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Brief communication

C9ORF72 and *UBQLN2* mutations are causes of amyotrophic lateral sclerosis in New Zealand: a genetic and pathologic study using banked human brain tissue

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ABSTRACT

Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disease, which causes progressive and eventually fatal loss of motor function. Here, we describe genetic and pathologic characterization of brain tissue banked from 19 ALS patients over nearly 20 years at the Department of Anatomy and the Centre for Brain Research, University of Auckland, New Zealand. We screened for mutations in *SOD1*, *TARDBP*, *FUS*, and *C9ORF72* genes and for neuropathology caused by phosphorylated TDP-43, dipeptide repeats (DPRs), and ubiquilin. We identified 2 cases with *C9ORF72* repeat expansions. Both harbored phosphorylated TDP-43 and DPR inclusions. We show that DPR inclusions can incorporate or occur independently of ubiquilin. We also identified 1 case with a *UBQLN2* mutation, which showed phosphorylated TDP-43 and characteristic ubiquilin protein inclusions. This is the first study of ALS genetics in New Zealand, adding New Zealand to the growing list of countries in which *C9ORF72* repeat expansion and *UBQLN2* mutations are detected in ALS cases.

1. Introduction

The Neurological Foundation of New Zealand Human Brain Bank at the Centre for Brain Research, University of Auckland was established in 1994 to collect brain and associated tissue from donors with neurologic diseases including amyotrophic lateral sclerosis (ALS). With a growing number of genetic causes of ALS identified by researchers internationally, we sought to examine the genetics and neuropathology of our ALS brain bank cases as a subset of ALS patients in New Zealand.

The genetic basis for approximately 18% of ALS cases is accounted for, with the GGGGCC expansion mutation in intron 1 of *C9ORF72* alone comprising approximately 10% of all ALS. Exonic mutations in *SOD1*, *FUS*, and *TARDBP*, along with rarer ALS genes (*UBQLN2*, *OPTN*, *TBK1*, *SQSTM1*, and so forth) found predominantly in familial ALS (FALS) cases, comprise the remaining cases (Renton et al., 2014). Despite the diverse genetic and environmental factors, approximately 97% of all ALS cases show a common neuropathology characterized by the deposition of phosphorylated TDP-43 aggregates (Neumann et al., 2006). The presence of additional protein aggregates can indicate specific genetic causes of ALS. For example, in addition to TDP-43 pathology, *C9ORF72* mutations also cause dipeptide repeat (DPR) protein aggregates in the cerebellum and hippocampus (Ash et al., 2013; Mori et al., 2013). Conversely, *FUS* and *SOD1* mutant cases do not show TDP-43 proteinopathy but rather deposition of the mutant proteins themselves (Mackenzie et al., 2007; Vance et al., 2009). Neuropathological analyses can therefore be used in conjunction with genetics to fully characterize ALS cases.

Here we examine genetic and pathologic features of ALS in New Zealand using banked brain tissue. We identify *C9ORF72* and

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UBQLN2 as genetic causes of ALS in New Zealand and confirm that cases with these mutations display characteristic neuropathological lesions. These findings will allow us to best utilize and share our existing bequests as well as establishing a platform for larger genetic and neuropathological studies of ALS in New Zealand.

2. Materials and methods

2.1. Subjects, ethics, and human tissue banking

Brain tissue was collected from 4 controls and 19 ALS cases through the Neurological Foundation of New Zealand Human Brain Bank from 1995–2013. Patient demographics are shown in Table 1. Clinical and neuropathological diagnoses were performed by consultant neurologists and neuropathologists at Auckland City or Middlemore Hospitals, Auckland, New Zealand. Informed written consent was obtained from all donors and next of kin. Ethical approval was granted by the University of Auckland Human Participants Ethics Committee.

2.2. DNA extraction from fixed and fresh brain tissue

One hundred milligrams fixed-frozen tissue and/or 300 mg fresh-frozen tissue from lateral cerebellum and/or rostral superior frontal gyrus was used. DNA was extracted from fresh tissue (n = 10 cases) using standard phenol-chloroform-isoamyl alcohol extraction, and DNA cleanup was performed using Microclean (Microzone). DNA was extracted from fixed-frozen tissue (n = 17 cases) using the QiaAmp FFPE Tissue Kit (Qiagen).

2.3. Sanger sequencing and repeat-primed polymerase chain reaction (PCR)

All coding exons and flanking sequence of *SOD1* (Refseq NM_000454), exon 6 of *TARDBP* (Refseq NM_007375), and exons 6, 14, and 15 of *FUS* (Refseq NM_001170937) were amplified using standard PCR and directly sequenced with Big-Dye Terminator v1.1 on an ABI3130 DNA analyzer (Applied Biosystems). Samples were screened for the *C9ORF72* GGGGCC repeat expansion mutation using repeat-primed PCR (DeJesus-Hernandez et al., 2011) and fragment analysis conducted on an ABI3130 DNA analyzer and peaks visualized using Genemapper 4.0.

2.4. Neuropathology

Fifty micron free-floating sections were taken from fixed-frozen sensory-motor cortex blocks or hippocampus blocks and immunohistochemistry performed using antibodies listed in Supplementary material (Waldvogel et al., 2006, 2008). Wide-field images were acquired using a Nikon Eclipse N*i* microscope (20× magnification, 0.13 NA) and confocal images sourced using a Zeiss LSM 710 inverted confocal microscope (63× magnification, 1.4 NA, Z-step 0.34 μm) with ZEN 2012 software (Carl Zeiss). Maximum intensity Z-projections and orthogonal projections were generated using ImageJ software (http://imagej.nih.gov/ij/).

3. Results

3.1. Demographics of ALS in our New Zealand cohort

Patient demographics are shown in Table 1.

A detailed clinical description of cases harboring mutations can be found in Supplementary Material.

3.2. Genetic analysis of banked ALS cases

One of our cases had tested positive for *UBQLN2* p.T487I mutation in life [patient IV:18 in (Williams et al., 2012)]. Another case tested negative for *C9ORF72*, *SOD1*, *TARDBP*, and *FUS* during life. All other cases were screened for these genes using DNA extracted from brain. Fresh-frozen brain DNA, available for 10 cases, gave a high rate of sequencing success. Fixed-frozen brain DNA gave only approximately 50% viable sequence. We identified 1 FALS patient (MN18) with *C9ORF72* repeat expansion (Fig. 1A). However, DNA was not available from other affected family members to test segregation.

3.3. Neuropathology of banked ALS cases

We next conducted immunohistochemistry on all 19 cases. All showed classical phosphorylated TDP-43 deposits in the motor cortex (Fig. 1B), thus ruling out *SOD1* and *FUS* gene mutations (Mackenzie et al., 2007; Vance et al., 2009). No phosphorylated TDP-43 inclusions were seen in controls nor when phospho-TDP-43 primary antibody was omitted. We also screened using an antibody to the poly-glycine-proline (poly-GP) DPR (Ash et al., 2013). The *C9ORF72*-positive case (MN18) showed hallmark deposition of DPR aggregates in the dentate gyrus and cornus ammonis regions of the hippocampus (Fig. 1C [a–d]). A second FALS case (MN2) also showed DPRs. For this case, *C9ORF72* repeat-primed PCR of fixed-frozen brain DNA had been unsuccessful. However, follow-up with the patient's family revealed an affected offspring had tested positive. No DPR staining was seen in control hippocampus, any *C9ORF72*-negative ALS cases, nor when primary antibody was omitted. Finally, by screening using an antibody to ubiquilin 2 we confirmed that the case with *UBQLN2* p.T487I mutation (MN17) showed deposition of ubiquilin in the hippocampus, particularly in the parahippocampal region and molecular layer of the dentate gyrus but not in the granular layer (Fig. 1D [a–d]). In cases with *C9ORF72* mutation, ubiquilin-positive inclusions were observed in the cornus ammonis regions and in both the granular and molecular layers (Fig. 1D [e–h]). Using double-label confocal microscopy,

Table 1
Demographics of ALS in our New Zealand cohort

Parameter	Controls (n = 4)	ALS cases		
		All cases (n = 19)	*C9ORF72*-negative (n = 17)	*C9ORF72*-positive (n = 2)
Male: female (n)	50% (2):50% (2)	47% (9):53% (10)	53% (9):47% (8)	0%:100% (2)
Family history of ALS (n)	—	26% (5)	18% (3)	100% (2)
Mean age at onset (years ± SD, range)	—	58.3 ± 14.9, 32–87	59.3 ± 15.9, 32–87	49.5 ± 3.5, 47–52
Mean age at death (years ± SD, range)	64.3 ± 2.9, 60–67	61.7 ± 13.5, 41–88	62.8 ± 14.3, 41–88	53.0 ± 0, 53–53
Mean postmortem delay (hours ± SD, range)	19.1 ± 6.9, 9–23.5	20.8 ± 17.1, 3–69[a]		

Key: ALS, amyotrophic lateral sclerosis.
[a] Excluding MN2 for which this information was unavailable.