Clinical spectrum of 4H leukodystrophy caused by *POLR3A* and *POLR3B* mutations

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Supplementary Data

Supplementary table e-1: Genetic findings in our patient cohort

Patient	Family	Consanguinity	Ethnicity	Gene	Mutation 1 (gDNA)	Mutation 1 (protein)	Exon	Mutation 2 (gDNA)	Mutation 2 (protein)	Exon	Published
4H-1	4H-F1	no	FC	*POLR3A*	c.1674C>G	p.Phe558Leu	13	c.3742insACC	p.1248insThr	28	Bernard et al., 2010; Bernard et al., 2011
4H-2	4H-F2	yes	FC	*POLR3A*	c.2015G>A	p.Gly672Glu	15	c.2015G>A	p.Gly672Glu	15	Bernard et al., 2010; Bernard et al., 2011
4H-3	4H-F2	yes	FC	*POLR3A*	c.2015G>A	p.Gly672Glu	15	c.2015G>A	p.Gly672Glu	15	Bernard et al., 2010; Bernard et al., 2011
4H-4	4H-F2	yes	FC	*POLR3A*	c.2015G>A	p.Gly672Glu	15	c.2015G>A	p.Gly672Glu	15	Bernard et al., 2010; Bernard et al., 2011
4H-5	4H-F3	suspected	FC	*POLR3A*	c.2015G>A	p.Gly672Glu	15	c.2015G>A	p.Gly672Glu	15	Bernard et al., 2010; Bernard et al., 2011
4H-6	4H-F4	yes	FC	*POLR3A*	c.2015G>A	p.Gly672Glu	15	c.2015G>A	p.Gly672Glu	15	Bernard et al., 2011
4H-7	4H-F5	no	Caucasian	*POLR3A*	c.2554A>G	p.Met852Val	19	c.2617-1G>A	p.Arg873fs	Intron 19	Bernard et al., 2011, Timmons et al., 2006
4H-8	4H-F6	no	African-American	*POLR3A*	c.1114G>A	p.Asp372Asn	8	c.2324A>T	p.Asn775Ile	17	Bernard et al., 2011
4H-9	4H-F7	no	Caucasian	*POLR3A*	c.2830G>T	p.Glu944*	21	c.3013C>T	p.Arg1005Cys	23	Bernard et al., 2011, Timmons et al., 2006
4H-10	4H-F8	no	Caucasian	*POLR3A*	c.2554A>G	p.Met852Val	19	c.2617-1G>A	p.Arg873fs	Intron 19	Bernard et al., 2011, Timmons et al., 2006
4H-11	4H-F9	no	Southeastern European	*POLR3A*	c.3014G>A	p.Arg1005His	23	c.3991G>A	p.Ala1331Thr	30	Potic et al., 2011
4H-12	4H-F10	no	Middle / Western European	*POLR3B*	c.1508C>A	p.Thr503Lys	15	c.1568A>T	p.Val523Glu	15	Tetreault et al., 2011

Patient	Family	Consanguinity	Ethnicity	Gene	Mutation 1 (gDNA)	Mutation 1 (protein)	Exon	Mutation 2 (gDNA)	Mutation 2 (protein)	Exon	Published
4H-13	4H-F11	no	Caucasian	*POLR3B*	c.1533delT	p.Ile511fs	15	c.1568A>T	p.Val523Glu	15	Tetreault et al., 2011
4H-14	4H-F12	no	Caucasian	*POLR3B*	c.1568A>T	p.Val523Glu	15	c.2686A>T	p.Lys896*	23	Tetreault et al., 2011
4H-15	4H-F13	suspected	FC	*POLR3A*	c.496G>A	p.Val166Ile	5	c.496G>A	p.Val166Ile	5	Daoud et al., 2013
4H-16	4H-F13	suspected	FC	*POLR3A*	c.496G>A	p.Val166Ile	5	c.496G>A	p.Val166Ile	5	Daoud et al., 2013
4H-17	4H-F14	yes	Mediterranean	*POLR3A*	c.2011T>C	p.Trp671Arg	15	c.2011T>C	p.Trp671Arg	15	Daoud et al., 2013
4H-18	4H-F14	yes	Mediterranean	*POLR3A*	c.2011T>C	p.Trp671Arg	15	c.2011T>C	p.Trp671Arg	15	Daoud et al., 2013
4H-19	4H-F15	no	Caucasian	*POLR3A*	c.1741insA	p.Val581fs	13	c.1804A>C	p.Ser602Arg	14	Daoud et al., 2013
4H-20	4H-F16	no	Caucasian	*POLR3A*	c.1160C>G	p.Ala387Gly	8	c.3781G>A	p.Glu1261Lys	29	Daoud et al., 2013
4H-21	4H-F17	no	Malaysian / Caucasian	*POLR3A*	c.1302insA	p.Tyr434*	10	c.2554A>G	p.Met852Val	19	Daoud et al., 2013
4H-22	4H-F18	no	Hispanic	*POLR3A*	c.272C>T	p.Pro91Leu	3	c.3013C>T	p.Arg1005Cys	23	Daoud et al., 2013
4H-23	4H-F19	no	Caucasian	*POLR3B*	c.1568T>A	p.Val523Glu	15	c.2084-6A>G	p.Gly695fs	Intron 19	Daoud et al., 2013
4H-24	4H-F20	no	North European	*POLR3B*	c.1568T>A	p.Val523Glu	15	c.2084-6A>G	p.Gly695fs	Intron 19	Daoud et al., 2013
4H-25	4H-F21	no	Middle European	*POLR3B*	c.1568T>A	p.Val523Glu	15	c.1579T>C	p.Cys527Arg	15	Daoud et al., 2013
4H-26	4H-F22	no	North European	*POLR3B*	c.1568T>A	p.Val523Glu	15	c.2817+30T>A		Intron 24	Daoud et al., 2013
4H-27	4H-F23	no	Caucasian	*POLR3B*	c.1324C>T	p.Arg442Cys	14	c.1568T>A	p.Val523Glu	15	Daoud et al., 2013
4H-28	4H-F24	no	Caucasian	*POLR3B*	c.312G>T	p.Leu104Phe	6	c.2570+1G>A	p.Gly818fs	22	Daoud et al., 2013
4H-29	4H-F25	no	North European	*POLR3B*	c.802A>G	p.Ser268Gly	10	c.1568T>A	p.Val523Glu	15	Daoud et al., 2013
4H-30	4H-F26	no	Caucasian	*POLR3A*	c2821A>C	p.Ser941Arg	21	c.3407G>A	p.Arg1136Gln	26	
4H-31	4H-F27	no	Caucasian	*POLR3A*	c.1186G>T	p.Val396Leu	9	c.2015G>A	p.Gly672Glu	15	
4H-32	4H-F28	no	Hispanic	*POLR3A*	c.364_366delAAG	p.Lys122del	4	c.1930G>A	p.Glu644Lys	15	
4H-33	4H-F29	no	Caucasian	*POLR3A*	c.1930G>A	p.Glu644Lys	15	c.1935G>C	p.Leu645Phe	15	
4H-34	4H-F30	no	Hispanic	*POLR3B*	c.1464+1G>A		Intron 14	c.1568T>A	p.Val523Glu	15	
4H-35	4H-F31	no	East European	*POLR3B*	c.1568T>A	p.Val523Glu	15	c.2084-6A>G	p.Gly695fs	Intron 19	
4H-36	4H-F32	no	N/A	*POLR3A*	c.550_553delinsAAT	p.Lys184fs	5	c.3014G>A	p.Arg1005His	23	

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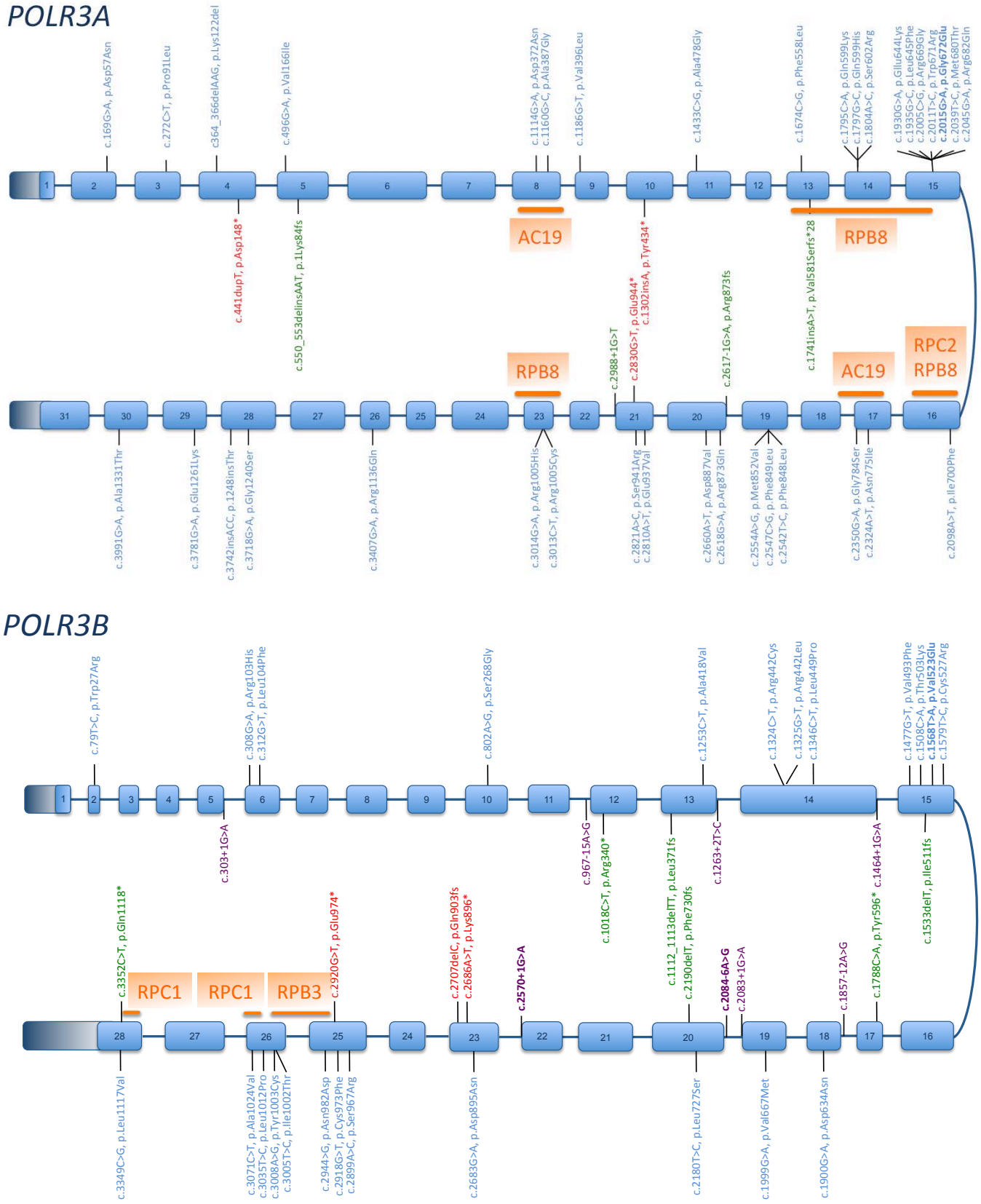
4H-37	4H-F33	no	African-American	*POLR3B*	c.1346T>C	p.Leu449Pro	14	c.2899A>C	p.Ser967Arg	25	
4H-38	4H-F33	no	African-American	*POLR3B*	c.1346T>C	p.Leu449Pro	14	c.2899A>C	p.Ser967Arg	25	
4H-F39	4H-F34	no	Caucasian	*POLR3B*	c.1568T>A	p.Val523Glu	15	not found			
4H-40	4H-F35	no	Caucasian	*POLR3B*	c.2918G>T	p.Cys973Phe	25	c.2920G>T	p.Glu974*	25	
4H-41	4H-F36	no	Mediterranean	*POLR3A*	c.3014G>A	p.Arg1005His	23	c.3781G>A	p.Glu1261Lys	29	
4H-42	4H-F37	no	FC	*POLR3A*	c.2015G>A	p.Gly672Glu	15	c.3718G>A	p.Gly1240Ser	28	
4H-43	4H-F38	no	FC	*POLR3A*	c.1674C>G	p.Phe558Leu	13	c.2015G>A	p.Gly672Glu	15	
4H-44	4H-F39	yes	Caucasian	*POLR3B*	c.1568T>A	p.Val523Glu	15	c.1568T>A	p.Val523Glu	15	
4H-45	4H-F39	yes	Caucasian	*POLR3B*	c.1568T>A	p.Val523Glu	15	c.1568T>A	p.Val523Glu	15	
4H-46	4H-F40	no	Western European	*POLR3A*	c.169G>A	p.Asp57Asn	2	c.2098A>T	p.Ile700Phe	16	Timmons et al., 2006
4H-47	4H-F41	no	Western European	*POLR3B*	c.1568T>A	p.Val523Glu	15	c.2707delC	p.Gln903fs	23	
4H-48	4H-F42	no	Middle European	*POLR3A*	c.1433C>G	p.Ala478Gly	11	c.2045G>A	p.Arg682Gln	15	Wolf et al., 2005
4H-49	4H-F43	no	Middle European	*POLR3B*	c.1568T>A	p.Val523Glu	15	c.2084-6A>G	p.Gly695fs	Intron 19	Wolf et al., 2007
4H-50	4H-F44	no	Middle European	*POLR3B*	c.1568T>A	p.Val523Glu	15	c.1900G>A	p.Asp634Asn	18	Wolf et al., 2005
4H-51	4H-F45	no	Middle European	*POLR3B*	c.1568T>A	p.Val523Glu	15	c.3352C>T	p.Gln1118*	28	Wolf et al., 2005
4H-52	4H-F46	no	Middle European	*POLR3B*	c.1568T>A	p.Val523Glu	15	c.2084-6A>G	p.Gly695fs	Intron 19	
4H-53	4H-F47	no	Middle European	*POLR3B*	c.967-15A>G		Intron 11	c.1568T>A	p.Val523Glu	15	Wolf et al., 2005
4H-54	4H-F48	no	Caucasian	*POLR3B*	c.1324C>T	p.Arg442Cys	14	c.1568T>A	p.Val523Glu	15	Wolf et al., 2007
4H-55	4H-F49	no	Western European	*POLR3B*	c.1325G>T	p.Arg442Leu	14	c.1568T>A	p.Val523Glu	15	
4H-56	4H-F49	no	Western European	*POLR3B*	c.1325G>T	p.Arg442Leu	14	c.1568T>A	p.Val523Glu	15	
4H-57	4H-F50	no	Western European	*POLR3B*	c.1788C>A	p.Tyr596*	17	c.2180T>C	p.Leu727Ser	20	
4H-58	4H-F51	no	Syrian	*POLR3B*	c.3005T>C	p.Ile1002Thr	26	c.3005T>C	p.Ile1002Thr	26	
4H-59	4H-F52	no	Middle European	*POLR3B*	c.1112_1113delTT	p.Leu371fs	13	c.1568T>A	p.Val523Glu	15	Wolf et al., 2007
4H-60	4H-F53	no	Caucasian	*POLR3B*	c.1568T>A	p.Val523Glu	15	c.2570+1G>A	p.Gly818fs	Intron 22	
4H-61	4H-F54	no	Caucasian	*POLR3B*	c.1263+2T>C		Intron 13	c.1568T>A	p.Val523Glu	15	

Patient	Family	Consanguinity	Ethnicity	Gene	Mutation 1 (gDNA)	Mutation 1 (protein)	Exon	Mutation 2 (gDNA)	Mutation 2 (protein)	Exon	Patient
4H-62	4H-F55	no	Western European	*POLR3B*	c.832_833dup	p.Thr279fs	10	c.1568T>A	p.Val523Glu	15	
4H-63	4H-F56	no	Caucasian	*POLR3B*	c.1018C>T	p.Arg340*	12	c.1568T>A	p.Val523Glu	15	
4H-64	4H-F57	no	Caucasian	*POLR3B*	c.1568T>A	p.Val523Glu	15	not found			
4H-65	4H-F57	no	Caucasian	*POLR3B*	c.1568T>A	p.Val523Glu	15	not found			
4H-66	4H-F58	no	Mediterranean	*POLR3A*	c.441dupT	p.Asp148*	4	c.2554A>G	p.Met852Val	19	
4H-67	4H-F59	no	Mediterranean	*POLR3A*	c.1795C>A	p.Gln599Lys	14	c.3718G>A	p.Gly1240Ser	28	
4H-68	4H-F60	no	Middle European	*POLR3B*	c.1568T>A	p.Val523Glu	15	c.2570+1G>A	p.Gly818fs	Intron 22	
4H-69	4H-F61	no	Western European	*POLR3B*	c.1263+2T>C		Intron 13	c.1568T>A	p.Val523Glu	15	
4H-70	4H-F62	no	Middle European	*POLR3B*	c.1999G>A	p.Val667Met	19	c.2084-6A>G	p.Gly695fs	Intron 19	
4H-71	4H-F63	no	Middle European	*POLR3B*	c.1568T>A	p.Val523Glu	15	c.2570+1G>A	p.Gly818fs	Intron 22	
4H-72	4H-F64	no	North European	*POLR3B*	c.1568T>A	p.Val523Glu	15	c.1857-12A>G		Intron 17	
4H-73	4H-F65	no	Mediterranean	*POLR3B*	c.1568T>A	p.Val523Glu	15	c.2683G>A	p.Asp895Asn	23	
4H-74	4H-F65	no	Mediterranean	*POLR3B*	c.1568T>A	p.Val523Glu	15	c.2683G>A	p.Asp895Asn	23	
4H-75	4H-F66	no	Mediterranean	*POLR3A*	c.2005C>G	p.Arg669Gly	15	c.2618G>A	p.Arg873Gln	20	Wolf et al., 2007
4H-76	4H-F67	no	Caucasian	*POLR3B*	c.1263+2T>C		Intron 13	c.1568T>A	p.Val523Glu	15	
4H-77	4H-F68	yes	Mediterranean	*POLR3A*	c.2547C>G	p.Phe849Leu	19	c.2547C>G	p.Phe849Leu	19	Vazquez-Lopez et al., 2008
4H-78	4H-F69	no	Mixed European	*POLR3B*	c.3008A>G	p.Tyr1003Cys	26	c.3008A>G	p.Tyr1003Cys	26	
4H-79	4H-F70	no	Caucasian	*POLR3A*	c.2039T>C	p.Met680Thr	15	c.2660A>T	p.Asp887Val	20	
4H-80	4H-F70	no	Caucasian	*POLR3A*	c.2039T>C	p.Met680Thr	15	c.2660A>T	p.Asp887Val	20	
4H-81	4H-F71	no	Caucasian	*POLR3B*	c.308G>A	p.Arg103His	6	c.1568T>A	p.Val523Glu	15	
4H-82	4H-F72	no	Caucasian	*POLR3B*	c.1325G>T	p.Arg442Leu	14	c.1568T>A	p.Val523Glu	15	
4H-83	4H-F73	no	Caucasian	*POLR3B*	c.1857-12A>G		Intron 17	c.1568T>A	p.Val523Glu	15	
4H-84	4H-F74	no	Western European	*POLR3B*	c.1101+1G>C		Intron 12	c.1568T>A	p.Val523Glu	15	
4H-85	4H-F75	no	Western European	*POLR3A*	c.2988+1G>T		Intron 21	c.3013C>T	p.Arg1005Cys	23	
4H-86	4H-F76	no	Western European	*POLR3A*	c.1930G>A	p.Glu644Lys	15	c.2350G>A	p.Gly784Ser	17	
4H-87	4H-F77	yes	Mediterranean	*POLR3B*	c.79T>C	p.Trp27Arg	2	c.79T>C	p.Trp27Arg	2	

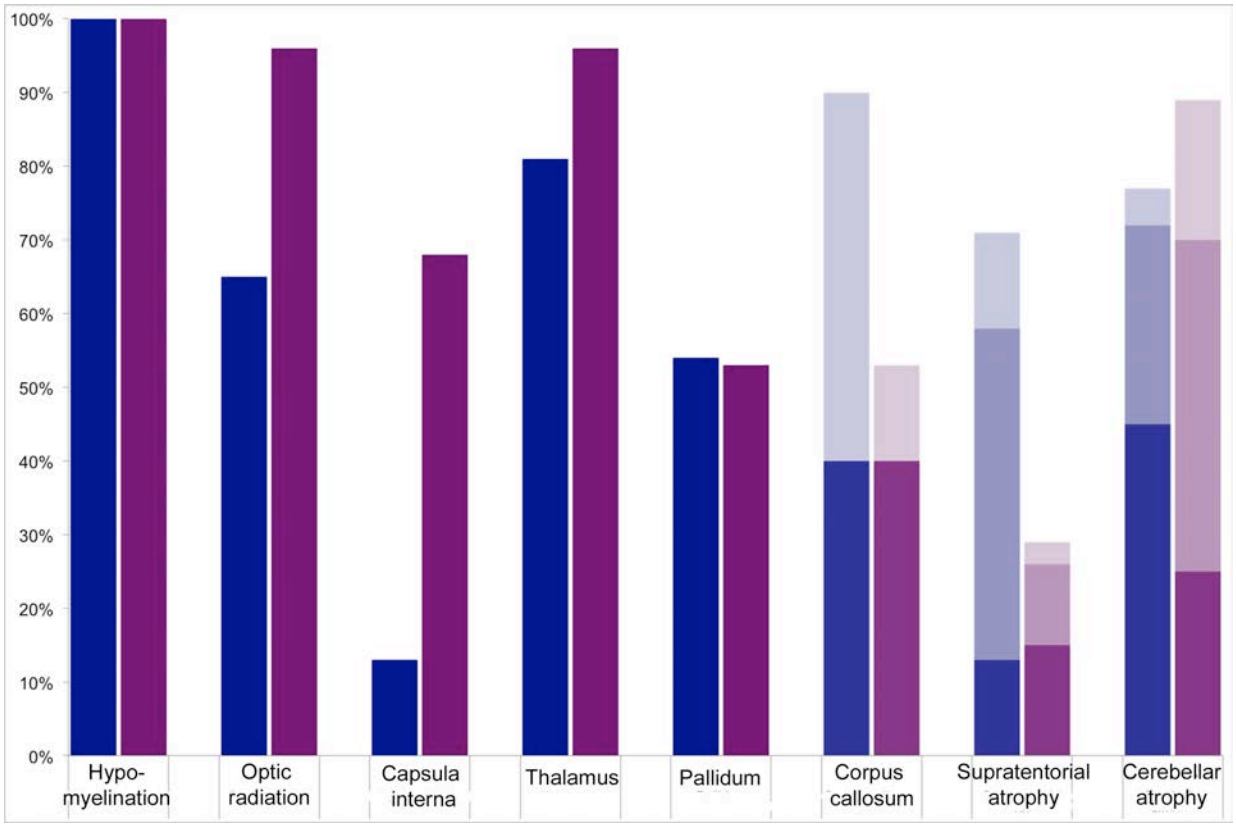
Patient	Family	Consanguinity	Ethnicity	Gene	Mutation 1 (gDNA)	Mutation 1 (protein)	Exon	Mutation 2 (gDNA)	Mutation 2 (protein)	Exon	Patient
4H-88	4H-F77	yes	Mediterranean	*POLR3B*	c.79T>C	p.Trp27Arg	2	c.79T>C	p.Trp27Arg	2	
4H-89	4H-F78	no	Mediterranean	*POLR3A*	c.2011T>C	p.Trp671Arg	15	c.2810A>T	p.Glu937Val	21	
4H-90	4H-F79	no	Middle European	*POLR3A*	c.1658C>T	p.Thr553Ile	13	c.2554A>G	p.Met852Val	19	
4H-91	4H-F80	yes	Mediterranean	*POLR3A*	c.1797G>C	p.Gln599His	14	c.1797G>C	p.Gln599His	14	Bekiesinska-Figatowska et al., 2010
4H-92	4H-F80	yes	Mediterranean	*POLR3A*	c.1797G>C	p.Gln599His	14	c.1797G>C	p.Gln599His	14	
4H-93	4H-F81	no	Western European	*POLR3B*	c.303+1G>A		Intron 5	c.1568T>A	p.Val523Glu	15	Ozgen et al., 2005
4H-94	4H-F82	no	Western European	*POLR3B*	c.1477G>T	p.Val493Phe	15	not found			
4H-95	4H-F83	no	Middle European	*POLR3B*	c.1568T>A	p.Val523Glu	15	c.2083+1G>A		Intron 19	
4H-96	4H-F84	no	Middle European	*POLR3B*	c.1568T>A	p.Val523Glu	15	c.3035T>C	p.Leu1012Pro	26	
4H-97	4H-F85	no	Mediterranean	*POLR3A*	c.2542T>C	p.Phe848Leu	19	c.2542T>C	p.Phe848Leu	19	
4H-98	4H-F86	no	North European	*POLR3B*	c.1568T>A	p.Val523Glu	15	c.3349C>G	p.Leu1117Val	28	
4H-99	4H-F87	no	North European	*POLR3B*	c.1568T>A	p.Val523Glu	15	c.2190delT	p.Phe730fs	20	
4H-100	4H-F88	no	Mediterranean	*POLR3A*	c.2015G>A	p.Gly672Glu	15	not found			
4H-101	4H-F89	no	East European	*POLR3B*	c.3071C>T	p.Ala1024Val	26	c.2944A>G	p.Asn982Asp	25	
4H-102	4H-F90	no	Middle European	*POLR3B*	c.1253C>T	p.Ala418Val	13	c.1253C>T	p.Ala418Val	13	
4H-103	4H-F91	no	Middle European	*POLR3B*	c.1568T>A	p.Val523Glu	15	c.2570+1G>A	p.Gly818fs	Intron 22	
4H-104	4H-F92	no	Caucasian	*POLR3B*	c.986G>A	p.Arg329Gln	12	c.1568T>A	p.Val523Glu	15	
4H-105	4H-F92	no	Caucasian	*POLR3B*	c.986G>A	p.Arg329Gln	12	c.1568T>A	p.Val523Glu	15	

Supplementary figure e-1: Mutations in *POLR3A* and *POLR3B*



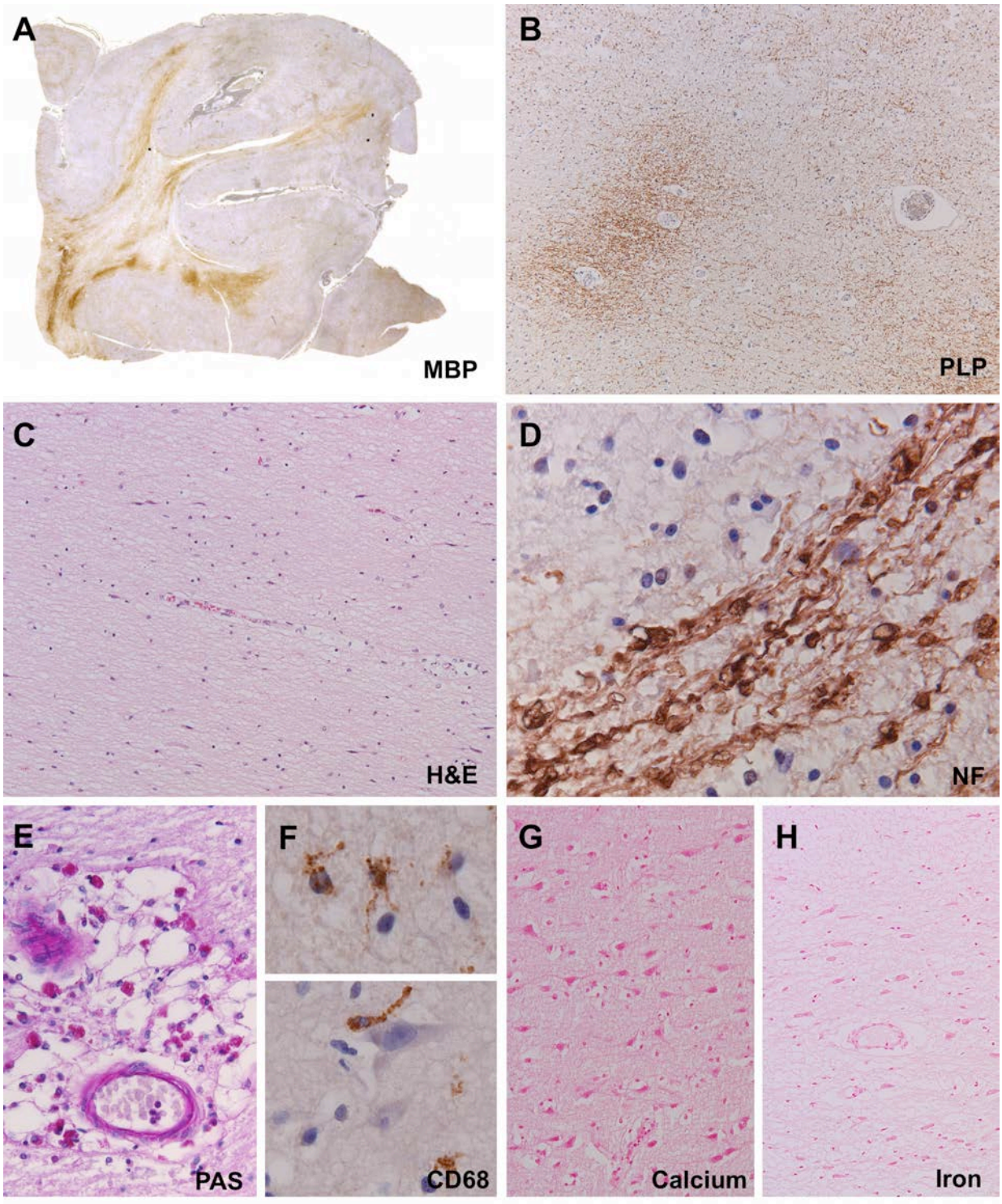
Localisation of the *POLR3A* (A) and *POLR3B* (B) mutations of our cohort in the gene structure. Mutations found in more than one unrelated patients are depicted in bold.

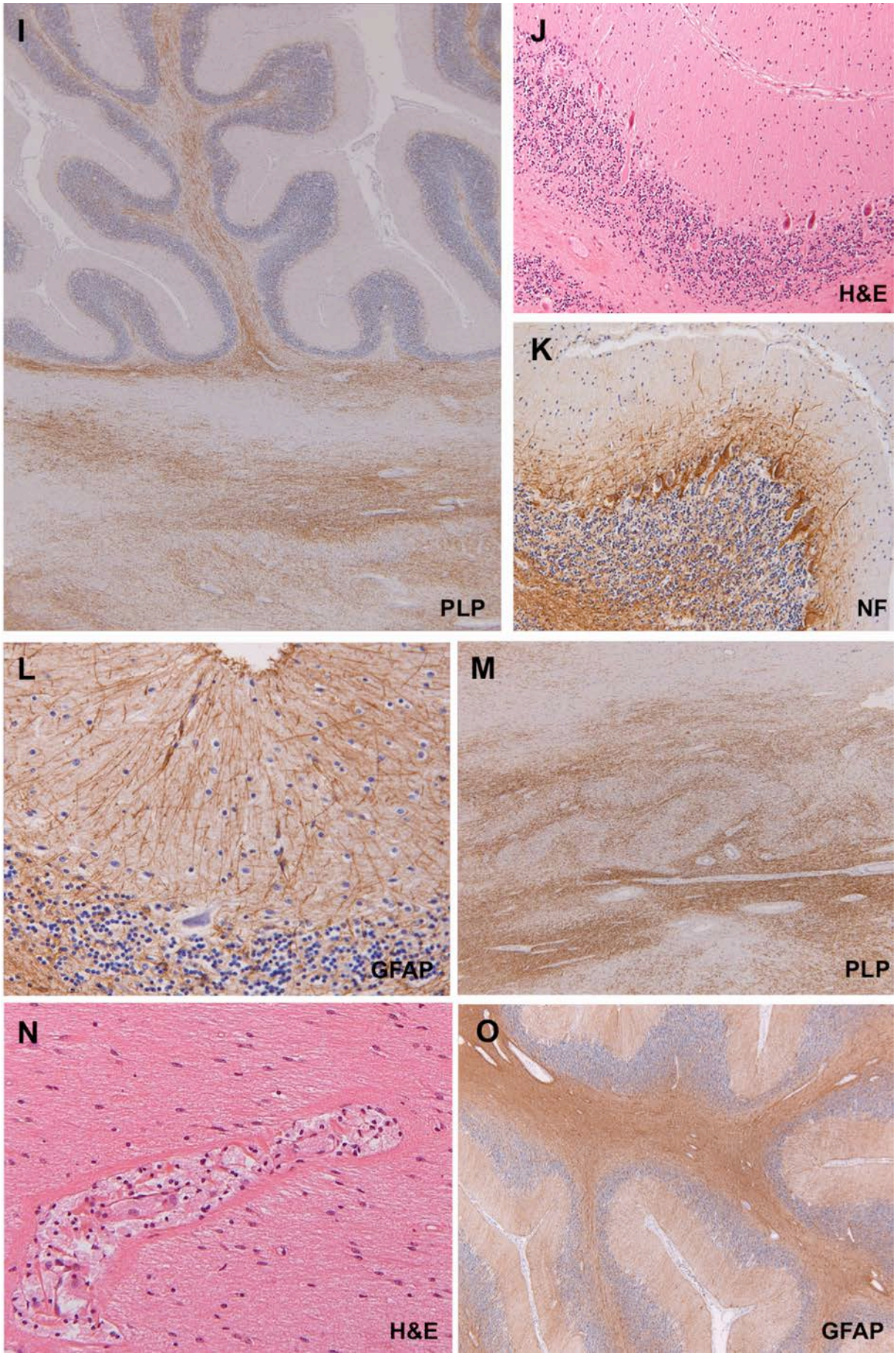
Supplemental figure e-2: MRI findings

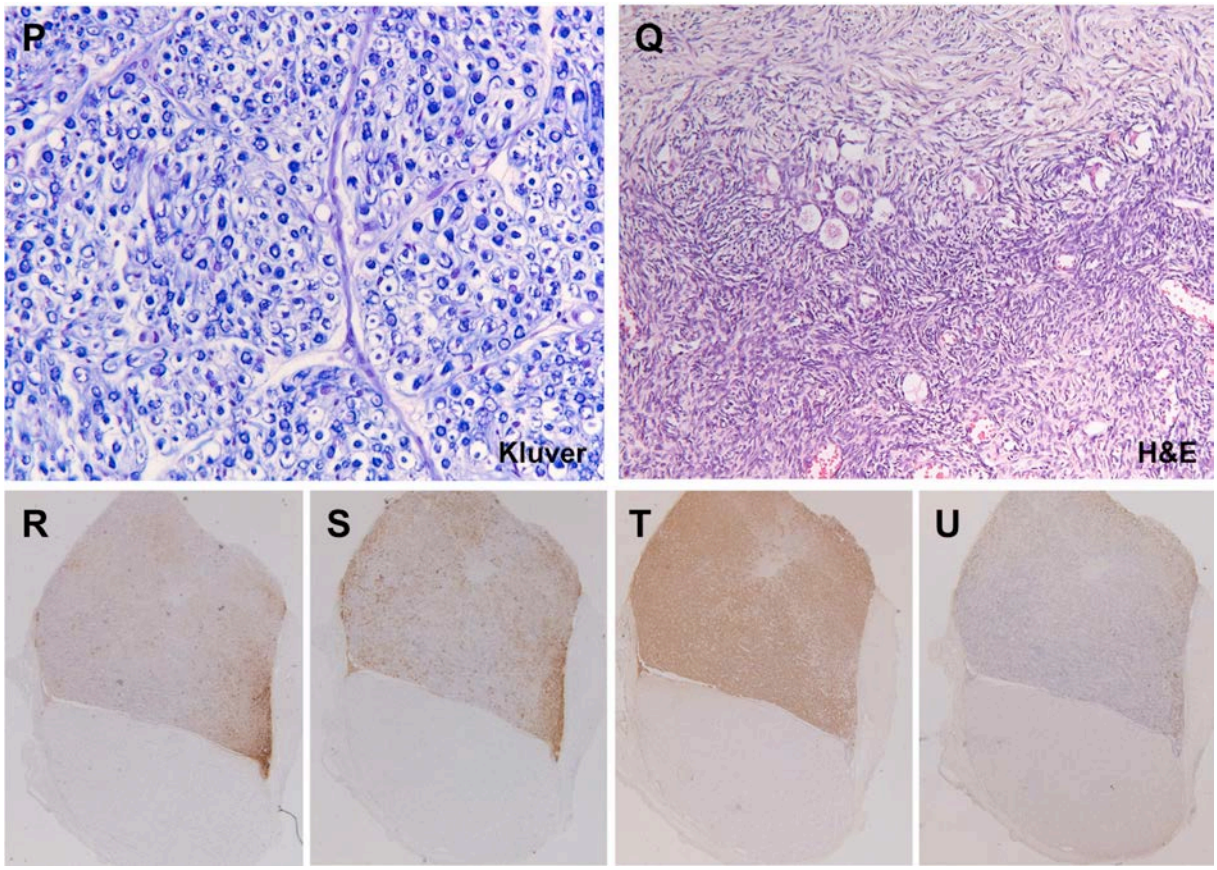


MRI findings in patients with mutations in POLR3A (blue shades) and POLR3B (violet shades). Optic radiation: preserved myelination in optic radiation. Capsula interna: partly myelinated internal capsule. Thalamus: relative T2 hypointense signal of ventrolateral thalamus. Globus pallidus: relative T2 hypointense signal of globus pallidus. Corpus callosum: decreased volume of the corpus callosum. Last three items (corpus callosum and supratentorial / cerebellar atrophy): dark shades – mild, medium shades – moderate, light shades – severe.

Supplementary figure e-2: Histopathological findings




I
PLP
J
H&E
K
NF
L
GFAP
M
PLP
N
H&E
O
GFAP



Whole mount of the second frontal gyrus stained for myelin basic protein (MBP) shows patchy lack of myelin extending to the U-fibers (A). In the centrum semiovale, the perivascular myelin is inconsistently better preserved in this proteolipid protein (PLP) stain (B). Myelin pallor is accompanied by reduced numbers of oligodendrocytes (C). Small axonal varicosities accumulating neurofilaments can be found in the relatively preserved white matter of the U-fibers, suggesting early secondary axonal degeneration (D). PAS-positive foamy macrophages cluster around small blood vessels indicating ongoing myelin breakdown (E). Activation of microglia is mild but CD68-expressing microglia cells are seen in close proximity to white matter oligodendrocytes (top) and cortical neurons (bottom) (F). The cortex is otherwise unaffected, without neuronal mineralization (G). No iron accumulation is seen in the globus pallidus (H).

Stain for proteolipid protein (PLP) shows patchy lack of myelin in the deep cerebellar white matter extending into the folia (A). The cerebellar cortex is mildly atrophic with some loss of granular neurons and, in places, Purkinje cells (B). Residual Purkinje cells have axonal and dendritic swellings accumulating neurofilaments (C), but the degree of reactive Bergmann gliosis is scanty (D). The myelin at the hilus of the dentate nucleus is relatively spared, correlating well with the MRI finding (E). Blood vessels are surrounded by foamy macrophages, suggesting ongoing myelin breakdown (F), and by a rim of reactive astrocytes (G). Original magnifications:

Semithin sections of the sural nerve stained with toluidine blue show mild loss of myelin (P). There are only a slightly reduced numbers of oocytes in the otherwise normal ovaries (Q). In the pituitary gland the immunoreactivity for FSH, LH, GH and prolactin is normal (R-U).

Original magnifications: (B) 50x; (C, G, H) 100x; (E) 200x; (D, F) 400x; (I) 12.5x; (J, K) 100x; (M, O) 25x; (L, N) 200x; (P) 200X; (Q) 100X; (R-U) 12.5X.

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