Supplementary Material

1.Supplementary Methods

Neuropsychological protocol included the CERAD battery, Rey-Osterrieth Complex Figure Test (copy and recall), Memory Impairment Screening (MIS), Buschke Memory Capacity Test (MCT), Phonological Verbal Fluency Test (letter F), Wisconsin Card Classification Test (short version), Raven Test Part A; Symbol Digit Test (WAIS), Trail Making Test, Functional assessment staging scale (FAST), Global Deterioration Scale (GDS), Katz, Barthel, Lawton-Brody, Yesavage depression scale, and a Memory Complaints questionnaire.

Extensive brain examination and staining were done for the middle frontal gyrus, superior temporal gyrus, medial temporal gyrus, inferior temporal gyrus, hippocampus, amygdala, insula, gyrus cinguli, lenticular nucleus, caudate nucleus, thalamus, inferior parietal lobule, occipital lobule, cerebellum, mesencephalon, pons, medulla oblongata, and spinal cord. The tissue was stained with hematoxylin and eosin (H&E) and Luxol fast blue/periodic acid of Schiff (LFB/PAS). Automatic immunostaining was performed with a Ventana Benchmark GX system (Roche AG, Basel, Switzerland) according to manufacturer instructions. Briefly, after dewaxing and inactivation of endogenous peroxidases (PBS/3% hydrogen peroxide), antibody-specific antigen retrieval was performed, and sections were blocked and incubated with the primary antibody. For detection of specific binding, the UltraView Universal 3,3′-Diaminobenzidine (DAB)

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Detection Kit (Ventana, Roche) was used which contains secondary antibodies, DAB stain, and counterstaining reagents. Immunohistochemical staining for phosphorylated neurofilament (NF) was performed on sections of the spinal cord using a Bond RX system (Leica) according to manufacturer instructions.

Used Antibodies					
Antibody	Source	Species	Isotype	Dilution	Specificity
11-9	COSMO BIO USA, Inc. Carlsbad, CA	Mouse	IgG1	1:1000	TDP-43 phospho Ser409/410
AT8	ThermoFisher Scientific, Dreieich, Germany	Mouse	IgG1, kappa	1:1200	phospho-Tau Ser202, Thr205
BAM-10	ThermoFisher Scientific, Dreieich, Germany	Mouse	IgG1	1:200	Aβ1-40
SMI-31	Sternberger-Meyers, Lutherville, MD	Mouse	IgG1	1:5000	phospho-NF
MJF-R13 (8-8)	Abcam, Waltham, MA	Rabbit	IgG	1:2000	Alpha-synuclein (phospho S129)

2.**Supplementary Tables**

2.1   **Supplementary Table 1**

	Case 1		Case 2		Case 3	
Age at the assessment (Years of formal education)	40 (5)		37 (6)		32 (5)	
	Raw Scores	Z score (%ile)	Raw Scores	Z score (%ile)	Raw Scores	Z score (%ile)
MMSE/30	26	-0.91 (18)	21	-4.08 (<1)	28	-0.49 (32)
Attention						
WAIS-III Digit and symbol	36	-0.34 (37)	6	-2.11 (2)	32	-0.47 (32)
Trail Making Test A (seconds)	87	-0.05 (47)	208	-4.21 (<1)	81	-0.06 (47)
Memory						
CERAD word list /30	11	-1.31 (9)	8	-2.17 (1)	11	-1.71 (4)
CERAD word list evocation /10	5	-0.44 (32)	1	-2.64 (<1)	1	-3.34 (<1)
Word List Recognition /10	10	0.43 (66)	3	-5.20 (-)	10	0.47 (68)
CERAD praxis evocation /11	4	-1.31 (9)	2	-2.44 (1)	4	-1.42 (8)
RCFT delayed recall /36	6	-1.25 (10)	2	-2.05 (2)	8	-0.92 (18)
MIS (free recall) /8	4	-1.10 (13)	0	-3.77 (<1)	2	-2.88 (<1)
MCT- 1 LIST /16	12	-1.09 (14)	9	-2.94 (<1)	15	0.18 (58)
MCT- 2 LIST /16	5	-1.73 (4)	2	-3.00 (<1)	5	-2.03 (2)
MCT List 1 Cued Recall 2 /16	12	-0.56 (30)	8	-2.34 (1)	10	-1.72 (4)
MCT List 2 Cued Recall 2 /16	7	-1.31 (9)	3	-2.87 (<1)	5	-2.17 (1)

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	Case 1		Case 2		Case 3	
MCT - Buschke free recall /16	8	-1.75 (4)	6	-2.16 (2)	6	-2.19 (1)
Praxias						
CERAD praxis copy /11	7	-1.52 (6)	6	-2.45 (1)	6	-2.16 (2)
RCFT copy /36	15.5	-1.37 (8)	15	-1.83 (4)	16	-1.87 (3)
Language						
CERAD shortened Boston Naming Test/15	8	-1.71 (4)	11	-0.77 (21)	11	-0.44 (32)
Semantic fluency (animals)	9	-1.84 (3)	7	-2.32 (1)	12	-1.10 (14)
Executive function						
WCST Categories /6	1	-1.17 (12)	2	-0.57 (27)	2	-0.54 (30)
WCST Perseverations	27	-0.75 (23)	34	-2.00 (2)	22	-0.26 (39)
Phonemic Fluency ("FAS")	17	-0.48 (32)	22	-0.25 (39)	18	-0.50 (32)
Raven's Progressive Matrices /12	6	-1.26 (10)	4	-2.39 (1)	6	-1.34 (9)
Functional scales						
Memory complaints scale: PC /45 versus CC /45	10 vs. 11		30 vs. 31		18 vs. 37	
FAST scale	3		4		3	
Lawton & Brody scale /8	7		4		8	
Yesavage depression scale /15	10		12		5	

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Neuropsychological battery performance in *PSEN1* c.851C>T p. Pro284Leu carriers according to their age groups and years of formal education.

All test scores were expressed as z-scores and the percentiles derived from normative data.

Scores ≥ 1.5 Standard Deviation (SD) below the age-adjusted normative value were considered abnormal. Scores between -1.5 and -2 SD are highlighted in blue. Scores > -2 SD are highlighted in red. ADL = Activities of Daily Living; CC = Caregiver's complaints; CERAD = Consortium to Establish a Registry for Alzheimer's Disease; FAST = Functional Assessment Staging Tool; GDS = Global Deterioration Scale; MCT = Memory Capacity Test; MIS = Memory Impairment Screen; MMSE = Mini-mental state examination; PC = Patient's complaints; RCFT = Rey Osterrieth Complex Figure Test; WAIS = Wechsler Adult Intelligence Scale; WCST = Wisconsin Card Sorting Test.

3. Supplementary Figures

3.1   Supplementary Figure 1
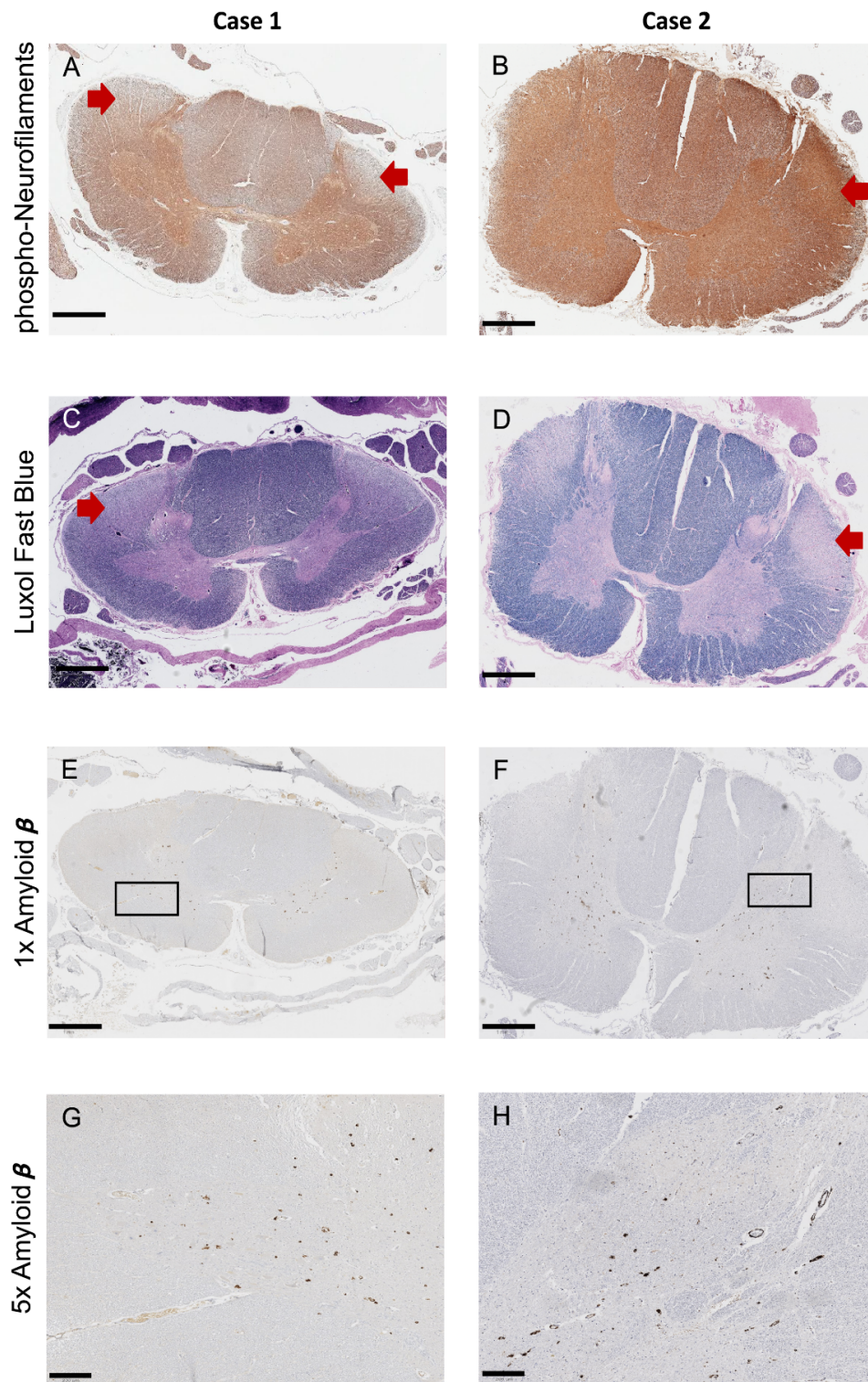
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Cross section of spinal cord demonstrating decreased immunohistochemical staining for phosphorylated neurofilaments (A) and Luxol fast blue staining for myelin (C) in the lateral corticospinal tracts, indicating loss of both axons (A) and myelin (C) in Case 1 (red arrows).
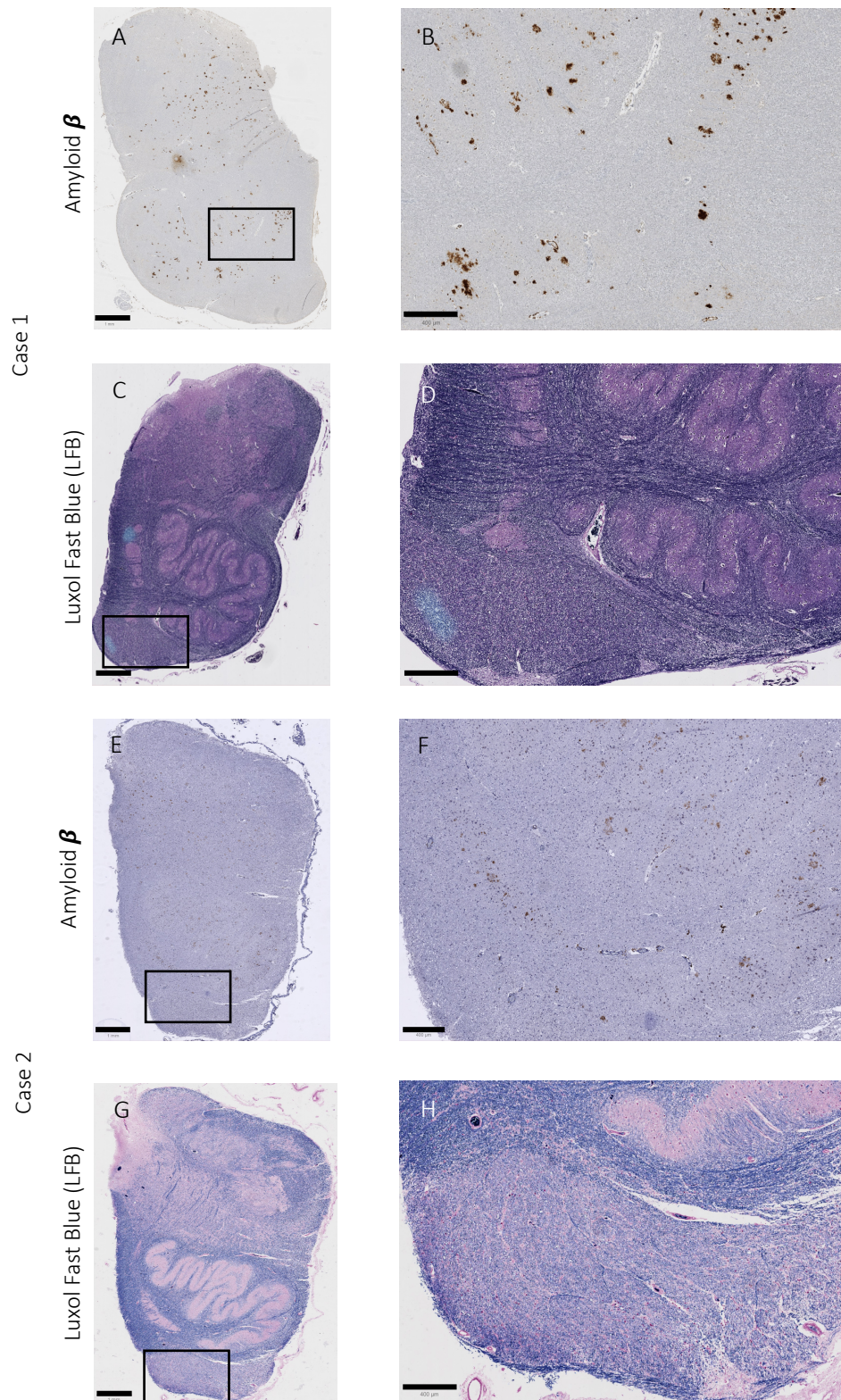
Cross section of spinal cord demonstrating decreased immunohistochemical staining for phosphorylated neurofilaments (B) and Luxol fast blue staining for myelin (D) in the lateral corticospinal tracts, indicating loss of both axons (B) and myelin (D) in Case 2 (red arrows). The axon loss demonstrated in B for Case 2 is not as dramatic as observed in A for Case 1.

Low (E) and high (G) power views of Aβ immunohistochemical staining in the anterior horn (gray matter) of the spinal cord from case 1.

Low (F) and high (H) power views of Aβ immunohistochemical staining in the posterior horn (gray matter) of the spinal cord from case 2.

(A-F) Scale bar 1mm. (G, H) Scale bar 200um.

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3.1.1.Supplementary Figure 2

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Low (A, C) and high (B, D) power views of Aβ immunohistochemical staining demonstrating frequent amyloid plaques in gray matter of the medulla from case 1 (A, B) and Luxol fast blue (C, D) stained sections of medulla demonstrating slightly decreased myelin staining in the pyramid (rectangle) of case 1.

Low (E, G) and high (F, H) power views of Aβ immunohistochemical staining demonstrating amyloid plaques in gray matter of the medulla from case 2 (E, F) and Luxol fast blue (G, H) stained sections of medulla demonstrating more significantly decreased myelin staining in the pyramid (rectangle) of case 2 compared to case 1.

A, C, E, G: Scale bar 1mm. B, D, F, H: Scale Bar 400um

Histological images from the comprehensive neuropathological analyses of both cases are available at the CIL Cell Image Library, DOI: https://doi.org/10.7295/W9P20489. These materials may be used and shared by anyone, provided that they are not modified, used for non-commercial purposes, and the original authors are duly credited and cited.

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