Letter

Isolated homozygous R217X *OPTN* mutation causes knock-out of functional C-terminal optineurin domains and associated oligodendrogliopathy-dominant ALS–TDP

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a heterogeneous neurodegenerative diseasecaused in a minority of individuals by mutations in more than one classical ALS-associated Mendelian gene, consistent with 'oligogenic' inheritance.[1] This observation complicates the dissection of precise genotype–phenotype relationships. In the absence of comprehensive genomic analysis (such as whole-exome sequencing) and molecular neuropathology, inferences of genotype–phenotype associations may be misleading, with potentially negative consequences for patient counselling, concepts of pathogenesis, disease modelling and patient selection for genomic therapeutics. Mutations in the autophagic adapter *OPTN* have been reported as causative of ALS[2] and are associated with diverse neuropathology, while also coexisting with other Mendelian ALS gene variants.[3 4]

To help clarify the role of *OPTN* variants in the pathogenesis of ALS, and refine genotype–phenotype associations, we provide a comprehensive genomic, neuropathological and biochemical analysis of an individual with a novel, isolated, homozygous R217X (c.649A>T) *OPTN* mutation and clinically upper motor neuron-dominant form of ALS-TDP with severe oligodendrogliopathy.

METHODS

The proband presented to the Oxford Motor Neuron Disease Clinic and enrolled in the brain donation programme of the Oxford Brain Bank, enabling integration of clinical observations with molecular neuropathological data, including whole exome-sequencing, repeat-primed PCR, *OPTN* mRNA and protein analyses, and comparison with both healthy brain tissue and that from sporadic (s) ALS-TDP patients. Please refer to online supplemental data for comprehensive methods.

RESULTS AND DISCUSSION

Clinical vignette

A middle-aged man presented with slowly progressive spastic dysarthria associated with an exaggerated jaw jerk and no other abnormal neurological findings. Dysarthria progressed to anarthria over 2 years and neuropsychometry reported mild abnormalities in executive function, but no evidence of language or behavioural abnormalities. Over the following 4 years, weakness with marked increase in tone but without wasting or fasciculations extended to all four limbs. Mild executive dysfunction continued but there was no progression to frontotemporal dementia. Tongue wasting and fasciculations, indicative of lower motor neuron involvement, only emerged in the last 6 months of life.

Whole-exome DNA sequencing

Whole-exome sequencing of DNA derived from frontal cortex revealed a novel, homozygous nonsense *OPTN* mutation (c.649A>T, p.R217X) which was absent from 368 simultaneously sequenced controls and from both the NCBI dbSNP and ExAC databases. No other relevant variants were identified.[5] In silico analysis predicted a stop-gain effect (SIFT, PolyPhen2), with a concomitant 62.4% reduction in protein length (figure 1A). The mutation meets multiple effect criteria making its pathogenic significance 'very strong' according to American College of Medical Geneticsguidelines.

Neuropathology

There was pronounced, symmetrical cortical atrophy of the primary motor cortex (figure 1C). Severe neuronal loss, gliosis and spongiosis of the motor cortex was associated with cortical and subcortical loss of myelin, which was absent from the sensory cortex (figure 1D–G). Immunohistochemistry (IHC) for TDP-43 hyperphosphorylated at serines 409/410 (pTDP-43) demonstrated an unusual pattern of oligodendroglia-dominant pTDP-43 proteinopathy (figure 1H–K). Motor cortical neuronal pTDP-43 pathology was less abundant but in keeping with that seen in classical sALS-TDP (granular 'preinclusions' merging with compact cytoplasmic inclusions (figure 1I) and short neurites). Minor neuronal pTDP-43 pathology was present in the lower motor neurons, including NXII (hypoglossal). Oligodendroglial pTDP-43 pathology was seen in white matter tracts such as the corpus callosum, corticospinal tract and also in cerebellar white matter (figure 1J,K). Rare, mostly pre-tangle, phospho-tau (AT8) pathology was seen in limbic and brainstem regions, consistent with primary age-related tauopathy (PART); there was no evidence of frontotemporal lobar dementia (FTLD)-Tau or FTLD-TDP. No other neurodegenerative disease-associated proteinaceous deposits were present (including *C9ORF72*-repeat or CAG-repeat expansion neuropathology).

Optineurin expression

Staining for C-terminal OPTN protein (using an antibody targeted against amino acids 233–577) was entirely absent in cortex, cerebellum and spinal cord using both western blot (figure 1U) and IHC (figure 1N–P and T). *OPTN* RNA was detectable, but severely reduced compared with normal brain (figure 1V).

The OPTN–TBK1–SQSTM1 axis in ALS–*OPTN* and sporadic ALS–TDP

The OPTN–TBK1–SQSTM1 axis is essential for protein and organelle homeostasis via regulation of endosomal–lysosomal processes and autophagy. Genetic evidence suggests that pathogenic variants in all three members of this pathway are sufficient to drive ALS–TDP.[6] As OPTN, TBK1 and SQSTM1 proteins are thought to function as an adapter complex that binds to proteins marked for degradation, we examined whether its constituents are recruited into pTDP-43 aggregates in our *OPTN* knock-out case or sALS–TDP. We also looked for obvious cell-type-specific expression patterns of OPTN protein that may provide clues to selective vulnerability to TDP-43 proteinopathy. We found that in R217X *OPTN* and sALS–TDP brain, SQSTM1 protein is consistently colocalised with compact (but not granular) pTDP-43 aggregates (figure 1L and online supplemental figure). Neither TBK1 nor OPTN colocalised to aggregates in a similar manner to SQSTM1 (figure 1M and online supplemental figure). Screening of normal human brain for differential expression of physiological OPTN protein in the absence of disease revealed evidence of strong expression in both Betz and anterior horn cells as well as the corticospinal tract (figure 1Q–S). This pattern is completely abolished in R217X *OPTN* spinal cord (figure 1T).

CONCLUSIONS

We report a novel, homozygous *OPTN* R217X mutation associated with upper motor neuron dominant ALS–TDP and pronounced oligodenrogliopathy. Our approach of comprehensive genomics

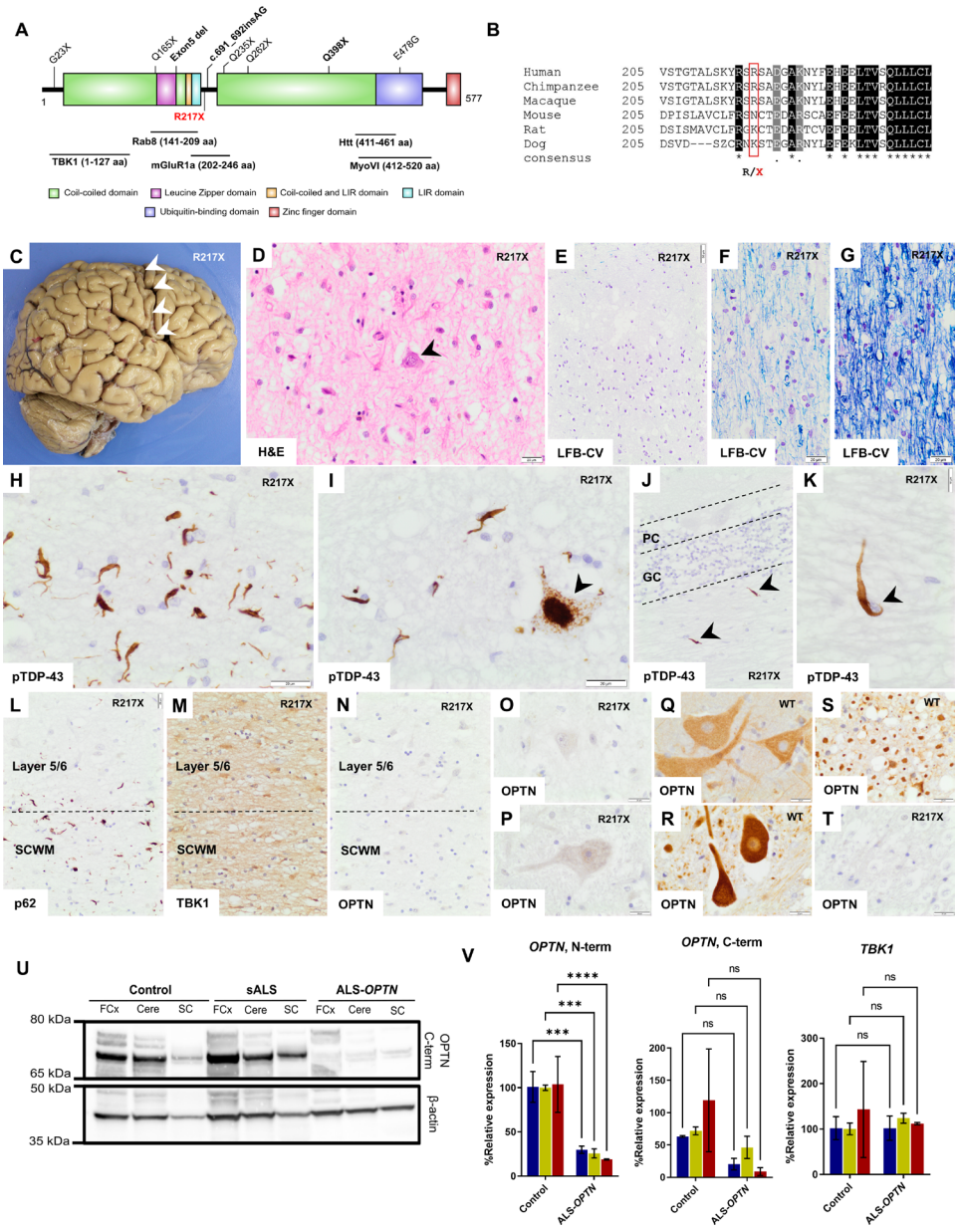


Figure 1 Genetics, neuropathology and biochemistry of the R217X *OPTN* mutation. Genetics: (A) The mutation affects the 217aa residue, between the LC3-interacting region (LIR) domain and the largest coil-coiled domain. Previously reported nonsense mutations are shown, homozygous mutations are in bold. The c.649A>T mutation (red) results in a premature stop codon, truncating the protein by 62.4% and preventing the translation of three C-terminal functional domains. (B) The mutation occurs at a residue conserved across primates but not other mammals (red box). Neuropathology: (C) Lateral view of the right hemisphere. Striking, highly selective atrophy of the primary motor cortex (arrows), with (D) near total loss of neurons; one shrunken presumed Betz cell is seen (arrow). Myelin pallor and spongiosis in motor cortex (E) and its subcortical white matter (F); compare with preservation of myelin (blue) in subcortical white matter of the primary sensory cortex (G). The great majority of pTDP-43 aggregates are present in oligodendroglia in the lower layers and subcortex of the motor cortex (H), medulla (I) and cerebellum (J, K, arrows). A granular/compact neuronal pTDP-43 inclusion is seen in a medullary neuron (I, arrow). p62, but not TBK1 or OPTN protein, colocalises with pTDP-43 aggregates in the *OPTN* R127X mutant motor cortex (L–N). Complete loss of C-terminal OPTN protein staining is highlighted in layer five motor cortex (O), alpha-motoneurons of the spinal cord (P) and lateral corticospinal tract (CST) (T). Contrast this with strong cytoplasmic OPTN expression in Betz cells (Q), alpha-motoneurons (R) and oligodendroglia and presumed corticospinal axons in the CST (S). Biochemistry: Western blotting for C-terminal OPTN protein confirms the immunohistochemical observations (U). qRT-PCR analysis (V) suggests *OPTN* expression is greatly reduced by the mutation. OPTN binding partner *TBK1* mRNA seems unaffected.

(which excluded oligogenicity) combined with analysis of *OPTN* mRNA and protein expression in brain makes it likely that *OPTN* R217X is the driver of the disease phenotype in this patient. Our data allow us to speculate that an intact C-terminal OPTN domain may be essential for maintenance of TDP-43 protein homeostasis in vulnerable cells of the human brain, inlcuding oligodendrocytes; however, this must await confirmation in the appropriate model systems. Finally, we observe that *OPTN* expression is not uniform across cells in the healthy adult brain and that SQSTM1 protein seems to be the only component of the OPTN–TBK1–SQSTM1 axis consistently and robustly colocalised with compact pTDP-43 protein aggregates in sALS–TDP (contrasting with previous observations[7]). Wethereforesuggest that a systematic - including mechanistic - analysis of this proteostatic pathway

in the context of ALS–TDP pathogenesis and selective vulnerability to TDP-43 proteinopathy is warranted, as this may yield tractable targets for therapy.

Matthew Nolan ,[1] **Paola Barbagallo,**[1] **Martin R Turner** ,[1] **Michael John Keogh,**[2] **Patrick F Chinnery,**[3] **Kevin Talbot** ,[1] **Olaf Ansorge**[1]

[1]Nuffield Department of Clinical Neurosciences, University of Oxford, Oxford, Oxfordshire, UK
[2]Biosciences Institute, Newcastle University, Newcastle upon Tyne, UK
[3]Department of Clinical Neurosciences, MRC Mitochondrial Biology Unit, Cambridge, UK

Correspondence to Dr Olaf Ansorge, Nuffield Department of Clinical Neurosciences, University of Oxford, Oxford OX1 2JD, UK; olaf.ansorge@ndcn.ox.ac.uk

Twitter Matthew Nolan @matthew__nolan

Acknowledgements We are grateful to the Oxford Brain Bank for providing the tissue used in this study, and thank the laboratory staff within the Academic Unit of Neuropathology, Oxford, as well as the donor and their family.

Contributors MN implemented the study and wrote the manuscript. PB performed the immunoblot and PCR analyses. OA conceived the study, performed neuropathological analysis and wrote the manuscript. KT was the diagnosing clinical neurologist and wrote the clinical summary. MJK and PFC performed the DNA analysis. Manuscript was contributed to and approved by MN, PB, OA, KT, MRT, MJK, PFC.

Funding This study was funded by Motor Neurone Disease Association (Ansorge/Oct14/977-792). MN was funded by a PhD studentship from the Motor Neurone Disease Association (grant # Ansorge/Oct14/977-792). KT receives funding from the Motor Neurone Disease Association, SMA Trust and Medical Research Council. We gratefully acknowledge support by the Motor Neurone Disease Association, the Medical Research Council, Brains for Dementia Research (Alzheimer Society and Alzheimer Research UK) and the National Institute for Health Research Oxford Biomedical Research Centre.

Disclaimer The views expressed are those of the authors and not necessarily those of the National Health Service (NHS), the National Institute for Health Research (NIHR) or the Department of Health. This work uses data provided by patients and collected by the NHS as part of their care and support and would not have been possible without access to this data. The NIHR recognises and values the role of patient data, securely accessed and stored, both in underpinning and leading to improvements in research and care.

Competing interests None declared.

Patient consent for publication Not required.

Provenance and peer review Not commissioned; externally peer reviewed.

► Additional material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/jnnp-2020-325803).

To cite Nolan M, Barbagallo P, Turner MR, *et al*. *J Neurol Neurosurg Psychiatry* 2021;**92**:1022–1024.

Received 14 December 2020
Revised 28 January 2021
Accepted 2 February 2021
Published Online First 16 March 2021

J Neurol Neurosurg Psychiatry 2021;**92**:1022–1024. doi:10.1136/jnnp-2020-325803

ORCID iDs
Matthew Nolan http://orcid.org/0000-0002-7204-5385
Martin R Turner http://orcid.org/0000-0003-0267-3180
Kevin Talbot http://orcid.org/0000-0001-5490-1697

REFERENCES

1 van Blitterswijk M, van Es MA, Hennekam EAM, *et al*. Evidence for an oligogenic basis of amyotrophic lateral sclerosis. *Hum Mol Genet* 2012;21:3776–84.
2 Maruyama H, Morino H, Ito H, *et al*. Mutations of optineurin in amyotrophic lateral sclerosis. *Nature* 2010;465:223–6.
3 Pottier C, Bieniek KF, Finch N, *et al*. Whole-genome sequencing reveals important role for TBK1 and OPTN mutations in frontotemporal lobar degeneration without motor neuron disease. *Acta Neuropathol* 2015;130:77–92.
4 Ayaki T, Ito H, Komure O, *et al*. Multiple proteinopathies in familial ALS cases with optineurin mutations. *J Neuropathol Exp Neurol* 2018;77:128–38.
5 Keogh MJ, Wei W, Wilson I, *et al*. Genetic compendium of 1511 human brains available through the UK medical Research Council brain banks network resource. *Genome Res* 2017;27:165–73.
6 Cirulli ET, Lasseigne BN, Petrovski S, *et al*. Exome sequencing in amyotrophic lateral sclerosis identifies risk genes and pathways. *Science* 2015;347:1436–41.
7 Hortobágyi T, Troakes C, Nishimura AL, *et al*. Optineurin inclusions occur in a minority of TDP-43 positive ALS and FTLD-TDP cases and are rarely observed in other neurodegenerative disorders. *Acta Neuropathol* 2011;121:519–27.

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.

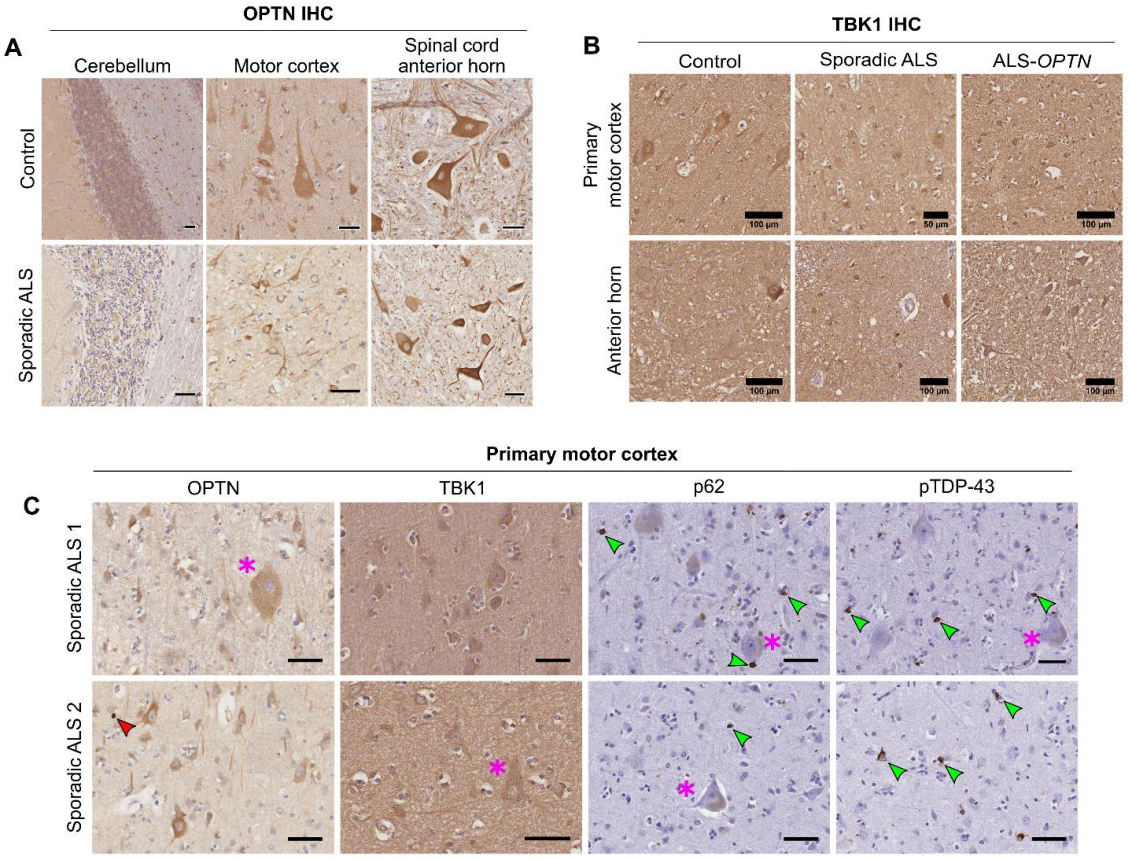
Primers

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.