An ALS-associated KIF5A mutant forms oligomers and aggregates and induces neuronal toxicity

Juri Nakano 1 | Kyoko Chiba 2 | Shinsuke Niwa 1,2

1Graduate School of Life Sciences, Tohoku University, Sendai, Japan
2Frontier Research Institute for Interdisciplinary Sciences (FRIS), Tohoku University, Sendai, Miyagi, Japan

Correspondence
Kyoko Chiba, Frontier Research Institute for Interdisciplinary Sciences (FRIS), Tohoku University, 6-3 Aramaki-Aoba, Aoba-ku, Sendai, Miyagi 980-0845, Japan. Email: kyoko.chiba.e7@tohoku.ac.jp

Shinsuke Niwa, Frontier Research Institute for Interdisciplinary Sciences (FRIS), Tohoku University, 6-3 Aramaki-Aoba, Aoba-ku, Sendai, Miyagi 980-0845, Japan. Email: shinsuke.niwa.c8@tohoku.ac.jp

Funding information
Japan Society for the Promotion of Science, Grant/Award Numbers: 19H04738, 20H03247, 20K21378, 21K20621; Ministry of Education, Culture, Sports, Science & Technology, Grant/Award Number: JPMXS0320200156; Kato Memorial Bioscience Foundation; Takeda Science Foundation; Uehara Memorial Foundation

Communicated by: Gohta Goshima

Abstract
KIF5A is a kinesin superfamily motor protein that transports various cargos in neurons. Mutations in Kif5a cause familial amyotrophic lateral sclerosis (ALS). These ALS mutations are in the intron of Kif5a and induce mis-splicing of KIF5A mRNA, leading to splicing out of exon 27, which in human KIF5A encodes the cargo-binding tail domain of KIF5A. Therefore, it has been suggested that ALS is caused by loss of function of KIF5A. However, the precise mechanisms regarding how mutations in KIF5A cause ALS remain unclear. Here, we show that an ALS-associated mutant of KIF5A, KIF5A(Δexon27), is predisposed to form oligomers and aggregates in cultured mouse cell lines. Interestingly, purified KIF5A(Δexon27) oligomers showed more active movement on microtubules than wild-type KIF5A in vitro. Purified KIF5A(Δexon27) oligomers showed more active movement on microtubules than wild-type KIF5A in vitro. Purified KIF5A(Δexon27) was prone to form aggregates in vitro. Moreover, KIF5A(Δexon27)-expressing Caenorhabditis elegans neurons showed morphological defects. These data collectively suggest that ALS-associated mutations of KIF5A are toxic gain-of-function mutations rather than simple loss-of-function mutations.

KEYWORDS
aggregation, ALS, KIF5A

1 | INTRODUCTION

Neuronal development, function, and maintenance depend on intracellular transport (Hirokawa et al., 2009). Kinesin superfamily proteins (KIFs) and cytoplasmic dyneins are molecular motors that enable anterograde and retrograde transport, respectively (Hirokawa et al., 2010; Kardon & Vale, 2009). Among KIFs, the Kinesin-1, -2, and -3 family members are the main anterograde transporters in neurons (Hall & Hedgecock, 1991; Niwa et al., 2008; Okada et al., 1995; Scholey, 2008; Vale et al., 1985; Xia et al., 2003). KIFs generally consist of a conserved motor domain, a dimerized coiled-coil domain, and a cargo-binding tail domain. The motor domain exhibits microtubule-stimulated ATPase activity, which allows the protein to move along microtubules (Hackney, 1995). Each KIF has a specialized tail domain (Vale, 2003) that binds to specific cargo vesicles and protein complexes (Hirokawa et al., 2009).
Advances in genomic sequencing technology have allowed identification of many disease-associated mutations in motor protein genes (Hirokawa et al., 2010; Holzbaur & Scherer, 2011). Mutations in KIFs and dynein subunits often cause motor neuron diseases. For example, mutations in Kinesin-3 family members, such as KIF1A and KIF1B, cause hereditary spastic paraplegia and Charcot–Marie–Tooth disease type 2A1 (Boyle et al., 2021; Budaitis et al., 2021; Chiba et al., 2019; Zhao et al., 2001). Mutations in dynein heavy chain 1 (DYNC1H1) cause Charcot–Marie–Tooth disease type 2O and spinal muscular atrophy-1 (Harms

(a) **human KIF5A genome**

| mRNA exon: | 26 | 27 | 28 | 29 |
|-----------|----|----|----|----|
| mRNA normal splicing | | | | |
| mRNA mutant splicing | | | | |
| KIF5A(wt) | | | | |
| KIF5AΔexon27 | | | | |

(b) **Legend on next page.**

(c) **mSca::KIF5A**

(d) **mSca::KIF5A**

(e) **mSca::KIF5AΔexon27**

(f) **Legend on next page.**

**FIGURE 1**
et al., 2012; Weedon et al., 2011). Mutations in the p150 subunit of dynactin (DCTN1), an activator of dynein, cause amyotrophic lateral sclerosis (ALS; Munch et al., 2004). Gain-of-function mutations in BICD2, a cargo adaptor protein for the cytoplasmic dynein complex, also cause spinal muscular atrophy (Huynh & Vale, 2017). KIF5A mutations are associated with hereditary spastic paraplegia (SPG) and ALS (Brenner et al., 2018; Nicolas et al., 2018; Reid et al., 2002). KIF5A transport protein complexes and membrane organelles such as neurofilaments, RNA granules and mitochondria (Hirokawa et al., 2010; Kanai et al., 2004; Uchida et al., 2009; Xia et al., 2003). The mutated residues differ between KIF5A-associated SPG and ALS. SPG is caused by motor domain mutations in KIF5A that abolish the motor activity of the KIF5A motor (Ebbing et al., 2008). However, ALS-associated KIF5A mutations commonly induce mis-splicing and deletion of exon 27 (Figure 1a; Brenner et al., 2018; Nicolas et al., 2018). The Δexon27 mutation induces frameshift, leading to abnormal C-terminal tail (Figure 1a). Previous studies have suggested that ALS-associated mutations in KIF5A are loss-of-function mutations because of the deletion of the cargo-binding tail domain (D. Brenner et al., 2018; Nicolas et al., 2018). However, the precise molecular mechanism of KIF5A-associated ALS has not been shown.

We show here that the product of ALS-associated KIF5A alleles, KIF5A(Δexon27), is predisposed to form oligomers and aggregates. Binding of KLC1, a cargo adaptor of KIF5A, was not affected by Δexon27. Interestingly, KIF5A(Δexon27) oligomers showed higher motility than those of wild-type KIF5A regardless of the presence of KLC1. Furthermore, exogenous expression of KIF5A(Δexon27) caused defects in the neuronal morphology of Caenorhabditis elegans. Collectively, these findings suggest that ALS-associated mutations in KIF5A are toxic gain-of-function mutations rather than simple loss-of-function mutations.

2 | RESULTS

2.1 | KIF5A(Δexon27) forms aggregates in the cell

To study the molecular mechanism of ALS caused by KIF5A mutations, we expressed mScarlet-fused KIF5A (mSca::KIF5A) and mScarlet-fused KIF5A(Δexon27) (mSca::KIF5A(Δexon27)) in a neuron-like cell line, CAD (Qi et al., 1997; Figure 1b–f). 20 hours after the transfection, mSca::KIF5A was mostly diffuse throughout the cell, but approximately 30% of mSca::KIF5A-expressing cells showed small aggregates in the cytoplasm (Figure 1c–f), which is consistent with our prior finding showing a propensity of KIF5A to form oligomers (Chiba et al., 2022). The proportion of cells showing aggregation was increased compared with cells expressing mScarlet-fused KIF5B, a homologue of KIF5A (Hirokawa et al., 2009). Only 10% of mSca::KIF5B-expressing cells exhibited aggregates. In contrast, mSca::KIF5A(Δexon27) formed many aggregates in the cytoplasm, as noted in 97% of cells. We observed that aggregates often accumulated in the tip of neurites in CAD cells (Figure 1f arrows), which is similar to the localization of KIF5A with constitutive-active mutations (Nakata et al., 2011). Few aggregates were found in mScarlet-expressing cells (Figure 1f). Formation of aggregates is not due to the higher expression of mSca::KIF5A(Δexon27) because most mSca::KIF5A(Δexon27)-expressing cells had aggregates even 6 h after the transfection. These data suggest that KIF5A is predisposed to form aggregates in the cell and that the ALS-associated mutation Δexon27 strongly enhances aggregate formation.
**FIGURE 2** Co-aggregation of amyotrophic lateral sclerosis-associated KIF5A. mScarlet fused to KIF5A or KIF5A (Δexon27) was co-expressed with enhanced green fluorescent protein (EGFP)-fused proteins in CAD cells. (a and b) Representative images showing mSca::KIF5A (a) and EGFP::KIF5A (b) co-expressing cells. When mSca::KIF5A did not form aggregates, EGFP::KIF5A did not co-aggregate in the same cell. No cells (0/70) showed co-aggregation. (c and d) Representative images showing mSca::KIF5A(Δexon27) (c) and EGFP::KIF5A (d) co-expressing cells. Note that EGFP::KIF5A co-aggregated with mSca::KIF5A(Δexon27). All cells (85/85, 100%) showed co-aggregation. (e and f) Representative images showing mSca::KIF5A (e) and EGFP::KIF5B (f) co-expressing cells. When mSca::KIF5A did not form aggregates, EGFP::KIF5B did not co-aggregate as well. No cells (0/70) showed co-aggregation. (g and h) Representative images showing mSca::KIF5A(Δexon27) and EGFP::KIF5B co-expressing cells. Note that EGFP::KIF5B co-aggregated with mSca::KIF5A(Δexon27). All cells (82/82, 100%) showed co-aggregation. (i and j) Representative images showing mSca::KIF5A(Δexon27) and EGFP co-expressing cells. Even when mSca::KIF5A(Δexon27) formed aggregates in the cytoplasm, no GFP aggregation was observed. No cells (0/54) showed co-aggregation. Bars, 50 μm
2.2 | KIF5A(Δexon27) co-aggregates with wild-type motors

We investigated whether the ALS-associated KIF5A protein co-aggregates with wild-type motors. mSca::KIF5A (Δexon27) was co-expressed with enhanced green fluorescent protein (EGFP)-labeled KIF5A (EGFP::KIF5A). Previous studies have shown that KIF5A forms a homodimer and moves along microtubules (Hackney, 1995; Hackney et al., 1991; Vai et al., 1996). As expected, mSca::KIF5A (Δexon27) co-aggregated with EGFP::KIF5A(wt) in the cell (Figure 2a–d), and aggregates accumulated at the tips of CAD cell neurites. Homo sapiens have three homologous kinesin heavy chain genes: KIF5A, KIF5B, and KIF5C (Hirokawa et al., 2009). These three proteins are highly conserved but do not form heterodimers (Kanai et al., 2000). Interestingly, mSca::KIF5A(Δexon27) co-aggregated with EGFP::KIF5B(wt) (Figure 2e–h). However, EGFP::KIF5B was diffuse in the cytoplasm when mSca::KIF5A did not form aggregates (Figure 2e,f), and EGFP::KIF5B(wt) uncharacteristically accumulated in neurite tips and co-aggregated with mSca::KIF5A(Δexon27) (Figure 2g,h). In contrast, mSca::KIF5A(Δexon27) did not co-aggregate with EGFP alone (Figure 2i,j). These data suggest that KIF5A (Δexon27) co-aggregates with wild-type KIF5A and KIF5B motors in the cell.

2.3 | KIF5A(Δexon27) oligomerizes in vitro

Kinesin-1 is a heterotetramer composed of two heavy chains (KIF5) and two light chains (KLC; Hackney et al., 1991). We have previously shown that KIF5A has a propensity to form oligomers in vitro (Chiba et al., 2022). To examine the effect of the Δexon27 mutation on the interaction of KIF5A with KLC1 and oligomerization, we next purified heavy chain dimers (KIF5A) and Kinesin-1 tetramers (KIF5A-KLC1) with or without the Δexon27 mutation. First, we expressed full-length KIF5A::mSca and KIF5A(Δexon27)::mSca in sf9 cells and purified them by affinity chromatography and size exclusion chromatography (SEC; Chiba et al., 2022). In SEC, wild-type KIF5A predominantly eluted at a single peak (Figure 3a,
blue shaded area). In addition, a small amount of wild-type KIF5A was recovered from fractions that were eluted before the major peak and from the void volume (Figure 3a, red and yellow shaded areas). SEC coupled with multi angle light scattering (SEC-MALS) analyses have shown that the main peak corresponds to KIF5A dimers and that the minor peak eluted before the main peak represents KIF5A oligomers (Chiba et al., 2022). Next, we examined KIF5A(Δexon27) and found that the Δexon27 mutation markedly changed the elution profile from that of wild-type KIF5A. The major elution peak of Δexon27 shifted toward a higher molecular weight, which corresponds to oligomerization (Figure 3a, red shaded area). Most KIF5A(Δexon27) was recovered from high molecular weight fractions but not from dimer fractions. Thus, the Δexon27 mutation induces oligomerization of KIF5A. We note that Δexon27 also increased the population recovered from the void volume, suggesting the potential of KIF5A(Δexon27) to form aggregates as well as oligomers (Figure 3a, yellow shaded area).

Next, to determine whether binding of the KLC subunit is affected, we co-expressed KLC1 with full length KIF5A or KIF5A(Δexon27). KLC1 was co-purified either with KIF5A(wt) or KIF5A(Δexon27) (Figure 3b,c). The ratio of heavy chains to light chains was almost 1:1 and was not markedly affected by Δexon27. Thus, binding to KLC is not abolished by the deletion of exon 27. The KIF5A-KLC1 complex showed an elution profile similar to that of KIF5A in the SEC analysis (Figure 3b, blue lines). With KIF5A(Δexon27)-KLC1, we again observed a peak shifted toward a higher molecular weight and an increased population recovered from the void volume. These results suggest that the propensity of KIF5A (Δexon27) to form oligomers and aggregates is not largely affected by KLC1.

2.4 KIF5A(Δexon27) oligomers are hyperactivated on microtubules

We analyzed the motility of purified microtubule motors at single-molecule resolution by total internal reflection fluorescence microscopy (Chiba et al., 2019; Chiba et al., 2022; McKenney et al., 2014). Purified full-length KIF5A(wt)::mSca and KIF5A(Δexon27)::mSca-KLC1 did not bind or move well on microtubules (Figure 4a,b) because of the autoinhibitory mechanism described previously (Chiba et al., 2022; Coy et al., 1999; Friedman & Vale, 1999; Hackney & Stock, 2000). In contrast, we found that full-length KIF5A(Δexon27)::mSca and full-length KIF5A(Δexon27)::mSca-KLC1 frequently bound to and moved along microtubules (Figure 4a–c). KLC1 suppresses the binding of KIF5A(Δexon27) onto microtubules (Figure 4c). Interestingly, Δexon27 mutation did not delete the IAK motif that is essential for the autoinhibitory mechanism of kinesin heavy chains (Figure 1b; Coy et al., 1999; Friedman & Vale, 1999; Hackney & Stock, 2000). The fluorescent intensities of KIF5A (Δexon27)::mSca and full-length KIF5A(Δexon27)::mSca-KLC1 were stronger than those of KIF5A(wt)::mSca and KIF5A(Δexon27)::mSca-KLC1 (Figure 4b), suggesting the formation of oligomers. On average, the binding rates of KIF5A(Δexon27)::mSca and KIF5A(Δexon27)::mSca-KLC1 were nine times higher than those of KIF5A(wt)::mSca and KIF5A(Δexon27)::mSca-KLC1, respectively (Figure 4c). We previously showed that the average run length and velocity of wild-type KIF5A are 1.0 μm and 1.1 μm/s, respectively (Chiba et al., 2022); however, we could not collect a sufficient number of samples to measure the run length and velocity of wild-type proteins under the present condition. The median run lengths of KIF5A(Δexon27)::mSca and KIF5A(Δexon27)::mSca-KLC1 were approximately 3 μm each (Figure 4d), which is much longer than that of wild-type KIF5A. No significant difference was detected between KIF5A(Δexon27)::mSca and KIF5A(Δexon27)::mSca-KLC1. The velocity of KIF5A(Δexon27)::mSca and KIF5A(Δexon27)::mSca-KLC1 was 0.4 μm/s (Figure 4e,f), which is slower than that of wild-type KIF5A. Taken together, these results suggest that KIF5A oligomers induced by Δexon27 are more active than those of wild-type KIF5A both in the presence and absence of the KLC subunit (Table 1).

2.5 KIF5A(Δexon27) oligomers tend to form aggregates

To examine the properties of KIF5A(Δexon27), we incubated purified mScarlet, KIF5A::mSca and KIF5A (Δexon27)::mSca at 37°C and measured the turbidity of protein solutions by determining the optical density at 600 nm, which was used to monitor the formation of protein aggregates in solution in a previous study (Schafheimer & King, 2013). Immediately before incubation, protein solutions were centrifuged and clarified. The turbidity of purified KIF5A(Δexon27)::mSca increased gradually while those of mScarlet and KIF5A::mSca did not (Figure 5a). Twenty-four hours later, the solution was again centrifuged, and purified mSca::KIF5A(Δexon27) formed a protein pellet (Figure 5b). At this concentration, purified mScarlet and KIF5A::mSca did not form visible pellets at all, even after the 24-h incubation. These data indicate that purified KIF5A(Δexon27) is predisposed to form protein aggregates in vitro.
KIF5A(Δexon27) is toxic in Caenorhabditis elegans neurons

Previous studies have shown that ALS is caused by toxic gain-of-function mutations (Bruijn et al., 1998; Johnson et al., 2009; Kwiatkowski Jr. et al., 2009). Therefore, we examined the toxicity of KIF5A(Δexon27) in C. elegans neurons. The morphology of mechanosensory neurons was compared after expressing human KIF5A and KIF5A (Δexon27) (Figure 6a–f). Mechanosensory neurons were analyzed because many studies have shown that these neurons have very stereotypical morphology and show***
small variations in the wild-type background (Gallegos & Bargmann, 2004; Ghosh-Roy et al., 2012). The morphology of posterior lateral microtubule (PLM) and posterior ventral microtubule (PVM) neurons was observed and compared in day 1 adults. PLM neurons had a long straight axon along the body in wild-type cells (Figure 6a,b).

Table 1: Measured parameters of KIF5A

| Summary of motility measurement of KIF5A(Δexon27) | Velocity (nm/s) | Landing rates (motors/μm/s/μM) | Run length (μm) |
|-----------------------------------------------|-----------------|---------------------------------|-----------------|
| KIF5A(1–420)                                 | 1048 ± 173a     | 162 ± 31a                       | 0.70a (0.55–0.93) |
| Wild-type                                    | 1182 ± 187a     | 0.85 ± 0.57                     | 1.00a (0.69–1.61) |
| Δexon27                                      | 377 ± 107       | 8.3 ± 2.3                       | 2.76 (1.50–4.54)  |
| KIF5A-KLC1 complex                           | 1051 ± 176a     | 0.20 ± 0.20                     | 1.49a (0.78–2.84) |
| Wild-type                                    | 378 ± 111       | 4.6 ± 0.80                      | 2.30 (1.44–4.03)  |
| Δexon27                                      |

Note: The motility parameters of tail-truncated KIF5A(1–420), full-length KIF5A and KIF5A-KLC1 complex. Velocities (mean ± SD), landing rates (mean ± SD), and run lengths (median and interquartile range) are shown. aValues are described in Chiba et al. (2022).
The PVM neuron extends an axon that grows ventrally and then turns anteriorly when it reaches the ventral nerve cord (Figure 6a,b). No significant differences were found between KIF5A-expressing neurons and control neurons (Figure 6c). In contrast, KIF5A(Δexon27)-expressing neurons showed morphological defects (Figure 6d–f). Approximately 60% of worms showed abnormal PLM and PVM morphologies (Figure 6d), but no stereotypical abnormalities were found. The PLM cell body was often mislocalized, and some worms had

---

**FIGURE 6** Legend on next page.
PLM neurons with multiple neuronal processes, whereas other worms had PLM neurons with bent axons. Approximately 20% of worms showed neuronal loss (Figure 6e). These data suggest that KIF5A (Δexon27) is toxic in neurons.

3 | DISCUSSION

Previous studies have suggested that KIF5A(Δexon27) causes ALS by a loss-of-function mechanism because the cargo-binding tail domain is deleted (Brenner et al., 2018; Nicolas et al., 2018). However, while several loss-of-function mutations have been found in the motor domain of KIF5A, these mutations cause SPG, rather than ALS (Ebbing et al., 2008; Reid et al., 2002). None of the motor domain mutations in KIF5A have been associated with ALS. These genetic data suggest that ALS mutations in KIF5A are not simple loss-of-function mutations. Protein aggregates are often associated with neurodegenerative disorders (Soto, 2003), and numerous ALS-associated mutations have been identified in other genes such as TARDBP (TDP-43 gene), SOD, and FUS (Aoki et al., 1993; Kabashi et al., 2008; Kwiatkowski Jr. et al., 2009; Vance et al., 2009). These mutations commonly induce aggregates that are toxic in cells (Bruijn et al., 1998; Johnson et al., 2009; Kwiatkowski Jr. et al., 2009). Our data suggest that the Δexon27 mutation in KIF5A induces toxic aggregates. Furthermore, while we were preparing this manuscript, a preprint supporting the same conclusion was posted on Biorxiv (Pant et al., 2022). The study showed that KIF5A(Δexon27) causes aggregate formation in cultured cells and is toxic when expressed in Drosophila. The study also showed that unpurified KIF5A (Δexon27) in cell lysates is more active than wild-type KIF5A. What induces the formation of KIF5A(Δexon27) aggregates? It is possible that unidentified proteins bind to the abnormal C-terminus of KIF5A(Δexon27) and induce hyperactivation and aggregation. However, our assays using purified proteins strongly suggest that KIF5A(Δexon27) is hyperactive and is predisposed to form aggregates without the involvement of other factors (Figures 3–5).

The Δexon27 mutation induces hyperactivation of KIF5A. KIF5 is inhibited by KLC-dependent and independent autoinhibitory mechanisms (Chiba et al., 2022; Hackney & Stock, 2000; Verhey et al., 1998). Binding with cargo vesicles or cargo complexes unlock the autoinhibition. The binding of KIF5A(Δexon27) onto microtubules is suppressed by KLC1 (Figure 4c), suggesting that KLC-dependent autoinhibitory mechanism works even in KIF5A(Δexon27). Interestingly, the IAK motif is not affected by the Δexon27 mutation (Figure 1b). It was shown that hydrophobic materials such as polystyrene beads and glass surface can mimic cargos and activate KIF5-KLC complex when they bind to the tail region (Vale et al., 1985). Δexon27 mutation induces the formation of large oligomers (Figure 3a,b). Large KIF5A (Δexon27) oligomers may mimic cargos and activate the motor. It is also possible that Δexon27 mutation disrupts previously unknown autoinhibitory mechanisms.

What induces toxicity in neurons? Toxicity in worm neurons would help to clarify neurotoxic mechanisms. One possibility is that hyperactivated KIF5A changes the distribution of cargo organelles and induces cellular toxicity. We have shown that another kinesin, human KIF1A, is functional in worm neurons (Chiba et al., 2019). We show here KIF5A(Δexon27) binds to the cargo-binding adaptor KLC1 (Figure 3c). Thus, cargo transport may be affected by KIF5A(Δexon27) even in worm neurons. Another possibility is that cargo transport is not directly related and KIF5A(Δexon27) aggregates are toxic in neurons as is the case in other ALS-associated mutations (Bruijn et al., 1998; Johnson et al., 2009; Kwiatkowski Jr. et al., 2009). Aggregates change cellular metabolisms and induces neuronal death (Soto, 2003). These possibilities will be discriminated by analyzing cargo transport and distributions in KIF5A(Δexon27)-expressed worm neurons. If aggregates...
are toxic, hyperactivation of KIF5A motor may enhance the toxicity because hyperactivation causes mis-
accumulation of KIF5A at axonal tips, leading to a high concentra-
tion of KIF5A and aggregate formation. This hypothesis is supported by the observation that KIF5A
(Δexon27) aggregates were often found at neurite tips in
CAD cells (Figure 1).

We have previously shown that KIF5A forms more oligomers than KIF5B and KIF5C in vitro (Chiba
et al., 2022). Additionally, KIF5A oligomers are more active than KIF5A dimers. However, the physiological
significance of these phenomena remains elusive. We show here that even wild-type KIF5A has a propensity to
form more aggregates in the cell than KIF5B (Figure 1).

A similar property has been found for TDP-43. TDP-43 is
intrinsically aggregation-prone, which implies that it may
be directly involved in the pathogenesis of sporadic ALS
enhanced by ALS-associated KIF5A mutations.

4 | EXPERIMENTAL PROCEDURES

4.1 | Molecular biology

Polymerase chain reaction (PCR) was performed using
KOD FX neo (TOYOBO) as described in the manual.
Restriction enzymes were purchased from New England
BioLabs Japan.

pAcebac1 plasmids containing human KIF5A (BC146670)
human KIF5B (BC126281), and KLC1 (BC008881) was
described previously (Chiba et al., 2022).

To generate mScarlet::KIF5A and mScarlet::KIF5B
expressing plasmids, pmScarletC1 plasmid was obtained
from Addgene. KIF5A and KIF5B were amplified by PCR
and transferred to pmScarletC1. To generate an EGFP::
KIF5B expressing vector, EGFP was amplified from
pEGFPN1::KIF1A (Niwa et al., 2008) and replaced with
mScarlet by using AgeI and XhoI sites. Δexon27 mutation
was introduced by Gibson assembly. cDNA fragment
encoding the mutated domain (Figure 1a) was synthe-
sized by gBlocks (Integrated DNA Technologies Japan)
and replated with the wild-type fragment by Gibson
assembly (Gibson et al., 2009). Plasmids used in this
paper is described in Table S1. Key plasmids were depos-
ited to Addgene.

4.2 | CAD cell experiments

CAD cells were obtained from European Collection of Cell
 Cultures and maintained as described (Qi et al., 1997). For
observation, cells were cultured on glass coverslips
(Matsunami, Tokyo, Japan). Plasmid transfection was per-
fomed by Lipofectamine LTX (Thermo Fisher Scientific)
as described in the manufacturer’s manual. 24 hours after
the transfection, mScarlet and GFP signals were observed
under Zeiss Axio Observer inverted microscope equipped
with LSM800 confocal unit (Carl Zeiss). ×40 water immers-
sion objective lens (Numerical Aperture: 1.1) was used for
imaging. ZEN software (Carl Zeiss) was used to control
the system.

4.3 | Purification of KIF5A

pAcebac plasmids were transformed to generate bacmid.
S9 cells were maintained as a suspension culture in Sf-
900II serum-free medium (Thermo Fisher Scientific) at
27°C. To prepare baculovirus, 1 × 10⁶ cells of S9 cells
were transferred to each well of a tissue-culture treated
6 well plate. After the cells attached to the bottom of the
dishes, about ~5 μg of bacmid were transsected using 6 μl
cellfectin II reagent (Thermo Fisher Scientific). Five
days after initial transfection, the culture media were
collected and spun at 3000 g for 3 min to obtain the superna-
tant (P1). For protein expression, 400 ml of S9 cells
(2 × 10⁶ cells/ml) were infected with 100 μl of P1 virus
and cultured for 65 hr at 27°C. Cells were harvested and
resuspended in 25 ml of lysis buffer (50 mM HEPES-
KOH, pH 7.5, 150 mM KCH₃COO, 2 mM MgSO₄, 1 mM
EGTA, 10% glycerol) along with 1 mM DTT, 1 mM
PMSF, 0.1 mM ATP and 0.5% Triton-X-100. After incubat-
ing on ice for 10 min, the lysates were centrifuged at
15,000 g for 20 min at 4°C. The resulting supernatant
were subject to affinity chromatography described below.

For affinity chromatography, the supernatants were put
over a column of Streptactin XT resin (IBA) at 4°C
The columns were then washed with excess lysis buffer
to remove unbound material and the proteins were
equilibrium in lysis buffer (50 mM HEPES-
KOH, pH 7.5, 150 mM KCH₃COO, 2 mM MgSO₄, 1 mM
EGTA, 10% glycerol) along with 1 mM DTT, 1 mM
PMSF, 0.1 mM ATP and 0.5% Triton-X-100. After incubat-
ing on ice for 10 min, the lysates were centrifuged at
15,000 g for 20 min at 4°C. The resulting supernatant
were subject to affinity chromatography described below.

For affinity chromatography, the supernatants were put
over a column of Streptactin XT resin (IBA) at 4°C
The columns were then washed with excess lysis buffer
to remove unbound material and the proteins were
equilibrium in lysis buffer (50 mM HEPES-
KOH, pH 7.5, 150 mM KCH₃COO, 2 mM MgSO₄, 1 mM
EGTA, 10% glycerol) along with 1 mM DTT, 1 mM
PMSF, 0.1 mM ATP and 0.5% Triton-X-100. After incubat-
ing on ice for 10 min, the lysates were centrifuged at
15,000 g for 20 min at 4°C. The resulting supernatant
were subject to affinity chromatography described below.

For affinity chromatography, the supernatants were put
over a column of Streptactin XT resin (IBA) at 4°C
The columns were then washed with excess lysis buffer
to remove unbound material and the proteins were
equilibrium in lysis buffer (50 mM HEPES-
KOH, pH 7.5, 150 mM KCH₃COO, 2 mM MgSO₄, 1 mM
EGTA, 10% glycerol) along with 1 mM DTT, 1 mM
PMSF, 0.1 mM ATP and 0.5% Triton-X-100. After incubat-
ing on ice for 10 min, the lysates were centrifuged at
15,000 g for 20 min at 4°C. The resulting supernatant
were subject to affinity chromatography described below.

4.4 | TIRF single-molecule motility
assays

TIRF assays were performed as described (Chiba
et al., 2019). Tubulin was purified from porcine brain as
described (Castold & Popov, 2003). Tubulin was labeled
with Biotin-PEG₂-NHS ester (Tokyo Chemical Industry) and AZDye647 NHS ester (Fluoroprobes) as described (Al-Bassam, 2014). To polymerize Taxol-stabilized microtubules labeled with biotin and AZDye647, 30 μM unlabeled tubulin, 1.5 μM biotin-labeled tubulin and 1.5 μM AZDye647-labeled tubulin were mixed in BRB80 buffer supplemented with 1 mM GTP and incubated for 15 min at 37°C. Then, an equal amount of BRB80 supplemented with 40 μM taxol was added and further incubated for more than 15 min. The solution was loaded on BRB80 supplemented with 300 mM sucrose and 20 μM taxol and ultracentrifuged at 100,000 g for 5 min at 30°C. The pellet was resuspended in BRB80 supplemented with 20 μM taxol. Glass chambers were prepared by acid washing as previously described (McKenney et al., 2014). Glass chambers were coated with PLL-PEG-biotin (SuSoS). Polymerized microtubules were flowed into streptavidin adsorbed flow chambers and allowed to adhere for 5–10 min. Unbound microtubules were washed away using assay buffer [90 mM Hepes pH 7.4, 50 mM KCH₃COO, 2 mM Mg(CH₃COO)₂, 1 mM EGTA, 20 μM tubulin, 1.5 μM tubulin labeled with biotin and AZDye647, 30 μM unlabeled tubulin, 0.1 mg/ml biotin–BSA, 0.2 mg/ml kappa-casein, 0.5% Pluronic F127, 2 mM ATP, and an oxygen scavenging system composed of PCA/PCD/Trolox. Purified motor protein was diluted to indicated concentrations in the assay buffer. Then, the solution was flowed into the glass chamber. An ECLIPSE Ti2-E microscope equipped with a CFI Apochromat TIRF 100XC Oil objective lens, an Andor iXion life 897 camera and a Ti2-LAPP illumination system (Nikon) was used to observe single molecule motility. NIS-Elements AR software ver. 5.2 (Nikon) was used to control the system.

4.5 | Worm experiments and strains

*C. elegans* strains were maintained as described previously (Brenner, 1974). Wild-type worm N2 and feeder bacteria OP50 were obtained from *C. elegans* genetic center (CGC; Minneapolis). Nematode Growth Media (NGM) agar plates were prepared as described (S. Brenner, 1974). Transformation of *C. elegans* was performed by DNA injection as described (Mello et al., 1991). 5 ng of *Pmec-7::KIF5A* or *Pmec-7::KIF5A(Δexon27)* plasmids were injected. Strains used in this study are described in Table S2.

Analysis of mechanosensory neurons in *Caenorhabditis elegans*

Mechanosensory neurons were visualized using *uIs31 [Pmec-7::gfp]* marker that was obtained from *C. elegans* genetic center (Chalfie et al., 1994). Strains expressing human KIF5A or KIF5A(Δexon27) were observed under Zeiss Axio Observer inverted microscope equipped with LSM800 confocal unit (Carl Zeiss). ×20 objective lens (Numerical Aperture: 0.8) was used for imaging. ZEN software (Carl Zeiss) was used to control the system. Fiji was used to analyze image files (Schindelin et al., 2012).

AUTHOR CONTRIBUTIONS

S.N. designed the study; J.N., K.C., and S.N. performed the study; J.N., K.C., and S.N. analyzed the data; J.N., K.C., and S.N. wrote the paper.

ACKNOWLEDGMENTS

We thank all of the members of the McKenney lab (UC Davis), Sugimoto lab (Tohoku University), and Niwa lab (Tohoku University) for helpful discussions. SN was supported by JSPS KAKENHI (20H03247, 19H04738, 20K21378), the Kato Memorial Bioscience Foundation, and Takeda Science Foundation. KC was supported by Uehara Memorial Foundation, JSPS KAKENHI (21K20621) and MEXT Leading Initiative for Excellent Researchers (JPMXS0320200156). Some *C. elegans* strains and OP50 were obtained from the CGC. We thank Lisa Kreiner, PhD, from Edanz (https://www.jp.edanz.com/ac) for editing a draft of this manuscript.

CONFLICT OF INTEREST

The authors have no conflicts of interest directly relevant to the content of this article.

ORCID

Shinsuke Niwa https://orcid.org/0000-0002-8367-9228

REFERENCES

Al-Bassam, J. (2014). Reconstituting dynamic microtubule polymerization regulation by TOG domain proteins. *Methods in Enzymology, 540*, 131–148. https://doi.org/10.1016/B978-0-12-397924-7.00008-X

Aoki, M., Ogasawara, M., Matsubara, Y., Narisawa, K., Nakamura, S., Itoyama, Y., & Abe, K. (1993). Mild ALS in Japan associated with novel SOD mutation. *Nature Genetics, 5*(4), 323–324. https://doi.org/10.1038/ng1293-323

Boyle, L., Rao, L., Kaur, S., Fan, X., Mebane, C., Hamm, L., Thornton, A., Ahrendsden, J. T., Anderson, M. P., Christodoulou, J., Gennerich, A., Shen, Y., & Chung, W. K. (2021). Genotype and defects in microtubule-based motility correlate with clinical severity in KIF1A-associated neurological disorder. *HGG Adv, 2*(2), 100026. https://doi.org/10.1016/j.hgga.2021.100026

Brenner, D., Yilmaz, K., Grehi, T., Petri, S., Meyer, T., Grosskreutz, J., Weydt, P., Ruf, W., Neuwirth, C., Weber, M., Pinto, S., Claey, K. G., Schrank, B., Jordan, B., Knehr, A., Gunther, K., Hubers, A., Zeller, D., … German, A. L. S. n. M. N. D. N. E. T. German ALS network MND-NET. (2018). Hot-spot KIF5A mutations cause familial ALS. *Brain, 141*(3), 688–697. https://doi.org/10.1093/brain/awx370
Brenner, S. (1974). The genetics of Caenorhabditis elegans. Genetics, 77(1), 71–94. https://www.ncbi.nlm.nih.gov/pubmed/4366476

Bruijn, L. I., Housewart, M. K., Kato, S., Anderson, K. L., Anderson, S. D., Ohama, E., Reaume, A. G., Scott, R. W., & Cleveland, D. W. (1998). Aggregation and motor neuron toxicity of an ALS-linked SOD1 mutant independent from wild-type SOD1. Science, 281(5384), 1851–1854. https://doi.org/10.1126/science.281.5384.1851

Budaitis, B. G., Jariwala, S., Rao, L., Yue, Y., Sept, D., Verhey, K. J., & Gennerich, A. (2021). Pathogenic mutations in the kinesin-3 motor KIF1A diminish force generation and movement through allosteric mechanisms. The Journal of Cell Biology, 220(4), e202004227. https://doi.org/10.1083/jcb.202004227

Castoldi, M., & Popov, A. V. (2003). Purification of brain tubulin through two cycles of polymerization-depolymerization in a high-molarity buffer. Protein Expression and Purification, 32(1), 83–88. https://doi.org/10.1016/S1046-5928(03)00218-3

Chalfie, M., Tu, Y., Euskirchen, G., Ward, W. W., & Prasher, D. C. (1994). Green fluorescent protein as a marker for gene expression. Science, 263(5148), 802–805. https://doi.org/10.1126/science.8303295

Chiba, K., Ori-McKenney, K. M., Niwa, S., & McKenney, R. J. (2022). Synergistic autoinhibition and activation mechanisms control Kinesin-1 motor activity. Cell Rep, in press.

Chiba, K., Takahashi, H., Chen, M., Obinata, H., Arai, S., Hashimoto, K., Oda, T., McKenney, R. J., & Niwa, S. (2019). Disease-associated mutations hyperactivate KIF1A motility and anterograde axonal transport of synaptic vesicle precursors. Proceedings of the National Academy of Sciences of the United States of America, 116(37), 18429–18434. https://doi.org/10.1073/pnas.1905690116

Coy, D. L., Hancock, W. O., Wagenbach, M., & Howard, J. (1999). Kinesin’s tail domain is an inhibitory regulator of the motor domain. Nature Cell Biology, 1(5), 288–292. https://doi.org/10.1038/13001

Ebbing, B., Mann, K., Starosta, A., Jaud, J., Schols, L., Schule, R., & Woehlke, G. (2008). Effect of spastic paraplegia mutations in KIF5A kinesin on transport activity. Human Molecular Genetics, 17(9), 1245–1252. https://doi.org/10.1093/hmg/ddn014

Friedman, D. S., & Vale, R. D. (1999). Single-molecule analysis of kinesin motility reveals regulation by the cargo-binding tail domain. Nature Cell Biology, 1(5), 293–297. https://doi.org/10.1038/13008

Gallegos, M. E., & Bargmann, C. I. (2004). Mechanosensory neurite termination and tiling depend on SAX-2 and the SAX-1 kinase. Neuron, 44(2), 239–249. https://doi.org/10.1016/j.neuron.2004.09.021

Ghosh-Roy, A., Goncharov, A., Jin, Y., & Chisholm, A. D. (2012). Kinesin-13 and tubulin posttranslational modifications regulate microtubule growth in axon regeneration. Developmental Cell, 23(4), 716–728. https://doi.org/10.1016/j.devcel.2012.08.010

Gibson, D. G., Young, L., Chuang, R. Y., Venter, J. C., Hutchison, C. A., 3rd, & Smith, H. O. (2009). Enzymatic assembly of DNA molecules up to several hundred kilobases. Nature Methods, 6(5), 343–345. https://doi.org/10.1038/nmeth.1318

Gibson, D. G., Young, L., Chuang, R. Y., Venter, J. C., Hutchison, C. A., 3rd, & Smith, H. O. (2009). Enzymatic assembly of DNA molecules up to several hundred kilobases. Nature Methods, 6(5), 343–345. https://doi.org/10.1038/nmeth.1318

Hackney, D. D. (1995). Highly processive microtubule-stimulated ATP hydrolysis by dimeric kinesin head domains. Nature, 377(6548), 448–450. https://doi.org/10.1038/377448a0

Hackney, D. D., Levitt, J. D., & Wagner, D. D. (1991). Characterization of alpha 2 beta 2 and alpha 2 alpha 2 forms of kinesin. Biochemical and Biophysical Research Communications, 174(2), 810–815. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/1825168

Hackney, D. D., & Stock, M. F. (2000). Kinesin’s IAK tail domain inhibits initial microtubule-stimulated ADP release. Nature Cell Biology, 2(5), 257–260. https://doi.org/10.1038/35010525

Hall, D. H., & Hedgcock, E. M. (1991). Kinesin-related gene unc-104 is required for axonal transport of synaptic vesicles in C. elegans. Cell, 65(5), 837–847. https://doi.org/10.1016/0092-8674(91)90391-b

Harms, M. B., Ori-McKenney, K. M., Scoto, M., Tuck, E. P., Bell, S., Ma, D., Masí, S., Allred, P., Al-Lozi, M., Reilly, M. M., Miller, L. J., Jan-Cacsadi, A., Pestrunk, A., Shy, M. E., Muntoni, F., Valle, R. B., & Baloh, R. H. (2012). Mutations in the tail domain of DYNC1H1 cause dominant spinal muscular atrophy. Neurology, 78(22), 1714–1720. https://doi.org/10.1212/WNL.0b013e3182556c05

Hirokawa, N., Niwa, S., & Tanaka, Y. (2010). Molecular motors in neurons: Transport mechanisms and roles in brain function, development, and disease. Neuron, 68(4), 610–638. https://doi.org/10.1016/j.neuron.2010.09.039

Hirokawa, N., Noda, Y., Tanaka, Y., & Niwa, S. (2009). Kinesin superfamily motor proteins and intracellular transport. Nature Reviews. Molecular Cell Biology, 10(10), 682–696. https://doi.org/10.1038/nrm2774

Holzbaur, E. L., & Scherer, S. S. (2011). Microtubules, axonal transport, and neuropathy. The New England Journal of Medicine, 365(24), 2320–2332. https://doi.org/10.1056/NEJMcibr1112481

Huynh, W., & Vale, R. D. (2017). Disease-associated mutations in human BICD2 hyperactivate motility of dynein-dynactin. Journal of Cell Biology, 216(10), 3051–3060. https://doi.org/10.1083/jcb.201703201

Johnson, B. S., Snead, D., Lee, J. J., McCaffery, J. M., Shorter, J., & Gitter, A. D. (2009). TDP-43 is intrinsically aggregation-prone, and amyotrophic lateral sclerosis-linked mutations accelerate aggregation and increase toxicity. The Journal of Biological Chemistry, 284(30), 20329–20339. https://doi.org/10.1074/jbc.M109.010264

Kabashi, E., Valdmanis, P. N., Dion, P., Spiegelman, D., McConkey, B. J., Vande Velde, C., Bouchard, J. P., Lacomblez, L., Pochigaeva, K., Salachas, F., Pradat, P. F., Camu, W., Meiners, V., Dupre, N., & Rouleau, G. A. (2008). TARDBP mutations in individuals with sporadic and familial amyotrophic lateral sclerosis. Nature Genetics, 40(5), 572–574. https://doi.org/10.1038/ng.132

Kanai, Y., Ohdama, N., & Hirokawa, N. (2004). Kinesin transports RNA: Isolation and characterization of an RNA-transporting granule. Neuron, 43(4), 513–525. https://doi.org/10.1016/j.neuron.2004.07.022

Kanai, Y., Okada, Y., Tanaka, Y., Harada, A., Terada, S., & Hirokawa, N. (2000). KIF5C, a novel neuronal kinesin enriched
in motor neurons. *The Journal of Neuroscience*, 20(17), 6374–6384. https://doi.org/10.1523/JNEUROSCI.20-17-06374.2000

Kardon, J. R., & Vale, R. D. (2009). Regulators of the cytoplasmic dynein motor. *Nature Reviews. Molecular Cell Biology*, 10(12), 854–865. https://doi.org/10.1038/nrm2804

Kwiatkowski, T. J., Jr., Bosco, D. A., Leclerc, A. L., Tamrazian, E., Vanderburg, C. R., Russ, C., Davis, A., Gilchrist, J., Kasarskis, E. J., Munsat, T., Valdmansis, P., Rouleau, G. A., Hosler, B. A., Cortelli, P., de Jong, P. J., Yoshinaga, Y., Haines, J. L., Pericak-Vance, M. A., Yan, J., ... Brown, R. H., Jr. (2009). Mutations in the FUS/TLS gene on chromosome 16 cause familial amyotrophic lateral sclerosis. *Science*, 323(5918), 1205–1208. https://doi.org/10.1126/science.1166066

McKenney, R. J., Huynh, W., Tanenbaum, M. E., Bhabha, G., & Vale, R. D. (2014). Activation of cytoplasmic dynein motility by dynactin-cargo adapter complexes. *Science*, 345(6194), 337–341. https://doi.org/10.1126/science.1254198

Mello, C. C., Kramer, J. M., Stinchcomb, D., & Ambros, V. (1991). Efficient gene transfer in C.elegans: Extrachromosomal maintenance and integration of transforming sequences. *The EMBO Journal*, 10(12), 3959–3970. https://www.ncbi.nlm.nih.gov/pubmed/1935914

Munch, C., Sedlmeier, R., Meyer, T., Homberg, V., Sperfeld, A. D., Kurt, A., Prudlo, J., Peraus, G., Hanemann, C. O., Stumm, G., & Ludolph, A. C. (2004). Point mutations of the p150 subunit of dynactin (DCTN1) gene in ALS. *Neurology*, 63(4), 724–726. https://doi.org/10.1212/01.wnl.0000134608.83927.b1

Nakata, T., Niwa, S., Okada, Y., Perez, F., & Hirokawa, N. (2011). Preferential binding of a kinesin-1 motor to GTP-tubulin-rich microtubules underlies polarized vesicle transport. *The Journal of Cell Biology*, 194(2), 245–255. https://doi.org/10.1083/jcb.201104034

Nicolas, A., Kenna, K. P., Renton, A. E., Ticozzi, N., Faghri, F., Chia, R., Dominov, J. A., Kenna, B. J., Nalls, M. A., Keagle, P., Rivera, A. M., van Rheenen, W., Murphy, N. A., van Vught, J., Geiger, J. T., Van der Spek, R. A., Pliner, H. A., Shankaracharya, Smith, B. N., ... Landers, J. E. (2018). Genome-wide analyses identify KIF5A as a novel ALS gene. *Neuron*, 97(6), 1268–1283 e1266. https://doi.org/10.1016/j.neuron.2018.02.027

Niwa, S., Tanaka, Y., & Hirokawa, N. (2008). KIF1Bbeta- and KIF1A-mediated axonal transport of presynaptic regulator Rab3 occurs in a GTP-dependent manner through DENDMADD. *Nature Cell Biology*, 10(11), 1269–1279. https://doi.org/10.1038/ncb1785

Okada, Y., Yamazaki, H., Sekine-Aizawa, Y., & Hirokawa, N. (1995). The neuron-specific kinesin superfamily protein KIF1A is a unique monomeric motor for anterograde axonal transport of synaptic vesicle precursors. *Cell*, 81(5), 769–780. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/7539720

Pant, C. D., Parmeswarn, J., Rao, L., Shi, L., Chilukuri, G., McEachin, T. Z., Glass, J., Bassell, J. G., Genrich, A. C., & Jiang, J. (2022). ALS-linked KIF5A ΔExon27 mutant causes neuronal toxicity through gain of function. Bioxriv. https://doi.org/10.1101/2022.03.05.483071

Qi, Y., Wang, J. K., McMillian, M., & Chikaraishi, D. M. (1997). Characterization of a CNS cell line, CAD, in which morphological differentiation is initiated by serum deprivation. *The Journal of Neuroscience*, 17(4), 1217–1225. https://doi.org/10.1523/JNEUROSCI.04-01217.1997

Reid, E., Kloo, M., Ashley-Koch, A., Hughes, L., Bevan, S., Svenson, I. K., Graham, F. L., Gaskell, P. C., Dearlove, A., Pericak-Vance, M. A., Rubinsztein, D. C., & Marchuk, D. A. (2002). A kinesin heavy chain (KIF5A) mutation in hereditary spastic paraplegia (SPG10). *American Journal of Human Genetics*, 71(5), 1189–1194. https://doi.org/10.1086/344210

Schälheimer, N., & King, J. (2013). Tryptophan cluster protects human gammaD-crystallin from ultraviolet radiation-induced photoaggregation in vitro. *Photochemistry and Photobiology*, 89(5), 1106–1115. https://doi.org/10.1111/php.12096

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J. Y., White, D. J., Hartenstein, V., Eliceiri, K., Tomancak, P., & Cardona, A. (2012). Fiji: An open-source platform for biological-image analysis. *Nature Methods*, 9(7), 676–682. https://doi.org/10.1038/nmeth.2019

Schley, J. M. (2008). Intraflagellar transport motors in cilia: Moving along the cell’s antenna. *The Journal of Cell Biology*, 180(1), 23–29. https://doi.org/10.1083/jcb.20070913

Soto, C. (2003). Unfolding the role of protein misfolding in neurodegenerative diseases. *Nature Reviews. Neuroscience*, 4(1), 49–60. https://doi.org/10.1038/nrn1007

Uchida, A., Alami, N. H., & Brown, A. (2009). Tight functional coupling of kinesin-1A and dynein motors in the bidirectional transport of neurofilaments. *Molecular Biology of the Cell*, 20(23), 4979–5006. https://doi.org/10.1091/mbc.E09-04-0304

Vale, R. D. (2003). The molecular motor toolbox for intracellular transport. *Cell*, 112(4), 467–480. https://doi.org/10.1016/s0092-8674(03)00111-9

Vale, R. D., Funatsu, T., Pierce, D. W., Romberg, L., Harada, Y., & Yanagida, T. (1996). Direct observation of single kinesin molecules moving along microtubules. *Nature*, 380(6573), 451–453. https://doi.org/10.1038/380451a0

Vale, R. D., Reese, T. S., & Sheetz, M. P. (1985). Identification of a novel force-generating protein, kinesin, involved in microtubule-based motility. *Cell*, 42(1), 39–50. https://www.ncbi.nlm.nih.gov/pubmed/3926325

Vance, C., Rogelj, B., Horthogayi, T., De Vos, K. J., Nishimura, A. L., Sreedharan, J., Hu, X., Smith, B., Ruddy, D., Wright, G., Ganesalingam, J., Williams, K. L., Tripathi, V., Al-Saraj, S., Al-Chalabi, A., Leigh, P. N., Blair, I. P., Nicholson, G., de Belleruche, J., ... Shaw, C. E. (2009). Mutations in FUS, an RNA processing protein, cause familial amyotrophic lateral sclerosis type 6. *Science*, 323(5918), 1208–1211. https://doi.org/10.1126/science.1165942

Verhey, K. J., Lizotte, D. L., Abramson, T., Barenboim, L., Schnapp, B. J., & Rapoport, T. A. (1998). Light chain-dependent regulation of Kinesin’s interaction with microtubules. *The Journal of Cell Biology*, 143(4), 1053–1066. https://doi.org/10.1083/jcb.143.4.1053

Weedon, M. N., Hastings, R., Caswell, R., Xie, W., Paszkiewicz, K., Antoniadi, T., Williams, M., King, C., Greenhalgh, L., Newbury-Ecob, R., & Ellard, S. (2011). Exome sequencing identifies a DYNC1H1 mutation in a large pedigree with dominant axonal Charcot-Marie-tooth disease. *American Journal of
Human Genetics, 89(2), 308–312. https://doi.org/10.1016/j.ajhg.2011.07.002

Xia, C. H., Roberts, E. A., Her, L. S., Liu, X., Williams, D. S., Cleveland, D. W., & Goldstein, L. S. (2003). Abnormal neurofilament transport caused by targeted disruption of neuronal kinesin heavy chain KIF5A. The Journal of Cell Biology, 161(1), 55–66. https://doi.org/10.1083/jcb.200301026

Zhao, C., Takita, J., Tanaka, Y., Setou, M., Nakagawa, T., Takeda, S., Yang, H. W., Terada, S., Nakata, T., Takei, Y., Saito, M., Tsuji, S., Hayashi, Y., & Hirokawa, N. (2001). Charcot-Marie-tooth disease type 2A caused by mutation in a microtubule motor KIF1Bbeta. Cell, 105(5), 587–597. https://doi.org/10.1016/s0092-8674(01)00363-4

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher’s website.

How to cite this article: Nakano, J., Chiba, K., & Niwa, S. (2022). An ALS-associated KIF5A mutant forms oligomers and aggregates and induces neuronal toxicity. Genes to Cells, 27(6), 421–435. https://doi.org/10.1111/gtc.12936