Received: 18 November 2022 | Accepted: 6 June 2023

DOI: 10.1111/bpa.13191

RESEARCH LETTER

Brain Pathology

# Increased expression of human antiviral protein MxA in FUS proteinopathy in amyotrophic lateral sclerosis

**Hiroyuki Honda**[1,2,3] | **Kaoru Yagita**[1] | **Hajime Arahata**[2] | **Hideomi Hamasaki**[1] | **Hideko Noguchi**[1] | **Sachiko Koyama**[1] | **Naokazu Sasagasako**[2]

[1]Department of Neuropathology, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan

[2]Division of Neurology, Department of Neurology, Neuro Muscular Center, National Hospital Organization, Omuta National Hospital, Fukuoka, Japan

[3]Neuropathology Center, National Hospital Organization, Omuta National Hospital, Fukuoka, Japan

**Correspondence**
Hiroyuki Honda, Neuropathology Center, National Hospital Organization, Omuta National Hospital, 1044-1 Tachibana, Omuta-city, Fukuoka 837-0911, Japan.
Email: honda.hiroyuki.vm@mail.hosp.go.jp; honda.hiroyuki.211@m.kyushu-u.ac.jp

**Abstract**

FUS mutations are one of the major mutations in familial amyotrophic lateral sclerosis (ALS). The pathological hallmark is FUS-positive neuronal cytoplasmic inclusions (FUS-NCI), known as FUS proteinopathy. Human myxovirus resistance protein 1 (MxA) is an IFN-induced dynamin-like GTPase that acts as antiviral factor. In this study, we examined the expression of MxA in neurons bearing FUS-NCI. We performed immunohistochemistry for FUS and MxA to examine the expression of MxA in two autopsy cases with different FUS gene mutations localized at the nuclear localization signal site (Case 1, H517P; Case 2, R521C). MxA. Most neurons bearing FUS-NCI have increased cytoplasmic MxA expression. Increased cytoplasmic MxA showed several distribution patterns in relation to FUS-NCIs such as the following: colocalization with NCI, distribution more widely than NCI, and different distribution peaks from NCI. Our results suggested that antiviral signaling IFNs are involved upstream in the formation of FUS-NCI in ALS-FUS patients.

**KEYWORDS**

ALS, FUS, MxA, myxovirus resistance protein, interferon

## 1 | INTRODUCTION

Fused in sarcoma (FUS), coded as *FUS*, is a heterogeneous nuclear ribonucleoprotein, and *FUS* mutations are one of the major mutations in familial amyotrophic lateral sclerosis (ALS-FUS) [1]. The pathological hallmark is FUS-positive neuronal cytoplasmic inclusions (FUS-NCI), known as FUS proteinopathy. Mutant FUS aggregates to form stress granules (SGs), which are initially membraneless organelles, cytoplasmic ribonucleoprotein condensates mediated by liquid–liquid phase separation (LLPS) of biomacromolecules [2, 3]. Recently, Shelkovnikova et al. reported that antiviral immune responses promote FUS accumulation, suggesting that viral infection can trigger FUS proteinopathy in ALS [4]. They also demonstrated that type I interferon (IFN), induced by a viral infection, promotes the accumulation of FUS.

Human myxovirus resistance protein 1 (MxA) is an IFN-induced dynamin-like GTPase that acts as an antiviral factor [5]. MxA expression depends on the induction of type I (alpha or beta) or III (lambda) INFs [6]. In this study, we examined the expression of human MxA in hippocampal neurons bearing FUS-NCI in two autopsy cases with different *FUS* gene mutations (Case 1, H517P; Case 2, R521C).

## 2 | METHODS

### 2.1 | Subjects

Case 1 (H517P) involved a 31-year-old man who experienced masseter muscle weakness as his initial symptom. Mechanical ventilation support was initiated at

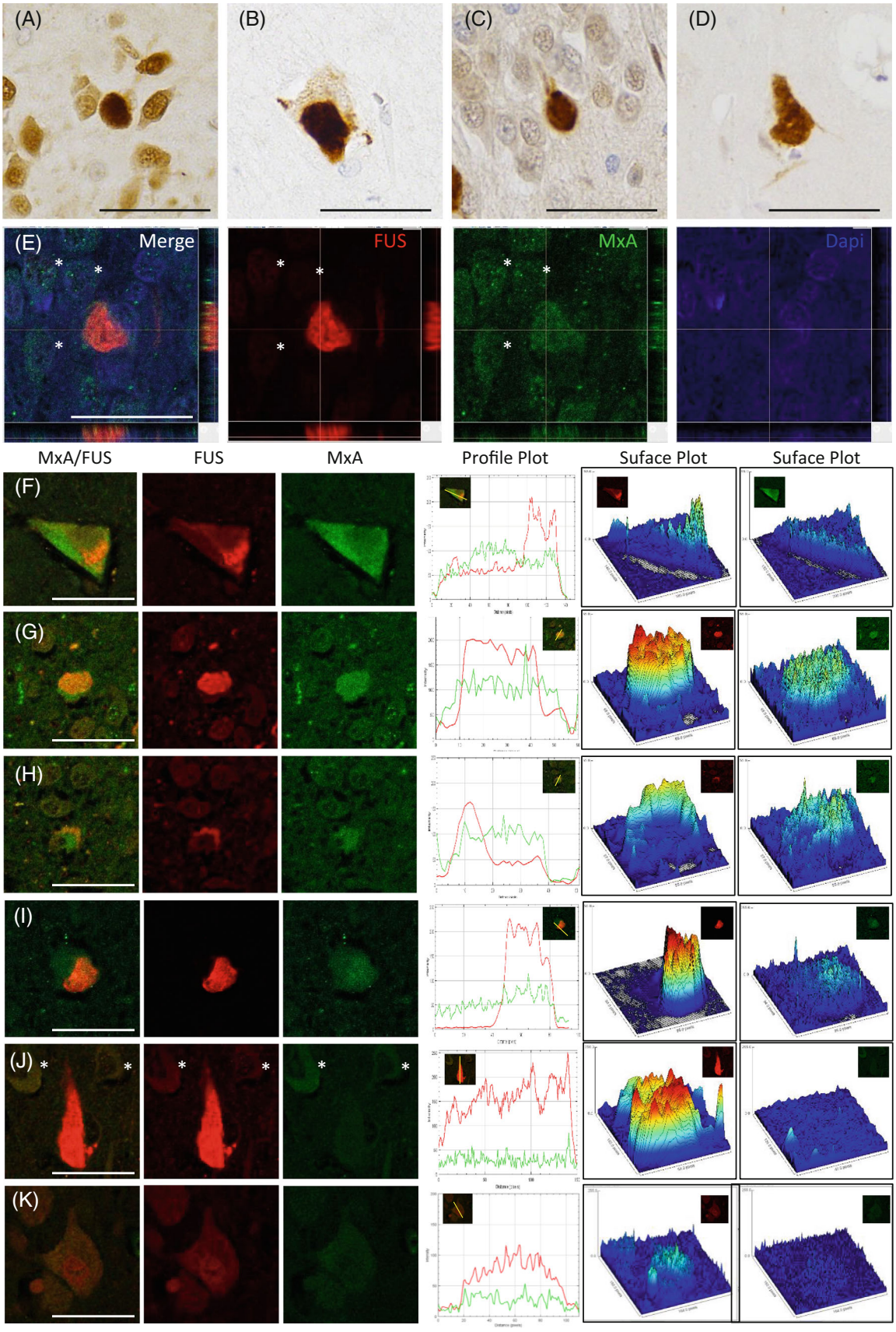


FIGURE 1 Legend on next page.

33 years. He became locked-in state at 36 years. He died of aspiration pneumonia at 44 years. Case 2 (R521C) (described as "*Patient 1(V-4)*" in a previous study [7]) showed muscle weakness in the right upper limb as an initial symptom at the age of 31. Seven months after the disease's onset, the patient became bedridden. Mechanical ventilation support was initiated 8 months after the disease's onset. He died of aspiration pneumonia at 45 years.

We obtained informed consent for autopsies from all patients or their next to kin in writing. All analyses were performed in accordance with Declaration of Helsinki and with the relevant guidelines and regulations. This study was approved by the Ethics Committee of the Faculty of Medicine, Kyushu University (#2020-167).

## 2.2 | Histochemistry

For histological examination, haematoxylin and eosin (HE), and Klüver–Barrera stains were performed. Immunohistochemistry was performed using the primary antibodies specific to anti-FUS antibody (rabbit polyclonal: 1:200; Novus Biologicals, Centennial, CO) and anti-Myxovirus resistance protein 1/2/3 (MxA) antibody (mouse monoclonal, 1:100; Santa Cruz Biotechnology, Dallas, TX). Double immunofluorescence for FUS and MxA was performed. AlexaFluor 488-labeled anti-mouse IgG (Invitrogen, Carlsbad, CA) and AlexaFluor 546-labeled anti-rabbit IgG (Invitrogen) were used as secondary antibodies. The specimens were visualized using a Nikon A1R-A1 confocal microscopy system (Nikon, Tokyo, Japan). To examine the detailed distribution of the expression of FUS and MxA in neurons, RGB profile and surface plots for each fluorescence were calculated using the ImageJ Fiji software (NIH).

# 3 | RESULTS

At autopsy, in Case 1, the brain weight was 885 g, with severe bilateral frontotemporal lobe atrophy. The cerebellum and spinal cord were both atrophic. For Case 2, the brain weighed 1070 g with severe bilateral frontotemporal lobe atrophy. Spinal cord was also atrophic. HE staining showed severe neuronal loss of the spinal anterior horns in both Cases 1 and case 2. While, neurons were relatively preserved in the dentate gyrus and pyramidal cell layer of the hippocampi in both cases. Immunohistochemistry for FUS revealed numerous FUS-NCIs in the neurons of the granular cell layers and pyramidal cell layers in the hippocampi (Figure 1A,B: case 1, Figure 1C,D: case 2). In the spinal cords, FUS-NCIs were rarely seen because of severe neuronal loss. Double immunofluorescence for FUS and MxA revealed increased MxA expression in neurons bearing FUS-NCI, and cross sections of z-stacked images revealed colocalization of FUS-NCI and MxA (Figure 1E: case 1). While, neurons without FUS-NCI did not show increasing of cytoplasmic MxA (Figure 1E, *). There were some variations in the localization of FUS and MxA. Almost all neurons bearing FUS-NCI showed increased MxA expression (Figure 1F: case 2, Figure 1G,H: case 1, Figure 1I: case 2). To examine the detailed distribution of FUS and MxA expression in neurons, profile and surface plots for each fluorescence were calculated using the ImageJ Fiji software. The profile and surface plots showed that the fluorescence peak of MxA almost coincided with that of FUS-NCI; additionally, the peak was slightly broader than that of NCI (Figure 1F: case 2, Figure 1G,H: case 1, Figure 1I: case 2). In contrast, some neurons showed very strong FUS fluorescence but almost no MxA fluorescence throughout the cytoplasm (Figure 1J: case 1). While, neurons without FUS-NCI showed normal cytoplasmic MxA (Figure 1J, *). Approximately 66.7% of neurons with FUS inclusion showed higher cytoplasmic MxA levels (percentage, neurons with high MxA / neurons with FUS inclusion; total (case 1 +case 2): 66.7%, 40/60, Case 1: 62.2%, 23/37; Case 2: 73.9%, 17/23). Neurons without FUS-NCI showed normal distribution, FUS localized in the cytoplasm and the nucleus, and MxA localized mainly in the cytoplasm (Figure 1K: control).

Neurons of hippocampal cornus ammonis 1 in Duchenne muscular dystrophy case showed no FUS inclusion and no increase in cytoplasmic MxA (Figure 2A). In both ALS-FUS cases 1 and 2, neuronal loss in the precentral gyrus and anterior horns of the spinal cords was severe. Very few neurons bearing FUS-NCI could be observed, and there was a mild increase in cytoplasmic MxA (Figure 2B: case 1, precentral gyrus, Figure 2C: case 1, anterior horn cell of spinal cord).

**FIGURE 1** Immunohistochemistry for FUS and MxA and their fluorescence intensity plots (Case 1: A, B, E, G, H, J; Case 2: C, D, F, I, Control: K). (A–D) Immunohistochemistry for FUS reveals FUS-positive neuronal cytoplasmic inclusions (FUS-NCIs) in the hippocampal neurons. (E) Double immunofluorescence for FUS and MxA reveals colocalization of FUS-NCI and MxA. Cross sections of *z*-stacked images also revealed colocalization of FUS-NCI and MxA. (F–I) Neurons bearing FUS-NCI often have a broader distribution of MxA expression than FUS. (J) Neuron that strongly expresses FUS has reduced MxA expression throughout the cytoplasm. (K) Normal FUS and MxA subcellular distribution in neurons without FUS-NCI are shown. Scale bars: 50 μm.

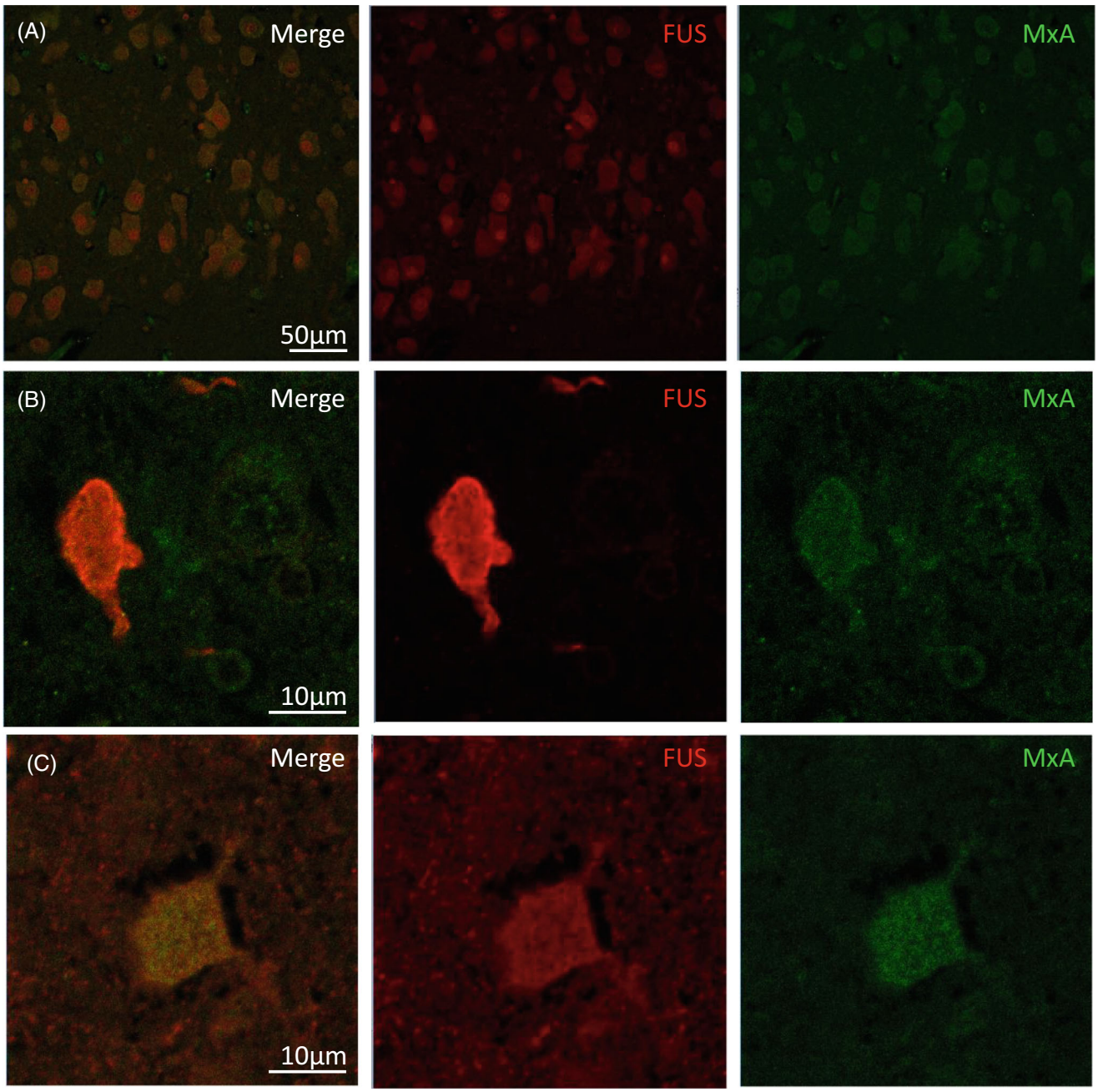


**FIGURE 2** Double immunofluorescence for FUS and MxA (Hippocampal cornus ammonis 1 of Duchenne muscular dystrophy (A), Case 1: precentral gyrus (B), anterior horns of spinal cord (C). (A) There are no FUS inclusions and no cytoplasmic MxA increasing. (B, C) Neurons bearing FUS-NCI shows mild increase of cytoplasmic MxA.

## 4 | DISCUSSION

MxA assembles into tetramers in the cytosol and it also assembles around viral ribonucleoprotein complexes into the oligomeric ring of MxA, which has antiviral effects [8]. Mutated FUS tends to spontaneously form the SGs [2]. In the early phase, FUS-positive SGs are membraneless organelles formed by LLPS which are reversible, and the involvement of IFN causes persistent SGs [4]. FUS physically interacts with the genomes of some viruses [9, 10].

In this study, most FUS-NCIs co-localized with MxA, and the subcellular distribution of MxA tended to be slightly broader than that of NCIs. This is probably because, as mentioned above, both FUS and MxA tend to aggregate as membraneless organelles formed by LLPS. In contrast, MxA expression was markedly reduced in some neurons with obvious FUS-NCI. Decreased MxA expression in neurons with FUS-NCI may suggest that NCI is at an advanced stage and neuronal dysfunction has occurred. In neurons bearing FUS-NCI in the precentral gyrus and anterior horn of the

spinal cord, we found a mild increase in cytoplasmic MxA, but the observable neurons were too few to make a strong association between FUS inclusion and cytoplasmic MxA yet. This study has some limitations. We have not been able to determine whether a viral infection occurs or which virus is present in such cases. Long-term monitoring of viral infection and INFs expression in patients with ALS-FUS are important.

In conclusion, our study demonstrated increased MxA expression in neurons bearing FUS-NCI in two autopsy cases of ALS-FUS. MxA and FUS are common in the sense that they have antiviral activity. Therefore, it is suggested that antiviral signaling type I or III IFNs are involved upstream in the formation of FUS-NCI in ALS-FUS patients.

## AUTHOR CONTRIBUTIONS

Hiroyuki Honda; study concept and design, postmortem examination, preparation for manuscript. Kaoru Yagita, Hideomi Hamasaki, Hideko Nobuchi and Sachiko Koyama; postmortem examination. Hajime Arahata and Naokazu Sasagasako; patient care, taking clinical care.

## ACKNOWLEDGMENTS

The authors greatly appreciate the patients and their families for their collaboration with this study. We would like to thank Dr. Toru Iwaki (Kyushu University, Fukuoka, Japan) for technical and conceptual assistance and Dr. Takaaki Kanemaru (Department of Morphology Core Unit, Kyushu University Hospital, Fukuoka, Japan) for technical assistance.

## FUNDING INFORMATION

This study has not received any funding.

## CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interests to declare.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## ETHICS STATEMENT

Informed written consent for autopsies was obtained from all patients or their next of kin. This study was approved by the Ethics Committee of the Faculty of Medicine, Kyushu University (#2020-167), and was performed in accordance with the ethical standards described in the fifth revision of the Declaration of Helsinki, 2000.

## ORCID

*Hiroyuki Honda* https://orcid.org/0000-0002-4225-7248

## REFERENCES

1. Kwiatkowski TJ Jr, Bosco DA, Leclerc AL, Tamrazian E, Vanderburg CR, Russ C, et al. Mutations in the FUS/TLS gene on chromosome 16 cause familial amyotrophic lateral sclerosis. Science. 2009;323(5918):1205–8.
2. Baradaran-Heravi Y, Van Broeckhoven C, van der Zee J. Stress granule mediated protein aggregation and underlying gene defects in the FTD-ALS spectrum. Neurobiol Dis. 2020;134:104639.
3. Ishiguro A, Lu J, Ozawa D, Nagai Y, Ishihama A. ALS-linked FUS mutations dysregulate G-quadruplex-dependent liquid-liquid phase separation and liquid-to-solid transition. J Biol Chem. 2021;297(5):101284.
4. Shelkovnikova TA, An H, Skelt L, Tregoning JS, Humphreys IR, Buchman VL. Antiviral immune response as a trigger of FUS Proteinopathy in amyotrophic lateral sclerosis. Cell Rep. 2019;29(13):4496–508.e4.
5. Haller O, Kochs G. Interferon-induced mx proteins: dynamin-like GTPases with antiviral activity. Traffic. 2002;3(10):710–7.
6. Holzinger D, Jorns C, Stertz S, Boisson-Dupuis S, Thimme R, Weidmann M, et al. Induction of MxA gene expression by influenza A virus requires type I or type III interferon signaling. J Virol. 2007;81(14):7776–85.
7. Tateishi T, Hokonohara T, Yamasaki R, Miura S, Kikuchi H, Iwaki A, et al. Multiple system degeneration with basophilic inclusions in Japanese ALS patients with FUS mutation. Acta Neuropathol. 2010;119(3):355–64.
8. Gao S, von der Malsburg A, Dick A, Faelber K, Schröder GF, Haller O, et al. Structure of myxovirus resistance protein a reveals intra- and intermolecular domain interactions required for the antiviral function. Immunity. 2011;35(4):514–25.
9. Ruggiero E, Frasson I, Tosoni E, Scalabrin M, Perrone R, Marušič M, et al. Fused in Liposarcoma protein, a new player in the regulation of HIV-1 transcription, binds to known and newly identified LTR G-Quadruplexes. ACS Infect Dis. 2022;8(5):958–68.
10. Xue YC, Ng CS, Mohamud Y, Fung G, Liu H, Bahreyni A, et al. FUS/TLS suppresses enterovirus replication and promotes antiviral innate immune responses. J Virol. 2021;95(12):e00304–21.

**How to cite this article:** Honda H, Yagita K, Arahata H, Hamasaki H, Noguchi H, Koyama S, et al. Increased expression of human antiviral protein MxA in FUS proteinopathy in amyotrophic lateral sclerosis. Brain Pathology. 2024;34(2):e13191. https://doi.org/10.1111/bpa.13191