDSS scores. Furthermore, performing a MLR using bootstrap procedure [Efron and Tibshirani, 1998] is a robust alternative approach to cope with deviations from normality and homoscedasticity. Bootstrapping (number of samples: 1000; confidence intervals: 95% using bias corrected accelerated methods) was applied to the MLR designed to investigate the relationship between the volume of thalamic nuclei and EDSS scores. No logarithmic transformation of the dependent variable was performed in this case. All the analyses were performed using SPSS 20 (IBM, New York).

Shape analysis

Three separate linear models were performed to investigate the relations between vertex-wise displacements and EDSS (after base-10 logarithmic transformation) for all segmented DGM structures. Influences of disease duration, whole brain WMLV and lobe-wise WMLV on the shape of the subcortical structures were investigated in separate linear models. Age and gender were used as nuisance covariates in all analyses. Results were corrected for multiple comparisons using false-discovery rate (FDR; [Genovese et al., 2002]) at alpha levels of 0.01 and 0.05. The mean displacement and standard deviation across subjects at the significant vertexes were reported. All analyses were performed using the RMINC package (R for Medical Imaging NetCDF; <https://wiki.phenogenomics.ca/display/MICePub/RMINC>), an image analysis software library developed for the R statistical environment (<http://www.r-project.org>).

TABLE II. White matter lesion volumes and DGM volumes (mm³)

Brain regions	White matter lesion volume	Standard deviation	Min	Max
Frontal lobe	2,802	3,184	0	17,749
Parietal lobe	1,548	1,746	0	8,919
Temporal lobe	1,356	1,660	0	9,536
Occipital lobe	244	394	0	2,036
Whole brain	5,952	6,362	0	3,0201
DGM	Normalized volume	Standard deviation	Min	Max
Globus pallidus	3,442	413	2,386	4,886
Striatum	21,524	1,799	16,115	27,686
Thalamus	13,126	1,590	8,894	17,327
<i>Thalamic nuclei</i>				
Lateral geniculate	343	53	227	487
Medial geniculate	463	56	329	635
Anterior	270	43	135	452
Central	531	56	349	681
Lateral dorsal	114	29	25	194
Lateral posterior	820	129	540	1,192
Medial dorsal	2,077	266	1375	2,900
Pulvinar	3,385	502	2,292	4,699
Ventral anterior	1,313	189	870	1,824
Ventral lateral	1,685	294	878	2,529
Ventral posterior	975	197	475	1,513

Volumes (combined left and right) of segmented subcortical structures and thalamic nuclei (mm³) and white matter lesion volumes (combined left and right; mm³) across all studied participants.

RESULTS

DGM Volumes

After visual quality control, we concluded that DGM structures were correctly segmented in all patients, indicating that the algorithm is robust even in the presence of lesions in close proximity to DGM structures. The WMLV, the normalized volumes of the DGM structures as well as of thalamic nuclei are reported in Table II. The mean nGMV was $753,554 \pm 66,727$ mm³ (range: 579,599–928,276 mm³).

In the first hierarchical MLR analysis, the relation between EDSS and DGM volumes was investigated. No correlation higher than $r = 0.6$ was observed among DGM structures. The full regression model ($R^2 = 0.29$, $F(5,112) = 9.14$, $P < 0.001$) showed that thalamic volume ($\beta = -0.23$, $t(112) = -2.1$, $P < 0.05$) together with nGMV ($\beta = -0.32$, $t(112) = -2.5$, $P < 0.05$) were significant predictors of EDSS scores. A second hierarchical MLR was designed to specifically investigate the relation between thalamic subnuclei and EDSS scores. The volumes of the ventral-lateral (VL), ventral-anterior (VA), and ventral-posterior (VP) nuclei were highly correlated (VL/VA: $r = 0.91$, $P < 0.001$; VL/VP: $r = 0.88$, $P < 0.001$). As these nuclei are known to relate to each other functionally and anatomically, they were combined for further analysis into one volume, termed ventral nuclear complex (VNC; Fig. 2). Thus, the hierarchical MLR model was performed with the VNC and the remaining thalamic nuclei in the second block. The full model explained 30% of the variance ($R^2 = 0.3$, $F(5,112) = 9.66$, $P < 0.001$), VNC volume ($\beta = -0.28$, $t(112) = -2.52$, $P < 0.05$), and nGMV ($\beta = -0.28$, $t(112) = -2.18$, $P < 0.05$) were significant predictors.

Figure 2.

Ventral nuclei of the thalamus as reported in Hirai and Jones nomenclature are shown and overlaid to high-resolution T1 MRI images (Colin27 MRI template). (a) Axial view and (b) coronal view. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

TABLE III. Mean DGM surface displacement

	Outward		Inward	
	Left	Right	Left	Right
Thalamus	0.8 ± 1.8	0.7 ± 2	1 ± 1.6	1.8 ± 1.3
Striatum	0.7 ± 1.4	0.7 ± 1.6	1.5 ± 1.8	1.1 ± 1.9
Globus pallidus	1.3 ± 1.7	1.1 ± 2	–	–

Mean DGM surface displacement (in millimetres $\pm$ SD) relating to WMLV across all studied participants.

Moreover, the regression analysis performed using the bootstrap procedure confirmed the results obtained with the previous model. The full model here explained 30% of EDSS variance ($F(5, 112) = 9.8$, $P < 0.001$), VNC volume (beta = -0.28 , $P < 0.01$; CI = -0.7 : -0.11), and nGMV (beta = -0.29 , $P < 0.05$; CI = -79 : -0.07) were significant predictors. The multinomial logistic regression results showed that the two independent variables included in the model (VNC volume and nGMV) differentially predicted the different FSS. Regarding cerebellar FSS (Nagelkerke $R^2 = 0.36$, $\chi^2(6) = 39$, $P < 0.001$) both VNC ($\chi^2(3) = 14.1$, $P < 0.01$) and nGMV ($\chi^2(3) = 10.1$, $P < 0.05$) were significant predictors. For the pyramidal FSS (Nagelkerke $R^2 = 0.24$, $\chi^2 = 29.4$, $P < 0.001$) and sensory FSS (Nagelkerke $R^2 = 0.16$, $\chi^2 = 19.8$, $P < 0.01$) only nGMV (pyramidal: $\chi^2(3) = 9.27$, $P < 0.05$; sensory: $\chi^2(3) = 13.8$, $P < 0.01$) was a significant predictor. All regression models were accurate for the sample and generalizable to the population level. All models showed a mean VIF of <1.5 indicating no multicollinearity. The residuals met linearity and homoscedasticity assumptions, were uncorrelated (D-W test ~ 1.7 for all models, uncorrelation interval: 1.63–2.23, $P < 0.05$, $n = 118$, $k = 5$) and normally distributed (K-S test was not significant).

DGM Shape Analysis

The shape analysis showed a significant relationship between whole brain WMLV and surface displacement of the thalamus ($F(3, 114) = 2.31$, $t = 3.12$), striatum ($F(3, 114) = 1.14$, $t = 2.79$) and globus pallidus ($F(3, 114) = 0.16$, $t = 3.21$). The thalamus showed outwards displacements in the anterior medial portion bilaterally and inwards displacements in the lateral medial portion bilaterally. The striatum showed outwards displacements in the body and tail of the medial caudate nuclei where caudate forms part of the floor of the anterior horn of the lateral ventricle and in the superior medial putamen. The lateral portion of the caudate nuclei showed inwards displacements in the body and tail. The globus pallidus showed outwards displacements in the anterior portion bilaterally, but no significant inwards displacements were observed. The observed displacements were primarily driven by frontal WMLV (thalamus: $F(3, 114) = 5.44$, $t = 3.59$; globus pallidus: $F(3, 114) = 5.16$, $t = 3.41$; striatum: $F(3, 114) = 5.44$, $t = 3.59$) and parietal WMLV (thalamus: $F(3, 114) = 5.37$, $t = 3.38$; globus pallidus: $F(3, 114) = 4.72$, $t = 3.1$; striatum: $F(3, 114) = 4.8$, $t = 3.17$). A significant relationship between striatal surface and temporal WMLV was observed ($F(3, 114) = 4.9$, $t = 3.4$). No significant relations were observed between surface displacements and occipital WMLV. Interestingly, the disease duration did not affect the shape of the subcortical structures (Table III and Fig. 3). All reported analyses are FDR = 0.01 corrected.

Notably, a significant relationship between bilateral outwards displacements of the thalamus and EDSS scores ($F(3, 114) = 1.73$, $t = 2.91$, FDR = 0.05; Fig. 4) was found. Specifically, the anterior portions were involved bilaterally (left: 1.12 ± 2.01 mm; right: 77 ± 2.11 mm). No inwards displacements showed a significant relationship to EDSS scores. Furthermore, no significant correlations between

Figure 3.

Relation between white matter lesion volume and subcortical surface displacement (outwards). Left side of the images corresponds to left side of the brain. Color-coded results of group statistics are overlaid on 3D models of the subcortical DGM areas: (a) thalamus, (b) striatum, and (c) globus pallidus. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Figure 4.

Relation between EDSS scores and thalamic surface displacement (outwards). Color-coded statistical maps of areas with significant relation between EDSS scores and thalamic shape are overlaid on a 3D model of the thalamus. (a) view from above (left side of the image corresponds to left side of the brain); (b) anterior view (right side of the figure corresponds to left side of the brain). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

DGM structures displacements and EDSS scores were observed in the striatum and the globus pallidus.

DISCUSSION

In this study, we investigated morphological changes of subcortical structures and their relationship to physical disability as measured by the EDSS in relapsing-remitting MS patients. The regression analysis showed that the thalamus was the most relevant DGM in predicting disability. Moreover, our results show that within the thalamus, the volume of the VNC (VA, VL, and VP) is the best predictor of EDSS scores. In addition, nGMV showed a significant relationship with EDSS scores. GM involvement and atrophy of DGM structures has been found in patients with MS already at early stages of the disease [Henry et al., 2008]. Various disease mechanisms could lead to atrophy of subcortical structures and specifically of the thalamus. Histological studies have shown that DGM structures can be directly affected by the disease [Cifelli et al., 2002; Vercellino et al., 2009] through demyelination and diffuse inflammatory alterations. Moreover, neuronal degeneration due to axonal damage may partially explain subcortical atrophy observed in MS [Dziedzic et al., 2010; Siffrin et al., 2010]. In this regard, neuronal loss could be related to both a lack of innervation due to damage of “afferent” axons (anterograde degeneration) as well as to the degeneration of parent neurons (retrograde degeneration). Due to its widespread connections with cortical and subcortical structures, the thalamus and particularly the VNC may be particularly vulnerable to such retrograde and anterograde degenerative mechanisms. Human post-mortem and primate studies have shown that the VL nucleus [Asanuma et al., 1983a; McFarland and Haber, 2002] possesses reciprocal connections with the primary motor cortex (M1),

supplementary motor area (SMA), and premotor cortex (PM). The VA nucleus has connections with preSMA, and PM [Asanuma et al., 1983b; McFarland and Haber, 2002] and the VP with the somatosensory cortex [Jones and Powell, 1970]. These thalamic nuclei also directly project to the striatum indicating that they play a primary role in modulating basal ganglia functions [Gerfen and Bolam, 2010]. Moreover, cortico-thalamic projections to the VNC are more extensive than thalamo-cortical projections [McFarland and Haber, 2002]. The VA receives nonreciprocal connections from the medial prefrontal cortex and the VL from rostral motor regions. The VL/VP nuclei receive afferents from deep cerebellar nuclei (dentate nucleus and nucleus interpositus; [Middleton and Strick, 2000]) and the VA/VL receive afferents from the internal segment of the globus pallidus as well as the substantia nigra (pars reticulata), representing the basal ganglia outflow pathways [Hoshi et al., 2005; Middleton and Strick, 2000].

Studies using diffusion tensor imaging (DTI) in humans have confirmed the conventional anatomical parcellation of the thalamus and the relation between thalamic nuclei and cortical regions as observed in primate and on histological series [Behrens et al., 2003; Zhang et al., 2010].

The thalamus is generally considered to relay information from the globus pallidus/substantia nigra to the cortex. During the last 10 years, a growing number of studies indicated that thalamic nuclei do more than just passively conveying information [Haber and Calzavara, 2009]. These nuclei are actively involved in modifying the dynamics of cortical processing in two ways. Thalamo-cortical projections to superficial cortical layers (I/II) and cortico-thalamo-cortical projections to deep cortical layers (V) directly modulate the cortico-cortical and cortico-subcortical flow of information [McFarland and Haber, 2002; Sherman and Guillery, 2002, 2011]. Thus, via several different cortical frontal lobe-subcortical pathways, the

VNC may serve as an important integrative center modulating behavior and more specifically motor output [Guillery and Sherman, 2002].

Previous studies showed ambiguous results regarding the potential relationship between EDSS scores and thalamic volume. Some authors have reported an inverse correlation between thalamic volume and EDSS scores [Bergsland et al., 2012; Ramasamy et al., 2009], but others did not find this association [Houtchens et al., 2007; Prinster et al., 2006]. These somewhat inconsistent results could be related to the specificity and accuracy of different segmentation methods used (e.g., manual, semiautomatic, and fully automatic). Moreover, the different methods have been applied on MRI images with different quality and different GM to WM contrast-to-noise ratios. Another source of variability may be related to DGM demyelination [Vercellino et al., 2009] and local iron deposition [Khalil et al., 2011] observed in MS. Such changes may affect the signal intensity of T1w images, which may in turn bias the DGM segmentation [Goto et al., 2013] increasing the variability within and between studies.

Nowadays, automated segmentation methods are commonly used. In case of model-based algorithms, these methods are usually based on one or more manual segmentations of a neuroanatomical structure by expert raters forming a template [Bajcsy et al., 1983; Collins et al., 1995]. The model labels are then warped to match the unique neuroanatomy of an individual subject/patient through the estimation of a high-dimensional nonlinear transformation. These types of segmentation may be limited in accuracy by errors in the transformation estimation, irreconcilable neuroanatomical differences between the anatomy of the template and the subject due to disease or age, and resampling errors after the application of the nonlinear transformation. The MAGeT Brain algorithm applied in our study accounts for these limitations through the implementation of a multitlas strategy while relying on a single input template derived from histological data. The generation of the template library from a subset of the input data distributes these possible errors across the input data set. The ability of the MAGeT brain algorithm to accurately identify thalamic subregions has been previously validated against manual segmentations [Chakravarty et al., 2009b], intraoperative recordings [Chakravarty et al., 2008] and functional magnetic resonance imaging findings [Chakravarty et al., 2009a]. Additionally, the atlas has previously been used to investigate the relationship between altered morphology and thalamic nuclei in attention deficit hyperactivity disorder [Ivanov et al., 2010] and Tourette syndrome [Miller et al., 2010].

Furthermore, the heterogeneity of patients with respect to disability levels could be another reason explaining variability among different studies. Indeed, the EDSS global score is a composite index and reflects a combination of subscales measuring different functional systems. Thus, at least in the lower EDSS range, similar increases of the global score could reflect different kinds of disability

(motor, sensory etc.). This could be an important source of variability between different groups of patients. Taking into account these considerations, we investigated which of the observed statistical predictors of global EDSS scores (i.e., VNC volume and nGMV) may predict specific EDSS FSS. Interestingly, this analysis showed that VNC volume predicts cerebellar FSS together with nGMV. However, pyramidal and sensory FSS were related only to nGMV. Thus, the VNC seems to rather relate to deficit of motor control, motor stability, and equilibrium (e.g. ataxia). These results are supported by the anatomical observation that the cerebellar-VL/VP thalamic nuclei-M1 circuit is involved in movement and sensory functions [Debaere et al., 2004]. At present, the dominant hypothesis is that it contributes to the preparation of movements in response to sensory input [Sommer, 2003; van Donkelaar et al., 1999]. Notably in our study, 90% of patients had a global EDSS score of less or equal than 4 and 86.4% had a pyramidal FSS of less or equal than 2. Moreover, 53% of patients had a cerebellar FSS of more than 1. This distribution of scores suggests that in our group of patients, the cerebellar FSS has a role in contributing to the global EDSS score and this could explain the relevance of the thalamus and specifically of the VNC in explaining global EDSS scores. Based on these observations, it is essential to highlight that VNC volume is related only to specific symptoms and it is not generally related to disability.

The performed shape analysis confirmed the relation between thalamic morphological changes and EDSS scores. Specifically, shape abnormalities in the anterior part of the thalamus showed a significant relation to disability. The morphological changes in this thalamic region seem to be driven by frontal and parietal WMLV. These results are in agreement with the previously described thalamo-frontal lobe anatomical network. Interestingly, a recent study on age-related changes in healthy subjects [Hughes et al., 2012] showed a relationship between anterior thalamic shape abnormalities and atrophy in thalamo-frontal pathways as measured using DTI. This is in line with our finding that thalamic abnormalities relate to white matter damage. Notably, we found a relationship between the shape of the investigated brain structures and WMLV in each of the DGM structures we assessed. These results are consistent with findings of early axonal pathology and Wallerian degeneration in MS plaques and perilesional white matter [Dziedzic et al., 2010] and the view that lesion-related secondary anterograde/retrograde degeneration at least partially explains neurodegenerative processes in MS.

An increasing number of studies on psychiatric [Levitt et al., 2004] and neurological diseases [Looi et al., 2010, 2011] have shown the sensitivity of shape analysis to investigate local morphological changes. The shape analysis approach used in our study describes how much a given structure differs from the reference atlas at the vertex-wise level in terms of inwards (the structure is deflated to match the atlas) and outwards displacements (the structure is inflated to match the atlas). Outwards displacements are consistent with

tissue loss. Moreover, studies that investigated developmental diseases showed shape abnormalities of medial structures due to tension effects during development [Van Essen, 1997]. Thus, shape analysis is complementary to volume analysis, because changes of shape may occur also with very small or even without changes of volume.

Due to the lack of healthy subjects, it is not possible to draw conclusions on the degree of atrophy in MS patients. This is a limitation of the study. However the aim of the present study was to investigate the relationship between brain morphology and disability in MS. Thus, the reported results are not invalidated by this limitation. Moreover, the segmentation of DGM using T1w images as only input may be a limitation. Indeed, it has been suggested that a multimodal imaging strategy (T1w, T2-weighted images and DTI) could improve the segmentation accuracy [Traynor et al., 2010, 2011]. To perform a multimodal analysis, each sequence has to be acquired using the same resolution in order to reduce possible segmentation biases due to partial volume effects. In our cohort, the PDw image has been acquired with lower resolution compared with the T1w data and DTI was not included in the MRI protocol. For this reason the segmentation was based only on the T1w images. Another limitation of our study relates to the cross-sectional study design. This is particularly true in MS studies due to the variability between patients, which may introduce confounding effects [Confavreux and Vukusic, 2006]. Thus, caution should be taken in generalizing the results and longer follow-up studies are needed to confirm the observed involvement of the VNC. Moreover, we wish to emphasize that our results are valid for RRMS, but are not directly applicable to primary and secondary progressive MS subtypes without further investigation.

According to our results, the studied EDSS predictors, e.g. volumes of thalamic nuclei, explain only a limited degree of EDSS variance. This may be due to several reasons. First, MS is an inflammatory-demyelinating disease and thus neuropathological features other than atrophy may contribute to disability. In this regard, MRI measures such as mean fractional anisotropy [Hulst et al., 2013] or magnetization transfer ratio [Fisniku et al., 2009] as well as electrophysiological measures including electroencephalography [Leocani et al., 2001] and evoked-potentials [Schlaeger et al., 2012] have been shown relate to disability. Moreover, the global EDSS reflects a variety of symptoms, while the function of thalamic subnuclei may be rather specific. Furthermore, other atrophy measures, which were not included in our study, have been previously related to disability such as spinal cord volume [Zivadinov and Bakshi, 2004]. Overall, these considerations point out the complexity of the relationship between pathophysiology and disability in MS. In order to shed light on this relationship a complex neuro-functional model that includes different structures and different measures may be needed. Nevertheless, understanding the involvement of specific brain

structures is the first step to define more complex models on the relationship between pathophysiology and disability.

In conclusion, our results show that this novel method for the analysis of subcortical volume and shape allows determining contributions of specific DGM structures to the development of neurological deficits in MS patients. The results emphasize the contribution of ventral thalamic nuclei to the accrual of physical disability in MS.

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