

## SHORT COMMUNICATION

# FALS with *FUS* mutation in Japan, with early onset, rapid progress and basophilic inclusion

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Mutations in the *fused in sarcoma* (*FUS*, also known as *translated in liposarcoma*) gene have been recently discovered to be associated with familial amyotrophic lateral sclerosis (FALS) in African, European and American populations. In a Japanese family with FALS, we found the R521C *FUS* mutation, which has been reported to be found in various ethnic backgrounds. The family history revealed 23 patients with FALS among 46 family members, suggesting a 100% penetrance rate. They developed muscle weakness at an average age of 35.3 years, followed by dysarthria, dysphagia, spasticity and muscle atrophy. The average age of death was 37.2 years. Neuropathological examination of the index case revealed remarkable atrophy of the brainstem tegmentum characterized by cytoplasmic basophilic inclusion bodies in the neurons of the brainstem. We screened 40 FALS families in Japan and found 4 mutations (S513P, K510E, R514S, H517P) in exon 14 and 15 of *FUS*. Even in Asian races, FALS with *FUS* mutations may have the common characteristics of early onset, rapid progress and high penetrance rate, although in patients with the S513P mutation it was late-onset. Degeneration in multiple systems and cytoplasmic basophilic inclusion bodies were found in the autopsied cases.

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Amyotrophic lateral sclerosis is an adult-onset neurodegenerative disorder characterized by the death of motor neurons.<sup>1</sup> Approximately 20% of familial amyotrophic lateral sclerosis (FALS) cases are caused by mutations in the *superoxide dismutase 1* gene.<sup>1,2</sup> Mutations in the *TARDBP* gene, which codes for TAR DNA-binding protein 43 (TDP-43), have been recently reported in FALS cases.<sup>3</sup> Very recently, two groups of investigators reported the autosomal dominant form of FALS caused by *fused in sarcoma* (*FUS*) mutations,<sup>4,5</sup> following several reports of both familial and sporadic cases from Europe.<sup>6–9</sup> *FUS* is a nucleoprotein that functions in DNA and RNA metabolism.<sup>10</sup> In this study, we found a large Japanese FALS family with mutations in the *FUS* gene with the characteristics of early onset. In addition, we report four mutations of the *FUS* gene in Japanese FALS.

## CASE REPORT OF THE INDEX CASE

The patient (indicated by arrow in Figure 1a) was a Japanese man with autosomal dominant hereditary burden as described in a previous report.<sup>11</sup> His family history revealed 23 patients with FALS over four generations (Figure 1a). He developed muscle weakness of the distal part of the right upper extremity at age 30, followed by dysarthria,

dysphagia, muscle weakness and atrophy in the four extremities, spasticity, hyperreflexia and Babinski's sign. His sensory, cerebellar and higher cortical functions were not affected. At age 31 (17 months after onset) he needed ventilatory support. At age 40, he died of bronchopneumonia.

## MATERIALS AND METHODS

All patients of FALS without the *superoxide dismutase 1* mutation and healthy controls provided informed consent, after which DNA extraction and genotyping were performed using standard protocols as described elsewhere.<sup>12</sup> We sequenced all exons in 40 unrelated FALS families with the characteristics of an autosomal dominant trait in Japan. Sections of 5 μm thickness were taken from the midbrain of the index case and stained as described elsewhere.<sup>5</sup> Rabbit polyclonal anti-TDP-43 (ProteinTech, Chicago, IL, USA) and rabbit polyclonal anti-ubiquitin (Abcam, Cambridge, MA, USA) antibodies were used. The study was approved by the ethics committee and all subjects gave written informed consent.

## RESULTS

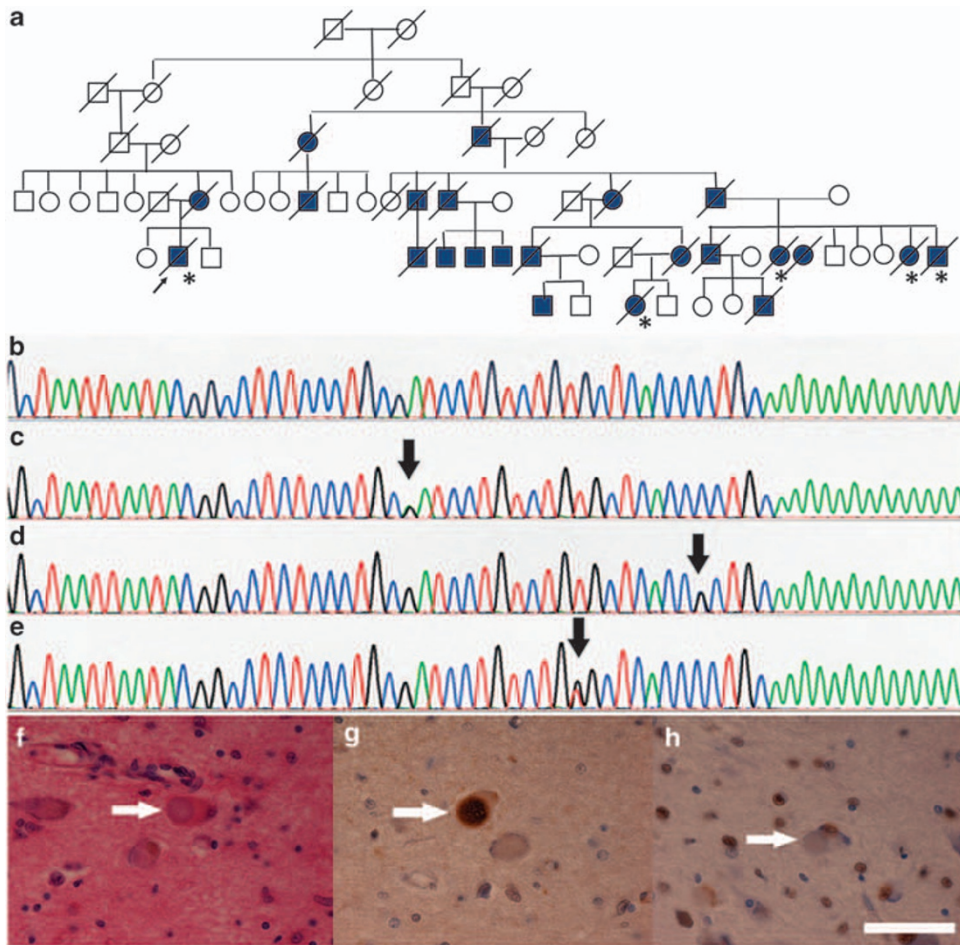
We detected a missense mutation (C1561T) in the *FUS* gene that substituted cysteine for arginine at residue 521 (R521C) (Figure 1c) in the index case. Family history revealed 23 patients with FALS among

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**Figure 1** (a) Pedigree of a Japanese family with FALS with *FUS* mutations. Males are represented by squares, females by circles. Affected members are represented by solid symbols, deceased individuals by diagonals. The proband is indicated by an arrow. Autopsied cases are marked with asterisks. (b–e) Sequence electropherogram of the *FUS* gene. (b) Sequence of a normal subject; (c) sequence of the index case with a missense mutation (C1561T) in exon 15 of the *FUS* gene that substituted cysteine for arginine at residue 521 (R521C); (d) arginine for serine at residue 514 (R514S); and (e) sequence of the other case with histidine for proline at residue 517 (H517P). The arrows indicate the substitution site. (f–h) Hematoxylin–eosin staining and immunohistochemistry of the midbrain from the index case. A few basophilic inclusion bodies were present in the neurons of the brain stem (f, arrow). These basophilic bodies were stained with ubiquitin (g, arrow), but not with TDP43 (h, arrow). Scale bar, 50  $\mu$ m.

46 family members, suggesting a 100% penetrance rate. The average age at disease onset was  $35.3 \pm 5.1$  years ( $n=12$ ). The disease duration (from onset to respiratory failure) of the cases with the R521C mutation was  $16.1 \pm 7.7$  months. The disease course was rapidly progressive and the average age at death was  $37.2 \pm 4.2$  years ( $n=12$ ). We found no case with cognitive impairment among the FALS patients with *FUS* mutations.

Neuropathological examination of the case (indicated by the arrow in Figure 1a) revealed not only neuronal loss in the upper and lower motor neuron systems and Clarke’s column, but also degeneration of the pyramidal tracts, middle root zone of the posterior column and the posterior spinocerebellar tract. Atrophy of the brainstem tegmentum was remarkable. Neuronal loss and astrogliosis in the globus pallidus, substantia nigra, locus ceruleus and subthalamic nucleus were associated with eosinophilic granular bodies. A few basophilic inclusion bodies were exclusively present in the neurons of the brainstem (indicated by the arrow in Figure 1f) as previously reported.<sup>11</sup> These basophilic bodies were immunohistochemically stained with ubiquitin (Figure 1g), not with TDP-43 (Figure 1h).

We also found four mutations, serine for proline at residue 513 (S513P), arginine for serine at residue 514 (R514S; Figure 1d), lysine for glutamate at residue 510 (K510E) in exon 14 and histidine for proline at residue 517 (H517P; Figure 1e) in exon 15 of *FUS*. These patients showed the characteristics of early onset at around age 30, and rapid progress, although the age at onset in patients with the S513P mutation was around 60. S513P, R514S and H517P are novel mutations. The lower motor neurons were mainly affected in these patients with *FUS* mutations. The case with the S513P mutation progressed slowly and was followed up as a case of spinal progressive muscular atrophy with family history. Exons 14 and 15 of the *FUS* gene were sequenced in 100 Japanese healthy controls and no mutations were detected.

## DISCUSSION

Mutations in the *FUS* gene have been reported to be associated with FALS in African, European and American populations.<sup>4–9</sup> We found the *FUS* mutation in a Japanese family with FALS with the characteristics of early onset, rapid progress, high penetrance, degeneration of

the multiple system, remarkable atrophy of the brainstem tegmentum and basophilic inclusion bodies in the autopsy. The immunohistochemical findings of the basophilic inclusion bodies of our case were recognized by using ubiquitin, but not by using antibodies to tau, neurofilament<sup>11</sup> or TDP-43. Very recently, an *FUS* abnormality was found in basophilic inclusion body disease including motor neuron disease.<sup>13,14</sup> Although the role of basophilic inclusion remains to be elucidated, this study underscores the importance of basophilic inclusion and *FUS* in the pathological process of amyotrophic lateral sclerosis. The basophilic inclusions were exclusively found in the brainstem tegmentum in our cases, although the number was small and the distribution of basophilic inclusion bodies may depend on the timing of autopsy. By using an animal model with mutations in exon 15 of *FUS*, it may be feasible to study the relationship between the disease course and basophilic inclusion.

The phenotype–genotype correlation in amyotrophic lateral sclerosis with *FUS* mutations is not well established.<sup>15</sup> Vance *et al.*<sup>4</sup> reported that the average age at onset was 44.5 years and average survival was 33 months in FALS with *FUS* gene mutations. Even in Asian races, FALS with *FUS* mutations may have the common characteristics of early onset and rapid progression, especially in patients with the R521C mutation, which is found in patients of various ethnic backgrounds. On the other hand, patients with the S513P mutation had late-onset disease. It is not clear why differences in the phenotypes among these mutations occurred, although all mutations were concentrated in the extreme C-terminus of the *FUS* gene. The accumulation of clinical, genetic and pathological information will be necessary to elucidate the pathomechanism of motor neuron death in FALS with *FUS* mutations.

#### CONFLICT OF INTEREST

The authors declare no conflict of interest.

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