AXONAL DAMAGE IN THE MAKING: NEUROFILAMENT PHOSPHORYLATION AND MAGNETIZATION TRANSFER IN MS NON-LESIONAL WHITE MATTER

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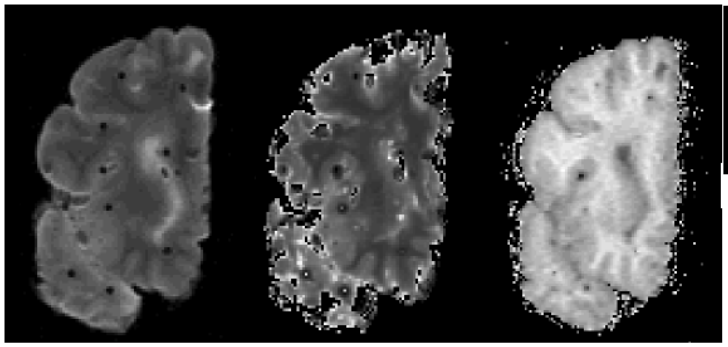
INTRODUCTION Multiple sclerosis (MS) affects the phosphorylation and thus the proton binding capacity of axonal neurofilament (Nf) proteins (1). Macromolecules can be quantified by measuring the exchange of magnetization between protons in the semisolid fraction of the tissue and protons in the mobile fraction. Using *post mortem* brain we explored whether in MS non-lesional white matter (NLWM) magnetization transfer ratio (MTR) is associated with Nf phosphoforms, thus potentially providing a biomarker of axonal phosphorylation in NLWM.

METHODS Unfixed *post mortem* brain slices of 10 women and 2 men (age: 56 years, standard deviation [SD] 14 years) with MS for 23 (SD: 10) years were used (table). Brain slices were retrieved 17 (SD: 6) hours after death and small snap frozen samples (n=128) of macroscopically NLWM obtained, homogenised, and protein levels quantified using enzyme linked immunosorbent assay (ELISA) for Nf heavy chain (NfH) phosphoforms, glial fibrillary acidic protein (GFAP), S100B and ferritin. **MRI** of the remaining tissue was performed 52 (SD: 23) hours *post mortem* on a Signa Horizon Echospeed 1.5T system to acquire (i) 2D dual spin-echo (SE) proton density (PD)- and T_2 weighted (TR: 2000 msec; TE1/TE2: 30/120 msec; flip angle: 90°; matrix size: 256 x 192 reconstructed to 256 x 256 over a field of view [FOV] of 24 x 24 cm^2), (ii) 2D PD- and T_1 GRE (TR/TE/flip angle: 1500 msec/11 msec/45° and 36 msec/11 msec/45°, respectively), from which T_1 maps were generated, and (iii) 2D dual SE images (TR/TE/flip angle: 1720 msec/80 msec/90°) obtained with and without a saturation prepulse (16 msec, 23.2 µT Hamming apodized three-lobe sinc pulse, applied 1 kHz off water resonance) from which MTR maps were generated. Matrix size for the T_1 and MTR maps was 256 x 256, and the FOV was 240 x 240 mm^2 (2) (fig 1). After scanning, tissue blocks were dissected, processed for embedding in paraffin, and sections stained for Luxol-Fast blue (myelin) and Bielschowsky's silver impregnation (axons) (fig 2). *T* tests and Spearman correlation coefficients were used for analyses.

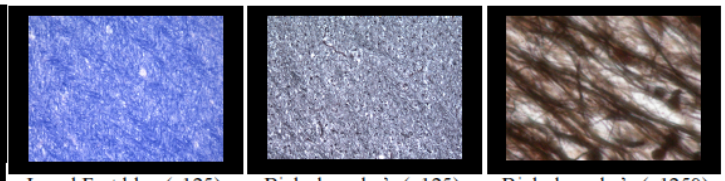
RESULTS Hyperphosphorylated NfH-SMI34 – but not total NfH-SMI35 – levels varied between individual patients' NLWM. Concentration of hyperphosphorylated NfH-SMI34 correlated with T_1 (r=0.7; p= 0.01) and inversely with MTR (r=-0.76 p<0.01) (fig 3), whereas NfH-SMI35 did not correlate with any MRI index.

FIGURE 1 MRI scanning



T_2 weighted T_1 map MTR map

FIGURE 2 Histology of non-lesional multiple sclerosis white matter



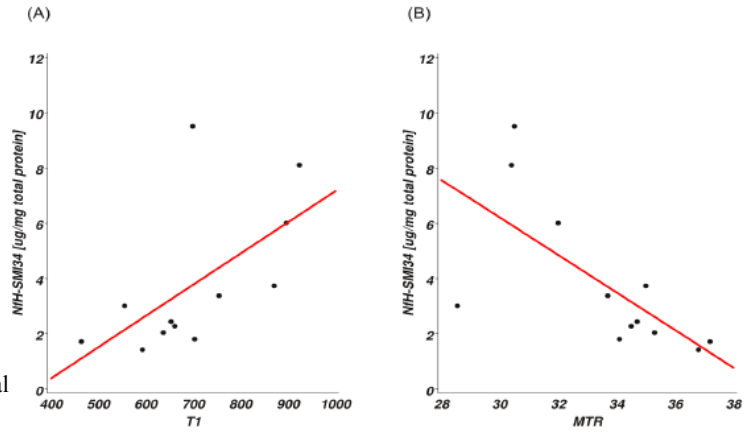
Luxol Fast blue (x125) Bielschowsky's (x125) Bielschowsky's (x1250)

TABLE Patient & tissue sample demographics

Case	Age	Gender	EDSS	Duration	Course	interval 1 (h)	interval 2 (h)	samples
#1	72	F	7	35	5	17.5	41.5	9
#2	49	F	9	14	2	16.5	44.5	18
#3	76	F	8.5	23	6	11	38	11
#4	52	M	7	24	6	13.5	23	11
#5	58	F	9	32	2	16.5	40	9
#6	82	F	8.5	37	2	15	77	8
#7	50	F	7.5	31	2	9	103	9
#8	34	F	9.5	12	2	12	33	4
#9	55	F	6.5	20	2	24	48	7
#10	44	F	9	16	2	18	83	18
#11	45	F	8.5	6	2	28	44	6
#12	56	M	8.5	29	6	25	48.5	18

(EDSS= expanded disability status scale score; duration= disease duration. Course: 2= secondary progressive MS, 5= benign, 6= undeterminded. Interval 1= time between death and tissue retrieval; interval 2= time between death and MRI; samples= n samples).

FIGURE 3 In MS non-lesional white matter the concentration of hyperphosphorylated neurofilaments (NfHSMI34) correlates with T_1 and (inversely) MTR.



CONCLUSION Post-translational modifications of axonal proteins such as phosphorylation and compactness of NfH are early signs of axonal damage. The association between hyperphosphorylated NfH (i) T_1 and (ii) (inversely) MTR suggests that early axonal changes on a proteomic level may be detectable in MS NLWM *in vivo*.

REFERENCES (1) Petzold A, et al. Exp Neurol 2008;213:326-35.; (2) Schmierer K, et al. J Magn Reson Imaging 2007;26:41-51.

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