Letter

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

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a heterogeneous neurodegenerative disease caused 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 ALS² 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 oligodendroglialopathy.

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 Genetics guidelines.

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 oligodendroglialopathy. 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* R217X 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, including 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 observations⁷). We therefore suggest that a systematic - including mechanistic - analysis of this proteostatic pathway

in the context of ALS-TDP pathogenesis and selective vulnerability to TDP-43 prote-inopathy 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 Ansorge¹

¹Nuffield Department of Clinical Neurosciences,
University of Oxford, Oxford, Oxfordshire, UK

²Biosciences Institute, Newcastle University, Newcastle
upon Tyne, UK

³Department 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.

Supplemental material This content has been
supplied by the author(s). It has not been vetted by
BMJ Publishing Group Limited (BMJ) and may not have
been peer-reviewed. Any opinions or recommendations
discussed are solely those of the author(s) and are
not endorsed by BMJ. BMJ disclaims all liability and
responsibility arising from any reliance placed on the
content. Where the content includes any translated
material, BMJ does not warrant the accuracy and
reliability of the translations (including but not limited
to local regulations, clinical guidelines, terminology,
drug names and drug dosages), and is not responsible
for any error and/or omissions arising from translation
and adaptation or otherwise.

OPEN ACCESS

Open access This is an open access article distributed
in accordance with the Creative Commons Attribution
Non Commercial (CC BY-NC 4.0) license, which permits
others to distribute, remix, adapt, build upon this work
non-commercially, and license their derivative works on
different terms, provided the original work is properly
cited, appropriate credit is given, any changes made
indicated, and the use is non-commercial. See: [http://
creativecommons.org/licenses/by-nc/4.0/](http://creativecommons.org/licenses/by-nc/4.0/).

© Author(s) (or their employer(s)) 2021. Re-use
permitted under CC BY-NC. No commercial re-use. See
rights and permissions. Published by BMJ.

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

- 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.
- Maruyama H, Morino H, Ito H, *et al.* Mutations of optineurin in amyotrophic lateral sclerosis. *Nature* 2010;**465**:223–6.
- 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.
- 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.
- 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.
- Cirulli ET, Lasseigne BN, Petrovski S, *et al.* Exome sequencing in amyotrophic lateral sclerosis identifies risk genes and pathways. *Science* 2015;**347**:1436–41.
- Hortobágyi T, Troakes C, Nishimura AL, *et al.* Optineurin inclusions occur in a minority of TDP-43 positive ALS and FTLT-TDP cases and are rarely observed in other neurodegenerative disorders. *Acta Neuropathol* 2011;**121**:519–27.