

From Mouse Models to Human Disease: An Approach for Amyotrophic Lateral Sclerosis

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Abstract. Amyotrophic lateral sclerosis (ALS) is a fatal adult-onset neurodegenerative disorder. There are several genetic mutations that lead to ALS development, such as chromosome 9 hexanucleotide repeat 72 (*C9ORF72*), transactive response DNA-binding protein (*TARDBP*), superoxide dismutase 1 (*SOD1*) and fused in sarcoma (*FUS*). ALS is associated with disrupted gene homeostasis causing aberrant RNA processing or toxic pathology. Several animal models of ALS disease have been developed to understand whether *TARDBP*-mediated neurodegeneration results from a gain or a loss of function of the protein, however, none exactly mimic the pathophysiology and the phenotype of human ALS. Here, the pathophysiology of specific ALS-linked gene mutations is discussed. Furthermore, some of the generated mouse models, as well as the similarities and differences between these models, are comprehensively reviewed. Further refinement of mouse models will likely aid the development of a better form of model that mimics human ALS. However, disrupted gene homeostasis that causes mutation can result in an ALS-like syndrome, increasing concerns about whether neurodegeneration and other effects in these models are due to the mutation or to gene overexpression. Research on the pleiotropic role of different proteins present in motor neurons is also summarized. The development of better mouse models that closely mimic human ALS will help identify potential therapeutic targets for this disease.

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Key Words: Amyotrophic lateral sclerosis, motor neuron diseases, mouse models, *C9ORF72*, *FUS*, *SOD1*, *TARDBP*, review.

Amyotrophic lateral sclerosis is a devastating adult neurodegenerative disorder characterized by motor neuron degeneration and death approximately 3 years after onset. Riluzole is currently the only treatment available, but only offers a slight survival benefit (1). The etiology of ALS is complex and is associated with several genes, making it difficult to study the etiopathogenesis of this disease (2) (Figure 1).

During the past decade, significant advances have been made in understanding this disease, from linkage analysis to isolation of defective genes and identification of their protein product (3). The development of animal models for the study of this disease, in particular, mouse models for ALS (1), has now made it possible to understand the molecular basis of this disease and has demonstrated the feasibility of using the whole affected gene, which is found in all patients with ALS, as a means of treating this disorder.

The most common form of ALS is sporadic ALS, which is an age-associated disease characterized by cytoskeletal abnormalities and the death of motor neurons (4). The familial form of ALS (FALS) is an inherited autosomal dominant disease linked to mutations in superoxide dismutase 1 (*SOD1*) gene, which manifest as inclusions and degeneration of motor neurons. It has been reported that 5-10% of all patients with ALS have the inherited form of ALS (5, 6).

A non-coding hexanucleotide *C9ORF72* gene repeat expansion is the most common mutation associated with frontotemporal dementia (FTD) and FALS (7). Transgenic ALS mouse models that express *SOD1* have been critical in furthering our understanding of the disease (8). Transactive response DNA-binding protein 43 (*TDP43*) ubiquitinated inclusions are a hallmark of ALS and FTD with ubiquitin-positive inclusions. However, mutations in the gene encoding these inclusions (*TARDBP*) are associated with only 3% of sporadic and FALS (8, 9). Moreover, mutations in the gene encoding the RNA-binding protein fused in sarcoma (*FUS*) can cause FALS but rarely FTD (10).

Experimental models of ALS are important for clarifying the complex functions of different proteins and the pathology of ALS (11). In order to examine the mechanisms underlying ALS, investigators have used a variety of animal models, including experimentally produced, spontaneously occurring, or genetically-engineered disease models. While these models have provided important insight into the underlying mechanisms of ALS, they are not without their limitations in that they allow study of different aspects of the disease.

This review discusses the major neuropathological features and mechanisms of ALS-associated mutations, as well as behavioral/neuropathophysiological features of recently developed mouse models, and the results of their biochemical mechanisms with the aim of potentially using these models to test new therapies for ALS.

The *C9ORF72* Mutation

Since its discovery in November 2011, the *C9ORF72* mutation has been reported as the most frequent mutation associated with ALS and FTD in Western countries (12, 13). The *C9ORF72* gene has a high penetrance, and most persons with the C9 expansion die due to a neurodegenerative disorder. Although some patients who carry this mutation develop a range of FTD disorders, others exhibit ALS symptoms; some patients have a mixture of both FTD and ALS at presentation (14). A number of important questions regarding this mutation remain unanswered, especially those pertaining to selective vulnerability and why ALS develops in one member of a family whereas another family member dies from FTD.

The discovery of *C9ORF72* repeat expansions has helped scientists understand the mechanism underlying neurodegeneration in ALS, which appears to be related to dysfunctional RNA processing (15). The repeat has been identified on a non-coding region of *C9ORF72*, which encodes a protein with no identified function that is expressed at high concentrations in the brain (15, 16). RNA aggregates are formed within the nucleus due to the very long hexanucleotide repeat, which is thought to range between 700 and 1,600 units (16). It is also thought that the long hexanucleotide repeat suppresses gene expression through abnormal RNA splicing. Another hypothesis that has been proposed to explain the mechanism of the disease is *C9ORF72* protein function loss (17). It will be challenging to develop therapies for *C9ORF72* and this might necessitate the development of new therapeutic strategies that involve suppressing the expression of the C9 repeat (17). The development of robust animal models and use of well-defined patient cohorts are crucial to understanding how the disease develops and help in discovering potential therapeutic targets (17, 18).

Mouse models. Besides inclusion formation, evidence suggests that patients with G4C2 repeat expansion have increased repressive histone hypermethylation at the *C9ORF72* repeat expansion locus (16). Scientists developed *C9ORF72* knockout cell and mouse models to understand the underlying mechanism in *C9ORF72*-associated ALS/FTD pathogenesis.

A decrease in *C9ORF72* transcript expression in animal models has been associated with neurodegenerative and behavioral deficits (17, 18).

Experiments on animal models have shown that a decrease in *C9ORF72* led to decrease in RNA foci and dipeptide repeat proteins (DPR), contrary to findings in patients with *C9ORF72*-associated ALS/FTD (19, 20). Nevertheless, in other models that do not support the loss of function (LOF) argument, the administration of antisense oligonucleotides against *C9ORF72* transcript did not cause adult mice to develop behavioral or neurodegenerative disorder (20). On the contrary, it reduced symptomatology (21). Moreover, patients with *C9ORF72*-associated ALS/FTD did not show a mutation in the coding region of the gene. Inclusions were, however, documented (22). Consequently, while abnormal *C9ORF72* repeat expansions may be implicated in the etiopathogenesis of ALS, there is no strong clinicopathological evidence that LOF leads to neurodegeneration in *C9ORF72*-associated ALS/FTD.

Given the difficulty in cloning GGGGCC (G4C2) expansions and the instability of these expansions in somatic cells, investigators face challenges in developing transgenic mouse models (23). However, much research has been ongoing in this area to assess the pathological functions of G4C2 expansion on *in vitro* and *in vivo* model systems. It was not until recently that scientists were able to develop transgenic mouse models that recapitulate the pathology and symptoms of human ALS/FTD (22).

Some investigators (23, 24) used bacterial artificial chromosome (BAC) to develop the first transgenic mice. These mice carried the G4C2 expansion and expressed approximately 500 and 1,000 repeats of the human C9 gene at moderate levels, respectively (23, 24). Although models exhibited histological phenotypes similar to human *C9ORF72*-associated ALS/FTD, the mice had normal phenotypes. Histological investigations demonstrated the production and deposition of RNA foci and poly-(glycine-proline) peptides in the brain and spinal cord (25). Other researchers used a virus-mediated gene-delivery system to develop transgenic mice harboring 66 repeats of G4C2 motifs or dipeptide repeat (DPR) proteins (26, 27). The mice expressed high levels of synthetic C9-RNA and DPR, and showed histopathological features and phenotypical neurodegenerative defects comparable to those observed in patients with ALS and FTD (26, 27). Of note, transgenic mice overexpressed synthetic peptides at levels exceeding the

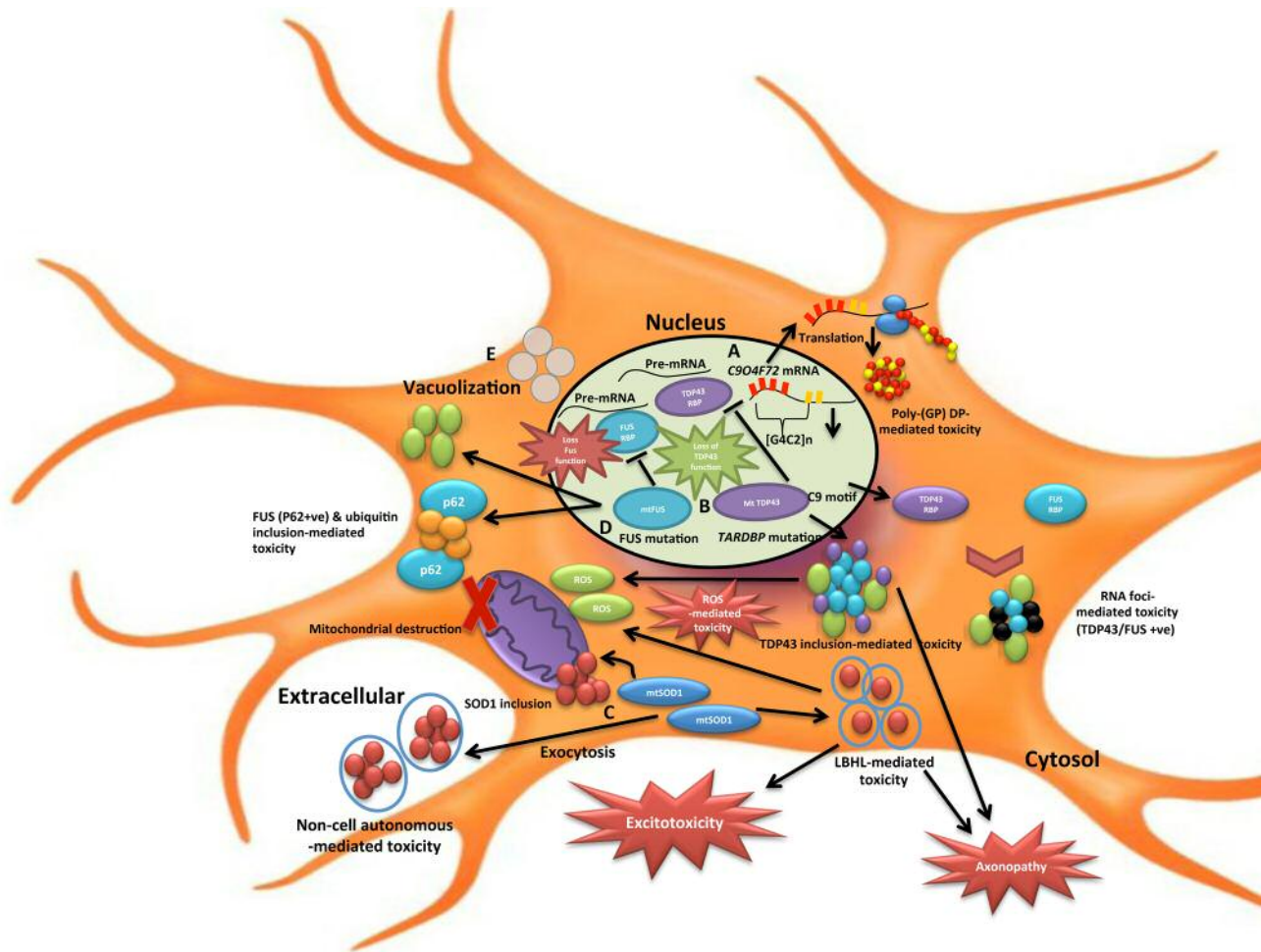


Figure 1. Pathogenesis of C9ORF72-, TARDBP-, SOD1-, and FUS-associated amyotrophic lateral sclerosis (ALS). A: Chromosome 9 hexanucleotide repeat72 (C9ORF72) 2 mutation acts through a gain-of-function (GOF) mechanism. GGGGCC[G4C2] is translocated to the cytosol and then either translated to form aggregates of poly-(GP) dipeptide-repeat proteins (DPR) or misfolded to form aggregates of ubiquitinated (U) RNA foci associated with TDP43 or FUS proteins, which both mediate neuronal toxicity. B: transactive response DNA-binding protein (TARDBP) mutation acts through both loss of function (LOF) and GOF mechanisms. Normal TDP43 function is lost due to mutant (mt) TDP43 proteins inhibiting normal TDP43 from binding to pre-mRNA. C: superoxide dismutase 1 (SOD1) mutation acts through a GOF mechanism. Mutant SOD1 dimers in the cytosol accumulate as SOD1 inclusions within mitochondria and Lewy-body-like hyaline (LBHL) inclusions in the cytosol where they can trigger mitochondrial reactive oxygen species (ROS) generation later, causing mitochondrial destruction. D: fused in sarcoma (FUS) mutation acts through both LOF and GOF mechanisms. Mutant FUS proteins cause LOF by inhibiting normal FUS from binding to pre-mRNA. E: Cytosol vacuolization is caused by all the above-mentioned mutations.

normal physiological levels noted in patients. Conversely, other investigators used BAC transgenic mice and successfully reproduced the histopathological, molecular and clinical features of the disease (28, 29). In the latter two studies, the investigators found that the number of RNA foci, DPR aggregates and neuronal loss were proportional to repeat length. Nevertheless Liu *et al.*, found that only symptomatic mice with acute end-stage disease presented cytoplasmic and nuclear TDP43 inclusions in the entirety of the denervated brain, hippocampus, and motor cortex (28, 29). The other

group contended that mice expressing up to 450 C9ORF72 RNAs (C9⁴⁵⁰ mice) did not display TDP43 mislocalization or aggregation, although higher levels of phosphorylated TDP43 were observed (28). Liu *et al.* reported a dramatic decrease in survival among mice from the C9-500/32 (two transgene copies, one with ~500 and the other with 32 repeats) and C9-500 (one copy with ~500 repeats), lines compared to non-transgenic controls (28, 29). Although the results of Jiang *et al.*'s study provide deep insight into critical drivers of disease pathogenesis and an understanding of the

molecular mechanisms of ALS and FTD (28), further research is warranted to elucidate the molecular basis of sex-specific differences. In addition, the data do not explain differences observed between the four *C9ORF72* BAC lines that were selected for analyses (28, 29). Put together, there is no solid basis to assume that the full-length gene construct is not obligatory for neurodegeneration since investigators used different constructs and observed dissimilar results. Jiang *et al.* (28), and Liu *et al.* (28, 29) for example, reported neurodegenerative disorders despite using part of the gene in their construct, whereas Rourke *et al.* did not observe behavioral abnormalities or neurodegeneration even at advanced ages (23).

The discrepancies in disease manifestation between the developed mouse models prompted the identification of models that closely mimic human ALS (Table I). A set of criteria were used to select these mouse models, including late-onset ALS, low expression of a misfolded protein, occurrence of gliosis or paralysis, and presence of cytosolic inclusions at presentation.

In most cases, *C9ORF72* models support the toxic gain of function (GOF) mechanism, which is evidenced by the accumulation of abnormal protein aggregates. On the other hand, no significant replication of ALS has been reported in knockdown models. Currently, research acknowledges the potential of the use of antisense oligonucleotides as a therapeutic approach for *C9ORF72*-related disease since they can target genes such as *SOD1* and *C9ORF72*, and alleviate toxicity due to G4C2 repeat while maintaining the normal function of *C9ORF72* (23).

The *SOD1* Mutation

In 1994, a breakthrough was achieved with the discovery of genetic mutations in the *SOD1* gene that were linked to FALS (30). *SOD1* mutations remained the only known cause of 'classical' ALS until causative mutations in the *TARDBP* gene were found (30, 31). During the subsequent two decades, more than 180 *SOD1* mutations have been identified, with most being missense point mutations, mainly substitution. However, insertion and deletion point mutations have also been identified (32).

Recent research on ALS genetics support the role of proteins in RNA metabolism and cytoskeletal organization, typically in the central nervous system (33). On the contrary, *SOD1* is expressed in several tissues, and is not limited to the spinal cord and motor neurons (34, 35). In addition, the level of *SOD1* in tissues is not developmentally regulated (36). The *SOD1* enzyme has a well-defined catalytic function, whereby it detoxifies the superoxide species in cells (36). Scientists quickly ruled-out deficient *SOD1* enzymatic activity in their quest to identify the possible underlying mechanism for ALS since mutations preserving

or abolishing *SOD1* activity were found to cause ALS disease (37). These factors by themselves suggest that there is no outright explanation for the involvement of *SOD1* in adult-onset neurodegenerative disease.

Here focus is placed on a few of the varied yet organized motor neuron toxicity due to the formation of aggregates caused by the instability of the *SOD1* protein (36). Among these are excitotoxicity, deficient axonal transport, and mitochondrial dysfunction (36). Like in all ALS-associated mutations, there is cytoplasmic accumulation of misfolded *SOD1* in the form of inclusions, namely Lewy-body-like hyaline inclusions (LBHI), which are the most frequent inclusions in *SOD1* mutants. These inclusions are located on mitochondrial neurons and astrocytes, and as a result, these suffer morphological damage (38, 39). LBHI is made up of several components, including mutated and wild-type *SOD1*, granule-coated fibrils, as well as ubiquitin (40). A disruption of Ca^{2+} intracellular reservoirs results from mitochondrial dysfunction, and, consequently, excess Ca^{2+} is stored in the mitochondria. This results in motor neurons becoming very sensitive to glutamate (excitatory neurotransmitter), causing excitotoxicity (39, 41). In TDP43 pathogenesis, it has been suggested that axonal transport disruption is attributed to damaged axonal cytoskeleton (42).

A growing body of evidence suggests that dysfunctional axonal transport plays a role in the pathogenesis of ALS (43). Defects in both anterograde and retrograde axonal transport have been described in *Sod1*^{G93A} transgenic mice (43). Previous research has demonstrated that one of the first axonal pathologies in *Sod1*^{G93A} transgenic mice was the block of axonal retrograde transport (43). This suggests that ineffective axonal transport is a major pathogenic driver of ALS. Furthermore, some investigators demonstrated impairment of both fast and slow axonal transport in transgenic mice that exhibited low levels of mutant *SOD1* (44). Severe defects in axonal transport have been observed in mice overexpressing human neurofilament heavy-subunit gene (45). Patients with sporadic ALS or FALS present point mutations of the p150 subunit of dynactin (46), which can cause a decrease in retrograde transport; however, the disease progresses at a slower rate than ALS in transgenic mice (47). Conversely, mutations in cytoplasmic dynein can cause pure sensory neuropathy or a sensory neuropathy with motor neuron involvement (48). It is thought that the progression of *SOD1*-associated ALS is caused by non-cell autonomous toxicity. Misfolded mutant *SOD1* is secreted in association with components of neurosecretory vesicles by motor neurons or glial cells (49). The secreted mutant *SOD1* will, in turn, affect other motor neurons and glial cells, especially astrocytes and microglia (50), causing the development of motor neuronal damage and, consequently, disease progression.

Contrary to health astrocytes, which have the capacity to block Ca^{2+} from entering motor neurons, mutant astrocytes

Table I. Mouse models for chromosome 9 hexanucleotide repeat 72 (C9ORF72)-related amyotrophic lateral sclerosis.

	C9ORF72 Mutation (Ref)					
Clinicopathological feature	(23)	(24)	(26)	(27)	(28)	(29)
No. of C9 repeats	100-1,000	500	NA	66	450	500
Promoter	BAC ¹	BAC ¹	NA ²	NA ²	BAC ¹	BAC ¹
Age of disease onset (months)	ND	ND	6	6	13	4
Cognitive deficit	No	No	Yes	Yes	Yes	Yes
Cortical MNL	No	No	Yes	Yes	No	Yes
Hippocampal MNL	No	No	Yes	No	Yes	Yes
Cerebral MNL	No	No	Yes	Yes	ND	Yes
Spinal cord MNL	No	No	No	No	No	Yes
Gliosis	No	No	No	Yes	No	Yes
Paralysis	No	No	No	No	No	Yes
Mechanism	ND	ND	GOF	GOF	GOF	GOF
Cytoplasmic inclusion	DPR (poly-GP), RNA foci	DPR (poly-GP), RNA foci	DPR, sparse phospho- TDP43	DPR (poly-GP), RNA foci, phospho-TDP43	DPR and RNA foci	DPR, RNA foci, TDP43e, vacuolization
Age of death (months)	ND	ND	ND	Unknown	ND	5-10

GOF: Gain of function; MNL: motor neuron loss; NA: not applicable; (ND): not described; DPR: dipeptide-repeat proteins; BAC: bacterial artificial chromosome. ¹This includes the promoter sequence of a human gene. ²Viral delivery.

lack the ability to regulate the GluR2 subunit, rendering motor neurons vulnerable to excitotoxicity (23, 51, 52). A site-specific recombinase technology (Cre-Lox system) has been used to remove *SOD1* mutants from astrocytes and microglia (51-53). This system slowed disease progression but did not prevent disease onset, although it has been demonstrated that the removal of mutant *SOD1* expression from neurons prevented disease onset (53). Thus, it is possible that astrocyte cell replacement therapy can improve survival in patients with ALS (53). Mutant *SOD1* causes other forms of neuronal damage, including endoplasmic reticulum stress, proteasome inhibition, and synaptic vesicle defects (54), which will not be covered in this review.

Mouse models. Many transgenic mouse models have been developed since the discovery of mutant *SOD1*-induced ALS. These models have helped scientists to understand the mechanism underlying ALS. It has now been established that *SOD1* gain and LOF can complement each other in the pathogenesis of ALS (55). In some models, *Sod1*^{-/-} knockout mice did not develop the ALS phenotype. Rather, these mice had signs of decreased fertility, axonal repair difficulties, higher oxidative stress, weakness, and in severe cases, early post-natal mortality without expressing any phenotypes of ALS up to 6 months of age (56-58).

Conversely, overexpression of human wild-type *SOD1* did not result in the development of symptoms typical of ALS. Thus, some mice showed late deficient hindlimb splaying at 8 months of age, whereas mutant *SOD1* transgenic mice exhibited the ALS phenotype at an earlier age (30, 35). Most

models in the literature were created by the administration of a 12-kb fragment of human genomic DNA that contains all regulatory elements as the transgene vector (59). Researchers have introduced the following mutant *SOD1* genes into mice: A4V, G93A, G37R, D90A, and G85R. Mice with these gene variants had phenotypes that mimicked several features of ALS (Table II).

Furthermore, these phenotypes depend on the patient's genetic profile, gender, mutation type, and accumulation of mutant *SOD1* protein. It was demonstrated that contrary to mice, patients with the A4V mutation had rapid disease progression and death within 1 year of symptom manifestation (60). Conversely, investigators noted that the disease was slowly progressive in both mice and humans who had D90A mutation (60). Furthermore, slow disease progression and prolonged survival were common among female mice (61). Similar observations were noted in humans (62). The mouse models most widely used by ALS researchers are those expressing approximately 25 copies of the transgene *Sod1*^{G93A} under the influence of a human *SOD1* promoter (3). These are ideal for studying the pathogenesis of ALS because they closely mimic the disease in patients with *SOD1*-related ALS. LBHI accumulation, neuronal loss in the ventral horns of the spinal cord, and reactive gliosis have been described in patients with *SOD1*-related ALS (62). Nevertheless, some features such as CNS vacuolization have only been observed in mutant *Sod1* mice and infrequently in humans (62). It is thought that these are due to the accumulation of remnants of damaged mitochondria. *Sod1*^{G93A} mice can live for approximately

Table II. Mouse models for superoxide dismutase 1 (SOD1)-related amyotrophic lateral sclerosis.

Clinicopathological features	Mutation (Ref)										
	h <i>SOD1</i> ^{wt} (30)	h <i>SOD1</i> ^{wt} (31)	<i>SOD1</i> ^{wtxG93A} (31)	¹ <i>SOD1</i> ^{G93A} (32-34)	<i>SOD1</i> ^{G93A-LOW} (35)	¹ <i>SOD1</i> ^{A4V} (36)	<i>SOD1</i> ^{D90A} (33)	¹ <i>SOD1</i> ^{G85R} (37)	<i>SOD1</i> ^{wtxG85R} (38)	¹ <i>SOD1</i> ^{G85R} (38)	¹ <i>SOD1</i> ^{G37R} (39, 40)
Age of disease onset (months)	9	15	5-6	3-4	10	ND	12	Variable ²	10	5	Variable ²
Cognitive deficit	ND	ND	ND	Yes	ND	ND	ND	ND	ND	ND	Learning deficit
Cortical MNL	ND	ND	ND	ND	No	No	No	No	ND	ND	No
Hippocampal MNL	ND	ND	ND	ND	No	No	No	No	ND	ND	No
Cerebral MNL	ND	Yes	Yes	Yes (before symptom onset)	No	No	No	No	ND	ND	No
Spinal cord MNL	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes (late onset)	Yes
Gliosis	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes
Paralysis	Yes	No	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes
Mechanism	GOF	GOF	GOF	GOF	GOF	No	GOF	GOF	GOF	GOF	GOF
Cytoplasmic inclusion	<i>SOD1</i> and vacuolization	<i>SOD1</i> , ubiquitin and vacuolization	<i>SOD1</i> , ubiquitin and high vacuolization	Detergent-resistant <i>SOD1</i> and vacuolization	Ubiquitinated LBHI, rare vacuolization and others	None	Detergent-resistant <i>SOD1</i> and vacuolization	LBHI in astrocyte, diffuse <i>SOD1</i> ubiquitin and eosinophilic	Stable insoluble <i>SOD1</i> , ubiquitin and mutant <i>SOD1</i>	Insoluble mutant <i>SOD1</i> surrounding defect	Diffused <i>SOD1</i> surrounding mitochondria, ubiquitin and vacuolization
Age of death (months)	13	ND	6-7	5	15	ND	14	2 weeks after onset	12	6	12

hSOD1: Human SOD1; GOF: gain of function; LBHI: Lewy-body-like hyaline inclusion; MNL: motor neuron loss; ND: not described. ¹Hemizygotes. ²Dependent on the mouse line used.

Table III. Mouse models for fused in sarcoma (FUS)-related amyotrophic lateral sclerosis.

Clinicopathological features	Mutation (Ref)						
	hFUS ^{wt} (71,75,85)	hFUS ^{wt} (71,75,85)	¹ hFUS ^{wt} (71,75,86)	² FUS ^{R521C} (72,73,82)	¹ FUS ^{R521C} (72,73,82)	² FUS ^{P525L} (71)	¹ FUS ^{R521G} (72,73,82)
Promoter	mPrp	tau	CAG	tau	hamPrp	tau	CAG
Age of disease onset (months)	1.0	ND	Approximately 0.1	2.0	1.0	1.0	Approximately 0.5
Cognitive deficit	NA	ND	NA	ND	ND	ND	Y (only mice that survived)
Cortical MNL	No	No	No	ND	Yes	ND	No
Hippocampal MNL	No	ND	ND	ND	ND	ND	ND
Spinal cord MNL	Yes	No	No	Yes	Yes	Yes	No
Gliosis	Yes	No	Yes	Yes	Yes	Yes	Yes
Paralysis	Yes	No	Yes	Yes	Yes	Yes	Yes
Mechanism	GOF	ND	LOF	GOF	LOF	GOF	GOF and LOF
Cytoplasmic inclusion	Diffused and intense FUS, perinuclear inclusions and ubiquitin	None	None	Diffused FUS	Low FUS aggregates	Highly diffused FUS	None
Age of death (months)	3	ND	<1	12	2-2.5	12	<1 (70% of mice)

hFUS: Human FUS; GOF: gain of function; LOF: loss of function; MNL: motor neuron loss; NA: not applicable; ND: not described. ¹Hemizygotes. ²Heterozygotes.

4 months after developing signs of motor neuron degeneration and paralysis. In previous reports, investigators have observed other signs such as muscle denervation at the neuromuscular junction (NMJ), microgliosis, and extensive inflammation of the brain (55, 57). Of note, motor neuron degeneration did not improve after eliminating microglia expressing mutant *Sod1*^{G93A}, whereas a decrease of mutant *Sod1*^{G37R} in astrocytes led to a significant prolongation of survival in transgenic mice (52, 63). Although significant progress has been made in identifying the mechanism underlying motor neuron degeneration in *SOD1*-related ALS, further study is warranted to understand disease progression among the different variants (64).

The FUS Mutation

The *FUS* mutation is the second most frequent mutation associated with ALS (64-66), accounting for 5% of cases in patients with FALS (64). Mutations in the FUS protein have been identified in patients with FALS, sporadic ALS, FTD, and frontotemporal lobar degeneration without motor impairment (64, 67). Clinically, the disease is aggressive and has been associated with young age (68, 69).

In most cases, mutations have been identified clustered in the C-terminal portion of the FUS protein, which consists of several domains, including the RNA-recognition motif, C-terminus, and nuclear localization signal (NLS) (70, 71). Researchers identified a new mutation that triggers juvenile FUS-associated ALS, located in the C-terminal amino acid

(Y526C) (72). FUS proteins play a role in controlling transcription, processing RNA, and repairing DNA (73). FUS proteins can translocate intracellularly between the nucleus and the cytoplasm similar to TDP43 (74).

In patients with *FUS* mutation, an impairment of DNA-repair pathways and RNA splicing can cause physiological defects such as dendritic growth retardation, NMJ denervation, and neuron dysfunction (75). However, it is believed that the impairment of DNA-repair mechanisms is not due to the binding of mutant FUS to the DNA foci but rather due to the interaction with histone deacetylase 1 (76-78). Juvenile ALS with basophilic inclusions is pathologically and genetically different from the classic form of sporadic ALS. Basophilic inclusions are positive for FUS protein; however, these are negative for TDP43 (78). Moreover, the intensity of basophilic inclusions, especially in the horn of the spinal cord and cortical neurons, is a reflection of disease severity (79).

The administration of rosiglitazone in *FUS* transgenic rats prevented neuronal loss by inhibiting the development of abnormal dendrites, thus preserving their spatial memory (80). This suggests that neuronal loss is downstream of dendritic disruption, and this might also be the case in NMJ denervation. The use of FUS transgenic rats can potentially help understand the mechanism underlying cortical dementia in frontotemporal lobar degeneration (80).

Mouse models. A mutation in the *FUS* gene can cause ALS by both LOF and toxic GOF, contrary to other forms of ALS (Table III).

Table IV. Mouse models for transactive response DNA-binding protein (TARDBP)-related amyotrophic lateral sclerosis

Clinicopathological features	Mutation (Ref)											
	hTARDBP ^{wt} (115-119)	hTARDBP ^{wt} (115-119)	hTARDBP ^{wt} (115-120)	TARDBP ^{A315T} (116,119,120)	¹ TARD BP ^{A315T} (116,120)	TARD BP ^{M337V} (122)	¹ TARD BP ^{M337V} (118,122)	¹ TARD BP ^{M337V} (118,122)	TARD BP ^{G348C} (116,120)	TARD BP ^{Q331K} (118,123)	TARD BP ^{wtQ331K} (110,123)	
Promoter	BAC	mPrp	mThy1.2	BAC2	mPrp	mThy1.2	mPrp	mPrp	BAC2	mPrp	mPrp	
Age of disease onset (months)	10.0	0.5	0.5-2.0	3	3.0-4.0	Variable ³	8.0	10.0	10.0	3.0	0.75	
Cognitive deficit	Yes	ND	ND	Yes	ND	ND	Yes	ND	Yes	ND	ND	
Cortical MNL	ND	ND	Yes	ND	Yes	Yes	Yes	Yes	ND	Yes	Yes	
Hippocampal MNL	ND	ND	Yes	ND	ND	Yes	No	ND	ND	ND	ND	
Spinal cord MNL	ND	No	Yes	ND	Yes	Yes	No	Yes	ND	Yes	Yes	
Gliosis	Yes	Yes	Yes	Yes	Yes	Yes	ND	ND	Yes	ND	Yes	
Paralysis	No	Yes	Yes	No	Yes	Yes	No	No	No	No	Yes	
Mechanism	GOF	GOF	GOF	GOF	LOF	GOF and LOF ⁴	GOF	GOF and LOF	GOF	GOF and LOF	GOF	
Cytoplasmic inclusion	Sparse peripherin aggregates and ubiquitin	Ubiquitinated phospho-TDP43, ubiquitin, eosinophilic, mitochondrial aggregates and vacuolization	Ubiquitinated phospho-TDP43, ubiquitin and vacuolization	peripherin aggregates, moderate ubiquitinated TDP43d and ubiquitin	Ubiquitin	TDP43, few phospho-TDP43, TDP43 CTF, eosinophilic, ubiquitin and mitochondrial aggregates	TDP43 in cytosol and mitochondria	None	Peripherin aggregates, high ubiquitinated TDP43d and ubiquitin	GOF and LOF Vacuolization	TDP43, phospho-TDP43, ubiquitin, p62 and vacuolization	
Age of death (months)	normal	1.0-2.0	1.0-6.7	3	5.0	Variable ³	16.0	normal	normal	normal	2.0-2.5	

TDP43: Transactive response DNA-binding protein 43; GOF: gain of function; MNL: motor neuron loss, ND: not described. ¹Hemizygotes. ²This includes the promoter sequence of a human gene. ³This depends on the mouse line used. ⁴GOF at disease-onset and LOF during disease progression.

In one report, antisense morpholino oligonucleotide was used to knock-down *fus* gene in zebrafish (81). Neurological disorders such as NMJ denervation associated with abnormal synaptic activity and motor neuronal excitotoxicity were observed in this model (81). Of note, the investigators also observed symptoms that mimicked ALS in another group of zebrafish overexpressing mutant FUS (17). These observations demonstrate that LOF is the key pathogenic mechanism in FUS-related ALS. Nevertheless, there was no evidence to exclude toxicity due to GOF. In a more recent study, investigators developed a transgenic mouse model expressing *FUS^{R521C}* mutation driven by the Syrian hamster prion promoter (82). While significant and progressive neuronal loss were observed in these mice, no cytoplasmic inclusions were noted. Conversely, in most cases, endogenous FUS was not able to function properly (83). Overall, by identifying defects in transcription and RNA splicing in knockdown mouse models, investigators have demonstrated that LOF should be considered as a mechanism of action in FUS-induced ALS (83). Nevertheless, additional research is warranted, as controversy continues to surround this hypothesis.

Other researchers hold that toxic GOF is involved in the development of FUS-related ALS (84). It was shown that homozygous mice overexpressing human wild-type FUS under the mouse prion promoter had progressive and lethal neurodegenerative disease despite having FUS protein levels only 1.7-fold higher than non-transgenic mice (85). Mice with FUS mutation developed tremors at 1 month of age and showed signs of spinal cord neuronal loss and gliosis (86). Nevertheless, researchers did not identify physiological alterations in the brain (87). Furthermore, the accumulation of FUS inclusions was observed in the cytoplasm of the anterior horn of the spinal cord and brain, but did not co-localize with ubiquitin inclusions (88). Of note, wild-type FUS is characteristic of FTD and not ALS, although the mice displayed signs typical of ALS (71). These observations support the hypothesis that the disease acts through a GOF mechanism due to cytoplasmic accumulation of FUS and the absence of inclusions in the nucleus (81). Other investigators did not observe significant motor neuron loss or NMJ denervation in wild-type transgenic mice (75). In their model, the mice expressed a single copy of human wild-type FUS using Cre-LoxP recombinant at the microtubule-associated protein tau (*MAPT*) locus (75). The investigators utilized the same promoter to develop transgenic mice overexpressing human mutant *FUS^{R521C}* and *FUS^{P525L}*, which have been reported to cause late- and early-onset disease in humans, respectively (71). Contrary to the healthy wild-type model, *FUS^{R521C}* and *FUS^{P525L}* mice exhibited denervated NMJ and progressive neurodegeneration at 2 and 1 month of age, respectively (71). This indicates that the *FUS^{P525L}* mutation causes more aggressive disease than the *FUS^{R521C}* mutation. In one report, the investigators found that mutant FUS, besides being more pathogenic, was more stable than human wild-type

FUS (72). In both mutant models, diffused condensed inclusions of human wild-type FUS were observed. In addition, researchers documented an abnormal accumulation of FUS in the cytosol rather than in the nucleus (72, 73, 82). *FUS^{P525L}* mice demonstrated more cytoplasmic FUS than *FUS^{R521C}*, indicating that the frequency of FUS accumulation is directly related to the severity of ALS (82). Furthermore, to support their argument that the disease acts through a GOF toxicity mechanism in these models, the investigators developed a conditional FUS knockout model to overcome the prenatal lethality of consecutive FUS knockouts (75). The investigators concluded that the toxicity observed in FUS-related ALS does not involve an excess of human FUS or require the interaction of the mutant gene and wild-type forms. Rather, it is thought that toxicity is only due to accumulation of mutant FUS (17, 73, 75).

Lastly, there is evidence that the disease acts through a GOF or LOF mechanism depending on the type of mutation (89). Investigators developed human wild-type FUS or *FUS^{R521G}* mice models by utilizing a Cre-inducible transgenic approach. Mortality rates were approximately 70% and 100% in mice expressing *FUS^{R521G}* and human wild-type FUS, respectively (82, 85). In both groups, mortality was due to loss of motor function, which occurred before the mice were 1 month old. *FUS^{R521G}* tat mice (Tat transgenic mice that express Tat protein; a number of studies have documented its neurotoxic property and its association with neurological diseases) survived and exhibited impaired motor function and mild behavioral disorders compared to their littermates (82, 85). Most mice expressing human wild-type FUS and *FUS^{R521G}* developed hindlimb clasping, NMJ denervation, and muscle atrophy prior to motor loss (82, 85). This finding contradicts those of previous researches that demonstrated that wild-type FUS was less severe than mutant FUS. Similarly to the model generated by Qiu *et al.* (82), cytoplasmic FUS proteins were not detected in these models. Nevertheless, both mutants were distinct in terms of their gene-expression profile and synaptic homeostasis. Mice that expressed human wild-type FUS exhibited low gene expression, implying that endogenous FUS was non-functional. Thus, the expression of human wild-type FUS was through the LOF mechanism. Mice that expressed *FUS^{R521G}*, contrary to transgenic mice that expressed human wild-type FUS, showed disrupted branching of dendrites, which was thought to be caused by toxic GOF. Nevertheless, it is worth mentioning that *FUS^{R521G}* mice also demonstrated low gene expression, a finding that is in line with that reported by Qiu *et al.* (82). This supports the hypothesis that FUS-related ALS acts through a partial LOF and GOF.

The *TARDBP* Mutation

TDP43 has been identified as the main component of cytoplasmic and intracellular inclusions in neurons and glia

of patients with sporadic and FALS (90). Over 30 distinct mutations in the gene encoding TDP43, *TARDBP*, have been identified, and 3-4% occurred in patients with FALS (91, 92). While FUS and TDP43 proteins share similar structures and functions, suggesting that they probably have the same disease mechanisms, their functions of both proteins, for the most part, uncharacterized.

More than 50 missense mutations in the *TARDBP* gene have been identified, and these are related to polymorphism or substitution. Mutations in the *TARDBP* gene cause TDP43 hyper-phosphorylation and ubiquitin aggregation (93). Of note, TDP43 inclusions are found in most patients with ALS and ALS-associated FTD, except for those who have mutations associated with *SOD1*. For example, TDP43 inclusions have been described in *progranulin* (*GRN*) gene, valosin-containing protein (*VCP*), and *C9ORF72* mutations (94). The presence of TDP43 inclusions can lead to a variety of proteinopathies, including axonopathy, mitochondrial degeneration, and abnormal RNA regulation (95). Under normal circumstances, TDP43 proteins help transport target mRNAs from the soma to distal axonal compartments as well as the NMJ (95). A deficiency of TDP43 causes retrograde movement of mRNA granules in *Drosophila* motor neurons and mouse cortical neurons (65).

Oxidative stress accumulation is also common in neurodegenerative diseases caused by protein mutation. Glutathione depletion triggers the formation of TDP43 inclusions (96), suggesting a relationship between reactive oxygen species and TDP43 impairment. Other investigators further demonstrated that TDP43 aggregates trigger oxidative stress and cause the formation of stress granules that recruit more TDP43, leading to the formation of large protein inclusions (97). These, then ubiquitinate mRNA molecules, including HDAC6 and fission factors, causing the degradation of mitochondria and subsequently, neurodegeneration (98, 99). Nevertheless, mitochondrial aggregation following the attachment of TDP43 did not require fission protein (100). Rather, this involved dysfunction of tau protein, which facilitates axonal transport of neurotransmitters (101). While these findings point towards a possible role of TDP43 inclusions in proteinopathies, researchers still have to determine whether these inclusions can cause neurodegeneration or are just a downstream consequence.

Mouse models. TDP43 affects many biological processes, including embryogenesis and neuronal development (102, 103). Cell dysfunction results if TDP43 does not function properly (104). That said, it was challenging for researchers to develop mouse models to study a single disease.

While some researchers successfully generated TDP43 models (105), the experiments had shortcomings in the sense that none could recapitulate the classical TDP43-associated

ALS-like phenotype. To understand whether TARDBP-related ALS acts through LOF, researchers generated knockout mouse models. They found that embryogenesis was impaired in homozygous knockout mice, suggesting that TDP43 played a part in disease pathogenesis (106). Conversely, there was evidence that TDP43 synthesis was autoregulated, as heterozygous knockout mice did not have symptoms of neuromuscular disease and had normal protein expression (106-108). Symptoms typical of ALS were not exhibited in any of the generated knockout models. Overall, it can be deduced that TDP43 aggregation is likely the main cause of ALS/FTLD through a GOF rather than LOF (109).

Furthermore, in order to study the nature of GOF toxicity in ALS and the nature of GOF toxicity in ALS in which high levels of *TARDBP* mRNA and protein in defected neurons have been reported, researchers generated transgenic mice overexpressing human wild-type TDP43 carried by exogenous promoters such as Cre, Thy1.2, Prp and CamkII α (108, 110). The mice in this model exhibited phenotypes similar to those observed in CamkII α mice. These include the presence of fragmented and phosphorylated TDP43 inclusions in the cytosol, early neuronal loss, gliosis, axonopathy, intestinal dysfunction, progressive paralysis, and death. However, these signs are not pathognomonic for ALS and may be observed in different types of TDP43 proteinopathies (66, 111, 112). These findings raise concerns regarding the reliability of these mouse models in the study of ALS pathogenesis.

Researchers have also attempted to understand the role of the structural compartments of TDP43 by generating transgenic mice models overexpressing only C-terminal fragments or containing defective NLS human TDP43 (37, 72, 113). Investigators observed only cognitive dysfunction and mild motor neurodegeneration in C-terminal fragments and defective NLS mice, respectively. This suggests that these domains may be involved in ALS (114), although they did not exactly mimic ALS.

Given that ALS-TDP43 cytoplasmic inclusions are typical in ALS, it is necessary to study these mutations, especially A315T, M337V, G348C, and A382T, reported to occur frequently (115-119). This review delves into the significance of these mutations in some transgenic lines (Table IV).

Some investigators generated human wild-type *TARDBP*^{A315T}, and *TARDBP*^{G348C} transgenic mouse models by injecting DNA fragments, encompassing its human endogenous promoter subcloned from *TARDBP*-BAC into mice (116). In all three models studied, TDP43 overexpression was about three-fold compared to endogenous levels. All the models under investigation, contrary to those with higher expression that used exogenous promoters (112, 119, 120), exhibited late disease onset at age 10 months. Of note, transgenic mice expressing *TARDBP*^{G348C} had more aggressive disease than mice with *TARDBP*^{A315T} and wild-

type *TARDBP* (120). For example, aggregates containing peripherin proteins, pathognomonic of ALS, were identified in high amounts in the hippocampus and the cortex of *TARDBP*^{G348C} mice compared with *TARDBP*^{A315T} and wild-type *TARDBP* mice. Similarly, investigators found that microgliosis and astrogliosis were prominent in *TARDBP*^{G348C} than in *TARDBP*^{A315T} and wild-type *TARDBP* mice. Other researchers generated knock-in mouse models expressing heterozygous human *TARDBP*^{A382T} or *TARDBP*^{G348C} mutations, and found that disease onset was late in these mice (121). This indicated that the use of an endogenous promoter can be better to recapitulate signs and symptoms characteristic of ALS. In addition, the researchers reported that the levels of mutant mRNA and proteins in astrocytes were higher in *TARDBP*^{A382T} than *TARDBP*^{G348C} mice. In other *TARDBP* transgenic mouse models carrying the endogenous promoter, late disease onset was documented in mice with mutant *TARDBP*^{M337V} and *TARDBP*^{Q331K} expressing exogenous promoters (118, 122). Janssens *et al.* demonstrated hindlimb clasping at less than 1 month of age in mice with mutant *TARDBP*^{M337V} driven by a Thy-1.2 promoter (118), whereas Wang *et al.* described cortical neuronal loss driven by a prion promoter in transgenic mice at 12 months of age (122). According to some investigators, the level of *TDP43*^{M337V} in hemizygous *TARDBP*^{M337V} mice was not enough to reach the minimum level required to cause motor neuron death (in the spinal cord), which is a typical feature necessary for the onset of ALS-like phenotypes such as muscle weakness and paralysis (122). However, this hypothesis should be further investigated. Conversely, other researchers described motor deficits at 3 and 10 months of age in *TARDBP*^{Q331K} and *TARDBP*^{M337V} mice, respectively; these were under the control of mouse prion promoter (110). The mice in this model did not exhibit cytoplasmic TDP43 aggregations and on the contrary, TDP43 proteins were localized in the nucleus. Nevertheless, aberrant splicing events were observed in some RNA targets and enhanced in others. Thus, it can be presumed that mutations in TDP43 cause ALS through LOF and GOF in the absence of toxicity. In yet another study, investigators used the Cre recombinase system to reduce *TDP43*^{Q331K} expression in neurons and discovered that disease onset was delayed; however, this did not stop it from progressing (123).

Overall, it can be deduced from the findings above that overexpression of TDP43 at very high levels, especially in mice with *A315T* mutation, can cause premature death, most likely due to gastrointestinal complications (124, 125). In addition, the use of either low protein expression or endogenous promoter correlates with late-onset motor dysfunction. Therefore, mice in these models develop signs that are reminiscent of TDP43-related ALS. Regarding the mechanism underlying neuronal toxicity, the subject remains a controversial issue, and further studies are warranted in this domain.

Conclusion

Much has been achieved in the field of genetic research, especially in understanding the pathogenesis of neurodegenerative diseases such as ALS. It was not until recently that researchers understood why it was important to determine the underlying mechanism of LOF and GOF. Despite advances in our knowledge of the mechanisms underlying the development of ALS, the disease remains fatal. Researchers have only been able to develop mouse models that closely mimic ALS in humans; however, none of these models mimic the exact pathophysiology of the disease in humans, and current treatments can only relieve symptoms, not provide a cure. This review provides a deep insight into the best mouse models that have been generated to date. These models can serve as a basis for further research into developing better models that can potentially help in identifying an effective therapy against ALS.

Acknowledgements

The Author would like to thank Dr. Aida Mohammedeid (Ph.D. of neuroscience) for her help in editing some parts of this article.

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Received April 14, 2018

Revised May 22, 2018

Accepted May 31, 2018