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Analysis of ageing-associated grey matter volume in patients with multiple sclerosis shows excess atrophy in subcortical regions

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ABSTRACT

Age of onset in multiple sclerosis (MS) exerts an influence on the course of disease. This study examined whether global and regional brain volumes differed between "younger" and "older" onset MS subjects who were matched for short disease duration, mean 1.9 years and burden as measured by the MS Severity Score and relapses.

21 younger-onset MS subjects (age 30.4 ± 3.2 years) were compared with 17 older-onset (age 48.7 ± 3.3 years) as well as age-matched controls ($n = 31$, 31.9 ± 3.5 years and $n = 21$, 47.3 ± 4.0 years). All subjects underwent 3D volumetric T1 and T2-FLAIR imaging. White matter (WM) and grey matter (GM) lesions were outlined manually. Lesions were filled prior to tissue and structural segmentation to reduce classification errors.

Volume loss versus control was predominantly in the subcortical GM, at >13% loss. Younger and older-onset MS subjects had similar, strong excess loss in the putamen, thalamus, and nucleus accumbens. No excess loss was detected in the amygdala or pallidum. The hippocampus and caudate showed significant excess loss in the younger group ($p < 0.001$) and a strong trend in the older-onset group.

These results provide a potential imaging correlate of published neuropsychological studies that reported the association of younger age at disease onset with impaired cognitive performance, including decreased working memory.

1. Introduction

Multiple Sclerosis (MS) is a chronic inflammatory disease of the central nervous system, most commonly presenting in young adults as relapsing remitting (RRMS) or later in life as progressive disease (either primary- or secondary-progressive: PPMS or SPMS, respectively), and associated, especially in the latter, with significant neurodegenerative pathological features. The notion of MS as a disease exclusively affecting the white matter (WM), with multifocal demyelination, is diminishing (Friese et al., 2014; Vigeveno et al., 2012). Indeed, WM lesion load detected by MRI is only weakly correlated with clinical symptoms (Zivadinov and Cox, 2007). There is a growing body of evidence from both pathology and MRI to suggest that grey matter (GM) degeneration is prevalent in MS (Bermel et al., 2003), (Bakshi et al., 2005), (Chard et al., 2004), (Sanfilipo et al., 2006) and may be a stronger predictor of clinical decline than WM measures (Fisniku et al., 2008; Pirko et al., 2007). Information pertaining to the relative atrophy rate of cortical and subcortical GM regions in early MS, and their role in the disease course, remains a topic of study (Bergsland et al., 2012).

Studies of GM atrophy in MS can be confounded by segmentation errors arising from the MS lesions, which result in misclassification of WM regions as GM, and vice versa. One way to circumvent this issue is to mask the lesion areas after global tissue segmentation, but the segmentation error could potentially extend beyond the locality of the lesion itself. Thus, lesion filling prior to segmentation of the anatomical MRI data has been proposed (Chard et al., 2010), and has shown superior segmentation results over retrospective lesion masking (Battaglini et al., 2012). However, lesion filling is not yet universally adopted in image analysis procedures.

The age of onset of MS is relatively varied and it is yet unclear what role GM changes play in this. An older age of RRMS onset is associated with an increased risk of conversion to the more disabling SPMS, independent of disease duration and early relapse frequency (Scalfari et al., 2011). Transformation from RRMS to SPMS may therefore be in

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part a consequence of ageing itself, rather than solely due to primary, disease-specific pathology. In other work, myelin integrity (as measured by magnetization transfer MRI) has been shown to be independently affected by age in early MS (Newbould et al., 2014), so GM may be similarly affected.

The objectives of this study are therefore three-fold: (i) to establish whether cortical or subcortical GM atrophy dominates in early MS; (ii) to investigate whether the degree of GM atrophy differs between younger and older-onset MS patients of matched clinical status – reflecting the effect of age on CNS damage and repair, independent of disease duration; and (iii) to propose an image analysis workflow for reproducible and standardized quantification of the required imaging endpoints for such analysis.

We recruited two age groups of MS patients with short disease duration from within a well-characterised research cohort. The only factor of significant demographic difference between the two MS groups was age. Comparison of these with 'younger' and 'older' groups of age-matched healthy controls enabled cross-sectional analysis of GM (and WM) atrophy in each age group.

2. Methods

2.1. Subjects

A total of 38 MS patients and 52 age-matched controls were involved in this study (Table 1). Subjects gave written informed consent, and the studies had ethical approval from the National Research Ethics Service.

2.2. MRI acquisition

All subjects were imaged on a 3T Verio clinical MR system (Siemens Healthcare, Erlangen, Germany; VB17), using a 12-channel phased-array head coil. A T1-weighted 3D MPRAGE volume acquisition was based on the ADNI-GO recommended parameters (Jack et al., 2008) but with 1 mm^3 isotropic resolution and parallel imaging (PI) factor of 2. A T2-weighted fluid-attenuated inversion recovery (T2-FLAIR) volume was acquired with 1 mm^3 isotropic resolution using a 3D T2w variable-refocusing angle turbo spin echo readout (Mugler and Brookeman, 2003), with 160 sagittal sections captured in a single 3D slab with the following parameters: echo time (TE) 395 ms, repetition time (TR) 5 s, inversion time (TI) 1800 ms, 250 × 250 mm field-of-view, and a PI factor of 2.

2.3. Data analysis

Images were processed following the analysis workflow depicted in Fig. 1. Each subject's T2-FLAIR volume was co-registered to their MPRAGE using the rigid-body transformation of FLIRT (Jenkinson et al., 2002). To improve the subsequent tissue segmentations (Popescu et al., 2012), the MNI152 template was aligned using the affine registration of FLIRT (Jenkinson et al., 2002) to the same MPRAGE. This transform was then applied to a rectangular mask covering the MNI152 template, and the transformed mask applied to the MPRAGE to remove excess neck that can corrupt brain extraction tools (Popescu et al., 2012). After masking, the anatomical scans were segmented using additional FSL tools: (i) SIENAX (Smith et al., 2002) for scaled tissue volumes of WM, GM, and cortical GM, normalized for subject head size; and (ii) FIRST (Patenaude et al., 2011) for subcortical grey matter volumes of the putamen, caudate, thalamus, hippocampus, amygdala, accumbens, and pallidum. Subcortical GM structures were multiplied by the SIENAX volume-correction factor to normalize the volumes across subjects. For the MS patients, additional processing steps were performed. WM and GM lesions were filled (Battaglini et al., 2012) using the manually defined lesion masks before the tissue segmentations. After segmentation, correct assignment of WM and GM lesions to WM and GM respectively was checked by masking the partial volume estimates inside the lesion masks. Finally, the recommended brain extraction tool (BET) parameters (of B and f = 0.1) for bias field estimation with SIENAX in MS subjects (Popescu et al., 2012) were used.

Lesion segmentation was performed using a semi-automated intensity-based thresholding technique with manual correction (Jim Version 6.0, Xinapse) by a trained observer and corroborated by a second experienced neuroradiologist, both blinded to subject age and clinical status. GM lesions were segmented from T2-weighted FLAIR images and confirmed on the T1-weighted MPRAGE images. FLAIR images were used for WM lesion definition, due to high lesion conspicuity and detectability on these scans. Fig. 2, top row, shows the segmentation results of the GM and WM lesions in one MS patient.

The scaled SIENAX output volumes (in units of mm^3) of GM, WM, and cortical GM (cGM) summed across each hemisphere are termed the 'global' tissue volumes in this study and were used for comparison of global tissue volumes across the different groups. We also computed a tissue volume for the subcortical and nonperipheral GM (scGM), by subtraction of the scaled cGM from the scaled GM volume, giving a fourth global tissue volume for group comparison (Fig. 2: bottom row). It should be noted that this scGM volume is not an accurate segmentation of subcortical deep grey matter structure volumes, but provides a first-pass indication of the subcortical tissue volume differences between the groups before formal segmentation of individual subcortical grey matter structure volumes (Fig. 2: bottom right). For example, the scGM includes the allocortex structures such as the hippocampus. The scaled FIRST output volumes for the defined individual deep grey matter regions (also in units of mm^3) were compared across groups, to investigate 'local' (subcortical) tissue volume changes.

Younger and older groups were combined to investigate tissue volume differences between controls and MS patients. Then, age-group related differences were explored. Differences for age were assessed using a univariate analysis of variance (ANOVA) with four groups. For the two MS groups, differences for the Expanded Disability Status Scale (EDSS), MS Severity Score (MSSS) (Roxburgh et al., 2005), and disease duration

Table 1
Demographics: four groups. Table of demographics for the four subject groups (both controls and MS patients, young and old) and results of group comparisons. Values reported as mean (SD). NA = not applicable.

	Younger Control	Younger MS	Older Control	Older MS	*p* (yC/yMS)	*p* (yC/oC)	*p* (yC/oMS)	*p* (yMS/oC)	*p* (yMS/oMS)	*p* (oC/oMS)
N	31	21	21	17						
Age (years)	31.9 (3.5)	30.4 (3.2)	47.3 (4.0)	48.7 (3.3)	0.86	<0.001 expected	<0.001 expected	<0.001 expected	<0.001 expected	1.00
Gender (M/F)	18/13	2/19	12/9	5/12	<0.001	1.00	0.75	0.003	0.21	0.11
D.D. (years)	NA	2.3 (1.6)	NA	2.4 (1.2)	NA	NA	NA	NA	0.89	NA
EDSS	NA	3.0 (1.2)	NA	4.1 (1.3)	NA	NA	NA	NA	0.008	NA
MSSS	NA	5.5 (1.6)	NA	6.3 (1.7)	NA	NA	NA	NA	0.13	NA
Relapses (#)	NA	2.0 (0.8) All RRMS	NA	1.8 (0.8) 15 RRMS 2 SPMS	NA	NA	NA	NA	0.50	NA