Scientific Correspondence

Does ALS-FUS without FUS mutation represent ALS-FET? Report of three cases

Abnormal cytoplasmic accumulation of fused in sarcoma (FUS) protein is the pathological hallmark of some cases of amyotrophic lateral sclerosis (ALS) with transactive response DNA-binding protein of 43KDa (TDP-43)-negative pathology that lack SOD1 mutations. FUS is an RNA-binding protein located predominantly in the nucleus and is involved in regulation of transcription, alternative splicing, RNA stability, microRNA biogenesis, apoptosis and cell division. FUS, Ewing’s sarcoma (EWS) and TATA-binding protein-associated factor 15 (TAF15) proteins constitute the FET (FUS/EWS/TAF15) family, highly conserved and ubiquitously expressed RNA-binding proteins that shuttle between nucleus and cytoplasm assisted by the nuclear import protein Transportin 1 (Trn1) [1].

Accumulation of FUS also occurs in other related neurodegenerative conditions such as atypical frontotemporal lobar degeneration with ubiquitinated inclusions (aFTLD), neuronal intermediate filament inclusion disease (NIFID) and basophilic inclusion body disease (BIBD), the three currently recognized forms of frontotemporal lobar degeneration with FUS pathology (FTLD-FUS) [2].

Recent work suggests different pathological processes underlie ALS-FUS and FTLD-FUS. First, most ALS-FUS cases are caused by FUS mutations [3], while most FTLD-FUS cases are not [2,4]. Neumann et al. described that in ALS-FUS, the cytoplasmic inclusions consist solely of FUS protein while in FTLD-FUS, the inclusions include other FET family proteins such as TAF15 or EWS [5]. In addition, they observed that Trn1, a protein involved in the nuclear transport, accumulates specifically in FTLD-FUS inclusions but not in ALS-FUS. These findings led the authors to suggest that ALS with FUS mutations is more restricted to FUS dysfunction, while in FTLD-FUS, there is a more global and complex dysregulation of all FET proteins. They suggest changing the nomenclature and recommended using the term FTLD-FET for FTLD-FUS but to preserve the term ALS-FUS [5].

We describe three cases of ALS-FUS with TAF15 and Trn1 accumulation in which FUS mutations were not detected. Brain donors and/or next of kin had given their written informed consent for the use of brain tissue for research, and the research protocol has been approved by the Ethics Committee of the Hospital Clinic Barcelona.

Patient 1, a 63-year-old man, developed slowly progressive weakness in the distal muscles, dysarthria and dysphagia. Neurological examination revealed symmetrical weakness and hyperreflexia, fulfilling the criteria for ALS. Cognitive and behavioural symptoms were not reported during follow-up. He died of respiratory failure at 69 years. After brain donation, the unfixed brain weight was 1390 g. A prominent atrophy of the medullary pyramids, anterior nerve roots and spinal cord was appreciated on gross examination, but without brain atrophy. Histologically, prominent neuronal loss of motor neurones of the anterior horn was observed at all levels of the spinal cord and was also present in the motor nuclei of the brain stem and the primary motor cortex. Degeneration of the corticospinal tracts was also observed. Several of the remaining spinal and cortical motor neurones showed relatively large cytoplasmic basophilic inclusions. These inclusions were also observed in nonmotor pyramidal neurones and were partly basophilic and partly fibrillar. These inclusions were immunoreactive for FUS protein, p62, TAF15 and Trn1 (Figure 1A–1S), and partially for ubiquitin, alpha-internexin and phosphorylated neurofilaments. These findings were consistent with ALS with FUS-positive basophilic and fibrillar inclusions.

Patient 2, a 71-year-old woman, presented with progressive weakness of lower extremities, dysarthria and dysphagia. Neurological examination revealed pyramidal signs. No lower motor neurone signs were found on examination, and she was diagnosed with primary lateral sclerosis. During the disease course, she developed an akinetic–rigid syndrome without response to levodopa. DAT-SPECT showed bilaterally reduced putaminal tracer uptake. No cognitive symptoms were reported. The patient died at the age of 83 years after...
a total disease duration of 12 years. After brain donation, the unfixed brain weight was 1500 g. Gross examination revealed mild brain atrophy with preferential involvement of the precentral and postcentral gyri. Histologically, loss of motor neurones was evident in the primary motor cortex, hypoglossal nuclei and also at all levels of the spinal cord. Moreover, in the pre- and postcentral regions as well as in the temporal cortex, laminar spongiosis and gliosis were evident in superficial cortical layers. While with H&E staining, inclusions were difficult to identify (Figure 1C1), immunohistochemistry for FUS showed frequent neuronal cytoplasmic inclusions (Figure 1C2), short neurites and few intranuclear inclusions. Inclusions were more abundant in the precentral gyrus, in the brainstem nuclei and in the spinal cord. They were also immunoreactive for TAF15 and Trn1 (Figure 1C4–C5) and were negative for TDP-43. The final diagnosis was ALS-FUS. In all three cases, granular neurones of the dentate gyrus were devoid of inclusions (Figure 1A3, B3, C3 and insets).

Figure 1. Representative neuropathological findings in the three cases: (A1, B1, C1) HE-stained sections show different types of intraneuronal inclusion bodies in the motor neurones of the frontal cortex, brainstem and spinal cord (arrows) varying in shape and tintorial properties (basophilic, pale, with a condensed centre or with fibrillar appearance). (A2, B2, C2) Inclusions are FUS-positive and appear either compact, more fibrillar or skein-like (inset) (immunohistochemistry for FUS; slightly counterstained with haematoxylin). (A3, B3, C3) There is no involvement of the dentate gyrus of the hippocampus, and granule cells are devoid of FUS/TAF15/Trn1 + inclusion bodies (immunohistochemistry for FUS (A3, B3 and insets), TAF15 (C3 and insets) and Transportin 1 (Trn1 (insets))). (A4, B4, C4) Intraneuronal inclusion bodies in motor cortex, brainstem and spinal cord neurones are also strongly immunoreactive for Transportin 1 and TAF15 (immunohistochemistry for Transportin 1 (Trn1) and TAF15 shown in the left and right panel, respectively; slightly counterstained with haematoxylin). A5: Double immunofluorescence for FUS (red, left panel), Trn1 (green, middle panel) and merged image (yellow-orange, right panel) shows codistribution of both proteins in the same inclusion body in patient 1. C5: Double immunofluorescence for TAF15 (red, left panel), Trn1 (green, middle panel) and merged image (yellow-orange, right panel) shows codistribution of both proteins in the same inclusion body in patient 3. A1–A5 are from patient 1, B1–B4 are from patient 2, and C1–C5 are from patient 3. Scale bars: A1, B1, C1, A2, B2, C2, A4, B4, C4: 20 μm, A3, B3, C3: 50 μm.

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found one patient harbouring the c.*41G>A rare heterozygous variant (rs80301724) [9]. Previous studies have reported this polymorphic variant to be equally present in ALS cases and controls, thus showing a lack of genetic association between this particular nucleotide change and ALS [8,10].

Here, we describe the clinicopathological phenotype of three ALS patients with abundant FUS-positive protein aggregates. The inclusion bodies were also immunoreactive for TAF15 and Trn1, and no mutation in the FUS gene was detected. Similar cases had been reported in Japan by Matsuoka et al., Fujita et al. and Takeuchi et al. (Table 1)[11–13]. Other possible genes that could have mutations include TPN1 and TAF15, among others, that were not tested in our cases.

These findings differ from the ALS-FUS cases previously reported by Neumann and behave immunohistochemically similar to FTLD-FET cases. Whether these cases might be specific to certain populations is unresolved. Based on our results, we confirm the concept that the presence of FET and Trn1 proteins within the inclusions is strong indicator of a lack of pathogenic mutations within FUS. However, this immunohistochemical profile does not differentiate between an ALS and FTLD phenotype. If we hypothesize that FTLD-FUS with FUS mutations will not show Trn1 or any other FET family protein than FUS, a change of the nomenclature in the ALS-FUS and FTLD-FUS with no mutations of FUS should be considered, and the use of the terms ALS-FET and FTLD-FET might be more appropriate.

ALS-FUS mutation cases seem to have different morphological phenotypes depending on the age of onset or disease duration; neuronal basophilic inclusions being more frequently detected in early juvenile forms, while fibrillary or tangle-like inclusions and glial inclusions tend to appear in late-onset cases [3]. Similar findings have been described in some sporadic FTLD-FUS cases [4,14]. Interestingly, ALS-FUS cases without FUS mutations seem to have an older age of onset and a less aggressive progression than cases with mutations [3].

While some reports have detected FUS mutations in juvenile ALS with basophilic inclusions’ [15], others have not found mutations in the adult-onset group [11,12]. It might be therefore that a subgroup of ‘adult-onset ALS with basophilic inclusions’ represents the ALS counterpart of basophilic inclusion body disease and may therefore be considered an ALS-FET subtype without FUS mutations.

Our study expands the clinicopathological spectrum of nongenetic ALS-FUS cases and reinforces the idea that not all ALS-FUS cases are secondary to FUS mutations. It also corroborates the usefulness of TAF-15 and Trn1 immunohistochemistry for the neuropathological diagnosis of nongenetic FTLD-FET and ALS-FET patients. Whether these cases represent a different pathogenetic subgroup among ALS-FUS is unclear and

| Table 1. Demographic and clinical features of ALS-FUS cases in the literature |
|-----------------|----------------|----------------|-----------------|----------------|----------------|
|                 | Present study | Fujita et al. [10] | Matsuoka et al. [11] | Takeuchi et al. [12] |
| Gender          | Male          | Male            | Male           | Female         | Female         |
| Family history  | No            | No              | No             | No             | No             |
| FUS mutation    | No            | No              | No             | No             | No             |
| Age at onset (y) | 63            | 71              | 43             | 73             | 75             |
| Age at death (y) | 69            | 83              | 48             | 75             | 79             |
| Motor neurone   | Yes           | Yes             | Yes            | Yes            | Yes            |
| Onset           | Spinal        | Spinal          | Spinal         | Spinal         | Spinal         |
| Dementia        | No            | No              | No             | No             | No             |
| Parkinsonism    | No            | Yes             | No             | No             | No             |
| Neupathology    | ALS-FUS       | ALS-FUS         | ALS-FUS        | ALS-FUS        | ALS-FUS        |
| FUS IHC         | +             | +               | +              | +              | +              |
| TAF15 IHC       | +             | +               | NE             | NE             | +              |
| TRN1 IHC        | +             | +               | NE             | NE             | NE             |

ALS, amyotrophic lateral sclerosis; FUS, fused in sarcoma; NE, not evaluated; y, years; IHC, immunohistochemistry.

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requires further detailed clinical, neuropathological and molecular studies.

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Authors’ Contributions

Study concept and design: SBE, RRG, EG; Acquisition of data: SBE, ECV, RRG, LCC, GR, JG, JB, EG; Analysis and interpretation of data: SBE, ECV, RRG, LCC, GR, JG, JB, EG; Drafting of the manuscript: SB, EG; Critical revision of the manuscript and editing: All authors.

Conflict of Interest

The authors do not report conflict of interests related to this article.

References

1 Law WJ, Cann KL, Hicks GG, TLS, EWS and TAF15: a model for transcriptional integration of gene expression. Brief Funct Genomic Proteomic 2006; 5: 8–14
2 MacKenzie IR, Munoz DG, Kusaka H, Yokota O, Ishihara K, Roeber S, et al. Distinct pathological subtypes of FTLD-FUS. Acta Neuropathol 2011; 121: 207–18
3 Mackenzie IR, Ansorge O, Strong M, Bilbao J, Zinman L, Ang LC, et al. Pathological heterogeneity in amyotrophic lateral sclerosis with FUS mutations: two distinct patterns correlating with disease severity and mutation. Acta Neuropathol 2011; 122: 87–98
4 Gelpi E, Lladó A, Clarimón J, Rey MJ, Rivera RM, Ezquerra M, et al. Phenotypic variability within the inclusion body spectrum of basophilic inclusion body disease and neuronal intermediate filament inclusion disease in frontotemporal lobar degenerations with FUS-positive inclusions. J Neuropathol Exp Neurol 2012; 71: 795–805
5 Neumann M, Bentmann E, Dormann D, Jawaid A, Dejesus-Hernandez M, Ansorge O, et al. FET proteins TAF15 and EWS are selective markers that distinguish FTLD with FUS pathology from amyotrophic lateral sclerosis with FUS mutations. Brain 2011; 134(Pt9): 2595–609
6 Montine TJ, Phelps CH, Beach TG, Bigio EH, Cairns NJ, Dickson DW, et al. National institute on aging-Alzheimer’s association guidelines for the neuropathologic assessment of Alzheimer’s disease: a practical approach. Acta Neuropathol 2012; 123: 1–11
7 Dini Modigliani S, Morlando M, Errichelli L, Sabatelli M, Bozzoni I. An ALS-associated mutation in the FUS 3’-UTR disrupts a microRNA-FUS regulatory circuitry. Nat Commun 2014; 5: 4335
8 Sabatelli M, Moncada A, Conte A, Lattante S, Marangi G, Luigetti M, et al. Mutations in the 3’ untranslated region of FUS causing FUS overexpression are associated with amyotrophic lateral sclerosis. Hum Mol Genet 2013; 22: 4748–55
9 Ticozzi N, Silani V, Le Clerc AL, Keagle P, Gellera C, Ratti A, et al. Analysis of FUS gene mutation in familial amyotrophic lateral sclerosis within an Italian cohort. Neurology 2009; 73: 1180–5
10 Groen EJ, van Es MA, van Vught PW, Spliet WG, van Engelzen-Lee J, de Visser M, et al. FUS mutations in familial amyotrophic lateral sclerosis in the Netherlands. Arch Neurol 2010; 67: 224–30
11 Matsuoka T, Fujii N, Kondo A, Iwaki A, Hironohara T, Honda H, et al. An autopsied case of sporadic adult-
onset amyotrophic lateral sclerosis with FUS-positive basophilic inclusions. *Neuropathology* 2011; 31: 71–6

12 Fujita Y, Fujita S, Takatama M, Ikeda M, Okamoto K. Numerous FUS-positive inclusions in an elderly woman with motor neuron disease. *Neuropathology* 2011; 31: 170–6

13 Takeuchi R, Toyoshima Y, Tada M, Shiga A, Tanaka H, Shimohata M, *et al.* Transportin 1 accumulates in FUS inclusions in adult-onset ALS without FUS mutation. *Neuropathol Appl Neurobiol* 2013; 39: 580–4

14 Molina-Porcel L, Iladó A, Rey MJ, Molinuevo JL, Martínez-Lage M, Esteve FX, *et al.* Clinical and pathological heterogeneity of neuronal intermediate filament inclusion disease. *Arch Neurol* 2008; 65: 272–5

15 Baumer D, Hilton D, Paine SML, Turner MR, Lowe J, Talbot K, *et al.* Juvenile ALS with basophilic inclusions is a FUS proteinopathy with FUS mutations. *Neurology* 2010; 75: 611–8

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