European Radiology (2019) 29:7027–7036
https://doi.org/10.1007/s00330-019-06268-8

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Structural and functional MRI correlates of T2 hyperintensities of brain white matter in young neurologically asymptomatic adults

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Received: 5 March 2019 / Revised: 25 April 2019 / Accepted: 7 May 2019 / Published online: 29 May 2019

Abstract

Objectives Although white matter hyperintensities (WMHs) are quite commonly found incidentally, their aetiology, structural characteristics, and functional consequences are not entirely known. The purpose of this study was to quantify WMHs in a sample of young, neurologically asymptomatic adults and evaluate the structural and functional correlations of lesion load with changes in brain volume, diffusivity, and functional connectivity.

Methods MRI brain scan using multimodal protocol was performed in 60 neurologically asymptomatic volunteers (21 men, 39 women, mean age 34.5 years). WMHs were manually segmented in 3D FLAIR images and counted automatically. The number and volume of WMHs were correlated with brain volume, resting-state functional MRI (rs-fMRI), and diffusion tensor imaging (DTI) data. Diffusion parameters measured within WMHs and normally appearing white matter (NAWM) were compared.

Results At least 1 lesion was found in 40 (67%) subjects, median incidence was 1 lesion (interquartile range [IQR] = 4.5), and median volume was 86.82 (IQR = 227.23) mm^3. Neither number nor volume of WMHs correlated significantly with total brain volume or volumes of white and grey matter. Mean diffusivity values within WMHs were significantly higher compared with those for NAWM, but none of the diffusion parameters of NAWM were significantly correlated with WMH load. Both the number and volume of WMHs were correlated with the changes of functional connectivity between several regions of the brain, mostly decreased connectivity of the cerebellum.

Conclusions WMHs are commonly found even in young, neurologically asymptomatic adults. Their presence is not associated with brain atrophy or global changes of diffusivity, but the increasing number and volume of these lesions correlate with changes of brain connectivity, and especially that of the cerebellum.

Key Points

- *White matter hyperintensities (WMHs) are commonly found in young, neurologically asymptomatic adults.*
- *The presence of WMHs is not associated with brain atrophy or global changes of white matter diffusivity.*
- *The increasing number and volume of WMHs correlate with changes of brain connectivity, and especially with that of the cerebellum.*

Keywords White matter · Healthy volunteers · Diffusion tensor imaging · Functional magnetic resonance imaging

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00330-019-06268-8) contains supplementary material, which is available to authorized users.

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Abbreviations

AD	Axial diffusivity
FA	Fractional anisotropy
FFE	Fast field echo
FLAIR	Fluid attenuation inversion recovery
ICC	Interclass correlation coefficient
IQR	Interquartile range
MD	Mean diffusivity
MS	Multiple sclerosis
NAWM	Normally appearing white matter
RD	Radial diffusivity
rs-fMRI	Resting-state functional MRI
TBSS	Tract-based spatial statistics
TE	Echo time
TR	Repetition time
TSE	Turbo spin echo
WM	White matter
WMHs	White matter hyperintensities

Introduction

White matter hyperintensities (WMHs) in T2-weighted MRI images are a common finding in neuroradiology. The prevalence of these lesions in neurologically asymptomatic individuals has been investigated by several authors with rather variable results ranging from 0.5% to more than 95% [1–4]. It is known that the prevalence of WMHs increases with age [5, 6]. Their presence is associated with several diseases or risk factors, such as hypertension [7], cognitive impairment [8, 9], and Parkinson's disease [10]. Comparatively few studies have focused on this finding in the populations under 40 years of age [4, 11]. This topic is interesting not only from the perspectives of aetiology, pathophysiology, and functional relationships of WMHs but also because WMHs constitute an important issue in differential diagnosis of several diseases. One of these is multiple sclerosis (MS), wherein the number and localisation of white matter (WM) lesions become crucial in terms of the McDonald criteria [12]. Because MS usually affects younger patients, investigating incidental WMHs in asymptomatic subjects of corresponding age may be beneficial.

Rapid development of MRI technology and data analysis techniques in recent years has opened up new possibilities for using such advanced methods as functional imaging (fMRI) or diffusion tensor imaging (DTI). The application of these modalities in relation to the presence of WMHs may contribute to knowledge about this topic, because the possible impact of incidentally found WMHs on structural and functional changes of the brain is not entirely known.

In this study, therefore, we set out to quantify the number and volume of WMHs in a sample of young, neurologically intact individuals and to evaluate the correlations between lesion load and changes in brain volume, diffusivity, and functional connectivity.

Methods

The study group included 60 neurologically asymptomatic individuals (21 men and 39 women) who had originally been recruited as a control group in another study concerning patients with demyelinating disease. Their mean age was 34.5 years (SD = 8.3 years) and ranged from 21.4 to 62.1 years. The exclusion criteria, as verified by questionnaire, included history of symptoms suggestive of MS lasting longer than 24 h, such as unilateral visual disturbance, diplopia, vertigo, facial nerve palsy, paresis, or abnormalities of skin sensitivity. Excluded also were those with history or suspicion of meningoencephalitis, stroke or transitory ischemic attack, epilepsy, and systemic inflammatory disease (e.g. systemic lupus erythematosus, rheumatic arthritis, or vasculitis), as well as subjects with known blood relatives suffering from MS. All participants entering the study signed an informed consent and the study was approved by an institutional ethical committee.

MRI brain scan on a 1.5-T scanner (Philips Achieva) was performed on all subjects. The protocol comprised sequences for structural imaging and subsequent volumetric analyses (T2, FLAIR 3D, and T1 3D), as well as DTI and resting-state functional MRI (rs-fMRI) sequences. The imaging protocol is detailed in Table 1. DTI was acquired with b factor 0 and 1000 s/mm^2 using 32 directions of the magnetic gradient; rs-fMRI data were measured at rest and awake for 7 min and 39 s in all subjects. A map of mean signal-to-noise ratio for rs-fMRI data is shown in the electronic supplementary material.

FLAIR images were evaluated in all subjects by two radiologists having more than 12 and 5 years of experience with MRI diagnostics (MK and JS, respectively). They searched for WHMs to differentiate the group of lesion-free individuals from those with at least 1 lesion. The two raters disagreed in four cases. Final decisions were then made by group consensus while including also the other co-authors (AŠ, MM). Both readers also recorded the presence of lesions in different zones of the WM (periventricular, paraventricular, and juxtacortical).

Furthermore, WMHs were segmented manually by MK in 3D FLAIR image using ITK SNAP v.3.4 (http://www.itksnap.org/pmwiki.php). The volume of segmented lesions was established in ITK SNAP and the number of segmented lesions was counted automatically using SPM toolbox function (spm_bwlabel). The latter labels connected areas by analysis of spatial relationships among 18 neighbouring voxels. A cluster of voxels was considered as one lesion if the voxels had mutual contact with at least one side or edge.

WMHs were segmented in ten randomly selected subjects independently by a second reader (JS) using the same methodology. The inter-observer variability as to the number and

Table 1 Parameters of MR imaging protocol

Sequence	Orientation	TR (ms)	TE (ms)	Acquisition voxel size (mm)	Reconstruction voxel size (mm)
T2 TSE	Transverse	4851	110	0.9 × 1.12 × 5	0.9 × 0.9 × 6
FLAIR 3D	Sagittal	8000	275	1.2 × 1.2 × 1.4	0.7 × 0.625 × 0.625
T1 3D FFE	Transverse	25	4.1	0.9 × 0.9 × 1.6	0.84 × 0.84 × 0.80
DTI	Transverse	21,000	62	2 × 2 × 2	1.75 × 1.75 × 2
rs-fMRI	Transverse	3000	50	3.4 × 3.5 × 3.8	2.85 × 2.85 × 3.8

TSE turbo spin echo, *TR* repetition time, *TE* echo time, *FLAIR* fluid attenuation inversion recovery, *FFE* fast field echo, *DTI* diffusion tensor imaging, *rs-fMRI* resting-state functional MRI

volume of WMHs between the two readers was evaluated by interclass correlation coefficient (ICC) analysis [13].

The number and volume of lesions were compared between males and females (Mann–Whitney *U* test) and correlated with the age of the subjects (Spearman's rank correlation coefficient).

The total brain volume and volumes of WM and grey matter were estimated with SienaX [14], which is part of FSL [15], using T1-weighted 3D images. The volumes were normalised using SienaX scaling factor to reduce head size–related variability between subjects. Pre-processing included also a lesion-filling procedure as described by Battaglini et al [16] to improve the accuracy of WM volume estimation. The number and volume of WMHs corrected for age and sex of the subjects were correlated with brain volumes using Spearman's rank correlation coefficient.

DTI data processing was done by FSL, starting with eddy current correction and calculation of maps of the following scalar parameters: fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD), and radial diffusivity (RD). The data were analysed by tract-based spatial statistics (TBSS) toolbox to find correlations of the scalar parameters with the number and volumes of lesions, while age and sex were set as further covariates. Furthermore, we registered FLAIR images to B0 images of DTI in individual subjects using linear registration (FSL toolbox FLIRT) and applied the transformation matrices to the segmentation masks of WMHs. We then registered atlas-based masks of WM (MNI 152 standard space) to T1 images in individual subjects using linear and non-linear registration (FSL toolboxes FLIRT and FNIRT) and subsequently registered the resulting WM masks to DTI space. Median values of the aforementioned diffusion parameters were calculated within the segmented lesions and normally appearing white matter (NAWM) in individual subjects and compared mutually using a paired *t* test. The median diffusion parameters measured within the entire NAWM were also correlated with the number and volume of WMHs using Spearman's rank correlation coefficient.

The rs-fMRI analysis was done using the MATLAB-based CONN toolbox [17]. One hundred twenty-eight regions of interest (ROIs) derived from the software's default brain parcellation atlas were used to extract rs-fMRI signals from various cortical and subcortical areas (average signals from 1-cm-diameter spheres centred at MNI coordinates described in the atlas). The signal denoising consisted in regressing out the movement parameters as well as the signals extracted from the cerebrospinal fluid and WM. This involved motion scrubbing and filtering using a 0.01–0.1-Hz band-pass filter. Using Pearson's correlation coefficient, 128 × 128 connectivity matrices were created among all the ROI signals. These matrices were used in subsequent analyses for calculating the main effect of the number and volume of WMHs on the connectivity values while correcting for age and sex of the subjects. FDR correction was used to control for the multiple comparisons problem, and thus, statistically significant connections correlating with the lesion load were identified.

Statistical analyses of rs-fMRI data and DTI TBSS analyses were performed using statistical modules of the aforementioned software tools. ICC estimates were calculated using R and irr software (https://CRAN.R-project.org/package=irr), and the remaining statistical tests were computed using Statistica 12 (StatSoft). The significance level for all statistical tests was set to $p < 0.05$.

Results

At least 1 lesion was found in 40 (67%) subjects, and median incidence in all subjects was 1 lesion (interquartile range [IQR] = 4.5) (Fig. 1). Separately in the group of subjects with WMHs, the median incidence was 3 lesions (IQR = 11.5). Results are detailed in Table 2.

At least 1 periventricular, paraventricular, or juxtacortical lesion was found in 11, 32, and 22 subjects, respectively, and in 5 (8.3%) subjects (3 women and 2 men of mean age 40.7 years), we observed simultaneous presence of at least 1 juxtacortical lesion and 1 periventricular lesion. Figure 2 presents a distribution map of the lesions.

ICC analysis of inter-observer variability of WMH segmentations found excellent reliability for the number of lesions (ICC, 0.985; 95% CI, 0.943–0.996), and moderate

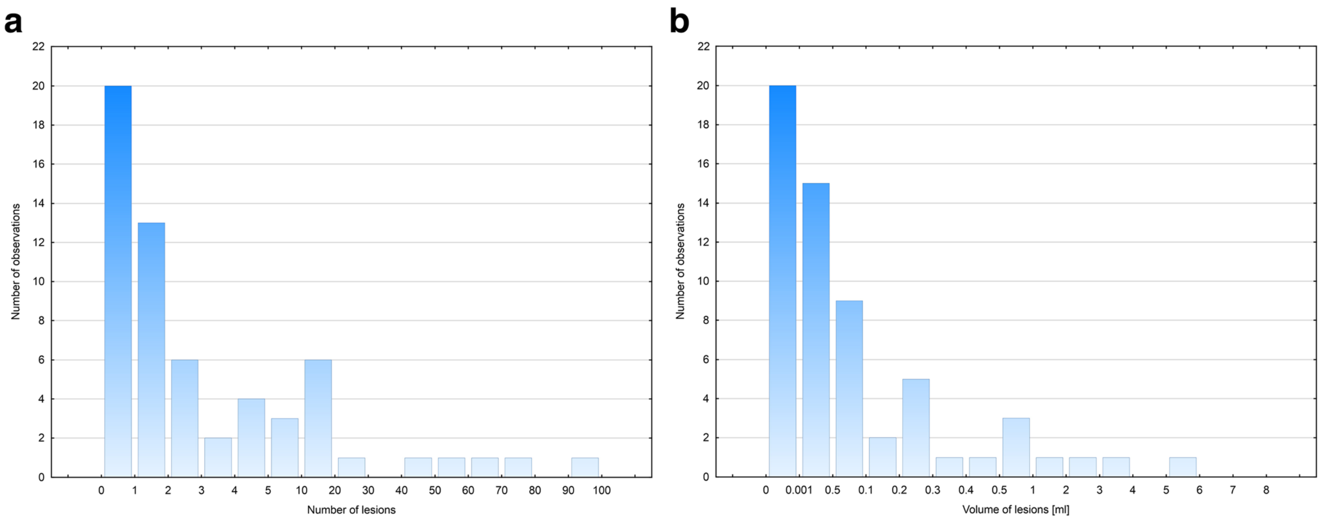

Fig. 1 Histograms demonstrating distribution of the number (**a**) and volume (**b**) of white matter hyperintensities in all subjects

reliability was observed concerning lesion volumes (ICC, 0.621; 59% CI, 0.087–0.887).

In the subgroup of subjects with WMHs, we revealed no statistically significant differences in the number or volume of the lesions or in the mean volume of 1 lesion between males and females. The parameters also did not correlate with the age of the subjects (Fig. 3).

Neither the number nor volume of WMHs correlated significantly with total brain volume or volumes of white and grey matter.

We also found no statistically significant correlation between lesion load and any of the DTI scalar parameters analysed by TBSS. There were no significant differences in diffusivity parameters between men and women; FA correlated negatively with the age of the subjects in extensive areas of WM (data shown in the electronic supplementary material).

Using analysis based upon region of interest, we found significantly higher MD values within WMHs (median 876.5×10^{-6}, IQR 143.5×10^{-6}) compared with all voxels of NAWM (median 745×10^{-6}, IQR 16.5×10^{-6}) by paired *t* test ($p < 0.0001$). The differences in other diffusion parameters (FA, AD, and RD) were not statistically significant (Fig. 4). Also, the median values of all measured diffusion parameters of NAWM were not significantly correlated with WMH load.

By analysis of functional connectivity within a matrix of 128 × 128 connections, we revealed significantly decreased functional connectivity between the cerebellar vermis and right cerebellar hemisphere with the right supplementary motor area in correlation with the number of lesions. Increasing volume of lesions correlated significantly with decreased connectivity between the regions of the right and left cerebellar hemispheres, between the left cerebellum and right caudate nucleus, and between the left postcentral gyrus and right paracingulate gyrus. The connectivity of the left frontal operculum with the right pallidum was significantly increased in correlation with WMH volume (Fig. 5).

Discussion

In this study, we quantified WMHs found in a randomly selected sample of a normal young population and investigated their impacts on various advanced modalities of MRI.

Table 2 Number and volumes of white matter hyperintensities (WMHs) in the group of 40 subjects with at least 1 lesion

Group	Number of WMHs	Volume of WMHs (mm^3)	Mean volume of one lesion (mm^3)
All ($n = 40$)	3 (11.5; 1–96)	86.82 (227.23; 13.12–5920.74)	24.02 (21.53; 10.97–168.98)
Females ($n = 25$)	3 (8; 1–64)	85.59 (225.31; 13.4–5920.74)	21.4 (22.22; 10.97–168.98)
Males ($n = 15$)	2 (20; 1–96)	88.87 (394.02; 13.12–3394.18)	27.69 (17.67; 13.12–44.43)
≤ 30 (F = 5; M = 6)	2 (19; 1–74)	202.89 (559.86; 13.39–2929.33)	39.59 (25.3; 13.4–168.98)
31–40 (F = 12; M = 4)	4 (8.25; 1–64)	77.11 (93.11; 13.12–1581.28)	16.41 (7.29; 10.97–44.43)
≥ 41 (F = 8; M = 5)	2 (6; 1–96)	73.28 (235.16; 18.04–5920.74)	31.72 (15.31; 15.7–109.64)

The values are presented as medians along with interquartile range, minimum, and maximum values. The last three rows demonstrate the distribution of lesions across different age groups

WMHs white matter hyperintensities, *F* females, *M* males

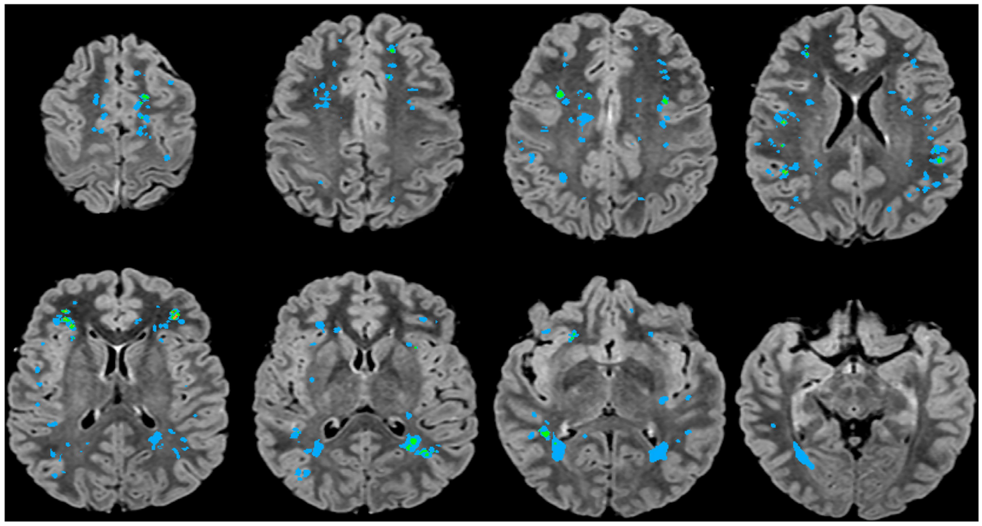

Fig. 2 Overlay of all co-registered segmentation masks of white matter hyperintensities (WMHs) over several axial images reformatted from 3D fluid attenuation inversion recovery. Different colours of the masks mark the areas with multiple occurrences of WMHs (blue = 1, green = 2, orange = 3, and red = 4 lesions)

We found relatively high incidence of WMHs, as at least 1 lesion was found in almost 67% of subjects investigated. Although WMHs constitute a commonly reported finding in asymptomatic elderly individuals [5, 6, 18], relatively fewer studies have focused upon this finding in younger populations. Generally lower prevalence is reported among the young. In a retrospective study, Katzmann et al report incidence of WMHs in only 0.5% of 1000 subjects with mean age 30.6 years (range 3–83 years) [2]. Other authors have found WMHs in 5.3% of healthy individuals with mean age 36.95 years (range 16–65 years) [4]. A comparatively higher number of WMHs was reported from a study by Wen et al that had focused upon an asymptomatic population aged 44–48 years. They had used automated segmentation and classification methods to evaluate the presence and location of WMHs, finding these in 50.9% of those subjects investigated [19].

The higher incidence of WMHs in our study may be due to our employing 3D FLAIR imaging sequence or generally by differences in imaging protocols between this study and the previous studies cited. Katzmann et al have not used a standardised imaging protocol evaluating T1- and T2-weighted images, they provide no details about the spatial resolution of the sequences, and proton density images were available only occasionally [2]. Similarly, Hopkins et al evaluated sagittal T1-weighted scans and T2-weighted axial scans obtained with slice thickness of 5-mm and 2-mm interspace gap [4]. Wen et al, who reported considerably higher prevalence of WMHs compared with both aforementioned studies, used FLAIR images acquired on a 1.5-T MR device with slice thickness of 4 mm and fairly high in-plane resolution of 0.898 × 0.898 mm [19]. On that account, we believe that the imaging protocol may strongly influence the sensitivity for the detection of WMHs, because especially the absence of FLAIR sequence might underestimate the number of periventricular and subcortical lesions. Most other recent studies dealing with WMHs in normal subjects or under various pathological conditions use conventional 2D T2 or FLAIR sequences with slice thickness ranging usually from 3 to 5 mm [19–23]. Inasmuch as there are a few studies reporting higher sensitivity of 3D FLAIR sequences for detecting WM lesions in

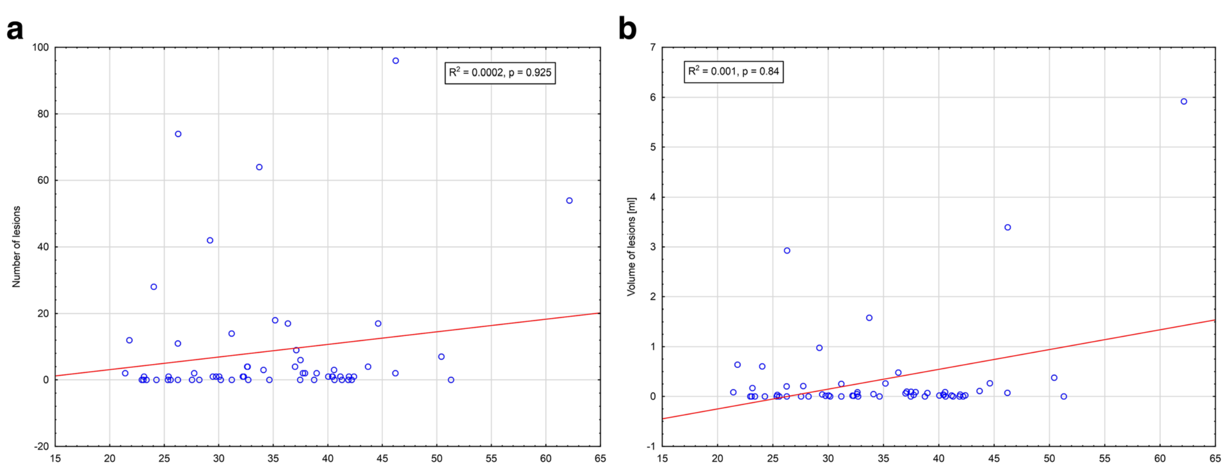

Fig. 3 Graphical representation of the number (**a**) and volume (**b**) of white matter hyperintensities as a function of age in all subjects

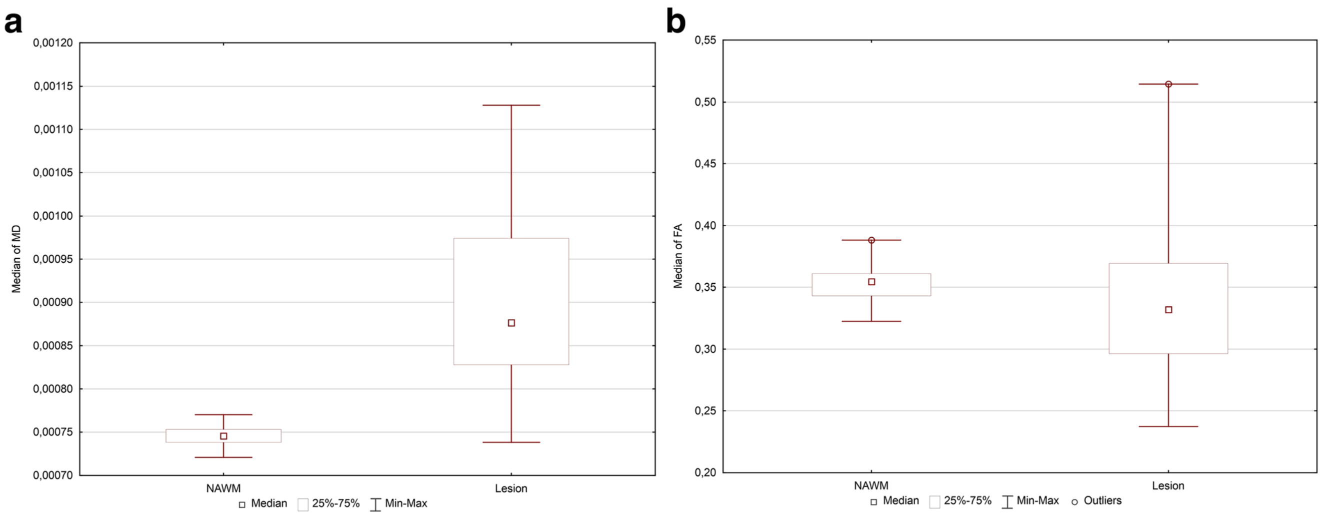

Fig. 4 Boxplot of mean diffusivity (**a**) and fractional anisotropy (**b**) values measured within the voxels of white matter hyperintensities and normally appearing white matter (NAWM). The differences in mean diffusivity values were statistically significant ($p < 0.0001$)

patients with MS [24–26] compared with conventional 2D techniques, we may analogously expect higher sensitivity also in the case of WMHs found incidentally in normal subjects. To our knowledge, however, no previous study has used 3D FLAIR technique in this field. Thus, a study directly comparing sensitivity of 2D and 3D FLAIR imaging for the detection of WMHs in healthy subjects would be beneficial.

Data about WMH volumes found in healthy populations are not uniform and are less available in the literature, because many authors evaluate only the prevalence of the lesions or

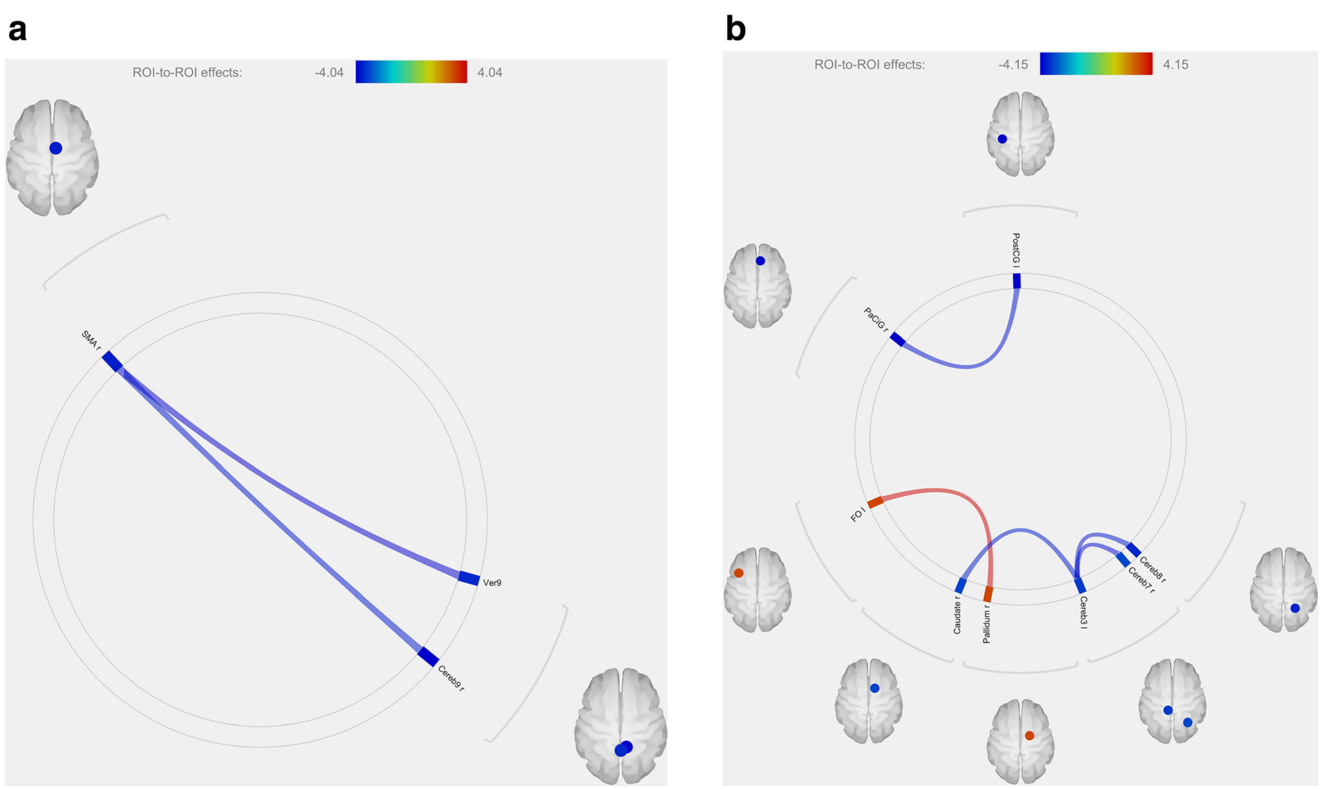

Fig. 5 Correlations of the number (**a**) and volume (**b**) of white matter hyperintensities with functional connectivity, corrected for the age and sex of the subjects. We found significantly decreased connectivity of the cerebellar vermis (Ver9) and right cerebellar hemisphere (Cereb9 r) with the right supplementary motor area (SMA r) in correlation with number of lesions. Increasing volume of lesions correlated significantly between the regions of the right and left cerebellar hemispheres (Cereb8 r, Cereb7 r, Cereb3 l), between the left cerebellum and right caudate nucleus (Caudate r), and between the left postcentral gyrus (PostCG l) and right paracingulate gyrus (PaCiG r). The connectivity of the left frontal operculum (FO l) with the right pallidum (Pallidum r) was significantly increased in correlation with WMH volume

use various semi-quantitative scaling systems. One of the studies roughly comparable with ours reports WMH volume of 547.12 mm^3 in a subgroup of subjects with at least 1 lesion [19]. We have found comparatively smaller mean lesion load (86.82 mm^3) in a corresponding group. One possible explanation for this may be higher sensitivity in our study for detecting and segmenting very small lesions, as that would generally increase the prevalence of WMHs while reducing the mean volume of WMHs in lesion-positive subjects. In fact, the segmented WMHs were mostly dot-like and with relatively small mean volume (23.02 mm^3). Even higher lesion load (4773 mm^3) was reported in another paper from Wen et al [27], but they had investigated a study group between 60 and 64 years of age, and thus these results are not really comparable with our sample. Similarly, some other studies using WMH volumometry deal with subjects of higher age compared with those in our study [28, 29]. Our data indicate that WMHs can be found commonly in younger age categories but that both their numbers and mean total volume are low in most subjects.

In contrast to previous studies [5, 30], our study found no significant correlation for the number or volume of WMHs with age. Habes et al had proven significant correlation of WMH volume with age on a large cohort of volunteers, but their mean age was higher (55.6 years) compared with our study and the authors had observed that WMH volumes larger than 2000 mm^3 started to appear after the fifth decade of life [31]. The lower age of our study participants, who were mostly under 40 years of age, may be considered as a possible explanation for our results in view of the fact that ischaemic changes may not yet play a significant role within this age group. Also playing a certain role may be the thresholding performed by Habes et al, who segmented only lesions larger than 25 mm^3 [31]. If WMHs in young subjects would tend to be smaller compared with larger ischaemic lesions in elderly subjects, then they could be underestimated when considering volume only above the given threshold. Although we have not proven significant correlation between mean volume of 1 lesion with age in our study group, this factor might become important in studies encompassing a wider age distribution of the subjects. For this reason, and considering that even the small lesions were quite clearly distinguishable in 3D FLAIR images, which was verified by double reading, we have chosen not to threshold the segmentations by volume.

The lesions were distributed within all zones of the brain's WM. Although the subjects were free of symptoms suggestive of MS, we did record in 5 subjects simultaneous presence of juxtacortical and periventricular lesions. This finding would fulfil the McDonald criteria for dissemination in space and evokes the question of radiologically isolated syndrome, which was first introduced in 2009 to define a relevant cohort of individuals routinely encountered in clinical practice who are at risk for future demyelinating events [32]. Although data concerning the population-based incidence and prevalence of radiologically isolated syndrome are scarce, that data available does suggest this to be uncommon. Forslin et al indicate in their population-based study a cumulative incidence of 0.1% [33]. Our study group is not large enough to draw definitive conclusions about incidence of radiologically isolated syndrome, but our results do suggest that the measured incidence may be higher than reported previously if high-resolution FLAIR imaging technique is employed.

There is some data in the literature pointing to alteration in diffusivity of the brain's WM in association with WMHs. We measured significantly higher MD values within WMHs compared with NAWM, which finding was similar to that from the study of Meninenga et al [34]. This may be given by structural disintegration of the WM tissue within the lesions in correlation with prior neuropathological findings of neuronal loss, demyelination, or gliosis [35], which result in enlargement of the extracellular water component and increase in isotropic diffusivity of water molecules. The aforementioned study also proved the diffusivity changes within NAWM in correlation with increasing WMH score [34]. In contrast, we revealed no significant association between the diffusion scalar parameters of NAWM and lesion load. A possible explanation for this discrepancy is the lower age of the subjects in our study group, in whom the WM structural changes seem to be limited only to the WMHs themselves rather than to be associated with widespread changes within NAWM. This fact may imply also a different pathophysiological background for these lesions relative to those in elderly individuals. The age of the subjects was the only significant determinant of FA values, and these were negatively correlated with age in many WM areas. This is consistent with previous studies dealing with diffusivity changes in normal ageing [36].

The lesion load in our study group did not significantly influence the volume of brain white or grey matter. This finding may correspond to the DTI analyses, which do not indicate structural changes within NAWM and, considering the fact that the volume of WHMs in most of the subjects was relatively low, the lesions were not able to influence global brain volumes. On the other hand, we may expect atrophic changes of the brain to be correlated with WMH load in older individuals, as some authors have proven significant reduction of grey matter to be linked with increasing lesion load in subjects 60 years of age and older [27, 37].

Given the significant alterations in functional connectivity of the brain, and especially significantly reduced functional connectivity between the cerebellum and several supratentorial regions, the presence of WMHs may affect the function of the brain's neural networks. Because the lesions in all subjects were located within the WM of the brain hemispheres, we might assume disruption of the supratentorial segments of the neural loops connecting the cerebellum with supratentorial structures.

The cerebellum has long been known to be involved in the motor control system [38], but there are several reports about its relationship to such other specific functions as cognitive processing or executive functions [39, 40]. Inasmuch as we have no functional clinical data, we may only speculate about the functional impacts of these findings. One possible impact could be modest deficit in motor functions. Sachdev et al proved an association between WMH volumes and poorer motor functions [41], and this may generally concur with our findings of decreased connectivity of the cerebellum. Only by further studies using specific neuropsychological testing, however, could these hypotheses be confirmed.

Only very sparse data is available in the literature regarding the impact of WMHs on brain connectivity as measured by rs-fMRI, and such data as are available are not fully comparable with ours. Shi et al proved a significant correlation between regional WMH volumes and intrinsic connectivity contrast maps in various regions of the brain, including the left cuneus, right superior occipital cortex, right superior corona radiate, and left superior occipital cortex [42]. A study by De Marco et al [43] using independent component analysis of rs-fMRI data demonstrates correlations between WMH load within the anterior default mode network (namely the left medial temporal lobe) and the salience network (right parietal cortex). Those findings do not anatomically correspond to the changes in connectivity found in our study, but it is also the case that those authors used a different methodology in their data analysis and studied an older population (mean age 61.98 years). Further studies are needed to confirm these findings and to stratify the functional connectivity changes in different age groups.

A question may arise regarding the approach to statistical correlation of lesion load with the modalities investigated. Alternatively, a comparison might be made between lesion-free subjects and those having at least one lesion. We do not consider such approach to be appropriate, however, due to the relatively large proportion of subjects having just one or two lesions that are not likely to cause significant changes in brain volume or in structural or functional connectivity. On the other hand, we are not able to establish a hypothesis for an alternative threshold to distinguish between "normal" and "abnormal" subjects from the perspective of those advanced MRI modalities. For this reason, we have chosen to correlate the number and volume of WMHs as continuous variables.

In addition to the aforementioned lack of specific clinical data, this study has some other limitations. One of them may be seen in the use of a 1.5-T MR device, considering that 3 T is widely used today in neuroimaging studies. Higher magnetic field strength could provide better signal-to-noise ratio and spatial resolution, thereby leading to more precise segmentations as well as even more robust results in terms of correlations with advanced modalities, especially rs-fMRI. Another limitation is the relatively small number of subjects, in which light this paper should not be regarded as an epidemiological study designed to reveal WMH prevalence. Our results nevertheless do suggest that the incidental finding of WMHs may be greater than previously reported when 3D FLAIR sequence is used. This fact may be especially important when evaluating examinations in patients suspected of having MS, for whom the 3D FLAIR imaging techniques are increasingly used. The topic thus deserves to be revisited from this perspective and analysed on larger study groups. Furthermore, although the exclusion criteria were set to filter out mainly those subjects with demyelinating or inflammatory CNS disease, we have no data about other comorbidities like diabetes, hypertension, or migraine that might also influence the load of WMHs. Thus, we are not able to discriminate the possible effect of these factors, and the subjects included in the study should be considered merely "neurologically intact" rather than completely "healthy". We believe, however, that in a vast majority of neuroimaging studies using cohorts of "healthy" subjects, the internal comorbidities are not set as exclusion criteria. Thus, one of the benefits of this study may be seen in its investigating impact of WMHs on the aforementioned MRI modalities in a population sample similar to those that are likely to be used in future neuroimaging studies comparing control subjects with patients.

In this regard, the question may arise as to whether the presence of incidentally found WMHs in cohorts of control subjects should be reflected in neuroimaging studies. According to our results, this is not the case for the global volumes of the brain, where we found no significant correlations with lesion load. Similarly, the impact of WMHs on the brain diffusivity seems to be relatively minor. Inasmuch as we have revealed significantly higher mean diffusivity within the lesions themselves compared with NAWM, some influence may become prominent in the case of ROI-based analyses in individual patients if ROI masks would be placed over the areas with high accumulation of WMHs. On the other hand, it seems that the lesion volume is too small compared with the overall WM volume to be able to affect global diffusivity changes, as revealed by our TBSS analysis. Thus, it appears that co-varying for the number or volume of WMHs in the studies using this methodology is not essential. Finally, according to our data, the load of WMHs seems significantly to affect functional connectivity of the brain, so the lesion load may be considered as a variable entering the rs-fMRI statistical analyses to eliminate this effect. Alternatively, those subjects with obviously high number of WMHs may be excluded. At this point, however, we are not able to differentiate whether the lesion load is really an independent predictor of connectivity changes or if the lesions are associated with, for example, cardiovascular risks or other internal comorbidities. In such a case, it would be advisable to reflect more carefully those factors so that they match within patient groups. Nevertheless, this hypothesis needs to be validated by further studies. To conclude, WMHs constitute a common finding even in young, neurologically asymptomatic adults. The analyses of multimodal MRI data revealed no impact of WMH load on the global brain volumes, and the changes of diffusivity seem to be limited to the lesions themselves rather than to the entire NAWM. The lesion load correlates significantly with changes in brain functional connectivity, and especially that of the cerebellum.

Acknowledgements This study was supported by grant project AZV-15-32133A of the Czech Health Research Council and by funds from the Faculty of Medicine MU to junior researcher (M. Keřkovský).

Funding This study has received funding by the Czech Health Research Council and by the Faculty of Medicine MU.

Compliance with ethical standards

Guarantor The scientific guarantor of this publication is Assoc. Prof. Marek Mechl, M.D., Ph.D., MBA.

Conflict of interest The authors of this manuscript declare no relationships with any companies whose products or services may be related to the subject matter of the article.

Statistics and biometry No complex statistical methods were necessary for this paper.

Informed consent Written informed consent was obtained from all subjects in this study.

Ethical approval Institutional Review Board approval was obtained.

Methodology
• Prospective
• Cross-sectional study
• Performed at one institution

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