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

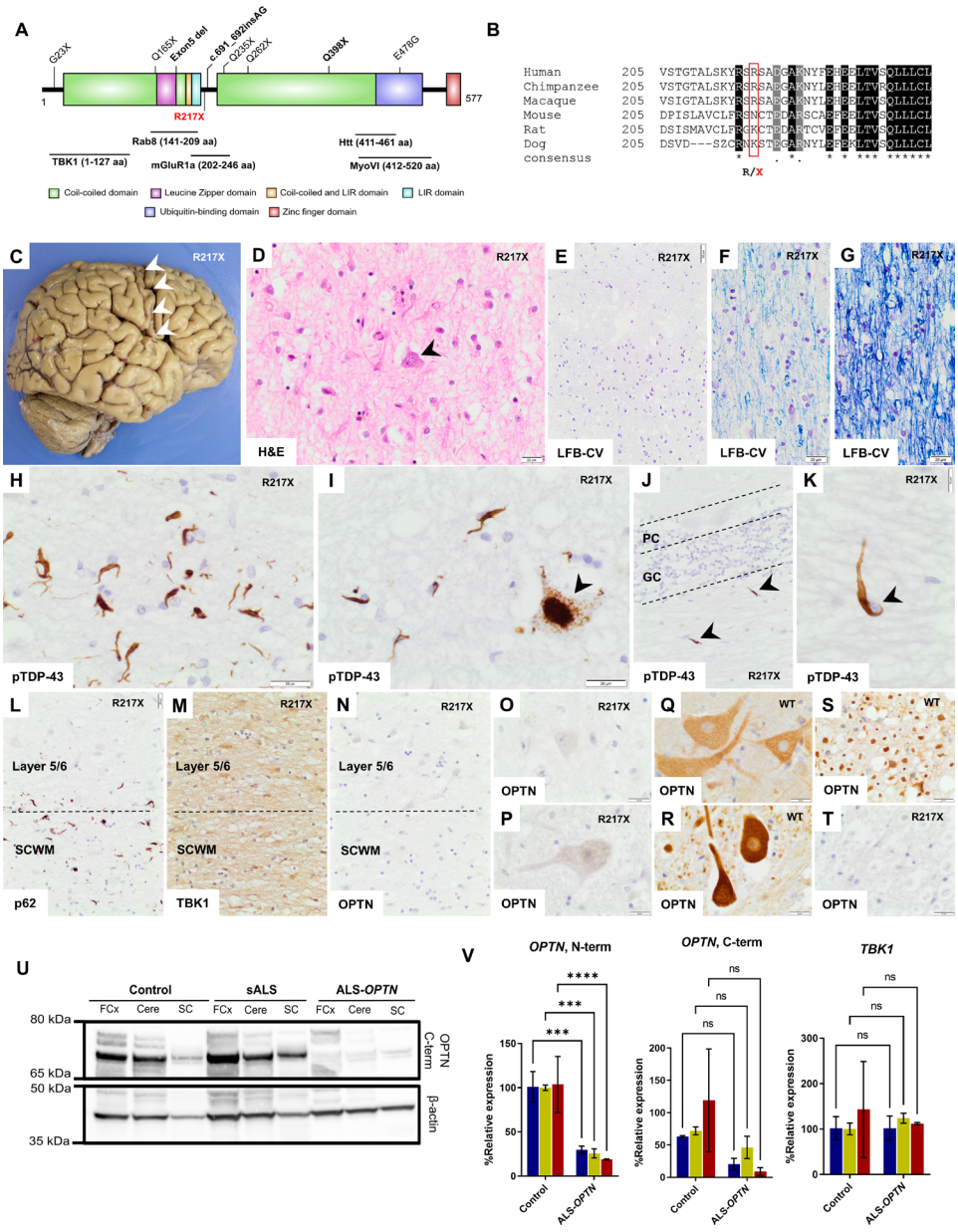

Figure 1 Genetics, neuropathology and biochemistry of the R217X *OPTN* mutation. Genetics: (A) The mutation affects the 217aa residue, between the LC3-interacting region (LIR) domain and the largest coil-coiled domain. Previously reported nonsense mutations are shown, homozygous mutations are in bold. 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.

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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.

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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.