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# *FUS* mutations in amyotrophic lateral sclerosis: clinical, pathological, neurophysiological and genetic analysis

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## ABSTRACT

**Objective** *FUS* gene mutations were recently identified in familial amyotrophic lateral sclerosis (ALS). The present studies sought to define the clinical, post-mortem and neurophysiological phenotypes in ALS families with *FUS* mutations and to determine the frequency of *FUS* mutations in familial and sporadic ALS.

**Methods** *FUS* was screened for mutations in familial and sporadic ALS cases. Clinical, post-mortem and neurophysiological features of large families with *FUS* mutations are described.

**Results and conclusions** *FUS* mutations were evident in 3.2% (4/124) of familial ALS, representing the second most common gene abnormality to be described in familial ALS after *SOD1*. No mutations were present in 247 sporadic ALS cases. The clinical presentation in 49 affected patients was consistent with a predominantly lower motor neuron disorder, supported by post-mortem findings. Upper motor neuron involvement varied, with Wallerian degeneration of corticospinal tracts present in one post-mortem case but absent in a second case from the same family. Features of cortical hyperexcitability demonstrated upper motor neuron involvement consistent with other forms of familial and sporadic ALS. One case presented with frontotemporal dementia (FTD) indicating that this may be a rare presenting feature in families with *FUS* mutation. Ubiquitin-positive cytoplasmic skein-like inclusions were present in lower motor neurons, but in contrast to sporadic ALS, no TDP-43 pathology was evident. Mutation-specific clinical features were identified. Patients with a R521C mutation were significantly more likely to develop disease at a younger age, and dropped-head syndrome was a frequent feature. Reduced disease penetrance was evident among most affected families.

## INTRODUCTION

Amyotrophic lateral sclerosis (ALS) leads to paralysis of voluntary muscles due to the progressive death of motor neurons in the brain and spinal cord.<sup>1,2</sup> ALS typically results in death within 3–5 years of first symptoms, usually related to respiratory complications. A proportion of patients may also develop clinical or subclinical frontotemporal dementia (FTD). The incidence of ALS in the population is around 1–2 per 100 000 and prevalence 4–6 per 100 000.<sup>3</sup> Ubiquitination of misfolded proteins that aggregate in affected cells are pathological hallmarks of ALS and FTD. Misfolded TAR DNA-binding protein (TDP-43),

which is involved in mRNA processing, is a principal component of these ubiquitin-positive inclusions in about 90% of cases.<sup>4–6</sup>

Approximately 10% of ALS cases have a positive family history (familial ALS) and appear clinically indistinguishable from sporadic ALS cases. Of these familial cases, 10–20% are caused by mutations in the superoxide dismutase 1 gene (*SOD1*).<sup>7,8</sup> The *TARDBP* gene, which encodes TDP-43, was also confidently linked to familial ALS, and rare mutations in this gene have now been described in both familial and sporadic ALS cases.<sup>9–11</sup> Mutations in the angiogenin (*ANG*) and dynactin (*DCTN1*) genes have also been reported in a small number of ALS cases. A mutation in the *VAPB* gene has been described in several families with atypical ALS linked to chromosome 20, although only one individual had classic ALS, and remaining individuals showed spinal muscular atrophy (SMA).<sup>12</sup> Mutations in the *CHMP2B* gene have been reported in a small number of atypical ALS cases,<sup>13</sup> but analysis of classic ALS cohorts suggest that this gene is not a common cause of familial or sporadic ALS.<sup>14</sup> Other ALS loci have been identified on chromosomes 18q21 and 20q13 with no discrete genes identified to date. Families with ALS-FTD have also been linked to chromosome 9.<sup>8</sup>

More recently, mutations in the fused in sarcoma gene (*FUS*, also known as translocated in liposarcoma, *TLS*), an RNA-processing gene that is functionally related to *TARDBP*, were identified in large ALS families linked to chromosome 16q12.<sup>15,16</sup> Mutations were also identified in several small ALS families and apparent sporadic cases supporting the pathogenic role of mutations in this gene.<sup>11,17–20</sup>

The present study was undertaken to establish the frequency of *FUS* mutations in familial and sporadic ALS patients. In addition, the clinical, post-mortem and neurophysiological phenotypes in several large multigenerational families with *FUS* mutations are described for the first time.

## METHODS

### Subjects

Australian ALS families were ascertained through neurogenetic clinics at Concord Hospital, Sydney, and the Molecular Medicine Laboratory, Concord Hospital, a referral centre for *SOD1* DNA diagnostic testing. Two hundred and forty-seven Australian sporadic ALS cases were obtained from the Australian MND DNA bank. Patients were

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predominantly of European descent. A clinical diagnosis of definite or probable ALS was based on the findings of neurological examination, particularly the development of progressive wasting and weakness. ALS families included in *FUS* mutation analysis were previously screened and shown to be negative for genes *SOD1*, *TARDBP*, *ANG*, *DCTN1*, *VEGF* and *CHMP2B*. Patients and family members provided informed written consent in accordance with protocols approved by the human research ethics committee of the Sydney South West Area Health Service.

### Genetic analysis

Genomic DNA was extracted from the peripheral blood of family members and genotyped using standard methods. Two-point and multipoint linkage analysis was performed as previously described.<sup>10</sup>

### Mutation screening

All exons and at least 100 bp of flanking intronic sequence of *FUS* were amplified by the PCR and sequenced using Big-Dye terminator sequencing and ABI 3730XL automated sequencer (Applied Biosystems, Foster City, California). Sequencing primers were the same as those used in PCR amplification. Primers and amplification conditions are available from the authors on request.

### Cortical excitability testing

Cortical excitability was assessed by applying transcranial magnetic stimulation (TMS) to the motor cortex by means of a 90 mm circular coil, with currents generated by two high-power magnetic stimulators which were connected via a BiStim device (Magstim Co., Whitland, UK). A threshold tracking paradigm was applied to record the resting motor threshold (RMT) and short interval intracortical inhibition (SICI) as previously described.<sup>21</sup> In addition, single stimulus TMS was used to record stimulus response (SR) curves, central motor conduction time (CMCT) and cortical silent period (CSP) duration.

Compound muscle action potential (CMAP) and motor-evoked potential (MEP) recordings were obtained as previously described.<sup>22</sup>

### Immunostaining

The following antisera were used for immunohistochemistry: rabbit polyclonal antibodies to Ubiquitin (Dako, Carpinteria, California) and TDP-43 (Proteintech Group, Chicago, Illinois), using a standard streptavidin-biotinylated immunoperoxidase technique. In brief, sections were dewaxed using xylene and rehydrated through alcohols, and antigen retrieval was performed using citrate buffer (pH 6) for Ubiquitin and EDTA (pH 8) for TDP-43. Slides were washed twice in PBS (pH 7.4), and then endogenous peroxidase activity was quenched. Non-specific proteins were blocked using normal horse serum for 20 min. Either antibody was applied at 1:4000 at room temperature overnight. Sections were washed twice in PBS, and then a biotinylated antirabbit secondary (Vector Laboratories, Burlingame, California) was applied for 60 min at room temperature. Following two PBS washes, slides were incubated for 1 h at room temperature with a streptavidin-conjugated peroxidase tertiary (Pierce, Rockford, Illinois). Sections were visualised using diaminobenzidine tetrahydrochloride (DAB), washed, counterstained with haematoxylin, dehydrated, cleared and mounted on glass slides.

### Statistical analysis

Age-dependent penetrance distributions were compared by constructing contingency tables and performing a  $\chi^2$  analysis. All other statistical analyses of clinical data were performed using an unpaired t test. A two-tailed p value was calculated with the nominal  $\alpha$  value set at 0.05. Tests were performed using GraphPad software (<http://www.graphpad.com>).

Cortical excitability studies in two patients with *FUS* mutations were compared with data from 55 normal controls (28 men; 23–73 years, mean: 46 years) and 53 clinically probable or definite sporadic ALS patients, defined according to the revised El Escorial criteria<sup>23</sup> (34 men; 26–71 years, mean 59 years). Results are expressed as mean  $\pm$  SE of the mean.

## RESULTS

### Mutation analysis

The overall ALS cohort comprised 124 ALS families and 247 sporadic ALS cases. Twenty-one of the ALS families were previously shown to carry *SOD1* mutations through our role as a referral centre for *SOD1* DNA diagnostic testing. A subset of ALS families were previously screened for *FUS* mutations with three positive families identified.<sup>15</sup> To complete analysis of this overall cohort, we screened *FUS* for mutations in affected individuals from a further 33 ALS families, as well as the 247 sporadic ALS cases by directly sequencing exons and at least 100 bp of flanking intronic regions. No mutations were identified in sporadic ALS cases. A missense mutation (accession NM\_004960, c.1562G  $\rightarrow$  A, R521H) was identified and shown to segregate with disease in a large multigenerational family (family ALS2, figure 1A).

We recruited additional family members to substantially extend three other families with *FUS* mutations (families ALS31, ALS53 and ALS156, figure 1A) that were in part described previously.<sup>15</sup> *FUS* mutations segregated with all affected individuals including three newly recruited ALS cases and an obligate-carrier (figure 1B).

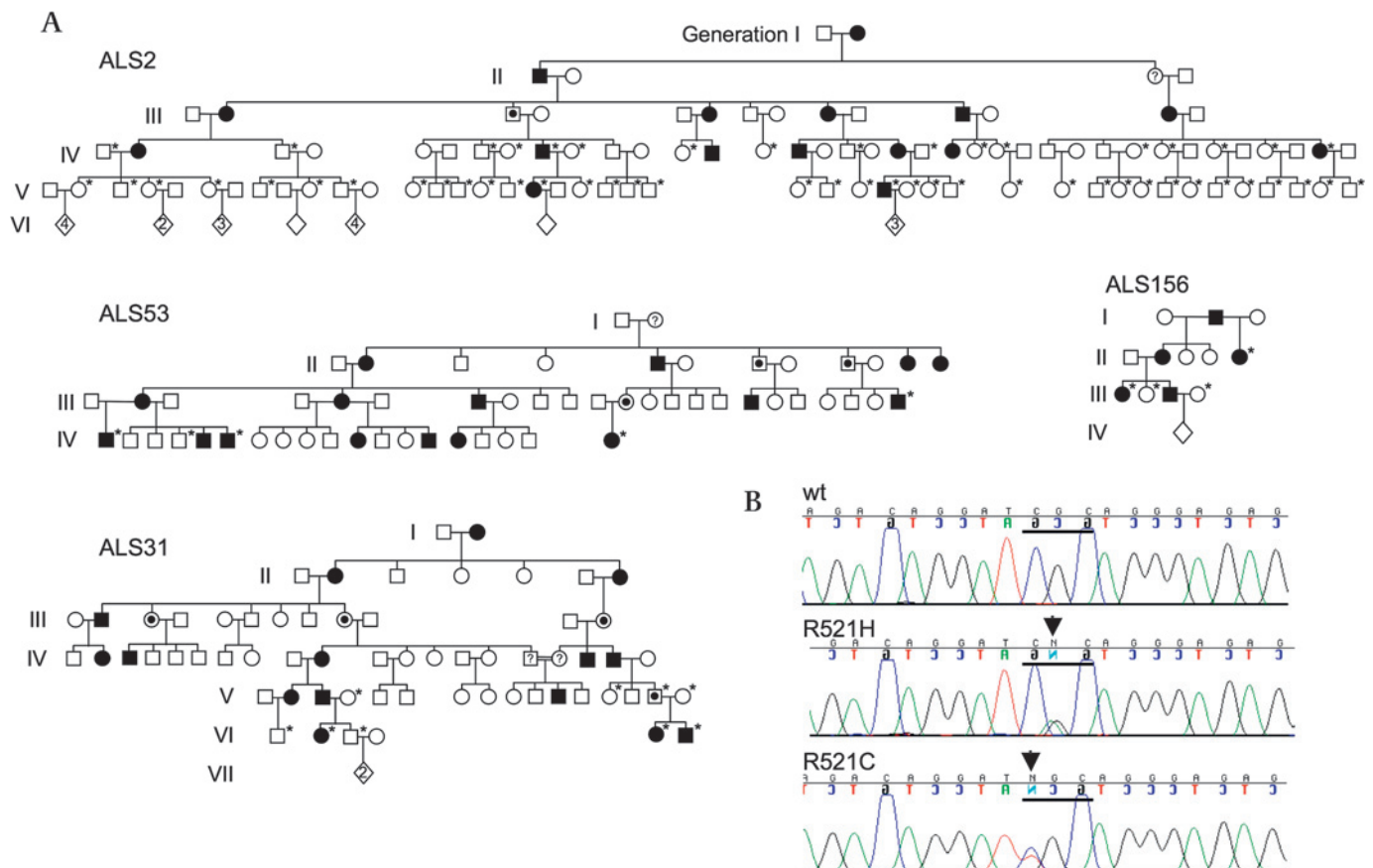
### Founder haplotype analysis

Linkage analysis in family ALS2 using microsatellite markers across the *FUS* locus confirmed that the disease was linked to the *FUS* R521H mutation (D16S690 lod=3.31, D16S753 lod=3.03, and D16S3044 lod=3.78; maximum multipoint lod score=4.54). Analysis of these microsatellite markers was undertaken across affected families (figure 1) to determine whether those that shared the same mutation were derived from the same common ancestor (founder effect). Minimum recombinant haplotypes for these markers were identified and compared (table 1). The families that share the R521C mutation shared no alleles for markers immediately adjacent to *FUS*, thereby providing no support for a common ancestor. Families that share the R521H mutation shared an allele for one marker (D16S685, allele 3) immediately adjacent to *FUS*. However, this shared allele was common in the population (43%).

### Illustrative case histories

#### Patient 1

Individual V-18, family ALS2 (figure 1 R521H mutation): 59-year-old woman presented with an 18-month history of progressive left foot weakness leading to frequent falls. The weakness progressed to involve proximal muscles in the lower limbs bilaterally. On examination, cranial nerves were intact. Upper limbs demonstrated symmetrically normal tone, power and reflexes. In the lower limbs, there was global weakness,



**Figure 1** *FUS* missense mutations in familial amyotrophic lateral sclerosis (ALS). (A) Pedigrees of ALS families that carry the R521H mutation (families ALS2 and ALS156) and R521C mutation (ALS31 and ALS53). Asterisks indicate DNA available for genotyping. Some individuals in recent generations have been omitted from the pedigrees for confidentiality. (B) Sequence traces from unaffected control and ALS cases illustrating the mutations that segregate with disease in these families. The arrowheads indicate the base positions at which *FUS* is mutated. The codon altered by the mutation is underlined.

more marked distally than proximally, with wasting of distal muscles and fasciculations. Reflexes were preserved in the lower limbs and plantars downgoing. The sensory examination was normal.

Neurophysiological investigations confirmed normal peripheral nerve conduction and reflex conduction (H-reflexes). F waves from the lower limbs demonstrated reduced persistence, consistent with loss of anterior horn cells. Electromyography demonstrated acute denervation particularly in the lower limbs, with limited evidence of reinnervation.

**Table 1** Comparison of chromosome 16 disease haplotypes from amyotrophic lateral sclerosis (ALS) families with *FUS* mutations

Marker	Mutation R521H		Mutation R521C		cM	Mb
	ALS2	ALS156	ALS31	ALS53		
D16S769	X				52.25	26.07
D16S690	289	X	X	X	56.77	27.87
D16S685	121	121	117	121	58.40	30.58
<i>FUS</i> mutation	A	A	T	T		31.11
D16S753	252	260	264	260	58.40	31.18
D16S3044	198	X	198	190	59.06	46.00
D16S541	154		150	150	63.37	49.15
D16S3034	X		X	X	67.57	51.70

Haplotype alleles are in base pairs.

X denotes an observed recombination event between the indicated marker and the disease within a family. For each family, the minimal haplotype that segregates with disease is boxed.

MRI scans of the brain and spinal cord confirmed no significant structural abnormality. Vasculitic screens were negative. Lumbar puncture demonstrated a mildly elevated CSF protein of 0.56 g/l (normal 0.15–0.45 g/l) with negative cytology. Oligoclonal bands were negative.

## Patient 2

From an individual VI-5, family ALS31 (figure 1, R521C mutation), a 35-year-old woman initially complained of neck pain. Three months later, she found that her head lagged when doing situp exercises. Two months later, she developed weakness of the right arm and shoulder followed by swallowing difficulties. After choking on food and developing weight loss, a gastrostomy tube was inserted. When seen 19 months after first symptoms, she had developed 'dropped head syndrome' and required a neck collar to hold her neck up. There was no wasting of the tongue or bulbar weakness. There was wasting of the neck and shoulder muscles with neck weakness MRC grade 3/5 and no movement at the shoulders. She could not elevate her arms above 45°. There was antigravity movement at the elbows, wrists and fingers. She could not sit up. There was MRC grade 4 hip flexion weakness, with normal power at the knees and ankle joints. The upper-limb reflexes were absent, and there were brisk lower-limb reflexes and flexor plantar responses. There was no sensory loss. Nerve-conduction studies demonstrated normal sensory responses in the upper and lower limbs. Compound motor action potentials were of reduced amplitude, without

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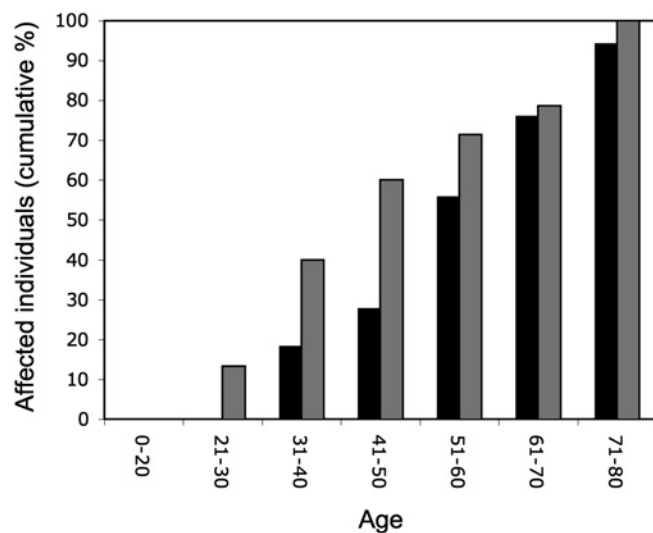
slowing of motor conduction or evidence of focal motor conduction block in clinically affected regions.

### Clinical features of FUS families

Family ALS2 was of Swedish ancestry, and families ALS31, ALS53 and ALS156 were of British ancestry. The clinical features seen in 49 affected individuals from these four families are described in the online supplementary table. Case histories and historical notes from families ALS2 and ALS31 are also provided in the online data supplement. The clinical presentation in all four families was consistent with a predominantly lower motor neuron disorder. Head weakness was evident as a common presenting feature among cases with the R521C mutation but was only seen as a later feature in one patient with the R521H mutation. One case in family ALS53 (individual IV-5) had FTD, and MRI showed atrophy of the frontotemporal regions.

The average age of disease onset in patients with the R521C and R521H mutations was 42.9 years and 50.7 years respectively, and these were not significantly different ( $p=0.14$ ). The average duration of disease in patients with the R521C and R521H mutation was 1.9 years and 3.0 years respectively, and these were also not significantly different ( $p=0.10$ ). There was a slight observed female bias among patients with *FUS* mutations, but this was not significant ( $p=0.50$ ; overall ratio 1.2:1; ranging from 1.0 to 1.4:1 across the four families).

A degree of non-penetrance of ALS was evident among the four families, although in some instances, unaffected individuals who carried a *FUS* mutation had died before attaining the average age of onset. The oldest known unaffected individual who carried a *FUS* mutation but showed no signs of ALS was aged 76 years. Ten unaffected individuals in family ALS2 were mutation carriers (including obligate carriers), and seven of these carriers were older than the average age of onset (52–76 years). The age-dependent penetrance of disease for each mutation was plotted (figure 2), taking into account the unaffected individuals who carried a *FUS* mutation (including obligate carriers) in each age category. This provided a likelihood estimate that an



**Figure 2** Plot of age-dependent penetrance of amyotrophic lateral sclerosis for individuals with *FUS* mutations from the four families shown in figure 1. Data for mutations R521H and R521C are shown in black and half-tone respectively. This provides a likelihood estimate that an individual who carries a mutation will be affected in a given age range. The age-dependent penetrance distributions for the R521H and R521C mutations are significantly different ( $p=0.0004$ ).

individual who carried a mutation would be affected in a given age range. The age-dependent penetrance distributions for the two described mutations were significantly different ( $p=0.0004$ ). Patients with the R521C mutation were significantly more likely to express the disease at a younger age than those with the R521H mutation.

### Assessment of cortical excitability

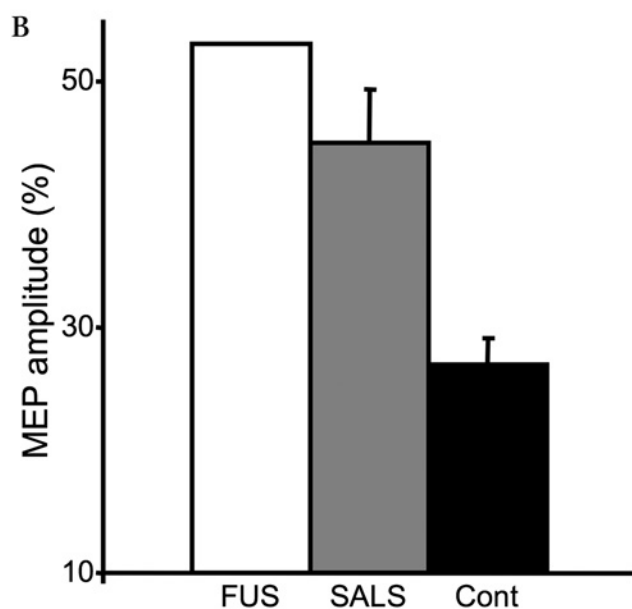
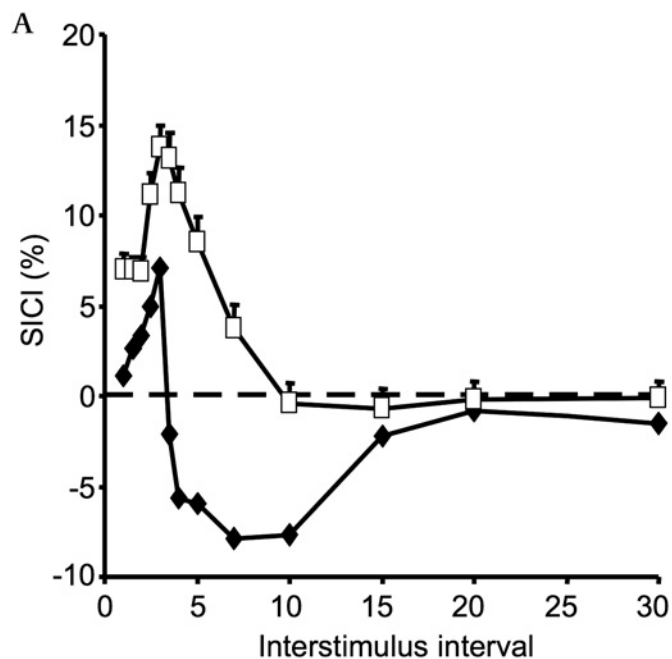
To clarify the pathophysiological mechanisms of neurodegeneration in patients with *FUS* mutations, assessment of cortical excitability was undertaken in two representative patients described earlier (family ALS2 individual V-18; and family ALS31 individual VI-5).<sup>24</sup> In these patients, SICI, defined as an increase in the test stimulus intensity required to track a constant target MEP response, was reduced (area under the curve,  $-0.3\%$ ) when compared with controls (area under the curve  $9.0\pm 0.8\%$ ,  $p<0.001$ ), although similar when compared with sporadic ALS patients ( $0.8\pm 1.2\%$ ) (figure 3A). Further, the MEP amplitude, expressed as a percentage of the CMAP amplitude recorded following electrical stimulation, was increased in the two patients with *FUS* mutations (53%) when compared with controls ( $27.0\pm 2.2\%$ , 95% CI 22.6 to 31.4%), and again similar when compared with sporadic ALS patients ( $45\pm 4.4\%$ , 95% CI 36.2 to 54.0%) (figure 3B).

The cortical silent period, defined as the period of electrical silence following an MEP response that interferes with ongoing EMG activity in a contracting muscle, was also assessed. In these affected patients, the increase in the CSP recruitment curve was non-linear, similar to the relationship established previously for sporadic ALS patients.<sup>21–25</sup> As stimulus intensity increased from 60 to 150% RMT, the CSP duration increased from 0 to 121 ms, and was shorter when compared with ALS patients ( $0-175\pm 8.8$  ms, 95% CI 157.4 to 192.6 ms) and controls ( $0-208.8\pm 4.8$  ms, 95% CI 199.2 to 218.4 ms). In contrast, RMT, defined as the unconditioned stimulus intensity required to produce and maintain the target MEP response, was similar in all groups (*FUS* patients, 50%; sporadic ALS  $57.9\pm 1.6\%$ ; controls  $60.7\pm 1.5\%$ ) as was the central motor conduction time (*FUS* patients, 4.8 ms; sporadic ALS,  $5.1\pm 0.2$  ms; controls,  $5.1\pm 0.2$  ms).

### Pathological features

A post-mortem examination was performed on patient III-2 from family ALS53. The brain was small. No gross lesions were externally visible or palpable. Coronal sections through the hemispheres showed no obvious gross abnormalities. The brainstem and cerebellum also appeared grossly normal. The spinal cord was small, and the anterior roots were very thin (sections taken at several levels). Examination of the medulla confirmed loss of neurons in hypoglossal and dorsal vagal nuclei. Neurons were sparse in the nucleus ambiguus. Anterior horn cells were very sparse. There was pallor of lateral and anterior white-matter columns and of cuneate tract at the upper cervical level. There was also pallor of lateral white-matter columns at mid-thoracic level with marked outfall of anterior horn cells. There were similar appearances in the lumbar cord. Overall, the appearance was that of progressive muscular atrophy with terminal bulbar palsy and little evidence of lateral sclerosis. The cause of death was defined as respiratory failure secondary to motor neuron disease.

Post-mortem examination was also performed on individual IV-5 of family ALS53 (the son of the above post-mortem subject) including immunostaining of brain and spinal cord tissue. This individual had progressive neurological decline of



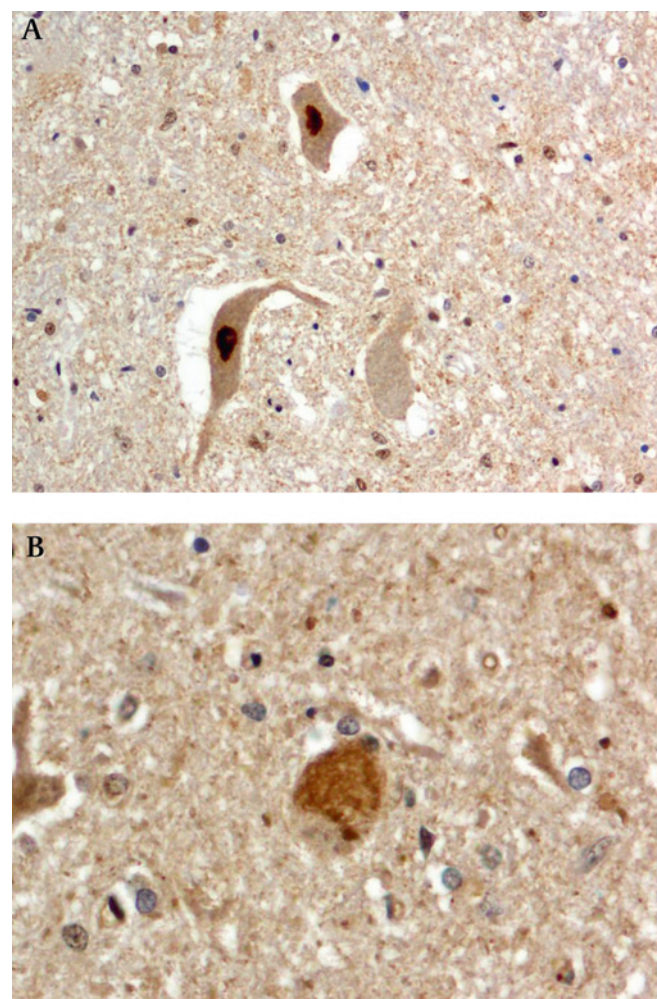
**Figure 3** Assessment of cortical excitability in amyotrophic lateral sclerosis (ALS) patients with *FUS* mutations. (A) Short-interval intracortical inhibition (SICI), defined as the stimulus intensity required to maintain a target output. This was reduced in familial ALS patients with *FUS* mutations (filled diamonds) when compared with controls (open boxes). (B) Motor-evoked potential (MEP), expressed as a percentage of compound muscle action potential (CMAP) amplitude. This was increased in familial ALS patients with *FUS* mutations and sporadic ALS (SALS) patients when compared with controls (Cont).

2-year disease duration including dysphagia, dystonia, dysarthria, unsteady gait and complex movement disorder. The spinal cord showed severe loss of anterior horn cells. There was no evidence of corticospinal degeneration. The midbrain showed pallor of the substantia nigra, and microscopic examination revealed severe loss of pigmented neurons with reactive gliosis. There were no Lewy bodies or  $\tau$  immunopositive neurofibrillar tangles. The basal ganglia (globus pallidus, putamen and caudate nuclei)

showed mild neuronal loss and reactive gliosis. TDP-43 immunostains showed normal nuclear staining (figure 4A) in all regions analysed including spinal cord segments, midbrain, medulla, hippocampus, frontal cortex, basal ganglia, subthalamic nucleus and thalamus. Ubiquitin immunostaining showed skein-like cytoplasmic staining (figure 4B) of some anterior horn cells, hypoglossal neurons, a subset of granule cells of the dentate gyrus and a small subset of pyramidal cells and neurites of the frontal cortex. Overall neuropathology established an unusual motor neuron and nigrostriatal degeneration with ubiquitin immunostaining (ubiquitinopathy), with no evidence of TDP-43 proteinopathy.

## DISCUSSION

The present series has identified the key clinical, neurophysiological and pathological findings across four large ALS kindreds with *FUS* mutations, incorporating data from 49 affected patients. *FUS* mutations accounted for the disease in 3.2% of familial ALS across the present cohort and thereby represent the second most common genetic abnormality to be established in familial ALS after *SOD1*. *SOD1* mutations were present in 16.9% of families consistent with previous studies of autosomal



**Figure 4** Immunostaining in *FUS* linked amyotrophic lateral sclerosis. (A) Surviving S3 anterior horn cells showing normal TDP-43 nuclear immunostaining ( $\times 200$  original magnification). (B) Hypoglossal motor neuron showing cytoplasmic ubiquitin immunoreactive skein-like staining ( $\times 400$  original magnification).

dominant familial ALS worldwide.<sup>8</sup> *FUS* mutations were established to be more common than TDP-43 mutations, which were present in 1.6% of families of the present cohort. The identified *FUS* mutations segregated with disease in each extended family, and the R521H mutation was strongly genetically linked to the disease in the largest kindred, ALS2. As such, data from the present series further support the pathogenic role of *FUS* mutations in familial ALS. In contrast, no mutations were identified in sporadic ALS cases. This is consistent with previous screens of sporadic ALS cohorts,<sup>11 20</sup> although rare mutations were recently identified in apparent sporadic cases from French and French Canadian populations.<sup>17</sup>

The clinical presentation across all families with *FUS* mutations was consistent with a predominantly lower motor neuron disorder, as supported by post-mortem findings. Nevertheless, some evidence of upper motor neuron involvement was provided by electrophysiological data that demonstrated cortical hyperexcitability, and post-mortem data that identified corticospinal tract degeneration in one case. In contrast, a second post-mortem case from the same family showed no corticospinal tract degeneration, demonstrating that upper motor neuron involvement is variably associated with *FUS*-linked ALS. Most cases (83%) with the R521H mutation had limb-onset disease. Disease onset associated with the R521C mutation was evenly distributed between bulbar and limb-onset cases. An inability to support the head at presentation, or dropped-head syndrome,<sup>26</sup> was a frequent feature in families with the R521C mutation. Despite this, further clinical studies are required to construct mutation-specific clinical phenotypes, as currently reported case numbers remain too small to make definitive conclusions.

FTD was evident in one individual from the current series. FTD may be associated with between 3 and 22% of all ALS cases, and families with comorbid ALS and FTD are increasingly being recognised,<sup>27</sup> with ALS-FTD loci identified on chromosome 9 (reviewed by Neumann *et al*<sup>28</sup>). *FUS* was recently identified as the pathological protein in a new subtype of FTD and neuronal intermediate filament inclusion disease.<sup>29 30</sup> Data from the present series indicated that FTD may also be a rare presenting feature associated with *FUS* mutations.

Reduced disease penetrance was evident across 75% of families. Incomplete mutation penetrance tended to be common among the *SOD1*-negative families within our overall cohort, and such families were generally smaller, presumably because of this fact. Although there was no statistical difference in the average age of disease onset among the four families, this analysis only included affected individuals and failed to take into account the numerous individuals who carried a *FUS* mutation but were unaffected (including obligate carriers). By taking into account these non-penetrant individuals, the present study has established that patients with the R521C mutation were significantly more likely to develop disease at a younger age.

Assessment of cortical function in ALS patients with *FUS* mutations established features indicative of cortical hyperexcitability, similar to changes observed in both familial ALS due to *SOD1* mutations and sporadic ALS patients, including those with flail limb, lower motor neuron presentations.<sup>22 25 31</sup> Together with the post-mortem data, this suggests a ubiquitous pathophysiological mechanism in ALS and further supports the hypothesis that a common mechanism underlies the motor neuron degeneration associated with mutant *FUS* and abnormal TDP-43 seen in most sporadic ALS cases.<sup>15 16</sup>

Consistent with previous findings,<sup>15</sup> post-mortem immunostaining in an affected family member with *FUS* mutation identified ubiquitin positive skein-like inclusions in lower motor

neurons, which were not TDP-43 immunoreactive. This contrasted with sporadic ALS where, for most cases, the hallmark cytoplasmic inclusions were immunoreactive for both TDP-43 and ubiquitin. As such, the disease mechanism underlying ALS associated with *FUS* mutation appears to be independent of TDP-43.

Haplotype analysis provided little or no evidence for the presence of founding mutations (common ancestor) among the families described in this study. This finding suggested that the mutations arose independently, perhaps due to the presence of a mutation hotspot at residue 521.

The mechanism through which *FUS* mutations lead to motor neuron death is unclear. Both *FUS* and TDP-43 are components of ribonucleoprotein complexes that regulate tissue-specific RNA processing and transport within most cells in the body. In the affected cells of most ALS cases, TDP-43 redistributes from the nucleus to the cytoplasm, where it forms aggregates.<sup>32</sup> Mutant *FUS* has also been shown to redistribute to the cytoplasm and form aggregates.<sup>15 16</sup> It remains unclear whether loss of *FUS* or TDP-43 nuclear function leads to motor neuron death, or whether there is a toxic gain of function in the cytoplasm.

A minority of ALS families carry mutations in *SOD1*, *TARDBP* and *FUS*. It is likely that many more ALS genes are yet to be discovered. A challenge for future studies will be to identify the complex interactions and common molecular pathways that lead to this devastating disease.

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**Competing interests** None.

**Patient consent** Obtained.

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**Provenance and peer review** Not commissioned; externally peer reviewed.

## REFERENCES

1. **Winhammar JM**, Rowe DB, Henderson RD, *et al*. Assessment of disease progression in motor neuron disease. *Lancet Neurol* 2005;**4**:229–38.
2. **Vucic S**, Burke D, Kiernan MC. Diagnosis of motor neurone disease. In: Kiernan MC, ed. *The motor neurone disease handbook*. Sydney: Australasian Medical Publishing Company, 2007:89–104.
3. **Kurtzke JF**. Epidemiology of amyotrophic lateral sclerosis. *Adv Neurol* 1982;**36**:281–302.
4. **Arai T**, Hasegawa M, Akiyama H, *et al*. TDP-43 is a component of ubiquitin-positive tau-negative inclusions in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Biochem Biophys Res Commun* 2006;**351**:602–11.
5. **Neumann M**, Sampathu DM, Kwong LK, *et al*. Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Science* 2006;**314**:130–3.
6. **Forman MS**, Trojanowski JQ, Lee VM. TDP-43: a novel neurodegenerative proteinopathy. *Curr Opin Neurobiol* 2007;**17**:548–55.
7. **Rosen DR**, Siddique T, Patterson D, *et al*. Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. *Nature* 1993;**362**:59–62.
8. **Valdmanis PN**, Rouleau GA. Genetics of familial amyotrophic lateral sclerosis. *Neurology* 2008;**70**:144–52.
9. **Gitcho MA**, Baloh RH, Chakraverty S, *et al*. TDP-43 A315T mutation in familial motor neuron disease. *Ann Neurol* 2008;**63**:535–8.
10. **Sreedharan J**, Blair IP, Tripathi VB, *et al*. TDP-43 mutations in familial and sporadic amyotrophic lateral sclerosis. *Science* 2008;**319**:1668–72.
11. **Lagier-Tourenne C**, Cleveland DW. Rethinking ALS: the *FUS* about TDP-43. *Cell* 2009;**136**:1001–4.

12. **Nishimura AL**, Mitne-Neto M, Silva HC, *et al*. A mutation in the vesicle-trafficking protein VAPB causes late-onset spinal muscular atrophy and amyotrophic lateral sclerosis. *Am J Hum Genet* 2004;**75**:822–31.
13. **Parkinson N**, Ince PG, Smith MO, *et al*. ALS phenotypes with mutations in CHMP2B (charged multivesicular body protein 2B). *Neurology* 2006;**67**:1074–7.
14. **Blair IP**, Vance C, Durnall JC, *et al*. CHMP2B mutations are not a common cause of familial or sporadic amyotrophic lateral sclerosis. *J Neurol Neurosurg Psychiatry* 2008;**79**:849–50.
15. **Vance C**, Rogelj B, Hortobágyi T, *et al*. Mutations in FUS, an RNA processing protein, cause familial amyotrophic lateral sclerosis type 6. *Science* 2009;**323**:1208–11.
16. **Kwiatkowski TJ Jr**, Bosco DA, Leclerc AL, *et al*. Mutations in the FUS/TLS gene on chromosome 16 cause familial amyotrophic lateral sclerosis. *Science* 2009;**323**:1205–8.
17. **Belzil VV**, Valdmanis PN, Dion PA, *et al*. Mutations in FUS cause FALS and SALS in French and French Canadian populations. *Neurology* 2009;**73**:1176–9.
18. **Chiò A**, Restagno G, Brunetti M, *et al*. Two Italian kindreds with familial amyotrophic lateral sclerosis due to FUS mutation. *Neurobiol Aging* 2009;**30**:1272–5.
19. **Ticozzi N**, Silani V, Leclerc AL, *et al*. Analysis of FUS gene mutation in familial amyotrophic lateral sclerosis within an Italian cohort. *Neurology* 2009;**73**:1180–5.
20. **Valdmanis PN**, Daoud H, Dion PA, *et al*. Recent advances in the genetics of amyotrophic lateral sclerosis. *Curr Neurol Neurosci Rep* 2009;**9**:198–205.
21. **Vucic S**, Howells J, Trevillion L, *et al*. Assessment of cortical excitability using threshold tracking techniques. *Muscle Nerve* 2006;**33**:477–86.
22. **Vucic S**, Nicholson GA, Kiernan MC. Cortical hyperexcitability may precede the onset of familial amyotrophic lateral sclerosis. *Brain* 2008;**131**:1540–50.
23. **Brooks BR**, Miller RG, Swash M, *et al*. El Escorial revisited: revised criteria for the diagnosis of amyotrophic lateral sclerosis. *Amyotroph Lateral Scler Other Motor Neuron Disord* 2000;**1**:293–9.
24. **Turner MR**, Kiernan MC, Leigh PN, *et al*. Biomarkers in amyotrophic lateral sclerosis. *Lancet Neurol* 2009;**8**:94–109.
25. **Vucic S**, Kiernan MC. Novel threshold tracking techniques suggest that cortical hyperexcitability is an early feature of motor neuron disease. *Brain* 2006;**129**:2436–46.
26. **Gourie-Devi M**, Nalini A, Sandhya S. Early or late appearance of 'dropped head syndrome' in amyotrophic lateral sclerosis. *J Neurol Neurosurg Psychiatry* 2003;**74**:683–6.
27. **Kiernan MC**. Identification of cognitive deficits in amyotrophic lateral sclerosis. *Clin Neurophysiol* 2009;**120**:645–6.
28. **Neumann M**, Kwong LK, Sampathu DM, *et al*. TDP-43 proteinopathy in frontotemporal lobar degeneration and amyotrophic lateral sclerosis: protein misfolding diseases without amyloidosis. *Arch Neurol* 2007;**64**:1388–94.
29. **Neumann M**, Rademakers R, Roeber S, *et al*. Frontotemporal lobar degeneration with FUS pathology. *Brain* 2009;**132**:2922–31.
30. **Neumann M**, Roeber S, Kretzschmar HA, *et al*. Abundant FUS-immunoreactive pathology in neuronal intermediate filament inclusion disease. *Acta Neuropathol* 2009;**118**:605–16.
31. **Vucic S**, Kiernan MC. Abnormalities in cortical and peripheral excitability in flail-arm variant amyotrophic lateral sclerosis. *J Neurol Neurosurg Psychiatry* 2007;**78**:849–52.
32. **Ule J**. Ribonucleoprotein complexes in neurologic diseases. *Curr Opin Neurobiol* 2008;**18**:516–23.





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