Supplementary material

Clinical description of proband

The patient was a 49 year old man who presented to a neurologist with a six month history of slowly progressive dysarthria. At this time there were no other neurological abnormalities on examination and an EMG study, including the tongue, was normal. He was reviewed one year later and his speech had deteriorated to the point of almost complete unintelligibility. An EMG was again normal. To direct questioning he admitted intermittent reflex emotional hypersensitivity, mostly pathological laughter. Two years after the initial onset of dysarthria he was anarthric. He underwent detailed neuropsychometry, which reported patchy abnormalities of uncertain significance in executive function, including in the perceptual index of the Wechsler Adult Intelligence Scale (WAIS), the executive subscores of the CANTAB battery and in the Trail Making Test. However there was no evidence of language or behavioural abnormalities. Three and a half years after onset he had developed frank weakness of his left upper and lower limb, associated with marked increase in tone with a mixed pyramidal/extrapyramidal quality, but without evidence of wasting or fasciculation. By four years after symptom onset he was confined to a wheelchair and fed through a percutaneous gastrostomy. Four and a half years after onset examination showed clear evidence of tongue wasting and fasciculation and spasticity was evident in all four limbs, with a left sided emphasis. He died five and a half years after the onset of an upper motor neuron predominant motor neuron syndrome, dominated initially by loss of speech, but spreading to the limbs, and associated with consistent but relatively mild loss of executive function.

Post-mortem neuropathology

A full post-mortem neuropathological assessment was conducted. On external examination there was very prominent focal cortical atrophy involving the entire motor region but without significant atrophy outside of the motor area (Fig 1C). Coronal slices revealed the grey/white matter was generally well demarcated. The lateral horn of the ventricle was not dilated and the hippocampi were of normal bulk. The substantia nigra was well pigmented. The aqueduct was patent and both the pons and medulla were within normal limits. The spinal cord was not particularly thin and the greying of anterior roots was not pronounced. Microscopic examination of pTDP-43, tau, β-amyloid, α-synuclein, CD68 and p62 on fourteen regions was

performed. No evidence of β-amyloid or α-synuclein was seen. There was severe reactive gliosis and transcortical spongiosis in the primary motor area, associated with significant neuronal loss including prominent depletion of the layer V Betz cells. p62 and pTDP-43 immunohistochemistry showed extensive white matter pathology which was at least as pronounced as grey matter pathology and consisted of neurites and perinuclear cytoplasmic inclusions as well as widespread glial inclusions (Fig 1H-K). No nuclear inclusions were seen. The hippocampus showed some intracellular neuronal tau accumulation but no apparent loss of neurons. There was very mild loss of substantia nigra neurons in the lateral pars compacta. The loci coerulei show no clear loss of pigmented neurons and there were no Lewy bodies. The cerebellum was relatively well preserved with possible mild Purkinje cell loss only, but with an abundance of p62-positive corpora amylacea within the molecular layer. The spinal cord showed severe degeneration of the corticospinal tract. Anterior horn cells were mildly depleted and there were only rare granular or cytoplasmic compact pTDP-43 inclusions. p62 highlighted glial aggregates which seem to be particularly prominent in the degenerated corticospinal tract.

Ethical approval

Tissue was donated to the Oxford brain bank, where consent and ethical approval for its use was provided under REC approval 15/SC/0639. DNA was extracted from post-mortem frontal cortex tissue and whole-exome sequenced as part of a separate study (Keogh et al, *Genome Research*, 2017)

Immunohistochemistry and western blotting

Immunohistochemistry and western blotting was performed on selected brain regions according to standard procedures. Primary antibody clones and dilutions are included in the supplementary material. RT-qPCR was performed on total RNA extracted from three regions of the index case and one neurologically normal control (Fig 1V; supplementary figure 1). After TRIzol extraction (Invitrogen), total RNA was treated with DNaseI (Invitrogen) and retro-transcribed (High capacity cDNA reverse transcription kit, Applied Biosystems). qPCR using Fast SYBR® Green Master Mix (Applied Biosystems) was run on a Roche LightCycler and melting curve analysis was performed on LightCycle Multicolor software. Target transcripts were *OPTN* and *TBK1*, and the internal reference was the combined expression of

TOPI, *RPL13A* and *ATP5B* whose stability has been previously described. Primers were designed based on the reference sequences in the National Centre for Biotechnology Information (NCBI) database and are listed in the supplementary material. Relative mRNA expression was calculated as $2^{-(\Delta Ct)}$ and plotted as a percentage.

Statistical analysis

Statistical analysis and graphing of gene expression was conducted using two-way ANOVA with Bonferonni multiple corrections in GraphPad prism software.

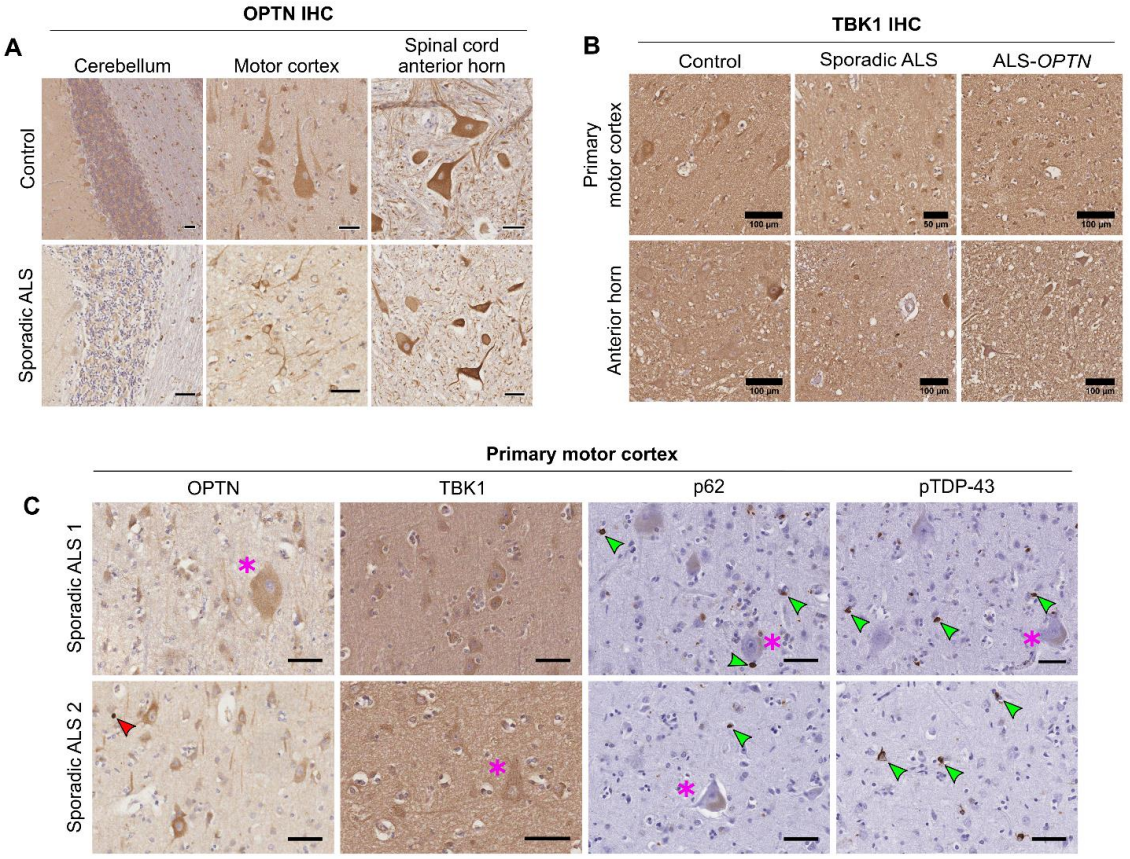
Primers

GENE	Forward	Reverse
TOP1 NM_003286.3	GTCCAAGCATAGCAACAGTGAAC	GCTCGAACCTTTTCGCTCTTTCG
RPL13A NM_012423.3	AGCTCATGAGGCTACGGAAAC	TTTATTGGGCTCAGACCAGGAG
ATP5B NM_001686.3	CCATTTGGCTGAGAGCACAG	TGCTGCACCAGAATCCAGTAC
OPTN (N-term) NM_001008211.1	ACTGAAAACGAGGACAGCCC	GCTTCTTTCACCTGGTCCTTCTC
OPTN (C-term) NM_001008211.1	AGACTGTTGGAAGCGAAGTG	CAAGGGCCTGACACTTTTCT
TBK1 NM_013254.3	GAAGAGGAGACAACAACAAGAC	TTCATTCCACCCACCACATC
TBK1 NM_013254.3	GGCTAAGGCAATAACAGGGGT	AATCAGCCATCGTATCCCCT

Primary antibodies

Antigen	Host	Clonality	Supplier	Catalogue #	IHC dilution	WB dilution
pTDP-43	Mouse	Monoclonal	Cosmo-bio	TIP-PTD-M01	1:40,000	-
p62	Rabbit	Polyclonal	Abcam	ab91526	1:1000	-
CD68	Mouse	Monoclonal	Dako	M0876	1:200	-
OPTN	Rabbit	Polyclonal	Proteintech	10837-1-AP	1:3000	1:1500
TBK1	Rabbit	Monoclonal	Abcam	ab40676	1:75	1:5000
GFAP	Mouse	Monoclonal	Sigma-Aldrich	C9205	1:2000	-
Tau	Mouse	Monoclonal	Innogenetics	90260	1:1500	-
β-amyloid	Mouse	Polyclonal	Biolegend	800709	1:24,000	-
α-synuclein	Mouse	Monoclonal	BD Biosciences	610786	1:1000	-

Use	Case	Regions	Age	Sex	PMD
WB, IHC, RT-qPCR	Control	Frontal cortex, spinal cord, cerebellum	57	M	46
	Sporadic ALS		65	M	48
	ALS-*OPTN*		54	M	72
IHC	Sporadic ALS	Motor cortex	65	M	Unknown
IHC	Sporadic ALS	Motor cortex	61	F	24



Supplementary figure:- Primers, antibodies and cases used listed. (A) Expression of OPTN is high across the brain in both control and sALS-TDP tissue in IHC, in contrast to OPTN expression in the index case. (B) TBK1 expression in this case was unaffected. (C) The staining patterns of p62 and pTDP-43, but not pTDP-43 and OPTN – are similar in sALS, suggesting the majority of pTDP-43-positive aggregates are OPTN negative. Green arrows highlight p62/pTDP-43 pathology, pink stars indicate Betz cells, red arrow indicates single example of OPTN-positive aggregate.