Alterations in sensorimotor and mesiotemporal cortices and diffuse white matter changes in primary progressive multiple sclerosis detected by adiabatic relaxometry

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Research

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Abstract

Background: The research of primary progressive multiple sclerosis (PPMS) has not been able to capitalize on recent progresses in advanced MRI protocols searching for disease-specific microstructural changes.

Methods: Conventional free precession T1 and T2, and rotating frame adiabatic T1ρ and T2ρ maps in combination with diffusion weighted parameters were acquired in 13 PPMS patients and 13 age and sex-matched controls.

Results: T1ρ, a marker of crucial relevance for PPMS due to its sensitivity to neuronal loss, revealed large-scale changes in mesiotemporal structures, sensorimotor cortex and cingulate, in combination with diffuse alterations in the white matter and cerebellum. T2ρ, particularly sensitive to local tissue background gradients and thus indicator of iron accumulation, concurred with similar topography of damage, but of lower extent. Moreover, these adiabatic protocols completely dwarfed the outcomes of both conventional T1 and T2 maps and diffusion tensor/kurtosis approaches – methods previously implicated in the MRI research of PPMS.

Conclusion: This study introduces adiabatic T1ρ and T2ρ as elegant markers confirming large-scale cortical grey matter, cerebellar and white matter alterations in PPMS invisible to other in vivo biomarkers.

Background

The recent years have seen a rapid evolution of advanced MRI imaging techniques in multiple sclerosis (MS) into viable surrogate biomarkers for various pathological processes associated with the disease, be it demyelination, inflammation or neurodegeneration. However, the research field seems to be dominated by studies focusing on relapse-remitting MS (RRMS), vastly overshadowing the primary progressive variant of MS (PPMS). True, large body of epidemiologic [1], imaging [2] and pathological studies [3] position PPMS into the opposite end of the same disease spectrum, but the differences in the dominant clinical phenotypes, clinical course [4] and ultimately therapeutic options [5] are far from subtle. Also the mechanisms responsible for the development of new focal lesions, a prominent sign in RRMS patients, might differ from the more insidious pathological processes involved in PPMS [4]. Although lesions are not infrequent in PPMS, the diffuse pathology of both white and grey matter (WM and GM, respectively) with neurodegeneration is more prominent [5]. The importance of GM pathology in PPMS, both in subcortical and cortical areas, has been repeatedly emphasized. Cortex suffers from demyelination, microglial activation, neuronal death, but is devoid of perivascular lymphocytic cuffs seen in white matter [6]. Indeed, cortical atrophy is prevalent in MS [7], and deep grey structures are not left unaffected in PPMS patients [8, 9]. Demyelinated axons, lacking structural and trophic support of myelin, seem to be more susceptible to chronic injury by inflammatory mediators, reactive oxygen species and iron compounds, with trans-synaptic degeneration due to distal axonal transection [10].

All the hypothesized mechanisms of neuronal damage, the clinical severity and tangible progression of the disease are in stark contrast with the paucity of MRI-detected activity in conventional clinical scans. These shortcomings call for the development of more advanced MRI protocols able to distinguish specific pathophysiological processes in PPMS patients. Magnetization transfer ratio (MTR) imaging has demonstrated sensitivity to "occult" WM damage in PPMS not visible to routine T1-weighted (T1w) and T2-weighted (T2w) MRI scans [11] and also to GM alterations correlating with clinical disability [12]. Furthermore, MTR may be a feasible marker of disease progression in PPMS, as lower baseline normally appearing WM (NAWM) values have been
reported to predict more adverse course of the disease [13]. Also diffusion weighted imaging (DWI) has been utilized in PPMS, showing differences between PPMS and healthy controls in various subcortical structures [14], diffusely abnormal white matter [15], with worsening over time [16]. Despite these advances, the complexity of these methods prevented further spread into the clinical practice and only a limited number of clinical trials utilized these MRI protocols as endpoints, achieving positive, but not very convincing results [17].

Facing the convoluted situation in PPMS MRI imaging, we have decided to capitalize on the technical developments in adiabatic rotating frame MRI relaxation protocols recently validated as methods receptive to both WM and GM damage in RRMS patients [18]. The sensitivity of adiabatic $T1\rho$ [19] and $T2\rho$ [20] to slow motional regimens detects a different water dynamics range, invisible to conventional protocols. To provide a more complete picture of relaxation metrics abilities in PPMS, we have added both conventional free precession T1 and T2 relaxation mapping protocols due to substantial sensitivity of these techniques in RRMS patients [21, 22]. The relationships of tissue microstructure and biochemistry with T1 and T2 relaxation time constants, which are particularly sensitive to dipolar fluctuations near the Larmor frequency in the MHz range, and $T1\rho$ and $T2\rho$, which provide information from spectral density in the kHz range, should in theory allow for more elaborate identification of eventual pathology.

The primary objective of the presented cross-sectional study was to compare the utility of the above listed relaxation metrics in PPMS in both GM and WM structures. To this end, high resolution T1w and T2w scans were utilized for GM/WM segmentation and construction of cortical maps and separate DWI scans were acquired to enable the reconstruction of relevant WM tracts further utilized as regions of interest (ROIs) for relaxometry analysis. Moreover, NAWM analysis utilizing relaxograms was performed to fully appreciate finer differences detectable by individual relaxation protocols in PPMS patients. The secondary, complementary objective of this study was to evaluate the yields of relaxation protocols against DWI metrics – repeatedly hypothesised as plausible candidates for PPMS monitoring.

**Methods**

**Subjects**

13 PPMS patients and 13 age and sex-matched healthy controls (HC) were enrolled into this study. The diagnosis of PPMS was based on the latest MAGNIMS criteria [23]. Relevant basic neurologic data (Expanded Disability Status Scale (EDSS)), including disease history were recorded, together with demographic data. The exclusion criteria were: presence of MRI contraindications, significant vascular or space occupying lesions in the MRI scans and comorbid neurological disorder other than PPMS. Every participant provided a written informed consent in accordance with the Declaration of Helsinki. The study protocol was approved by the ethics committee of the University Hospital of St. Anne.

**Imaging protocol and data analysis**

For the full imaging protocol, data analysis, and statistical approach, see the supplementary material.

Briefly, MRI acquisition was performed in a 3 T Siemens Prisma system. The imaging protocol consisted of T1-weighted (T1w), T2-weighted (T2w) high-resolution scans, conventional free precession T1, T2, adiabatic $T1\rho$ and adiabatic $T2\rho$ maps and DWI scans. The processing pipeline for structural T1w and T2w images and DWI was based on the human connectome project (HCP) minimal preprocessing pipeline with minor modifications.
Processed DWI data was used to calculate the standard diffusion tensor imaging (DTI) parameters [fractional anisotropy (FA), axial diffusivity (AD), radial diffusivity (RD) and mean diffusivity (MD) maps], mean kurtosis (MK) and to perform probabilistic tractography to reconstruct 3 main motor function related tracts – cerebellum-thalamo-cortical, cortico-spinal and cortico-striatal, separately for the left and the right side. Relaxation time constants for $T1^\rho$, $T2^\rho$ and $T2$ maps were calculated with custom routines utilizing 2-parameter non-linear fitting. $T1$ maps were available as the direct output of the utilized MP2RAGE sequence for $T1w$ acquisition. NAWM masks were created utilizing a hybrid semiautomatic approach where a $T2w$ intensity threshold was individually selected for each PPMS patient from the FreeSurfer-derived WM ROI in prescan-normalized $T2w$ image (see Fig. 1).

The group analysis was performed using separate approaches for GM and WM. In the WM analysis, relevant masks (6 tractography-derived masks, FreeSurfer-based whole WM, NAWM mask created as described above) were co-registered to the scans with lower resolution (i.e. $T2$, $T1^\rho$, $T2^\rho$, FA, AD, RD, MK) and thresholded to include only voxels with at least 0.9 probability of inclusion in the relevant ROI to limit partial volume effects. Furthermore, we constructed relaxograms (histograms of relaxation time constants) for whole WM in both PPMS and HC and for NAWM in PPMS. For GM analysis of all relevant parameters of interest ($T2$, $T1^\rho$, $T2^\rho$, FA, MD, MK), the cortical GM voxels in native space were mapped to cortical surfaces of each subject and resampled to the standard HCP greyordinate space. The MNI-warped subcortical GM volume images were then combined with cortical surface maps to create CIFTI files for further analysis. While the cross-subject alignment in deep cerebral regions is usually of reasonable precision, this approach benefits from crucial improvement of cortical area correspondence in inter-subject analyses compared to inconsistency-prone MNI coregistration of cerebral cortex due to high inter-individual variability in cortical folding patterns.

**Statistical analyses**

Two one-sided t-test (TOST) procedure was utilized to evaluate equivalence of sex and age between PPMS patients and HC, with 5-year and 33% difference considered significant and the significance level $\alpha$ of the test set at 0.05.

General linear models (GLMs) were used to compare PPMS and HC. Separate GLMs were constructed for the primary objective ($T1$, $T2$, $T1^\rho$, $T2^\rho$ maps) and the secondary objective (DWI parameters). For GM analysis, voxel/vertex-wise approach with CIFTI files was utilized and for WM analysis, median values of relevant ROIs (NAWM, whole WM in a separate model and 6 tracks in another separate model) were considered. Median was chosen as the measure of central tendency due to significant departures from normality in multiple metrics. Furthermore, 2 more GLMs for the analysis of kurtosis in NAWM and whole WM separately for relaxation and for DWI metrics was created. All the GLMs (6 in total) included sex and age as covariates of non-interest. And lastly, we performed a complementary analysis searching for any correlations between EDSS and relevant MRI metrics.

Permutation-based non-parametric analysis as implemented in the Permutation Analysis of Linear Models package [24] was utilized with non-parametric combination (NPC) approach across the individual modalities to perform joint inference. For CIFTI files (cortical and deep GM analysis), a type I error of 0.05 was implemented after family-wise error (FWE) voxel/vertex-wise correction, minimal cluster size of 25 voxels (subcortical) and 100 mm$^2$ (cortical). For ROI-based WM analysis, we considered the results statistically significant at the predetermined level of $p < 0.05$ with false discovery rate (FDR) correction over modalities and contrasts in each GLM model.
Results

TOST confirmed the equivalence of both age and sex distribution in PPMS and HC. Demographic information and basic clinical data are provided in the Table 1.

Table 1
Demographics and neurologic data of patients with primary progressive multiple sclerosis and healthy controls

|                      | PPMS (n = 13) | Healthy controls (n = 13) |
|----------------------|---------------|--------------------------|
| Sex (M/F)            | 6/7           | 7/6                      |
| Age (years)          | 60 [40–66]    | 58 [40–69]               |
| Neurologic data      | 46 [35–60]    | –                        |
| Age at the onset      | 11 [1–30]     | –                        |
| Disease duration     | 5.5 [3.5–7.5] | –                        |
| EDSS                 | –             | –                        |

The values are stated in the format median [range]. Abbreviations: PPMS – primary progressive multiple sclerosis; F – female; M – male; EDSS – Expanded Disability Status Scale

The NPC analysis of relaxometry CIFTI data revealed substantial differences between PPMS and HC in the cerebellum and bilateral mesiotemporal cortex (see Table 2, Fig. 2). These alterations were driven by increased relaxation times predominantly in T1\(\rho\) (diffuse changes in whole cerebellum, brainstem and in primary sensorimotor, premotor, cingulate and mesiotemporal cortical structures) and T2\(\rho\) (the posterior cerebellar lobe, right sensorimotor cortex and bilateral mesiotemporal cortices). T1 failed to detect any inter-group differences. T2 found only a smaller cluster in the area of left fusiform gyrus (not depicted in Fig. 2). DWI CIFTI analysis was far less fruitful, as only MK was able to detect changes in the right amygdala, hippocampus, and brainstem (see Table 2, not depicted in Fig. 2).

Table 2: Anatomical localization of 3D volume and 2D surface clusters with median [10\(^{\text{th}}\)–90\(^{\text{th}}\) percentile] MRI metric values over each cluster. 2 GLMs (separately for relaxation and DWI metrics) – permutation analysis with NPC joint inference across modalities. Analyses failing to provide significant results (relaxometry – T1 map; DWI – NPC, FA, MD) are not provided in the table. Clusters are significant at \(p < 0.05\) family-wise error voxel/vertex-wise corrected, cluster threshold of 25 contiguous voxels (subcortical) and 100 \(\text{mm}^2\) (cortical clusters). Only structures providing the highest overlap with individual clusters are listed in the table.

Abbreviations: GLM – general linear model; FWE – family-wise error; NPC – non-parametric combination; L – left; R – right; lob – cerebellar lobule
### Relaxometry

| Cl. no. | Structure                  | PPMS / HC Median [10th–90th percentile] | Volume (voxels) | – log p (FWE) |
|---------|----------------------------|----------------------------------------|----------------|--------------|
|         |                            |                                       |                |              |
| **T1ρ map [ms]** |                          |                                       |                |              |
| 1       | R lob VI, L lob VI        | 160 [139–181] / 154 [134–172]         | 7,146          | 3.11         |
| 2       | L lob IX, L lob VIIb      | 83 [79–91] / 80 [76–86]               | 3,321          | 2.20         |
| 3       | Vermis VIIa, R lob VIIb   | 79 [69–96] / 75 [60–87]               | 60             | 1.44         |

### T2ρ map [ms]

| Cl. no. | Structure                  | PPMS / HC Median [10th–90th percentile] | Volume (voxels) | – log p (FWE) |
|---------|----------------------------|----------------------------------------|----------------|--------------|
|         |                            |                                       |                |              |
| 1       | R lob VI, L lob VI        | 160 [139–181] / 154 [134–172]         | 7,146          | 3.11         |
| 2       | L lob VI, R lob VI        | 83 [79–91] / 80 [76–86]               | 3,321          | 2.20         |
| 3       | Brainstem                 | 79 [69–96] / 75 [60–87]               | 60             | 1.44         |

### DWI

**Mean kurtosis**

| Cl. no. | Structure                  | PPMS / HC Median [10th–90th percentile] | Volume (voxels) | – log p (FWE) |
|---------|----------------------------|----------------------------------------|----------------|--------------|
|         |                            |                                       |                |              |
| 1       | R amygdala                 | 0.65 [0.61–0.71] / 0.69 [0.64–0.76]    | 476            | 1.62         |
| 2       | R hippocampus              | 0.67 [0.61–0.75] / 0.71 [0.65–0.81]    | 188            | 1.47         |
| 3       | Brainstem                  | 0.86 [0.76–1.05] / 0.9 [0.8–1.1]       | 98             | 1.51         |

### DWI

**Mean kurtosis**

| Cl. no. | Structure                  | PPMS / HC Median [10th–90th percentile] | Volume (voxels) | – log p (FWE) |
|---------|----------------------------|----------------------------------------|----------------|--------------|
|         |                            |                                       |                |              |
| 1       | R amygdala                 | 0.65 [0.61–0.71] / 0.69 [0.64–0.76]    | 476            | 1.62         |
| 2       | R hippocampus              | 0.67 [0.61–0.75] / 0.71 [0.65–0.81]    | 188            | 1.47         |
| 3       | Brainstem                  | 0.86 [0.76–1.05] / 0.9 [0.8–1.1]       | 98             | 1.51         |

WM analysis detected statistically significantly higher relaxation time constants in PPMS in the whole WM ROI in all the relaxation metrics (see Table 3, Fig. 3), but not in NAWM. Compared to HC, PPMS patients had significantly lower kurtosis in NAWM (mesokurtic in PPMS and leptokurtic in HC), but no inter-group differences in kurtosis were found in whole WM (leptokurtic in both PPMS and HC). On the other hand, DWI metrics failed to show any differences between PPMS and HC, both in the whole WM and NAWM.
Table 3
MRI metrics in whole WM and NAWM; 4 GLMs (separately for relaxation/DWI metrics and for medians/kurtoses) – permutation analysis with NPC joint inference across modalities. Median [10th – 90th percentile] values over each ROI, with percentual differences between PPMS and HC, and non-excess kurtosis are provided, with FDR correction across modalities and contrasts in each GLM; significance level $\alpha$ at 0.05. Statistically significant results written in **bold** and marked with an asterisk.

| Cl. no. | Structure                        | PPMS / HC Median [10th – 90th percentile] | Volume (voxels) | $- \log p$ (FWE) |
|---------|----------------------------------|-------------------------------------------|-----------------|-----------------|
|         | relaxometry NPC                   |                                            |                 |                 |
| 1       | R lob VI, L lob VI               |                                            |                 |                 |
| 2       | L lob IX, L lob VIIb             |                                            |                 |                 |
| 3       | Vermis VIIIa, R lob VIIb         |                                            |                 |                 |
|         | T1ρ map [ms]                     |                                            |                 |                 |
| 1       | R lob VI, L lob VI               | 160 [139–181] / 154 [134–172]              | 7,146           | 3.11            |
| 2       | R lob VI, R lob VI               | 83 [79–91] / 80 [76–86]                    | 3,321           | 2.20            |
| 3       | Brainstem                        | 79 [69–96] / 75 [60–87]                    | 60              | 1.44            |
|         | DWI                              |                                            |                 |                 |
| 1       | R amygdala                       | 0.65 [0.61–0.71] / 0.69 [0.64–0.76]        | 476             | 1.62            |
| 2       | R hippocampus                    | 0.67 [0.61–0.75] / 0.71 [0.65–0.81]        | 188             | 1.47            |
| 3       | Brainstem                        | 0.86 [0.76–1.05] / 0.9 [0.8–1.1]           | 98              | 1.51            |
|         | Surface                           |                                            |                 |                 |
|         | relaxometry NPC                   |                                            |                 |                 |
| 1       | L fusiform, L lingual, L parahipp|                                            |                 |                 |
| 2       | R fusiform, R parahipp            |                                            |                 |                 |

Note that the analysis compared NAWM in PPMS patients to whole WM in HC, i.e. the “NAWM” values in HC correspond to respective whole WM values.

Abbreviations: WM – white matter; NAWM – normally appearing white matter; GLM – general linear model; PPMS – primary progressive multiple sclerosis; HC – healthy controls; FDR – false discovery rate; DWI – diffusion weighted imaging; NPC – non-parametric combination; FA – fractional anisotropy; AD – axial diffusivity; RD – radial diffusivity; MK – mean kurtosis.
| Cl. no. | Structure                          | PPMS / HC | Volume (voxels) | − log p (FWE) |
|--------|------------------------------------|-----------|----------------|---------------|
|        |                                    | Median [10th − 90th percentile] |                |               |
| 3      | L posterior cingulate              |           | 407            | 1.34          |

**T1p map [ms]**

|        |                          |           |                |               |
| 1      | L precentral, L superior frontal, L postcentral, L fusiform, L cingul. | 176 [158–207] / 168 [154–191] | 22,695 | 1.91 |
| 2      | R precentral, R superior frontal, R postcentral, R fusiform, R cingul.  | 174 [157–204] / 166 [152–189] | 17,717 | 1.97 |

**T2p map [ms]**

|        |                          |           |                |               |
| 1      | R precentral, R postcentral | 87 [75–110] / 81 [73–98] | 5,674 | 1.37 |
| 2      | R superior temporal, R middle temporal | 85 [75–103] / 81 [73–92] | 3,891 | 1.38 |
| 3      | R lateral occipital, R lingual | 80 [72–95] / 76 [70–86] | 3,654 | 1.55 |
| 4      | L lateral occipital | 79 [72–90] / 73 [69–82] | 1,097 | 1.42 |

**T2 map [ms]**

|        |                          |           |                |               |
| 1      | L fusiform | 104 [90–144] / 95 [87–111] | 885 | 1.36 |

Note that the analysis compared NAWM in PPMS patients to whole WM in HC, i.e. the “NAWM” values in HC correspond to respective whole WM values.

In the analysed motor tracts (see Table 4; for complete analyses, see Supplementary table 1), relaxation metrics were again able to detect significant differences between PPMS and HC, with clear dominance of T1p and T2p (both significant for all the 6 considered tracts). Significant inter-group differences in T1 relaxation time constants were found only in the left side tracts. For T2 maps, the inter-group differences reached the predetermined significance threshold only for the left cortico-spinal, left cortico-striatal and right cortico-thalamo-cerebellar tract. In the other tractography-derived ROIs, the inter-group comparison of T2 relaxation time constants fell short of surviving the multiple comparison correction. The analysis of DWI metrics did not yield any statistically significant results.

Abbreviations: WM – white matter; NAWM – normally appearing white matter; GLM – general linear model; PPMS – primary progressive multiple sclerosis; HC – healthy controls; FDR – false discovery rate; DWI – diffusion weighted imaging; NPC – non-parametric combination; FA – fractional anisotropy; AD – axial diffusivity; RD – radial diffusivity; MK – mean kurtosis.
Table 4

Differences between PPMS and HC in the medians of individual relaxation and DWI metrics over predetermined track masks (cortico-spinal, cortico-striatal, cortico-thalmo-cerebellar tract, each separately for the left and right hemisphere). 2 GLMs (separately for relaxation/DWI metrics) – permutation analysis with NPC joint inference across modalities. The table provides -log(p) values of relevant tests with FDR correction across modalities and contrasts in each GLM. The statistically significant cells (-log(p) > 1.30) highlighted bold. Only the dominant direction contrast presented, i.e. PPMS > HC for all relaxometry parameters, NPC, AD and RD in DWI parameters; and HC > PPMS for FA and MK. For full table including medians and ranges, see the Supplementary table 1.

| ROI     | Metrics | PPMS / HC | % Δ  | - log p (FDR) median | PPMS/HC kurtosis | - log p (FDR) kurtosis |
|---------|---------|-----------|------|----------------------|------------------|------------------------|
|         |         |           |      |                      | PPMS > HC       | HC > PPMS             | PPMS > HC       | HC > PPMS             |
| Relaxometry | whole WM | NPC | – | – | 2.25* | 0.00 | – | 0.17 | 0.17 |
|          |         | T1 [ms] | 894 [857–930] / 868 [832–908] | 3.0% | 1.58* | 0.00 | 5.11/5.26 | 0.21 | 0.00 |
|          |         | T1p [ms] | 142 [138–148] / 136 [132–144] | 4.3% | 1.97* | 0.00 | 2.98/2.91 | 0.21 | 0.00 |
|          |         | T2 [ms] | 88 [85–93] / 85 [81–89] | 4.6% | 1.97* | 0.00 | 5.42/6.02 | 0.00 | 0.14 |
|          |         | T2p [ms] | 76 [73–79] / 72 [71–77] | 4.7% | 1.97* | 0.00 | 3.65/4.21 | 0.00 | 0.24 |
| NAWM    | NPC | – | – | 0.19 | 0.00 | – | 0.00 | 3.10* |
|          | T1 [ms] | 873 [837–897] / 868 [832–908] | 0.6% | 0.19 | 0.00 | 2.87/5.26 | 0.00 | 2.97* |
|          | T1p [ms] | 138 [134–143] / 136 [132–144] | 1.2% | 0.25 | 0.00 | 2.08/2.91 | 0.00 | 2.97* |

Abbreviations: GLM – general linear model; PPMS – primary progressive multiple sclerosis; HC – healthy controls; FDR – false discovery rate; NPC – non-parametric combination; DWI – diffusion weighted imaging; FA – fractional anisotropy; AD – axial diffusivity; RD – radial diffusivity; MK – mean kurtosis.
|                |                | T2 [ms] | T2p [ms] |
|----------------|----------------|---------|----------|
|                |                | 87 [83– 91] / 85 [81–89] | 73 [70– 75] / 72 [71–77] |
|                |                | 2.8% 0.65 0.00 3.19/6.02 0.00 | 1.7% 0.13 0.00 2.68/4.21 0.00 |
|                |                | 2.97* | 2.49* |

### DWI

|                | whole WM       | NPC | – | – | 1.18 | 1.18 | – | 0.50 | 0.07 |
|----------------|----------------|-----|----|----|------|------|----|------|-----|
| FA             |                | 0.44 [0.42–0.48] / 0.46 [0.44–0.49] | -5.9% 0.00 1.25 1.84/1.83 0.14 0.00 |
| AD × 10^-3     |                | 1.06 [1.01–1.08] / 1.03 [0.99–1.08] | 2.7% 0.81 0.00 2.11/1.97 0.17 0.00 |
| RD × 10^-3     |                | 0.52 [0.47–0.54] / 0.49 [0.45–0.51] | 7.4% 1.25 0.00 2.96/2.65 0.01 0.17 |
| MK             |                | 0.88 [0.84–0.93] / 0.91 [0.87–0.96] | -3.1% 0.00 1.25 2.40/2.08 0.43 0.00 |

|                | NAWM           | NPC | – | – | 0.01 | 0.46 | – | 0.12 | 0.34 |
|----------------|----------------|-----|----|----|------|------|----|------|-----|
| FA             |                | 0.46 [0.45–0.48] / 0.46 [0.44–0.49] | -1.0% 0.01 0.06 1.83/1.83 0.24 0.01 |
| AD × 10^-3     |                | 1.02 [0.98–1.05] / 1.03 [0.99–1.08] | -0.6% 0.00 0.44 1.96/1.97 0.01 0.28 |

Abbreviations: GLM – general linear model; PPMS – primary progressive multiple sclerosis; HC – healthy controls; FDR – false discovery rate; NPC – non-parametric combination; DWI – diffusion weighted imaging; FA – fractional anisotropy; AD – axial diffusivity; RD – radial diffusivity; MK – mean kurtosis.
The complementary analysis correlating EDSS and individual MRI metrics failed to reveal any statistically significant correlations at the predetermined alpha.

**Discussion**

Despite the dramatic advances in MRI protocols in RRMS searching for disease-specific microstructural changes, the research in the field of PPMS seems to have been frustrated by the infrequency of the condition. The consequent limited extent of our knowledge about this disease combined with the bitter paucity of therapeutic options [5] clearly calls for targeted research specifically focused on PPMS. The presented study is the first one to evaluate the utility of four different MRI relaxation metrics in combination with DWI parameters in PPMS. T1ρ and T2ρ have proven to be exceptionally sensitive to the differences between PPMS and HC – both in cortical areas and in WM. T1ρ revealed large-scale changes in mesiotemporal structures, sensorimotor cortex and cingulate, in combination with substantial alterations in the white matter and cerebellum. T2ρ maps concurred, even though detecting differences of lower extent, but still mimicking the finding in the cerebellum, mesiotemporal structures and right-side sensorimotor and cingulate cortex.

Surprisingly, these adiabatic relaxation protocols completely dwarfed the results achieved using methods more established in the field of PPMS MRI research, be it T1 and T2 mapping or DTI metrics. Only diffusion kurtosis imaging, a recently developed technique expressing the degree of “non-Gaussianity” of water diffusion [25], found similar changes in the brainstem, but also in hippocampus and amygdala. FA and diffusivity measures (MD implemented for GM; AD and RD utilized for WM) failed to detect any significant differences in relevant ROIs, partly in contrast with previous reports based on higher numbers of subjects [16, 26]. Considering the high quality of DWI data utilized in this study with state-of-the art advanced processing, it is highly unlikely that the quality of data would cause the lack of significant findings. Indeed, our study found a 5.9% inter-group difference in FA in the whole WM ROI, but the combination of relatively low number of PPMS patients with the multiple comparison correction over several modalities and contrasts implemented in our study probably lead to FA falling just short of our predetermined significance level.

The conventional T1 and T2 maps underperformed when compared to the adiabatic T1ρ and T2ρ, too. T1 maps have been proposed as a viable indicator of NAWM affection in PPMS [27], based on measures of central
tendency and/or histogram shape analysis. In our study, MP2RAGE-derived T1 maps have yielded the least convincing outcomes out of the relaxometry analyses. Before condemning this metric, one should consider the method of calculation utilized by the protocol – MP2RAGE estimates T1 relaxation times and fits the relaxation curve based on 2 measured points only. Even though it provides expectable ranges in healthy brain tissue, the inferences on the precision of this method in pathologically altered conditions might be premature.

All in all, adiabatic relaxation protocols were clear winners, confirming their prime position among MRI biomarkers for MS previously established in RRMS [18, 28]. However, while relaxation metrics seem to be exquisitely sensitive to tissue alteration, they are notoriously non-specific, affected by a wide range of processes. Ergo, the results require careful interpretation. T1ρ has been previously associated with neuronal cellular density [29] – a notion truly intriguing when given into the context of the large-scale cortical T1ρ differences between PPMS and HC, since neuronal and axonal loss seems to be the pathological substrate of progressive disability [30] and the reduction of cortico-spinal tract axons, not the extent of demyelination, has been reported to correlate with motor disability [30, 31]. The topography of T1ρ differences affecting primary sensorimotor cortex, premotor cortex, cingulate and mesiotemporal structures points to wide-spread alterations with possibly dire clinical implications and interference with a large spectrum of functions. On the other hand, T2ρ has been reported to correlate with iron load [32], a trace metal implicated in neurodegeneration [33], oxidative injury leading to mitochondrial dysfunctions in both neurons and glia [34] and mechanisms crucial for the proper function of oligodendrocyte progenitors with possible therapeutic implications [35]. And last, but not least, the acquisition requirements for these methods are much lower than those of high-quality DWI sequences, opening a window for the implementation into the clinical practice.

However, several limitations need to be considered in the context of this study, with the first and most obvious one being the cross-sectional character. Since both the clinical course and the underlying pathophysiological processes in PPMS show certain inter-individual variability [4], the need for long-term follow-up studies able to properly assess the progression of the disease and sensitivity of individual methods is dire. Secondly, we used a relatively rough scale to measure clinical disability, as common in the routine clinical practice. The very character of the scale, with substantial emphasis on the ability to walk in the range above 4.0, makes it a problematic measure for correlation analyses, which presume continuous character of input variables. There is of course the possibility to use disease duration as a potential covariate in the utilized GLM, but this approach is far less informative about the progression of the disease and hence presumably the damage to the central nervous system than the clinical score, providing dubious inferences highly confounded by the age of the subject. And lastly, no formal testing of cognitive deterioration has been performed, which could definitely shed light on the nature and eventual clinical relevance of the substantial alterations in mesiotemporal cortex and cingulate. Nonetheless, it is exceedingly difficult to provide inferences about the deterioration of motor function and higher cognitive processes and their relation to MRI metrics based on cross-sectional studies due to substantial inter-individuality in the disease course. Hence, any plausible hypotheses on causal associations between the detected MRI changes and clinical functional measures should stand on longitudinal data as well.

**Conclusion**

Advanced MRI imaging techniques are a rapidly evolving field, slowly increasing their value as surrogate biomarkers for relevant pathophysiological processes in virtually all the diseases of the central nervous system. Although still requiring further validation in longitudinal studies with standardized descriptions of motor and
cognitive performance, T1ρ and T2ρ have been confirmed as elegant markers able to differentiate large-scale cortical GM, cerebellar and WM alterations. Their ability to detect neuronal loss and iron deposition might be of major importance and provide for suitable outcome measures for future clinical trials in PPMS.

**Abbreviations**

MRI – magnetic resonance imaging; PPMS – primary progressive multiple sclerosis; RRMS – relapse-remitting multiple sclerosis; GM – grey matter; WM – white matter; HC – healthy controls; NPC – non-parametric combination; FA – fractional anisotropy; MD – mean diffusivity; AD – axial diffusivity; RD – radial diffusivity; MK – mean kurtosis; MNI – Montreal Neurological Institute.

**Declarations**

**Ethics approval and consent to participate**

Every participant provided a written informed consent in accordance with the Declaration of Helsinki. The study protocol was approved by the ethics committee of the University Hospital of St. Anne.

**Consent for publication**

Not applicable.

**Availability of data and materials**

The raw data set of this study is available from the corresponding author upon reasonable request. The data are not publicly available due to their sensitive nature to protect the privacy of research participants.

**Competing interests**

None of the authors has any competing interest to disclose.

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**Authors' contributions**

Pavel Filip was responsible for the study design, analysis and interpretation of data and preparation of manuscript. Michal Dufek was responsible for study design, patient enrollment and he edited the manuscript. Silvia Mangia, Shalom Michaeli and Martin Bareš participated in the study design and editing of the manuscript.
Daniel Schwarz was responsible for the study design, statistical analyses and editing of the manuscript. Lubomír Vojtíšek was responsible for the study design, data acquisition and interpretation and he edited the manuscript.

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Figures

Figure 1

Axial image from a representative primary progressive multiple sclerosis subject: A. native T2-weighted image. B. superimposed white matter mask of the T2-weighted image with separate colour palette (T2-weighted intensity range 150–400), allowing for easy detection of diffusely abnormal white matter and lesions. C. Resulting normal appearing white matter mask after manual visual validation and thresholding in 1-mm isotropic resolution of the original T2-weighted scan (orange) and 1.5-mm isotropic resolution of the diffusion weighted image (yellow) after further inclusion probability thresholding at the level of 0.9 to avoid partial volume effects in lower resolution scans. Laterality conventions where the right side of the figure corresponds to the right side of the brain is used.
Results of voxel/vertex-wise comparison between primary progressive multiple sclerosis patients and healthy controls for relaxation metrics, including the non-parametric combination joint inference across modalities. Results overlaid over the average FreeSurfer cortical parcellation. Only the results of NPC, T1_ρ and T2_ρ analysis are presented (T1 map analysis failed to provide significant results, T2 map analysis results not depicted, as only one cortical cluster of limited extent was detected). See Table 2 for further information. Clusters are significant at p <0.05 family-wise error voxel/vertex-wise corrected, cluster threshold of 25 contiguous voxels (subcortical) and 100 mm² (cortical clusters). Laterality conventions where the right side of the figure corresponds to the right side of the brain is used.
Figure 3

Relaxograms (T1, T2, T1ρ, T2ρ) in white matter in primary progressive multiple sclerosis (PPMS) patients (red) and healthy controls (HC) (green). Full lines correspond to median values in whole white matter (WM), shadows to 10th–90th percentile range in whole WM in the respective group; red dashed line without shadow depicts the median values in normally appearing WM in PPMS patients. X axis provides relaxation time constants in ms, Y axis normalised pixel counts.

Supplementary Files

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- RelaxometryinPPMSv5supplementarymaterial.docx