



Minireview

RNA-Binding Proteins in Amyotrophic Lateral Sclerosis

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Significant research efforts are ongoing to elucidate the complex molecular mechanisms underlying amyotrophic lateral sclerosis (ALS), which may in turn pinpoint potential therapeutic targets for treatment. The ALS research field has evolved with recent discoveries of numerous genetic mutations in ALS patients, many of which are in genes encoding RNA binding proteins (RBPs), including TDP-43, FUS, ATXN2, TAF15, EWSR1, hnRNPA1, hnRNPA2/B1, MATR3 and TIA1. Accumulating evidence from studies on these ALS-linked RBPs suggests that dysregulation of RNA metabolism, cytoplasmic mislocalization of RBPs, dysfunction in stress granule dynamics of RBPs and increased propensity of mutant RBPs to aggregate may lead to ALS pathogenesis. Here, we review current knowledge of the biological function of these RBPs and the contributions of ALS-linked mutations to disease pathogenesis.

Keywords: ALS, RNA-binding proteins

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder characterized by progressive degeneration of motor neurons in the brain and spinal cord, leading to muscle weakness, paralysis and death (Rowland and Shneider, 2001). Although the underlying cause is unknown for most ALS cases, advances in sequencing technology have allowed

the identification of more than one hundred genes associated with ALS, including approximately thirty potential ALS-causing drivers (Al-Chalabi et al., 2017; Guerreiro et al., 2015; Wroe et al., 2008). Interestingly, many of the genes implicated in ALS encode RNA-binding proteins (RBPs), which include transactive response DNA-binding protein 43 (TDP-43), fused in sarcoma/translocated in liposarcoma (FUS/TLS or FUS), ataxin-2 (ATXN2), TATA-box binding protein associated factor 15 (TAF15), Ewing's sarcoma breakpoint region 1 (EWSR1), heterogeneous nuclear ribonucleoprotein A1 (hnRNPA1), heterogeneous nuclear ribonucleoprotein A2/B1 (hnRNPA2/B1), matrin 3 (MATR3) and T-cell-restricted intracellular antigen-1 (TIA1). As shown in Fig. 1, these RBPs share structural similarities; they all contain one or more RNA-binding domains (e.g., RRM, Lsm or LsmAD), a glycine (Gly)-rich region (except for MATR3 and ATXN2), and a nuclear localization signal (NLS) (except for TIA1 and ATXN2). In addition, these RBPs share functional similarities, since they are involved in RNA metabolism and many localize to stress granules upon cellular stress. Stress granules are membrane-less discrete cytoplasmic structures containing mRNA and associated proteins that form as a protective response to stress (Buchan and Parker, 2009; Kedersha et al., 1999; Monahan et al., 2016). Interestingly, the stress granule proteome was shown to be enriched for proteins encoding predicted prion-like or low-complexity (LC) domains, including many ALS-linked RBPs (Jain et al., 2016; Monahan et al., 2016). Mutations within these LC domains have been

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shown to interrupt stress granule dynamics and increase aggregation or fibrillization, which suggests a potential mechanism for ALS pathogenesis. Indeed, an increasing number of mutations identified in RBPs strongly suggest that abnormal RBP function and dysregulated RNA homeostasis also lead to ALS pathogenesis. Much progress has been made in understanding the mechanisms of how mutant RBPs exert toxicity and lead to neurodegeneration in ALS. In this review, we summarize studies that have investigated the biological function of the potential ALS-driving RBPs and the mechanisms by which mutations in these RBPs cause ALS. Where available, we discuss what we have learned from ALS models, with a focus on mouse models.

TDP-43

TDP-43 is an RBP containing two RNA recognition motifs (RRMs), an NLS, a nuclear export signal (NES), and a Gly-rich region at the C-terminus (Fig. 1) (Buratti and Baralle, 2001; Winton et al., 2008). TDP-43 plays a role in transcription, alternative splicing and mRNA stability and is involved in various cellular processes, such as apoptosis, cell division and axonal transport (Buratti and Baralle, 2008). TDP-43 primarily binds to UG-rich regions in the introns of pre-

mRNA, particularly long introns (Polymenidou et al., 2011; Sephton et al., 2011; Tollervey et al., 2011; Xiao et al., 2011), and its depletion leads to significant changes in alternative splicing and cryptic splicing (Ling et al., 2015; Polymenidou et al., 2011; Tan et al., 2016; Tollervey et al., 2011). TDP-43 is primarily localized in the nucleus; however, upon cellular stress, it localizes to stress granules (Buratti and Baralle, 2008; Colombrita et al., 2009; Li et al., 2013).

In 2006, TDP-43 was identified as a major component of protein inclusions in the cytoplasm of ALS-affected motor neurons, marking it as the first RBP associated with ALS (Arai et al., 2006; Mackenzie et al., 2007; Neumann et al., 2006). Corresponding with abnormal cytoplasmic inclusion, nuclear clearance of TDP-43 was also observed (Neumann et al., 2006; Van Deerlin et al., 2008). This finding raises the question of whether TDP-43 pathogenicity is due to loss of nuclear function, gain of cytoplasmic function, or both. Numerous *TDP-43* mutations have been identified in ALS patients, accounting for approximately 5% of familial and less than 1% of sporadic cases (Taylor et al., 2016). Most mutations are clustered in the Gly-rich domain (Fig. 1) (Kapeli et al., 2017; Sreedharan et al., 2008). The Gly-rich, LC domain has been shown to be necessary for TDP-43 assembly into stress granules (Colombrita et al., 2009) and

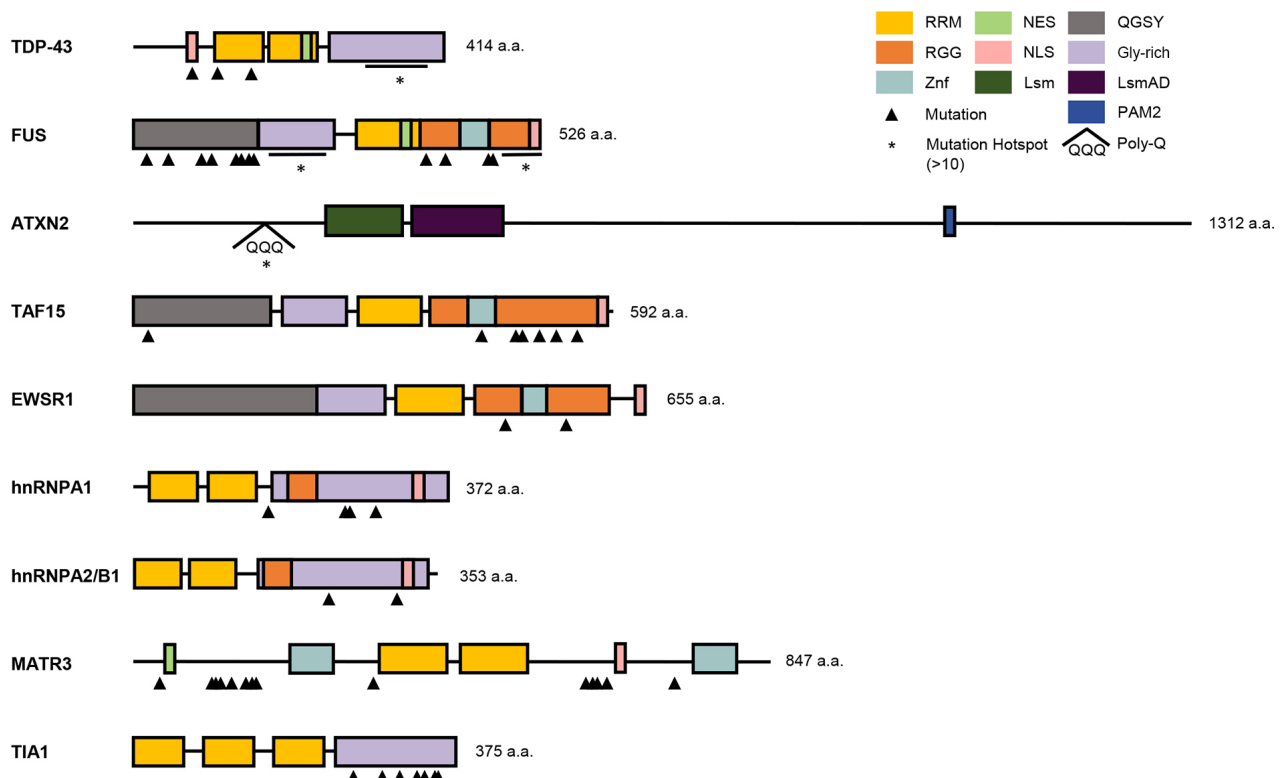


Fig. 1. Functional domains and ALS-linked mutations in RNA binding proteins. The arrowheads indicate single mutations and asterisks denote mutation hotspots found in both familial and sporadic ALS cases. RRM, RNA recognition motif; RGG, arginine/glycine-rich region; Znf, Zinc finger motif; NES, nuclear export signal; NLS, nuclear localization signal; Lsm, like-Sm protein domain; QGSY, glutamine, glycine, serine and tyrosine-rich region; Gly-rich, glycine-rich region; LsmAD, Lsm-associated domain; PAM2, PABP-interacting motif 2; Poly-Q, polyglutamine repeat; a.a., amino acids.

for undergoing liquid-liquid phase separation (or phase separation) (Aguilera-Gomez and Rabouille, 2017; Anderson and Kedersha, 2009; Brangwynne et al., 2009; Chong and Forman-Kay, 2016; Conicella et al., 2016). This process is dynamic, as proteins can transition between liquid and gel-like states (recently reviewed in (Purice and Taylor, 2018)). However, TDP-43 proteins that harbor disease mutations in the LC domain were shown to favor the gel-like, rigid state and form aggregates (Conicella et al., 2016). Additionally, these mutant TDP-43 proteins increased the formation of stress granules upon oxidative stress induced by sodium arsenite (Liu-Yesucevitz et al., 2010). As many other studies have demonstrated that ALS mutations in the LC domain results in increased conversion to aggregates (Kapeli et al., 2017; Purice and Taylor, 2018), phase separation disruption is a possible mechanism leading to ALS. In addition to full length TDP-43, low molecular weight species containing the LC domain (e.g., TDP-35) were also found to localize to cytoplasmic inclusions in ALS motor neurons (Xiao et al., 2015), further supporting that this LC domain is critical for aggregation. In parallel, other studies have shown that dysregulation in RNA splicing due to loss of TDP-43 function may be involved in ALS pathogenesis. One example demonstrated by Ling and colleagues showed that cryptic exon inclusion induced by loss of TDP-43 is found in ALS patients presenting TDP-43 pathology (Ling et al., 2015).

Many transgenic mice and rats expressing mutant TDP-43 have been generated; most of which reproduce clinical features seen in ALS patients including impaired motor function, degeneration of motor neurons, and accumulation of ubiquitinated TDP-43 cytoplasmic aggregates (Liu et al., 2013; Picher-Martel et al., 2016; Wegorzewska et al., 2009). Loss of TDP-43 was found to cause age-dependent motor neuron degeneration in mice and many other animal models (Iguchi et al., 2013; Vanden Broeck et al., 2014), suggesting that loss of TDP-43 function is involved in ALS pathogenesis. However, it is still not clear to what magnitude gain-of-function and loss-of-function contribute to neurodegeneration.

FUS

FUS was first identified as an oncogene that causes liposarcomas, where chromosomal translocation fuses the FUS transactivation domain to transcription factors (Crozat et al., 1993; Kapeli et al., 2017; Rabbitts et al., 1993). In addition to its role in transcription, FUS plays a role in RNA processing events. Cross linking immunoprecipitation coupled with high-throughput sequencing (CLIP-seq) analysis revealed that FUS binds to RNAs that contain an enriched GUGGU motif (Lagier-Tourenne et al., 2012) or GUU motif (Rogelj et al., 2012). FUS-binding sites are clustered in RNAs with exceptionally long introns, and depletion of FUS leads to downregulation of long intron-containing FUS targets (Lagier-Tourenne et al., 2012).

Mutations in *FUS* were reported to cause ALS in 2009 (Kwiatkowski et al., 2009; Vance et al., 2009). *FUS* is normally localized primarily in the nucleus, but in ALS-affected brains and spinal cords, *FUS* is often found aggregated in

the cytoplasm. Interestingly, TDP-43 pathology is absent in ALS patients with *FUS* mutations (Vance et al., 2009). *FUS* mutations account for 5% of familial ALS and less than 1% of sporadic ALS cases (Taylor et al., 2016). The average age of onset for ALS cases with *FUS* mutations is 43.6 ± 15.8 years, which is relatively earlier than that of patients with *SOD1* or *TDP-43* mutations (Shang and Huang, 2016; Yan et al., 2010). In addition, many *FUS* mutations were found in juvenile ALS cases (late teens and early 20s) (Baumer et al., 2010; Huang et al., 2010; Liu et al., 2017b). Most of the mutations identified in *FUS* are clustered in either the N-terminal glutamine-glycine-serine-tyrosine (QGSY)-rich and Gly-rich regions or the C-terminal region within the arginine-glycine-glycine (RGG)-rich domain and NLS (Fig. 1). Several groups have demonstrated that ALS mutations accelerate the phase transition of *FUS* and readily lead to formation of nuclear and cytoplasmic aggregates (Murakami et al., 2015; Patel et al., 2015). In addition, ALS-linked *FUS* mutants have shown increased localization to stress granules upon cellular stress (Bosco et al., 2010). Recently, the detailed structural and molecular mechanism of the phase behavior of *FUS* has been further investigated (Hofweber et al., 2018; Luo et al., 2018; Murray et al., 2017; Qamar et al., 2018; Yoshizawa et al., 2018), providing additional support for aberrant phase transition as a molecular mechanism leading to disease.

Several mouse and rat models that express mutant *FUS* recapitulate ALS features, including cytoplasmic inclusions, muscle atrophy, and motor neuron degeneration (Qiu et al., 2014; Sephton et al., 2014; Sharma et al., 2016; Shelkovi-kova et al., 2013). Similarly, mice overexpressing wild-type *FUS* also exhibit ALS features (Mitchell et al., 2013; Sephton et al., 2014), suggesting that increased levels of *FUS* contribute to toxicity and neurodegeneration. Interestingly, neither widespread postnatal *FUS* deletion nor motor neuron-specific *FUS* knockout mice develop motor neuron degeneration (Sharma et al., 2016), suggesting that loss of *FUS* function does not contribute to ALS. However, studies in other animal models such as *Drosophila* and *C. elegans* suggest that loss of *FUS* function may be involved in pathogenesis (Lanson and Pandey, 2012; Shang and Huang, 2016). Qiu and colleagues performed RNA-seq on spinal cord tissue of *FUS*-R521C mice and found RNA splicing defects, particularly in genes that are involved in dendritic growth and synaptic functions (Qiu et al., 2014), suggesting that aberrant RNA splicing caused by mutant *FUS* contributes to neurodegeneration. In addition, RNA analysis of motor neurons derived from induced pluripotent stem cells (iPSCs) with ALS mutations in *FUS* revealed that aberrant gene expression and splicing changes are associated with mutant *FUS* (De Santis et al., 2017; Ichiyangi et al., 2016). These results suggest that dysregulation of RNA metabolism is involved in ALS pathogenesis.

ATXN2

ATXN2 plays diverse roles in the regulation of RNA metabolism, including mRNA stability, polyadenylation and translational activation (Auburger et al., 2017; Carmo-Silva et al., 2017; Lee et al., 2018a; Ostrowski et al., 2017). ATXN2 con-

tains an N-terminal like-Sm protein domain (Lsm) and Lsm-associated domain (LsmAD), which bind to the 3' UTR of mRNA and promote mRNA stability (Yokoshi et al., 2014). In addition, ATXN2 is involved in translational regulation by associating with polyribosomes and poly (A)-binding protein (PABP) through its C-terminal PABP-interacting motif 2 (PAM2) domain (Ralser et al., 2005; Satterfield and Pallanck, 2006). ATXN2 is predominately found in the cytoplasm and localizes to stress granules upon stress (Nonhoff et al., 2007; Ralser et al., 2005). ATXN2 is also involved in reorganization of the actin cytoskeleton and regulation of calcium signaling, cellular metabolism and circadian rhythm (Auburger et al., 2017; Carmo-Silva et al., 2017; Lee et al., 2018b; Lim and Allada, 2013; Ostrowski et al., 2017).

ATXN2 normally contains ~22 polyglutamine (polyQ) repeats in the N-terminus (Fig. 1), and an expansion of >34 polyQ repeats causes spinocerebellar ataxia type 2 (SCA2) (Imbert et al., 1996). In 2010, intermediate-length polyQ repeats (27-33), which were found in up to 4.7% of ALS cases, were identified as a significant risk factor for ALS, although the cutoff length of the polyQ expansion varies between populations (Conforti et al., 2012; Elden et al., 2010; Lee et al., 2011; Lu et al., 2015; Ross et al., 2011; Van Damme et al., 2011). Similar to other ALS-linked RBPs, distinct cytoplasmic accumulation of ATXN2 was observed in spinal cord autopsies of ALS patients, which differs from the diffuse pattern observed in control tissue (Elden et al., 2010). The link between ATXN2 and ALS was first discovered using yeast two-hybrid screens, which identified ATXN2 as a TDP-43 modifier (Elden et al., 2010). Up-regulation of ATXN2 enhanced TDP-43 toxicity in yeast and flies, while a decrease in ATXN2 levels suppressed TDP-43 toxicity, a feature also observed in mice (Becker et al., 2017; Elden et al., 2010), strongly suggesting that reducing ATXN2 levels is an attractive therapeutic strategy to mitigate ALS. Similarly, expression of mutant ATXN2 enhances mutant FUS toxicity (Farg et al., 2013). A very recent study has identified domains in ATXN2 that are required for phase separation and stress granule assembly as well as long-term memory and neurodegeneration (Bakthavachalu et al., 2018). Relative to the other RBPs discussed, the distinct structure and function of ATXN2 speaks to the complex molecular mechanism of ALS.

TAF15

TAF15 is composed of an N-terminal QGSY-rich domain, Gly-rich region, an RRM, a zinc finger motif (Znf), a C-terminal RGG repeat region, and an NLS (Fig. 1) (Andersson et al., 2008; Bertolotti et al., 1996; Morohoshi et al., 1996). Although TAF15 is found primarily in the nucleus, it localizes to stress granules upon cellular stress (Andersson et al., 2008; Marko et al., 2012). TAF15 has been implicated in DNA damage repair and RNA processing (Bertolotti et al., 1996; Ibrahim et al., 2013; Izhar et al., 2015; Jobert et al., 2009). Several studies were unable to identify a consensus RNA-binding sequence and proposed that TAF15 binds to stem-loop structures within intronic regions (Hoell et al., 2011; Kashyap et al., 2015). However, other studies have suggested that TAF15 binds to the CUG motif or

GGUA motif (Ibrahim et al., 2013; Kapeli et al., 2016). Interestingly, TAF15 and FUS share common RNA targets (Hoell et al., 2011; Ibrahim et al., 2013; Kapeli et al., 2016), supporting that TAF15 and FUS are structurally and functionally similar.

In 2011, mutations in *TAF15* were identified in familial ALS cases (Ticozzi et al., 2011). Couthouis and colleagues identified additional mutations within the RGG-rich domain in sporadic ALS patients (Couthouis et al., 2011). Punctate granular cytoplasmic TAF15 staining was observed in sporadic ALS postmortem spinal cord tissue, whereas in postmortem controls, TAF15 is localized in the nucleus (Couthouis et al., 2011). ALS-linked mutations were also shown to increase the aggregation propensity of TAF15 compared to that of wild-type TAF15 (Couthouis et al., 2011). Furthermore, Couthouis and colleagues found that overexpression of wild-type TAF15 led to shortened lifespan in *Drosophila*, and ALS-associated mutations reduced lifespan even further (Couthouis et al., 2011), suggesting a possible gain-of-function mechanism.

EWSR1

EWSR1 or EWS was originally identified in Ewing's sarcoma, in which chromosomal translocation fuses the N-terminus of EWSR1 to a transcription factor (Delattre et al., 1992). EWSR1 is composed of an N-terminal QGSY-rich domain, a Gly-rich domain, an RRM, a C-terminal RGG-region divided by a Znf, and an NLS (Fig. 1) (Bertolotti et al., 1996; Ohno et al., 1994; Zakaryan and Gehring, 2006). Like other RBPs detailed above, EWSR1 is primarily found in the nucleus but can localize to stress granules upon cellular stress (Andersson et al., 2008; Neumann et al., 2011). One study failed to identify an RNA consensus sequence for EWSR1 and proposed that it recognizes a stem-loop structure within intronic sequences, similar to TAF15 (Hoell et al., 2011). However, several studies have suggested that EWSR1 recognizes a guanine (G)-rich sequence (Ohno et al., 1994; Paronetto et al., 2014). EWSR1 regulates alternative splicing and gene expression for genes involved in many cellular processes, including the DNA damage response, cell growth and apoptosis (Duggimpudi et al., 2015; Huang et al., 2012; Paronetto et al., 2011; 2014).

Two mutations within the RGG-rich domain of EWSR1 were identified in sporadic ALS cases in 2012 (Couthouis et al., 2012). The ALS-associated mutations were found to promote cytoplasmic accumulation of mutant EWSR1 in primary mouse neuron cultures and increase aggregation kinetics when compared to the wild-type protein. In *Drosophila*, overexpression of wild-type EWSR1 leads to neurodegeneration; however, overexpression of mutant EWSR1 does not exacerbate the phenotypes. In postmortem tissue from sporadic ALS patients, EWSR1 was present in cytoplasmic punctate granular structures (Couthouis et al., 2012). Together, these results suggest that EWSR1 has a potential role in conferring toxicity, but further experiments on these mutations and additional studies to identify novel mutations in *EWSR1* are required.

HnRNPA1

HnRNPA1 belongs to a family of at least 20 human hnRNPs, which bind to and regulate nascent RNA polymerase II transcripts (Dreyfuss et al., 1993). Structurally, hnRNPA1 contains two RRM domains and a Gly-rich domain at the C-terminus (Fig. 1) (Dreyfuss et al., 1993). HnRNPA1 is highly expressed in the nucleus but can shuttle between the nucleus and cytoplasm (Dreyfuss et al., 2002; Michael et al., 1995; Mili et al., 2001; Nakielnny et al., 1996; Pinol-Roma and Dreyfuss, 1992; Pollard et al., 1996). HnRNPA1 preferentially binds to UAGGGA/U sequences and cooperates with other hnRNPs to regulate splicing, including splicing of its own transcripts, and pathological splicing events (Chabot et al., 1997; Clower et al., 2010; Huelga et al., 2012; Mayeda and Krainer, 1992; Mohagheghi et al., 2016; Yang et al., 1994). In addition to its role in splicing, hnRNPA1 also has functions in mRNA stability, export, transport, localization, and translation as well as miRNA processing (Alarcon et al., 2015; Cammas et al., 2007; Dreyfuss et al., 2002; Guil and Caceres, 2007; Kwon et al., 1999; Villarroya-Beltri et al., 2013). Moreover, hnRNPA1 is recruited to stress granules upon stress (Guil et al., 2006).

Mutations in *hnRNPA1* underlie less than 1% of ALS cases (Taylor et al., 2016). Kim and colleagues identified *hnRNPA1* missense mutations in a family affected by ALS and in two families with multisystem proteinopathy (MSP) affecting the brain, motor neurons, muscle and bone (Kim et al., 2013). Cytoplasmic accumulation and nuclear clearance of mutant hnRNPA1 were observed in patient muscle tissue (Kim et al., 2013). Interestingly, hnRNPA1 staining in the postmortem tissue of sporadic ALS patients was reduced in the nuclei of motor neurons relative to that of control tissue, and did not colocalize with TDP-43 inclusions (Honda et al., 2015). ALS-associated mutations in the Gly-rich LC domain of hnRNPA1, which mediates phase separation (Molliex et al., 2015), have been shown to increase incorporation into stress granules, strengthen steric zipper motifs and accelerate fibrillization compared to wildtype hnRNPA1 (Kim et al., 2013; Molliex et al., 2015). Additional mutations in *hnRNPA1* were later identified by targeted sequencing of sporadic ALS patients and of an ALS family with flail arm syndrome (Couthouis et al., 2014; Liu et al., 2016). HnRNPA1 knockout mice are embryonic lethal, while heterozygous animals display a cardiac phenotype and show many changes in alternative splicing of muscle development-related genes (Liu et al., 2017a). This evidence supports an important role for hnRNPA1 in alternative splicing, but further animal studies are required to investigate its role in ALS pathogenesis.

HnRNPA2/B1

HnRNPA2/B1, another core hnRNP, is similar to hnRNPA1 in structure, expression, and function (Dreyfuss et al., 1993). HnRNPA2 and hnRNPA1 are two isoforms expressed from the same *hnRNPA2/B1* gene. By preferentially binding UAGG motifs, this RBP plays a role in alternative splicing and alternative polyadenylation (Alarcon et al., 2015; Burd and Dreyfuss, 1994; Huelga et al., 2012; Martinez et al., 2016).

In addition, hnRNPA2/B1 regulates RNA export, transport, localization of transcripts, and miRNA processing (Alarcon et al., 2015; Gao et al., 2008; Munro et al., 1999; Shan et al., 2000; Shan et al., 2003).

ALS-associated mutations in *hnRNPA2/B1* are extremely rare (< 1%) (Taylor et al., 2016). Exome sequencing and linkage revealed a single mutation in *hnRNPA2/B1* in an MSP family (Kim et al., 2013). Targeted sequencing revealed an additional mutation in sporadic ALS patients (Couthouis et al., 2014). Mutant hnRNPA2/B1 displays a similar pathology as mutant hnRNPA1 in patient muscle, where it was cleared from nuclei and accumulated in cytoplasmic inclusions in ~10% of muscle fibers (Kim et al., 2013). Similarly, electroporation of hnRNPA2 into mouse tibialis anterior muscle revealed that while wild-type hnRNPA2 was localized in the nuclei, mutant hnRNPA2 was excluded from nuclei and accumulated in cytoplasmic inclusions (Kim et al., 2013). *Drosophila* models that express hnRNPA2/B1 also show features reminiscent of myopathy and cytoplasmic protein inclusions (Kim et al., 2013; Li et al., 2016). ALS-associated hnRNPA2/B1 mutant iPSC-derived motor neurons also displayed hnRNPA2/B1 localization to cytoplasmic granules, abnormal splicing changes, and decreased survival in long-term culture (Martinez et al., 2016). Furthermore, hnRNPA2/B1 knockdown in mice induced alternative splicing changes, neuronal dendrite loss, and learning and memory impairments (Berson et al., 2012). Altogether, the evidence suggests that *hnRNPA2/B1* mutations confer susceptibility to ALS. However, further experiments and identification of additional mutations are required to fully support this claim.

MATR3

MATR3 is a component of the nuclear matrix (Berezney and Coffey, 1974; Nakayasu and Berezney, 1991) and contains two RRM domains, two Znfs, an NLS and an NES (Fig. 1) (Belgrader et al., 1991; Hibino et al., 2006; Hisada-Ishii et al., 2007). By binding to DNA and RNA, MATR3 regulates transcription, RNA processing, and mRNA export and stability (Banerjee et al., 2017; Boehringer et al., 2017; Coelho et al., 2015; Salton et al., 2011; Skowronska-Krawczyk et al., 2014; Zhang and Carmichael, 2001). Several studies have provided evidence that MATR3 preferentially binds to pyrimidine-rich sequences within intronic regions and controls alternative splicing (Coelho et al., 2015; Uemura et al., 2017).

A *MATR3* mutation (S85C) was first identified in autosomal-dominant distal myopathy (Senderek et al., 2009). However, in 2014, myopathy in patients with the S85C mutation was reclassified as ALS, and other missense mutations in *MATR3* were identified in familial ALS cases (Johnson et al., 2014). Since then, several other missense and splicing mutations have been identified in familial and sporadic ALS cases, although mutations in *MATR3* account for less than 1% of all ALS cases (Leblond et al., 2016; Lin et al., 2015; Marangi et al., 2017; Origone et al., 2015; Taylor et al., 2016; Xu et al., 2016). None of these mutations are found in known domains; instead, they are clustered in two regions as shown in Fig. 1. MATR3 staining in ALS postmortem spinal cord tissue is primarily observed in the nucleus. However,

in some cases, diffuse cytoplasmic MATR3 or MATR3 inclusions have been observed (Johnson et al., 2014; Tada et al., 2018). For example, intense nuclear and diffuse cytoplasmic MATR3 staining was observed in a patient harboring a *MATR3* F115C mutation (Johnson et al., 2014). A very recent study found MATR3 in a subset of cytoplasmic TDP-43-positive inclusions (Tada et al., 2018). However, several studies demonstrated that wild-type or mutant MATR3 was mostly localized in the nucleus even upon stress and that only a small subset of cells overexpressing MATR3 show cytoplasmic puncta resembling stress granules (Gallego-Iradi et al., 2015). A recent paper showed that fibroblasts from S85C myopathy patients exhibited no significant changes in mutant MATR3 localization but showed impaired stress granule formation in response to stress, suggesting that mutant MATR3 may indirectly impact stress granule formation (Mensch et al., 2018). Several recent studies have attempted to identify protein interactors of both wild-type and mutant MATR3; one study found an enrichment of proteins involved in mRNA nuclear export to interact with wild-type MATR3, and these proteins show altered interactions with the mutant form (Boehringer et al., 2017). In contrast, another study did not find significant differences in the interactions with binding partners between wild-type and mutant MATR3 (Iradi et al., 2018). Further studies are required to determine how *MATR3* mutations confer pathogenicity.

TIA1

TIA1 is a major component of stress granules (Kedersha et al., 1999; 2000). As shown in Fig. 1, TIA1 is composed of three RRM domains and a C-terminal Gly-rich LC domain, which undergoes phase separation (Gilks et al., 2004; Mackenzie et al., 2017). TIA1 binds to uridine (U)-rich sequences (Aznarez et al., 2008; Dember et al., 1996; Piecyk et al., 2000) and is implicated in RNA processing and translation regulation (Del Gatto-Konczak et al., 2000; Dixon et al., 2003; Forch et al., 2000; Kedersha et al., 1999; Piecyk et al., 2000). TIA1 has also been shown to regulate genes involved in multiple processes, including apoptosis, inflammation, and cell proliferation (Dixon et al., 2003; Forch et al., 2000; Heck et al., 2014; Reyes et al., 2009; Sanchez-Jimenez and Izquierdo, 2013).

In 2017, several mutations were identified within the C-terminal LC domain of TIA1 in familial and sporadic ALS cases, and several other mutations were found in 2018 (Mackenzie et al., 2017; Yuan et al., 2018; Zhang et al., 2018). Brain and spinal cord autopsy tissue samples with *TIA1* mutations revealed cytoplasmic TDP-43 pathology and hyaline Lewy body-like inclusions, but, interestingly, no TIA1 pathology (Hirsch-Reinshagen et al., 2017; Mackenzie et al., 2017). Functional studies revealed that these mutations increased phase separation, delayed stress granule disassembly and promoted accumulation of stress granules harboring TDP-43 (Mackenzie et al., 2017), providing evidence that *TIA1* mutations confer pathogenicity. A homozygous TIA1 knockout mouse line exhibited 50% lethality before 3 weeks postpartum, while surviving mice exhibited no gross abnormali-

ties and had normal lifespans (Piecyk et al., 2000). Animal studies will further provide clues as to whether TIA1 gain-of-function, loss-of-function or both lead to ALS pathogenesis.

CONCLUSION

Several RBPs have been identified to be strongly linked with ALS. Many of these proteins share structural and functional properties that mediate their role in the disease process. The most striking structural property shared by many of the RBPs are the LC domains. When harboring ALS-linked mutations in these domains, these RBPs are associated with increased aggregation or fibrillization propensity, cytoplasmic mislocalization, and dysregulation of stress granule dynamics, suggesting that LC domains play an important role in ALS pathogenesis. However, not all ALS-linked RBPs have a defined LC domain (e.g., MATR3 and ATXN2), suggesting that alternative pathogenic mechanisms may exist. By definition, these RBPs play functional roles in RNA metabolism, including transcription, RNA processing, mRNA export and stability, and translation regulation. As such, ALS-linked mutations in these proteins have the potential to affect gene expression and thereby impact certain cellular processes, including the DNA repair response, apoptosis, and cell growth and proliferation. However, it is difficult to pinpoint a single or a few pathway(s) or mechanism(s) by which all these RBPs converge to cause ALS. A better understanding of the normal function as well as pathological significance of these RBPs will be critical to illuminate the biology behind this devastating disease.

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