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Research article

Evidence of axonal damage in cerebellar peduncles without T2-lesions in multiple sclerosis

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ABSTRACT

Background and aim: Cerebellar peduncles (CP) can be probed by diffusion tensor imaging (DTI) to evaluate the integrity of cerebellar afferent and efferent networks. Damage to the CP in multiple sclerosis (MS) could lead to serious cognitive and mobility impairment. The aim of this study was to investigate the extent and the clinical impact of CP damage in MS.
Methods: Sixty-eight MS patients were included in this study along with 27 healthy controls (HC) and underwent an MRI on a 1.5T including T1, T2, FLAIR and DTI. Using DTI, the microstructural integrity within the CP regions (superior (SCP), inferior (ICP) and middle (MCP)) was probed while controlling for focal T2-lesions presence or absence. A general linear model was performed to test for associations between clinical scores and DTI metrics for each CP.
Results: Significantly decreased fractional anisotropy (FA) and increased radial diffusivity (RD) were found in the CP of all MS patients compared to those of HC, but to a lesser extent in non-lesioned CP than those with lesions. Axial diffusivity (AD) was significantly and similarly increased in both non-lesioned and lesioned CP, but only in the SCP and ICP. Expanded disability status scale (EDSS) significantly correlated with MCP's FA ($p < 0.05$) and RD ($p < 0.05$), while MS functional composite (MSFC) significantly correlated with SCP's FA ($p < 0.01$) and RD ($p < 0.01$).
Conclusion: The diffusion changes (FA and RD) measured in lesioned CP are probably directly related to the presence of inflammatory and/or demyelinating lesions. In contrast, the microstructural alterations reflected by AD increase in non-lesioned CP may result either from remote effects of cerebral white matter injury (diaschisis) or primary axonal degeneration, that are associated with cognitive, sensory and motor impairments of MS patients.

Abbreviations: 9-HPT, 9-Hole peg test; AD, Axial diffusivity; CP, Cerebellar peduncle; CSF, Cerebro-spinal fluid; DD, Disease duration; DTI, Diffusion tensor imaging; EDSS, Expanded Disability Status Scale; FA, Fractional anisotropy; FSL, Functional magnetic resonance imaging of the brain software library; GM, Gray matter; HC, Healthy controls; ICBM, International consortium of brain mapping; ICP, Inferior cerebellar peduncle; MCP, Middle cerebellar peduncle; MD, Mean diffusivity; MRI, Magnetic resonance imaging; MS, Multiple sclerosis; MSFC, Multiple sclerosis functional composite; NAWM, Normal appearing white matter; PASAT, Paced auditory serial addition test; PPMS, Primary progressive multiple sclerosis; RD, Radial diffusivity; RRMS, Relapsing remitting multiple sclerosis; SCP, Superior cerebellar peduncle; SPMS, Secondary progressive multiple sclerosis; TBSS, Tract-based spatial statistics

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1. Introduction

Multiple sclerosis (MS) is characterized by recurrent formation of inflammatory demyelinating lesions that might show reactive astrogliosis, phagocytic activity, oligodendroglial loss, and axonal pathology. By allowing *in vivo* detection of focal lesions, MRI has become an essential tool for an early diagnosis of MS [1]. However, lesion-load estimation using conventional MRI often fails to correlate with patients' disease severity, a phenomenon known as "clinical/MRI dissociation" [2]. This observation could be partially explained by several factors, including lesion location, microscopic alterations in normal appearing white matter (NAWM) or degenerative processes involving gray matter (GM). In assessing subtle microstructural changes occurring in both white matter lesions and NAWM, diffusion tensor imaging (DTI) provides more sensitive measures of clinically-relevant brain abnormalities, such as fractional anisotropy (FA), axial (AD) and radial (RD) diffusivities [3].

MS patients consistently show decreased FA in lesions and NAWM [4], whereas subcortical GM injury is usually characterized by increased FA [5]. While most DTI studies reported abnormalities in NAWM or supratentorial white matter, only a few have specifically targeted infratentorial white matter. DTI studies of the cerebellum have shown clinically relevant abnormalities in MS [6,7] that were characterized by decreased FA and increased RD in the three CP sets of densely packed fibers – superior (SCP), middle (MCP) and inferior (ICP), and to a lesser extent increased AD in the MCP and the SCP. CP alterations were also associated with speed of walking and working memory performance of primary progressive MS (PPMS) patients [8].

DTI thereby constitutes a sensitive tool to evaluate the integrity of cerebellar networks that are involved in temporal and spatial integration of neural inputs from both descending cortical afferents (cortico-ponto-cerebellar projections) and ascending spinal afferents (proprioceptive information via spino-cerebellar projections) with regards to cognitive and mobility impairments [7]. Furthermore, these microstructural changes were associated with lower cerebellar white matter volume and motor deficits [6]. However, it remains unclear whether the observed diffusion abnormalities reflect only damage associated with focal lesions or they contribute *per se* to neurological deficits. We therefore propose in this study to first test the hypothesis that diffusion abnormalities are detectable in the CP's NAWM without focal T2-lesions, and second to analyze the T2-lesions's level of contribution in lesioned and non-lesioned CP, and to estimate their effect on clinical outcomes of MS patients.

2. Methods

2.1. Subjects

Twenty-four relapsing-remitting (RRMS), 26 secondary progressive (SPMS) and 18 PPMS patients with definite MS according to the McDonald's criteria [9] were included in this study. Clinical history was collected and neurological examination, including expanded disability status scale (EDSS – it quantifies and monitors the levels of disability in MS; it ranges from 0 to 10 in 0.5 unit increments that represent higher levels of disability), and MS functional composite (MSFC – it is a three-part, standardized, quantitative assessment in MS; it produces scores for each of the three individual measures as well as a composite score), was performed by a board-certified neurologist in all patients. MRI scans were acquired on the same day as the clinical assessment. Twenty-seven healthy volunteers with no history or signs of neurological disorders served as healthy controls (HC) (Table 1). Local ethics committee approval and written informed consent from all participants were obtained.

2.2. Image acquisition

All patients and HC underwent MR examination on a 1.5 T MRI (Sonata Siemens, Erlangen, Germany) with an 8-element phased-array head-coil. MRI protocol included a 3DT1-weighted MPRAGE sequence (TR/TE/TI = 1970/3.93/1100 ms; flip angle = 15°; matrix = 256 × 256; FOV = 256 x 256 mm; slice thickness = 1 mm; voxel size = 1 × 1 x 1 mm; acquisition time = 4.62 min), a TSE with proton density and T2-weighted contrasts (TR/TE1/TE2 = 3020/12/85 ms; flip angle = 180°; matrix = 192 × 256; FOV = 240-240 mm; slice thickness = 3 mm; voxel size = 0.9 × 0.9 × 3 mm; acquisition time = 4.55 min) and a FLAIR sequence (TR/TE/TI = 8000/105/2200 ms; flip-angle = 150°; matrix = 192 × 256; FOV = 240 × 240 mm; slice thickness = 3 mm; voxel size = 0.9 × 0.9 × 3 mm; acquisition time = 4.57 min).

DTI protocol was based on 2D multi-slice spin-echo echo-planar imaging sequence (TR/TE = 6900/86 ms, acquisition time = 7 min). Fifty-one contiguous, 2.5 mm thick, axial slices aligned to the AC/PC plane, were acquired. Twenty-four diffusion gradient directions ($b = 1000s/mm^2$) were applied. A nominal isotropic $2.5\,mm^3$ resolution was obtained using a matrix size of 96 × 96 over a FOV of 240 × 240 mm. The b0 image was acquired four times to increase signal-to-noise ratio, while other directions were acquired twice.

2.3. Data processing

Cerebellar peduncles were divided in two groups, based on the presence or absence of T2-lesions by a senior neuroradiologist (FC) on T2-weighted images. The presence of one lesion was sufficient to include the peduncle in the lesioned group.

DTI data were analyzed using the Functional Magnetic Resonance Imaging of the Brain (FMRIB) Software Library (FSL-v.5.0). Eddy current correction using FMRIB's Diffusion Toolbox (FDT) was first applied followed by a brain extraction step. FA, AD, and RD maps were then generated using FDT. Individual DTI maps were visually inspected for significant residual motion or other artifacts.

To extract DTI metrics from each peduncle, an atlas-based definition of each peduncle was combined with tract-based spatial statistics (TBSS) of DTI images. TBSS analysis is described elsewhere [10]. In summary, a mean common skeletal representation of fiber tracts was first generated by combining FA maps from all subjects. A non-linear registration aligning all FA images is therefore applied followed by an affine registration to align them to the MNI152 standard space. The mean of all FA images is then created and fed into the skeletonisation program to create a mean FA skeleton. This process was then applied to AD and RD in order to obtain a mean AD and a mean RD skeletons [10]. Finally, for each subject, the mean skeletal values for the different DTI metrics within each peduncle of interest were extracted (Fig. 1). To do so, the FMRIB's linear image registration tool was used to align and resample the FA template image of the International Consortium of Brain Mapping (ICBM) DTI-81 atlas to the TBSS FA template. The resulting affine transformation was then applied to the ICBM DTI-81 atlas label map using nearest-neighbor interpolation. This resulted in the identification of skeletal segments that fell within each ROI of the DTI-81 atlas (Fig. 1). Finally, the mean values of FA, AD, and RD for each ROI of the subjects CP were extracted. The ICBM DTI-81 atlas provided left and right masks of the SCP and the ICP that were merged to form one SCP and one ICP mask, and one mask for the whole MCP.

2.4. Statistical analysis

Statistical analysis was performed using R (R v.3.4.2). One-Way ANOVA with Bonferroni correction was applied to study the differences in the DTI metrics in a given peduncle between HC subjects and MS patients. General linear model was performed to test for associations of EDSS and MSFC scores with disease duration, age at disease onset, gender, and disease type. DTI metrics and the presence or absence of

Table 1
Patients and healthy control subjects' demographics.

	HC	RRMS	SPMS	PPMS
Number of patients	27	24	26	18
Patients' sex (M/F)	9/18	4/20	16/10	7/11
Mean age (years ± SD)	37.03 ± 9.67	34.84 ± 7.66	42.38 ± 4.23	41.38 ± 5.87
Mean age at onset (years ± SD)	–	27.78 ± 8.03	27.60 ± 7.59	34.92 ± 5.73
Mean DD (years ± SD)	–	7.06 ± 4.20	14.79 ± 5.83	6.45 ± 2.77
Median EDSS (min-max)	–	2.8 (0.0–4.5)	5.3 (4.0–7.0)	4.0 (2.5–6.0)
Median MSFC (min-max)	–	0.4 (−0.5–1.1)	−0.6 (−31.6–0.4)	−0.2 (−2.1–0.6)

HC: Healthy controls; RRMS: Relapsing-remitting MS patients; SPMS: Secondary progressive MS patients; PPMS: Primary progressive MS patients; DD: Disease Duration; EDSS: Expanded Disability Status Scale; MSFC: Multiple Sclerosis Functional Composite.

lesions for each CP were then added to the model to investigate the association between diffusion abnormalities and disability scores, controlling for demographics, disease type and duration, and presence of lesions within the CP. Additionally, to take into account the possible relation between the MS clinical course and the DTI metrics, an interaction term was added to the model. Similarly, a term accounting for the interaction between the presence of lesions in the region, the DTI metrics and the MS clinical course was added. A result was considered significant at a p-value lower than 0.05.

3. Results

The study participants were assessed in three different ways: 1) as a whole (All-MS), 2) in sub-groups according to MS clinical forms (RRMS, SPMS, and PPMS), and 3) stratified by the presence or absence of T2-lesions within individual peduncles (Table 2). T2-lesions were found in 51 out of 68 patients in the MCP. Thirteen patients had T2-lesions in the ICP. Six patients had bilateral lesions, while seven patients had T2-lesions either in the right (N = 3) or left (N = 4) ICP. Twenty-three patients had T2-lesions in the SCP. Nine patients had bilateral lesions, while fourteen patients had T2-lesions either in the right (N = 3) or left (N = 11) SCP.

3.1. Diffusion changes in MS patients as a whole and between MS clinical forms

When comparing HC and All-MS groups, FA was decreased, while AD and RD were significantly increased ($p < 0.001$) in all CP regions, except for AD in the MCP (Table 2).

When comparing HC to MS patients' sub-groups based on their clinical forms, FA was significantly decreased in progressive MS (SPMS and PPMS) in all three CP regions. AD was significantly increased only in SCP and ICP of RRMS and SPMS patients. RD was also significantly increased in SCP and ICP of all MS patients' sub-groups, but only in MCP of SPMS. When compared to RRMS, both progressive MS groups showed significantly lower FA ($p < 0.001$) and higher RD (SPMS ($p < 0.001$) and PPMS ($p < 0.01$)) in ICP. SPMS, but not PPMS patients, showed similar differences in MCP ($p < 0.01$). Compared to PPMS, SPMS showed significant higher AD values in ICP ($p < 0.01$) and increased RD values in MCP ($p < 0.05$) (Table 2).

3.2. Effect of T2-lesions on diffusion metrics

When comparing HC and All-MS groups, FA was significantly decreased, while AD and RD were significantly increased in both lesioned and non-lesioned SCP and ICP. FA and RD were also significantly

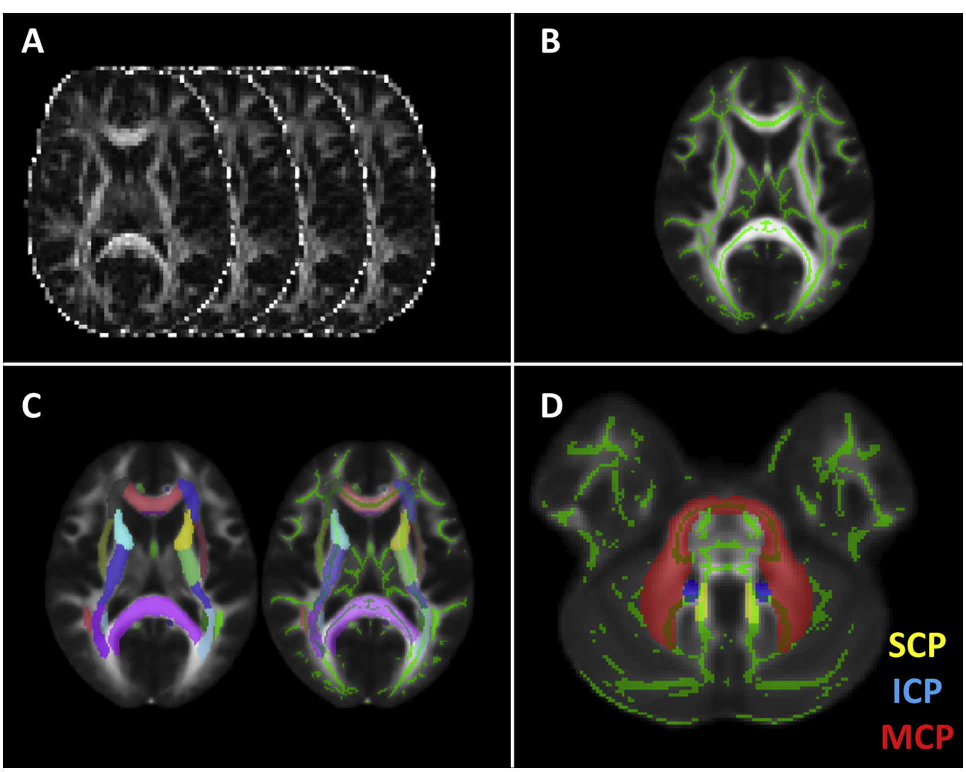

Fig. 1. A) FA maps created using the FDT tool of the FSL software. B) Tract-Based Spatial Analysis (TBSS) application (FSL) was applied to enhance reproducibility and ensure overlap of narrow white matter structures between subjects. TBSS defines a white matter skeleton from the DTI data by finding continuous tracts of local maxima. FA, AD and RD maps were then propagated on the skeleton. C) Registration of the ICBM-81-DTI atlas to the TBSS MNI152 FA template using an affine registration. D) The FA, AD and RD values of the SCP (yellow), ICP (blue) and MCP (red) were obtained for each patient and control subjects using the ICBM CP masks and the white matter skeleton. Both masks were merged to select only the skeleton white matter voxels within the three peduncles ROIs.

Table 2
DTI metrics of the cerebellar peduncles of HC compared to those of MS patients as a whole, as well as divided according to the presence of T2 lesions or to the disease clinical phenotype.

ROI		HC	All MS	Non-lesioned	Lesioned	RRMS	SPMS	PPMS
N		27 SCP 27 ICP 27 MCP	68 SCP 68 ICP 68 MCP	45 SCP 55 ICP 17 MCP	23 SCP 13 ICP 51 MCP	24 SCP 24 ICP 24 MCP	26 SCP 26 ICP 26 MCP	18 SCP 18 ICP 18 MCP
FA	**SCP**	0.509 ± 0.024	0.486 ± 0.037***	0.492 ± 0.040**	0.474 ± 0.028***+	0.495 ± 0.023	0.484 ± 0.048**	0.476 ± 0.033***
	ICP	0.441 ± 0.024	0.400 ± 0.040***	0.403 ± 0.039***	0.386 ± 0.044***	0.423 ± 0.018	0.389 ± 0.046***	0.385 ± 0.042***
	MCP	0.481 ± 0.012	0.447 ± 0.035***	0.471 ± 0.019	0.439 ± 0.035***+++	0.462 ± 0.018	0.432 ± 0.039***	0.449 ± 0.038**
AD	**SCP**	1.267 ± 0.042	1.298 ± 0.053***	1.293 ± 0.051*	1.306 ± 0.055**	1.303 ± 0.051**	1.306 ± 0.051***	1.277 ± 0.055
	ICP	1.127 ± 0.036	1.152 ± 0.047***	1.150 ± 0.048*	1.159 ± 0.039*	1.153 ± 0.036*	1.166 ± 0.050***	1.130 ± 0.047
	MCP	1.065 ± 0.034	1.083 ± 0.047	1.076 ± 0.041	1.085 ± 0.049	1.087 ± 0.038	1.094 ± 0.055	1.063 ± 0.042
RD	**SCP**	0.544 ± 0.031	0.591 ± 0.056***	0.582 ± 0.062***	0.609 ± 0.036***++	0.580 ± 0.033**	0.598 ± 0.076***	0.595 ± 0.045***
	ICP	0.553 ± 0.017	0.614 ± 0.057***	0.609 ± 0.058***	0.637 ± 0.052***+	0.585 ± 0.024**	0.636 ± 0.076***	0.621 ± 0.043***
	MCP	0.483 ± 0.013	0.528 ± 0.051***	0.499 ± 0.018	0.538 ± 0.054***++	0.512 ± 0.019	0.551 ± 0.065***	0.516 ± 0.046

Results are presented as Mean ± SD. HC: Healthy controls; FA: Fractional Anisotropy; AD: Axial Diffusivity; RD: Radial Diffusivity. (*p < 0.05; **p < 0.01; ***p < 0.001 when comparing DTI values between MS patients and HC; +p < 0.05; ++p < 0.01; +++p < 0.001 when comparing lesioned to non-lesioned CP).

altered only in the lesioned MCP, whereas AD was not significant in neither lesioned nor non-lesioned MCP.

When comparing lesioned to non-lesioned CP, FA was significantly decreased in the SCP and the MCP, whereas RD was significantly increased in all CP regions (Table 2).

3.3. Correlation between diffusion metrics and clinical data

EDSS was significantly lower in RRMS patients compared to PPMS and SPMS patients ($p < 0.001$) while MSFC was only significantly different between the two progressive clinical forms (SPMS vs. PPMS; $p < 0.01$). The linear model analysis showed significant correlation between EDSS and disease duration (correlation slope = 0.1175; $p < 0.01$), and age at disease onset (correlation slope = 0.0529; $p < 0.05$). When adding diffusion metrics to the model, EDSS was significantly correlated with FA of the MCP (correlation slope = -10.5482; $p < 0.05$) and RD (correlation slope = 8.2794; $p < 0.05$), while MSFC was significantly correlated with FA (correlation slope = 65.1126; $p < 0.01$) and RD (correlation slope = -49.3980; $p < 0.01$) of the SCP (Supplementary Fig. 1). When comparing the correlation slopes of MSFC with FA, AD and RD in the MCP (FA: $p < 0.05$; AD: $p < 0.001$, RD: $p < 0.05$) and the ICP (FA: $p < 0.05$; AD: $p < 0.01$), the association within the SPMS group was significantly more pronounced than the PPMS group. A significant MSFC interaction with AD was also found in the MCP when comparing SPMS to RRMS ($p < 0.001$).

4. Discussion

Our study showed significant diffusivity changes in all CP regions, regardless of the presence or absence of T2-lesions, but to a lesser extent in non-lesioned compared to lesioned CP. While diffusivity changes in lesioned CP could be attributed to the lesions' effect on diffusion, the observed diffusion abnormalities in non-lesioned CP may reflect either local white matter microstructural damage or antero/retro-grade degeneration from brain lesions at a distance. Our findings of reduced FA and increased AD and RD in all CP regions is in line with previous results reporting widespread diffusion abnormalities across the cerebellum [3], regardless of the presence of focal T2-lesions.

To our knowledge, our findings are the first to demonstrate significant AD increases only in ICP and SCP (not in the MCP) of MS patients. Interestingly, when comparing lesioned and non-lesioned CP, both groups showed a significant increase in AD compared to the CP of HC. In contrast to FA and RD, AD did not show significant changes between CP with and without T2-lesions. This finding may result from reduced sensitivity of this metric to inflammatory processes related to the presence of T2-lesions and may reveal a more severe axonal degeneration occurring with or without inflammatory and/or demyelinating lesions.

In the presence of white matter lesions, the "outer unit" of the neuron, i.e. oligodendrocyte and/or myelin membranes, is the first to be damaged as a result of demyelination processes, whereas the "inside unit", i.e. axon, becomes vulnerable and degenerates with time [11]. In contrast, in the absence of focal lesions, diffusivity alterations in patients result either from local axonal degeneration or distant cerebral white matter lesions or cerebellar GM lesions occurring along afferent/efferent pathways, leading to antero/retro-grade neurodegeneration [5]. Indeed, GM lesions are frequent in the cerebellum and account for a greater area of affected cerebellum than do white matter lesions [12]. Axonal injury may therefore precede demyelination in MS, in which case neurodegeneration may be caused by specific insults to axons or neurons. Such a "direct attack" may induce axonal retraction, and thus empty myelin sheaths, which then degenerate secondarily [13]. This notion of cerebellar abnormalities was also attributed to remote effects of demyelinating lesions, i.e crossed cerebellar diaschisis [14]. This mechanism consists of an interruption of cerebro-ponto-cerebellar pathways that causes de-afferentation and transneural metabolic depression of the contralateral cerebellar hemisphere [15].

AD changes are considered to reflect axonal degeneration rather than demyelination and/or inflammation [16]. However, AD directionality change is inconsistent throughout different studies. While MS animal models studies reported decreased AD [17], previous studies in humans reported increases in AD. The discrepancies between human and animal studies may be due to the fact that the latter might not have taken into account all pathological processes taking place in MS [18]. AD increase was noted in several regions of various neurological diseases such as the SCP and the dentate nuclei of Friedreich's Ataxia patients [19], or the corpus callosum of Huntington's disease patients. The latter was associated with faster molecular movement parallel to the axons due to decreased axonal calibre that caused an increase in extra-axonal space [20]. Some studies attributed this diffusivity increase to changes in axonal attenuation and packing, axonal diameter [21], or axonal loss [22]. Others have attempted to explain it by various biophysical phenomena, including atrophy of white matter fibers contributing to an extracellular water content increase, flux associated with cytoskeletal content accumulation or breakdown, axonal water content alterations, as well as glial alterations [23]. However, the various hypotheses proposed above, have been challenged by Wheeler-Kingshott and colleagues [24]. Indeed, AD direction may not be preserved in pathological tissues and is not always aligned with the underlying tissue structures [25]. Furthermore, AD, is affected by noise, pathology and shape of calculated diffusion ellipsoid. Consequently, it is difficult to interpret changes in eigenvalues of diffusivities in the context of axonal loss or demyelination, especially when compared to control subjects [24].

Our study also identified significant diffusion changes in MS patients' cerebellar peduncles according to their clinical forms. Indeed, FA was decreased, while AD and RD increased in SPMS and PPMS compared to HC. These results are in accordance with previous studies that have demonstrated damages within the cerebellum of MS patients [26–28]. However, all of these studies performed a global brain analysis and did not target the cerebellum directly. To our knowledge, only two DTI studies focused on cerebellar peduncles in MS patients [6,7]. These studies reported fiber coherence reduction in the main cerebellar connections and linked cerebellar white matter damage to motor deficits in PPMS.

While all three regions of non-lesioned CP presented significant alterations, no FA changes were observed in the CP of RRMS (Table 2). As previously mentioned, these alterations found in non-lesioned CP result probably from axonal degeneration and/or distant lesions acting remotely. However, this result could be biased by the fact that non-lesioned CP include all three clinical forms. Indeed, SPMS and PPMS are clinically more advanced than RRMS (as demonstrated by the clinical scores in Table 1), and hence, have more lesions that could induce distant effects to CP regions [29]. The lack of FA differences in RRMS patients could genuinely reflect the non-occurrence of cerebellar damage when compared with HC subjects.

When comparing lesioned and non-lesioned CP to those of HC subjects and when comparing the CP of MS patients with different clinical forms to those of HC subjects, no significant differences were observed for AD values in MCP, whereas FA and RD were significantly altered. This negative result could be related to MCP size. Indeed, the lesions effect in MCP could be diluted due to the large size of MCP in comparison with ICP and SCP. Lesions could alter AD but not sufficiently to become significant. In order to better characterize these changes in future studies, histogram or voxel-based analysis may be performed.

By showing damage to the CP, our work supports the notion that cerebellar connections, which contribute to the control of numerous brain functions such as associative, limbic, memory, sensory and motor functions [30] are altered in MS [31]. FA decrease and RD increase in all CP regions as well as AD increase in MCP and ICP, were significantly correlated to EDSS and MSFC scores. Indeed, MCP is primarily responsible for regulating the body's movement, whereas SCP plays a major role in the coordination of ipsilateral arms and legs, and ICP is mainly concerned with integrating proprioceptive sensory input with motor vestibular functions such as balance and posture maintenance. Thus, any damage to these regions could result in a motor, sensory and cognitive dysfunction, thereby explaining the reported correlations.

The dichotomic separation of CP according to the presence or not of T2-lesions, especially for larger white matter tracts (e.g. MCP), could be considered as a limitation. Indeed, a small lesion could have a smaller role in influencing DTI measures in the whole tract compared to the smaller ICP and SCP tracts. We tried to overcome the potential partial volume effects technical issues in the computation of DTI indices of SCP and ICP by performing a TBSS analysis. SCP and ICP may entail substantial partial volume effects by including tissue compartments adjacent to the structures of interest, such as isotropic CSF (FA < 0.1) or low anisotropic GM (FA < 0.3). These effects could interfere and distort significantly DTI metrics values, and more particularly AD and RD. In our study, we aimed to overcome this problem by using the TBSS analysis technique coupled with the ICBM DTI atlas for a better reproducibility. In fact, before applying TBSS, we observed abnormal AD and RD that most likely reflected CSF contamination. Thus, ICBM masks and TBSS skeleton were merged to select only the skeleton's white matter voxels within the three peduncles ROIs, hence reducing potential contamination of adjacent voxels (Fig. 1). The use of ICBM DTI atlas coupled with TBSS was the result of the relatively low spatial resolution (due in part to the use of 1.5 T MR imaging) and the geometric distortions of DTI data due to EPI acquisition. This technique presented a high level of reproducibility demonstrated by the similar variations observed within the different ROIs. Further work using more advanced DTI methodology, such as high angular and high resolution acquisition sequences as well as high resolution post-processing techniques [32] could improve significantly the spatial resolution and resolve these limitations, particularly for the investigation of narrow ROIs of the cerebellar peduncles.

5. Conclusion

In conclusion, our findings confirmed previous reports of cerebellar changes in MS patients by showing diffusion abnormalities that reflect the effect of both local and distant pathology. Moreover, the AD abnormalities, reported here for the first time in both non-lesioned and lesioned CP of MS patients, seem particularly relevant, since they most likely reflect early axonal damage that may affect cognitive, sensory and motor functions as shown by the observed association with clinical and disability outcomes.

Author declaration

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property.

We further confirm that any aspect of the work covered in this manuscript has been conducted with the ethical approval of all relevant bodies and that such approvals are acknowledged within the manuscript.

We understand that the Corresponding Author is the sole contact for the Editorial process (including Editorial Manager and direct communications with the office). He is responsible for communicating with the other authors about progress, submissions of revisions and final approval of proofs.

Signed by all authors as follows:

Salem Hannoun, Gabriel Kocevar, Françoise Durand-Dubief, Claudio Stamile, Amal Naji, Francois Cotton, Michele Cavallari, Charles RG Guttmann, Dominique Sappey-Marinier

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Appendix A. Supplementary data

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