Mouse models for the motor neuron disease Amyotrophic Laterals Sclerosis (ALS)

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Abstract: Lou Gehrig's disease, also known as Amyotrophic lateral sclerosis (ALS), is a neurodegenerative disease that affects motor neurons. It causes muscle atrophy and paralysis, and gradually worsens over time, typically lasting for around three to five years before death. People with ALS have a weak grip, problems of breathing and swallowing and difficulty moving and walking. The condition worsens over time, and up to date there is no cure. Familial ALS (FALS) accounts for around 10% of all cases. Mutations in superoxide dismutase 1 (SOD1) gene are known to cause a large proportion of these FALS cases, and some research has indicated that defects in SOD1 may also underlie other forms of sporadic ALS. The main function of SOD1 is to remove superoxide radicals, preventing their accumulation that will cause oxidative damage to the cell. However, the mutant diversity of SOD1 takes on a new, toxic function that finally leads to motor neurons death that control muscle movement. Although there are already numerous SOD1 ALS mouse models in existence, all overexpress the human gene, producing excessive amounts of the mutant protein. However overexpression of the *non-mutant* SOD1 gene can result in an ALS-like syndrome as well. This increases concerns about whether the neurodegeneration and other effects in these models are actually due to the mutation, or due to gene overexpression. This review compares the various mouse models generated to date and summarizes the research on the pleiotropic role of different proteins present in motor neurons. It is believed that these observations will help identify potential therapeutic targets of this disease.
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Introduction

Human neurodegenerative disorders, including motor neuron diseases such as amyotrophic lateral sclerosis, are characterized by selective involvement of select populations of neurons in certain regions of the brain and spinal cord (Tripolszki et al., 2017).

Amyotrophic lateral sclerosis is a devastating adult neurodegenerative disorder that is characterized by motor neuron degeneration and death at around 3 years from onset. So far, riluzole is the only treatment available, but it only offers a slight increase in survival(Mitsumoto et al., 2014). The etiology of ALS is complex and is associated with several genes, which makes its study difficult (Scarrott et al., 2015).

In the last decade, there have been significant advances in the information available about this disease, from linkage analysis to isolation of the defective gene and identification of its protein product(Deitch et al., 2014). The development of animal models of the disease, in particular, mouse models of ALS(Mitsumoto et al., 2014). With the development of these animal models, it is now possible to further understand the molecular basis of this disease and demonstrate the feasibility of using the intact gene, which is found in all ALS patients, 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(Karch et al., 2009). Another form of ALS, familial ALS, is an inherited autosomal dominant disease that is linked to mutations in the superoxide dismutase 1 (*SOD1*) gene; it is manifested by inclusions and the degeneration of motor neurons. It appears that 5–10% of all ALS patients have the inherited form of ALS(Milanese et al., 2014, Tripolszki et al., 2017).

To examine the mechanisms underlying ALS, investigators have used a variety of animal models, including experimentally produced, spontaneously occurring, or genetically engineered disease models. This review discusses the behavioral/neuropathological features, the results of investigations on the mechanisms of motor neuron disease in model systems, and the potential utility of some of these models for testing new therapies (Price et al., 1997).

Here, we will seek to summarize most current mouse models developed in gene therapy targeting ALS by describing the main techniques that are being generated, with attention to the potential for their future clinical application.

Genetics of ALS

Over 60% of FALS cases and 11% of sporadic ALS cases are associated with mutations of various genes. Four of the most prominent genetic subtypes of

ALS are C9orf72, SOD1, TDP-43 and fused-in-sarcoma (FUS), and there are several hypotheses about the possible pathogenesis of each genetic variant(Donnelly et al., 2013). In addition to these prominent genes, mutations in many other genes have been associated with ALS, including VCP, OPTN, UBQLN2, SQSTM1 and PFN1 (Renton et al., 2014, Scarrott et al., 2015, Tripolszki et al., 2017).

Animal Models of ALS

In addition to cellular models, animal models have also been used to understand the pathogenesis of ALS such as zebra fish and rodent.

Experimental models of ALS are important for clarifying the complex functions of different proteins and the pathology of ALS(Pitzer et al., 2008). Several different models have been used, each of which has certain advantages and disadvantages with regard to studying different aspects of the disease.

ALS Mouse Models

C9-BAC500 (Brown model)

Species	Genes	Mutations	Modification	Strain Name	Genetic Background	Availability
Mouse	C9ORF72	Hexanucleotide repeat in C9ORF72	C9ORF72: Transgenic	N/A	SJL/B6	Available through Robert Brown

Summary

This transgenic mouse expresses the C9orf72 repeat expansion (GGGGCC), which is associated with ALS and FTD. This model, generated by Robert Brown and colleagues, uses a bacterial artificial chromosome (BAC) to deliver a sizeable chunk of the human C9orf72 sequence, including about 500 copies of the repeat expansion (Peters et al., 2015). Hemizygous C9BAC mice are viable, fertile, and born at expected Mendelian frequencies. They live a normal lifespan and do not develop overt behavioral abnormalities. However, they do recapitulate distinctive histopathological features of C9orf72 ALS/FTD, including intranuclear RNA foci and dipeptide repeats within the nervous system.

Male C9BAC mice live a normal lifespan with no overt behavioral abnormalities. Assessment of Rotarod performance and grip strength revealed no motor deficits over a wide range of ages (three to 24 months). Social behavior also appeared normal as assessed by the intruder assay. Survival analysis and behavioral data for females have not yet been reported.

Consistent with the absence of motor impairment, spinal motor axons were normal with respect to overall number and morphology. Furthermore, there was no evidence of increased denervation of neuromuscular junctions in 24-month-old male mice.

In the brain, there was no evidence of increased microgliosis, astrogliosis, or neuronal loss. Likewise, dendritic spine density in layer 2/3 of the prefrontal cortex was unaffected and TDP-43 was not mislocalized to the cytoplasm. Notably, C9BAC mice develop prominent RNA foci and dipeptide repeats. Intranuclear RNA foci were detectable as early as three months of age, and were abundant throughout the CNS, including the spinal cord and motor cortex, by 10 to 24 months of age. RNA foci were observed in both neurons and glia. Foci containing sense strand RNA were more abundant than foci containing antisense RNA.

The mice also express dipeptide repeat proteins produced by repeat-associated non-ATG (RAN) translation. Specifically, poly-glycine-proline peptides (poly-GP) were observed in the brain and spinal cord by four months of age. The poly-GP peptides, which are synthesized from both sense and antisense transcripts, were primarily soluble in young mice, but aggregated into small perinuclear inclusions in older mice, leading to a decrease in levels of soluble protein. At four months of age, poly-GP peptides were most abundant in the cerebellum. It is not yet clear whether, and to what extent, other RAN translation products (e.g., GA, GP, GR, PR, and PA) may be present.

In summary, these mice model certain pathological aspects of disease (e.g., RNA foci and dipeptide repeats). The fact that they do not develop neurodegeneration or behavioral impairment suggests that RNA foci and poly-dipeptides alone are not sufficient to drive these phenotypes.

Modification Details

The BAC construct was constructed from DNA isolated from a familial FTD/ALS patient carrying the C9 expansion. The construct included about 140 kb upstream sequence, exons 1 to 6 of the C9orf72 gene, including part of the 3′UTR of the short isoform V1, and 20 kb downstream of the locus. The construct contained about 500 GGGGCC motifs between exons 1 and 2. The repeat sizes were relatively stable across tissues (i.e., minimal somatic instability), and appeared to be stable across generations as well, although this should be monitored.

Publication

A non-coding hexanucleotide repeat expansion in the C9ORF72 gene is the most common mutation

associated with familial amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). To investigate the pathological role of C9ORF72 in these diseases, we generated a line of mice carrying a bacterial artificial chromosome containing exons 1 to 6 of the human C9ORF72 gene with approximately 500 repeats of the GGGGCC motif. The mice showed no overt behavioral phenotype but recapitulated distinctive histopathological features of C9ORF72 ALS/FTD, including sense and antisense intranuclear RNA foci and poly (glycine-proline) dipeptide repeat proteins. Finally, using an artificial microRNA that targets human C9ORF72 in cultures of primary cortical neurons from the C9BAC mice, we have attenuated expression of the C9BAC transgene and the poly (GP) dipeptides. The C9ORF72 BAC transgenic mice will be a valuable tool in the study of ALS/FTD pathobiology and therapy. (Peters et al., 2015)

C9-BACexp (Baloh/Lutz model)

Species	Genes	Mutations	Modification	Strain Name	Genetic Background	Availability
Mouse	C9ORF72	Hexanucleotide repeat in C9ORF72	C9ORF72: Transgenic	C57BL/6J-Tg (C9orf72_i3)112Lutzy/J	C57BL/6J	The Jackson Lab: Stock# 023099; Live

Summary

This transgenic mouse expresses the repeat expansion (GGGGCC) within the C9ORF72 locus, which is associated with ALS and FTD. This model, generated by Cat Lutz at Jackson Labs and Robert Baloh at Cedars-Sinai, uses a bacterial artificial chromosome (BAC) to deliver the full-length C9ORF72 sequence with the disease-associated expansion (O'Rourke et al., 2015). The model contains multiple insertions of the transgene, each expressing ~100-1,000 hexanucleotide repeats. This entry specifically describes C9BACexp (line 112), which is well-characterized into advanced age and is available through the Jackson Lab.

Hemizygous C9BACexp line 112 mice are viable, fertile, and born in Mendelian ratios. They develop core pathologic features observed in C9ORF72 expansion carriers, including RNA foci in the nervous system. By three months of age, 40-80 percent of the cells throughout the brain exhibit sense and antisense foci. The distribution and frequency of the foci remain relatively stable as the animals age.

In addition to RNA foci, C9BACexp mice express poly-dipeptides translated from sense and antisense transcripts via repeat-associated non-ATG-dependent (RAN) translation. The mice express poly-glycine-proline peptides (poly-GP) in the brain and spinal cord. The poly-GP peptides are primarily soluble in six-month-old mice, but increasingly aggregate into inclusions as the mice age. Within the nervous system, poly-GP peptides were most abundant in the cerebellum and least abundant in in the spinal cord. Poly-glycine-alanine (poly-GA) peptides were also detected and displayed similar aggregation over time (Robert Baloh, personal communication, Dec 2015).

Despite the early and widespread appearance of RNA foci and poly-dipeptides, C9BACexp mice display very little neuropathology. Even at an advanced age (e.g., 18 months), they do not develop neurodegeneration, and likewise, gliosis, inflammation, and synapse loss are absent. Furthermore, there is no evidence of abnormal accumulation of TDP-43 or ubiquitin. One exception is that mild nucleolar dysfunction was observed in the form of dispersal of nucleolin from the nucleus.

Behaviorally, the mice are also appear remarkably normal. Even at an advanced age (18 months) they perform similarly to non-Tg controls in a battery of tests designed to assess motor performance (e.g., grip strength, Rotarod, open-field testing), social behavior (three-chamber test), and memory (Y-maze). To date, only behavioral data on male mice has been reported.

In summary, these mice model certain pathological aspects of disease (e.g., RNA foci, dipeptide repeats). The fact that they do not develop neurodegeneration or behavioral impairment suggests that RNA foci and poly-dipeptides alone are not sufficient to drive these phenotypes at levels seen in human tissue.

Modification Details

The BAC construct contains DNA isolated from an ALS patient carrying the C9 expansion (~800 repeats). The construct includes about 110kb upstream and 20kb downstream of the locus. The repeat sizes were comparable across tissues and brain regions, suggesting minimal somatic instability.

Publication

Noncoding expansions of a hexanucleotide repeat (GGGGCC) in the C9orf72 gene are the most common cause of familial amyotrophic lateral sclerosis and frontotemporal dementia. Here we report transgenic mice carrying a bacterial artificial chromosome (BAC) containing the full human C9orf72 gene with either a normal allele (15 repeats) or disease-associated expansion (□100-1,000 repeats;

C9-BACexp). C9-BACexp mice displayed pathologic features seen in C9orf72 expansion patients, including widespread RNA foci and repeat-associated non-ATG (RAN) translated dipeptides, which were suppressed by antisense oligonucleotides targeting human C9orf72. Nucleolin distribution was altered, supporting that either C9orf72 transcripts or RAN dipeptides promote nucleolar dysfunction. Despite early and widespread production of RNA foci and RAN dipeptides in C9-BACexp mice, behavioral abnormalities and neurodegeneration were not observed even at advanced ages, supporting the hypothesis that RNA foci and RAN dipeptides occur presymptomatically and are not sufficient to drive neurodegeneration in mice at levels seen in patients.(O'Rourke et al., 2015)

C9ORF72(AAV) (G4C2)66

Species	Genes	Mutations	Modification	Strain Name	Genetic Background	Availability
Mouse	C9ORF72	Hexanucleotide repeat in C9ORF72	C9ORF72: Virus	N/A	C57BL/6	Viral construct available through Leonard Petrucelli

Summary

When it was reported in 2015, this model was heralded as the first mouse model of ALS/FTD due to the repeat expansion in C9ORF72. In this model, an adeno-associated viral (AAV) vector is used to express the repeats in the CNS of wild-type mice. The construct contains 66 copies of the hexanucleotide motif, GGGGCC. The mice develop several pathological features of ALS/FTD, including nuclear RNA foci, dipeptide aggregates, and cytoplasmic inclusions of phosphorylated TDP-43. Subtle behavioral changes were also noted. By six months of age, the mice demonstrated anxiety-like behavior, antisocial beahvior, and hyperactivity. (Chew et al., 2015).

By six months of age, the mice develop a number of brain pathologies reminiscent of C9ALS/FTD. RNA foci develop in the nuclei of neurons throughout the brain. Approximately 50 percent of the cells in the cortex, motor cortex, hippocampus, and cerebellar Purkinje cells contained RNA foci. Foci were also observed, albeit to a lesser extent, in the ventral horn of the spinal cord and other areas of the brain.

In addition to neuronal RNA pathology, the mice develop globular inclusions of RAN-translated dipeptides in neurons. Specifically, dipeptide repeats translated from sense RNA were detected; anti-sense dipeptides were not detected. Dipeptides from all frames were observed, including polyGA, polyGP, and polyGR. The majority of dipeptide inclusions were ubiquitin-positive and observed in neurons, although astrocytes had some as well.

Neurons also developed inclusions of phosphorylated TDP-43. Inclusions occurred in about 8 percent of cells in the cortex and hippocampus. Inclusions were primarily nuclear, with occasional cytoplasmic inclusions. TDP-43 pathology was largely restricted to cells containing RNA foci.

Neurodegeneration and gliosis were observed by six months of age. Compared with mice expressing just two repeats, the 66-repeat mice had 17 percent fewer neurons in the cortex and 11 percent fewer Purkinje cells in the cerebellum. At this age the neurons of the hippocampus, thalamus, and spinal cord were not affected. The number of GFAP-positive reactive astrocytes increased in the cortex. Overall, there was a modest decrease in brain weight compared with 2-repeat mice.

Subtle behavioral changes reminiscent of behavioral variant FTD (bv-FTD) were evident at six months of age. The 66-repeat mice displayed anxiety-like behavior in the open-field test, hugging the walls of the enclosure. They traveled faster and farther overall, indicating hyperactivity. They also showed abnormalities in social behavior, preferring an empty chamber to interaction with peers.

A subtle motor deficit was also seen at six months of age. On the Rotarod, 66-repeat mice performed as well as 2-repeat mice on the first day of testing. However, their performance failed to improve on subsequent trials, suggesting impairments in coordination and/or motor learning.

Modification Details

An adeno-associated viral (AAV) vector was used to deliver 66 repeats of the hexanucleotide GGGGCC motif. The construct does not contain the full-length C9ORF72 sequence. Virus was injected directly into the ventricles of wild-type pups on postnatal day 0. The β-actin promoter drives widespread expression in the brain and spinal cord.

Publication

The major genetic cause of frontotemporal dementia and amyotrophic lateral sclerosis is a G4C2 repeat expansion in C9ORF72. Efforts to combat neurodegeneration associated with "c9FTD/ALS" are hindered by a lack of animal models recapitulating disease features. We developed a mouse model to mimic both neuropathological and clinical c9FTD/ALS phenotypes. We expressed (G4C2)66 throughout the murine central nervous system by means of somatic brain transgenesis mediated by

adeno-associated virus. Brains of 6-month-old mice contained nuclear RNA foci, inclusions of poly (Gly-Pro), poly (Gly-Ala), and poly (Gly-Arg) dipeptide repeat proteins, as well as TDP-43 pathology. These mouse brains also exhibited cortical neuron and cerebellar Purkinje cell loss, astrogliosis, and decreased weight. (G4C2)66 mice also developed behavioral abnormalities similar to clinical symptoms of c9FTD/ALS patients, including hyperactivity, anxiety, antisocial behavior, and motor deficits.(Chew et al., 2015)

C9orf72 Knock-out

Species	Genes	Mutations	Modification	Strain Name	Genetic Background	Availability
Mouse	C9ORF72	Knock-Out	C9ORF72: Knock-Out	3110043O21Riktm1(KOMP)Mbp	C57BL/6N	Available through the UC Davis Knockout Mouse Project (KOMP) Repository, gene 3110043021Rik; Cryopreserved

Summary

These mice have a targeted deletion of 3110043O21Rik, the murine homologue of human C9ORF72. No C9ORF72 gene product (mRNA or protein) is detected in homozygous null animals (O'Rourke et al., 2016). Homozygous null animals are viable, normal in size, and have a normal life span. They show no signs of motor neuron disease up to 17 months of age. Close examination reveals that homozygous null animals have enlarged spleens and lymph nodes. Macrophages and microglia show evidence of defective endosomal trafficking and increased expression of inflammatory markers such as IL-1β, IL-6, IL-10. Behaviorally, homozygous C9orf72 knockouts exhibit decreased exploration in the open-field test. Phenotypes of hemizygous animals are largely similar to wildtype controls. No phenotypic differences were observed between male and female mice, when examined.

Modification Details

The mouse 3110043O21Rik gene (homologue of human C9ORF72) was inactivated by deleting a region containing exons 2-6, which includes the start codon. The targeting vector contained expression cassettes, flanked by FRT sites, for lacZ and neo as selectable markers.

Publication

Expansions of a hexanucleotide repeat (GGGGCC) in the noncoding region of the C9orf72 gene are the most common genetic cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia. Decreased expression of C9orf72 is seen in expansion carriers, suggesting that loss of function may play a role in disease. We found that two independent mouse lines lacking the C9orf72 ortholog (3110043O21Rik) in all tissues developed normally and aged without motor neuron disease. Instead, C9orf72 null mice developed progressive splenomegaly and lymphadenopathy with accumulation of engorged macrophage-like cells. C9orf72 expression was highest in myeloid cells, and the loss of C9orf72 led to lysosomal accumulation and altered immune responses in macrophages and microglia, with age-related neuroinflammation similar to C9orf72 ALS but not sporadic ALS human patient tissue. Thus, C9orf72 is required for the normal function of myeloid cells, and altered microglial function may contribute to neurodegeneration in C9orf72 expansion carriers (O'Rourke et al., 2016).

Endogenous Sod1 D83G

Species	Genes	Mutations	Modification	Strain Name	Genetic Background	Availability
Mouse	SOD1	D83G in SOD1	SOD1: Other	B6(C3H)-Sod1m1H/J	C57BL/6J	The Jackson Lab: Stock# 020440; Cryopreserved

Summary

In contrast to most SOD1 mouse models, which overexpress either mutant or wild-type human SOD1, this model has a mutation in the endogenous murine Sod1 gene (Joyce et al., 2015). Although human SOD1 is not present, the A>G point mutation carried by these mice corresponds to the D83G mutation in human SOD1, which causes familial ALS.

The mutation in these mice did not arise spontaneously, but was the result of treatment with the chemical N-ethyl-N-Nitrosourea (ENU), which causes random mutagenesis. Both homozygous and heterozygous mice are viable. ALS-related phenotypes are more pronounced in homozygotes, which are the focus of this entry. Mice carrying the mutation exhibited reduced levels of Sod1 protein in the spinal cord. Homozygous D83G mice had protein levels only about 12 percent of levels in wild-type mice, whereas heterozygotes had intermediate protein levels (about 70 percent of wild-type). Augmenting this loss of function, is the fact that the D83G mutation alters SOD1's zinc-binding site, destabilizing the protein and inactivating the enzyme.

Homozygous D83G mice exhibit ALS-related neuropathology (e.g., upper and motor neuron loss, degenerating neuromuscular junctions, gliosis) and progressive motor impairment (e.g., tremor, muscle weakness, gait abnormalities). They do not develop

paralysis, although humane euthanasia around 16 months of age may preclude end-stage motor impairment. Euthanasia is necessitated primarily by excessive weight loss, probably due to the corresponding development of hepatocellular carcinomas. In homozygotes, body weight started to diverge from wild-type mice as early as four weeks of age and worsened with age. Males reached end-stage sooner than females (495 ± 22 versus 588 ± 24 days).

In terms of neuropathology, lower motor neuron loss appears first, between 6 and 15 weeks of age. It then appears to stabilize and is no more severe at 52 weeks. In the cerebral cortex, neuronal numbers were comparable to wild-type at 15 weeks of age, but cellular degeneration occurred by 29 weeks in layer V of the motor cortex, which showed selective vulnerability. Astrocytosis and microgliosis were evident by 15 weeks, and became more severe with age.

Denervation of a hindlimb muscle, the extensor digitorum longus (EDL), was detected by 52 weeks of age. A decreased number of motor units were seen in the EDL muscle of homozygous D83G mice compared with wild-type littermates.

D83G homozygous mice also develop progressive motor symptoms, including gait abnormalities, tremor, and impaired performance on motor tasks. Grip strength was reduced in both male and female mice at 6 weeks of age. Tremors developed by about five months of age in both sexes, and became more severe over time. Deficits in Rotarod performance were also apparent, at 23 and 67 weeks in females and males, respectively. Select sensory deficits were also observed, such as to a noxious heat stimulus.

Due to the random-mutagenesis technique used to generate these mice, they also carry other mutations throughout the genome that theoretically could contribute to phenotypic abnormalities. However, random mutations in the initial ENU-treated gametes were diluted by at least 10 generations of backcrossing to wild-type C57BL/6J. In addition, the majority of the ENU-induced genomic changes are not linked to the SOD locus, and therefore do not co-segregate with SOD1, further diluting their potential effects.

Modification Details

This mouse has a point mutation (A>G) in the endogenous murine Sod1 gene, resulting from exposure to the chemical mutagen N-ethyl-N-nitrosourea (ENU). The endogenous murine Sod1 promoter drives expression of the mutant protein, which carries a D83G missense mutation.

publication

Transgenic mouse models expressing mutant superoxide dismutase 1 (SOD1) have been critical in furthering our understanding of amyotrophic lateral sclerosis (ALS). However, such models generally overexpress the mutant protein, which may give rise to phenotypes not directly relevant to the disorder. Here, we have analysed a novel mouse model that has a point mutation in the endogenous mouse Sod1 gene; this mutation is identical to a pathological change in human familial ALS (fALS) which results in a D83G change in SOD1 protein. Homozgous Sod1(D83G/D83G) mice develop progressive degeneration of lower (LMN) and upper motor neurons, likely due to the same unknown toxic gain of function as occurs in human fALS cases, but intriguingly LMN cell death appears to stop in early adulthood and the mice do not become paralyzed. The D83 residue coordinates zinc binding, and the D83G mutation results in loss of dismutase activity and SOD1 protein instability. As a result, Sod1(D83G/D83G) mice also phenocopy the distal axonopathy and hepatocellular carcinoma found in Sod1 null mice (Sod1(-/-)). These unique mice allow us to further our understanding of ALS by separating the central motor neuron body degeneration and the peripheral effects from a fALS mutation expressed at endogenous levels.(Joyce et al., 2015)

FUSΔ14 (FUSd14)

Species	Genes	Mutations	Modification	Strain Name	Genetic Background	Availability
Mouse	FUS	FUS Δ14	FUS: Virus	N/A	B6C3F1	Viral construct available through Thomas Kukar

Summary

This model uses adeno-associated virus (AAV) to introduce cDNA encoding mutant human FUS into the brains of wild-type mice. It is considered a model of the neuronal pathology observed in FUS proteinopathies, such as ALS and FTD (Verbeeck et al., 2012).

This model was created alongside two other AAV-mediated FUS models, one expressing wild-type human FUS and the other carrying the R521C mutation. Among these three models, FUSΔ14 mice had the most severe neuropathology when assessed three months after viral injection. Notably, FUSΔ14 mice exhibited significant mislocalization of FUS protein into the cytoplasm along with the formation of neuronal cytoplasmic inclusions (NCIs), reminiscent of those observed in a subset of ALS cases.

Transgene expression ramps up in the three weeks post-injection, and by three months of age the mice expressed transgenic FUS protein throughout the

brain. The highest expression levels were observed in the cerebral cortex and hippocampus. Expression in the spinal cord is unknown. Compared to the other AAV models, FUSΔ14 mice showed the greatest cytoplasmic redistribution of FUS as well as the most inclusion pathology. FUS inclusions were present in about 20 percent of neurons in the cortex and hippocampus and often co-labeled with ubiquitin. TDP-43 pathology was not observed.

The mice appeared healthy when they were sacrificed at three months of age. They showed no obvious motor impairment, although it is unknown whether behavioral impairments would develop at more advanced ages. At three months of age, at least, neurodegeneration and reactive gliosis were not observed in the brain.

Modification Details

In this model, recombinant adeno-associated virus (AAV) is used to express mutant human FUS in the brain. The virus is injected into the ventricles of P0 pups. Expression of human FUS is driven by the cytomegalovirus enhancer/chicken β-actin promoter. The cDNA encodes C-terminal truncation, which lacks exon 14, and thus the entire nuclear localization signal. The AAV construct is AAV1-human p.G466VfsX14, as originally described in DeJesus-Hernandez et al., 2010.

Publication

Background:

Mutations in the gene encoding the RNA-binding protein fused in sarcoma (FUS) can cause familial and sporadic amyotrophic lateral sclerosis (ALS) and rarely frontotemproal dementia (FTD). FUS accumulates in neuronal cytoplasmic inclusions (NCIs) in ALS patients with FUS mutations. FUS is also a major pathologic marker for a group of less common forms of frontotemporal lobar degeneration (FTLD), which includes atypical FTLD with ubiquitinated inclusions (aFTLD-U), neuronal intermediate filament inclusion disease (NIFID) and basophilic inclusion body disease (BIBD). These diseases are now called FUS proteinopathies, because they share this disease marker. It is unknown how FUS mutations cause disease and the role of FUS in FTD-FUS cases, which do not have FUS mutations. In this paper we report the development of somatic brain transgenic (SBT) mice using recombinant adeno-associated virus (rAAV) to investigate how FUS mutations lead to neurodegeneration.

Results:

We compared SBT mice expressing wild-type human FUS (FUSWT), and two ALS-linked mutations: FUSR521C and FUSΔ14, which lacks the nuclear localization signal. Both FUS mutants accumulated in the cytoplasm relative to FUSWT. The degree of this shift correlated with the severity of the FUS mutation as reflected by disease onset in humans. Mice expressing the most aggressive mutation, FUSΔ14, recapitulated many aspects of FUS proteinopathies, including insoluble FUS, basophilic and eosiniphilic NCIs, and other pathologic markers, including ubiquitin, p62/SQSTM1, α-internexin, and the poly-adenylate (A)-binding protein 1 (PABP-1). However, TDP-43 did not localize to inclusions.

Conclusions:

Our data supports the hypothesis that ALS or FTD-linked FUS mutations cause neurodegeneration by increasing cyotplasmic FUS. Accumulation of FUS in the cytoplasm may retain RNA targets and recruit additional RNA-binding proteins, such as PABP-1, into stress-granule like aggregates that coalesce into permanent inclusions that could negatively affect RNA metabolism. Identification of mutations in other genes that cause ALS/FTD, such as C9ORF72, sentaxin, and angiogenin, lends support to the idea that defective RNA metabolism is a critical pathogenic pathway. The SBT FUS mice described here will provide a valuable platform for dissecting the pathogenic mechanism of FUS mutations, define the relationship between FTD and ALS-FUS, and help identify therapeutic targets that are desperately needed for these devastating neurodegenerative disorders.(Verbeeck et al., 2012)

FUS-R521C

Species	Genes	Mutations	Modification	Strain Name	Genetic Background	Availability
Mouse	FUS	FUS R521C	FUS: Transgenic		Transgene injected into B6SJL oocytes. Maintained on C57BL/6, therefore subsequent generations have a higher percentage of C57BL/6.	The Jackson Lab: Stock# 026406; Live

Summary

At a young age, this transgenic mouse develops severe motor impairment and other ALS-related phenotypes. Notably, it develops robust neuronal loss in the spinal cord, denervation of neuromuscular junctions, and muscle atrophy. Phenotype development is swift—detectable within weeks of birth—and the mice decline rapidly. Most mice in the original N1F1 generation reached end-stage within three months (Qiu et al., 2014).

At one month of age, the mice express mutant FUS at levels approximately equal to endogenous FUS levels in the brain and spinal cord. The majority of the transgenic FUS-R521C protein in these mice is nuclear. Cytoplasmic protein is occasionally detected; however, less than 10 percent of spinal motor neurons

contain cytoplasmic FUS inclusions. Endogenous FUS protein, but not transgenic protein, appears in dendrites as a punctate pattern. Similar to endogenous FUS, FUS-R521C protein is detected in astrocytes and oligodendrocytes, but not in microglia.

The mice develop prominent neuronal loss in the spinal cord. At birth, the number of spinal motor neurons is normal. However, by day 16, the number of ChAT-positive neurons in the anterior horn of the spinal cord is reduced by 20 percent. Degeneration continues, and by end stage, only about half of the neurons remain. The surviving motor neurons have reduced dendritic complexity, synaptic defects, and DNA damage. There is no detectable loss of cortical neurons; however, neurons in the sensorimotor cortex have reduced dendritic complexity and reduced synaptic density.

Despite modest transgene expression, FUS-R521C mice exhibit a variety of motor impairments from a young age, including spastic paraplegia and abnormal hindlimb clasping when lifted by the tail. They also have gait abnormalities, including reduced distance between their hind paws during walking. Performance on the Rotarod is poor.

The majority of mice in the original N1F1 generation reached end stage by postnatal day 100. Mice in subsequent generations (e.g., N2F2, N2F3), which have greater C57BL/6 contribution, live longer; about 40 percent reached end stage by postnatal day 200. Both male and female mice show similar disease phenotypes. Hemizygous males may be sterile, or at least have a much reduced breeding capacity (Eric Huang, personal communication, March 2016).

Data on this page refer to hemizygous mice. The phenotype of homozygous mice have not been reported.

Modification Details

The transgene in this model encodes human FUS with the R521C mutation near the C-terminus. It uses a flag-tagged construct driven by the Syrian hamster prion protein promoter.

publication

Autosomal dominant mutations of the RNA/DNA binding protein FUS are linked to familial amyotrophic lateral sclerosis (FALS); however, it is not clear how FUS mutations cause neurodegeneration. Using transgenic mice expressing a common FALS-associated FUS mutation (FUS-R521C mice), we found that mutant FUS proteins formed a stable complex with WT FUS proteins and interfered with the normal interactions between FUS and histone deacetylase 1 (HDAC1). Consequently, FUS-R521C mice exhibited evidence of DNA damage as well as profound dendritic and synaptic phenotypes in brain and spinal cord. To provide insights into these defects, we screened neural genes for nucleotide oxidation and identified brain-derived neurotrophic factor (Bdnf) as a target of FUS-R521C-associated DNA damage and RNA splicing defects in mice. Compared with WT FUS, mutant FUS-R521C proteins formed a more stable complex with Bdnf RNA in electrophoretic mobility shift assays. Stabilization of the FUS/Bdnf RNA complex contributed to Bdnf splicing defects and impaired BDNF signaling through receptor TrkB. Exogenous BDNF only partially restored dendrite phenotype in FUS-R521C neurons, suggesting that BDNF-independent mechanisms may contribute to the defects in these neurons. Indeed, RNA-seq analyses of FUS-R521C spinal cords revealed additional transcription and splicing defects in genes that regulate dendritic growth and synaptic functions. Together, our results provide insight into how gain-of-function FUS mutations affect critical neuronal functions.(Qiu et al., 2014)

hFUS (+/+) (PrP-hFUS)

Species	Genes	Mutations	Modification	Strain Name	Genetic Background	Availability
Mouse	FUS		FUS: Transgenic	Tg (Prnp-FUS)WT3Cshw/J	C57Bl/6/SJL founder mice backcrossed to C57Bl/6	The Jackson Lab: Stock# 017916; Cryopreserved

Summary

This transgenic mouse model of ALS overexpresses wild-type human FUS. Motor symptoms appear at a young age, accompanied by degeneration of spinal motor neurons. End-stage disease occurs by 12 weeks of age in homozygous mice, and is characterized by severe motor dysfunction that includes hindlimb paralysis (Mitchell et al., 2013).

In this model, the mouse prion protein promoter (PrP) regulates transgene expression, driving expression preferentially in the brain and spinal cord, with much lower levels elsewhere. Even in the CNS, transgenic protein levels were relatively low. Compared to levels in non-Tg mice, total FUS protein was elevated in hemizygous and homozygous mice by 1.4- and 1.7–fold, respectively. Homozygous mice are the focus of this entry because hemizygous mice were fairly normal. They gained weight normally, displayed no significant motor dysfunction or signs of ill health, and appeared to have a normal lifespan (>104 weeks).

Homozygous mice, on the other hand, rapidly develop severe motor neuron disease. Although indistinguishable from littermates at birth, they

develop tremor by about four weeks of age and a stilted gait. At that time, their growth tapers off and they have difficulties maneuvering on the Rotarod. By eight weeks, they have reduced locomotion and progressive hindlimb weakening, manifested by an inability to raise the pelvis off the ground. Homozygous mice were euthanized by 10 to 13 weeks of age due to hindlimb paralysis and/or a 25 percent weight loss. Specifically, the average survival was 82 ± 12 days.

The severe motor impairment manifested by these mice is accompanied by significant motor neuron pathology and degeneration. Despite relatively mild over-expression, transgenic FUS protein accumulates in the cytoplasm of homozygous mice. Furthermore, some cytoplasmic FUS accumulates into neuronal inclusions in both the brain and spinal cord. Inclusions in the spinal cord were described as "globular" and "granular." No apparent loss of nuclear FUS was observed. Ubiquitin-positive inclusions were also observed, but did not co-localize with FUS immunoreactivity.

Although the human FUS transgene was expressed in both the brain and spinal cord, neuronal degeneration appears specific to the spinal cord in this model. By end stage, about 60 percent of lumbar spinal neurons were lost in the anterior horn. Neuronal degeneration was accompanied by astrogliosis and microgliosis as well as denervation of hindlimb muscles and focal muscle atrophy.

In this model there is no indication of gender differences in phenotype onset or progression. The mice do not appear to be affected by gastrointestinal dysfunction even by end-stage, although pathological assessment of the myenteric plexus has not been carried out (personal communication, Jacqueline Mitchell, Nov 2015).

Modification Details

The transgene in this model expresses wild-type human FUS with an N-terminal HA-tag. The mouse prion protein promoter (PrP) drives transgene expression.

Publication

Amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD) are relentlessly progressive neurodegenerative disorders with overlapping clinical, genetic and pathological features. Cytoplasmic inclusions of fused in sarcoma (FUS) are the hallmark of several forms of FTLD and ALS patients with mutations in the FUS gene. FUS is a multifunctional, predominantly nuclear, DNA and RNA binding protein. Here, we report that transgenic mice overexpressing wild-type human FUS develop an aggressive phenotype with an early onset tremor followed by progressive hind limb paralysis and death by 12 weeks in homozygous animals. Large motor neurons were lost from the spinal cord accompanied by neurophysiological evidence of denervation and focal muscle atrophy. Surviving motor neurons in the spinal cord had greatly increased cytoplasmic expression of FUS, with globular and skein-like FUS-positive and ubiquitin-negative inclusions associated with astroglial and microglial reactivity. Cytoplasmic FUS inclusions were also detected in the brain of transgenic mice without apparent neuronal loss and little astroglial or microglial activation. Hemizygous FUS overexpressing mice showed no evidence of a motor phenotype or pathology. These findings recapitulate several pathological features seen in human ALS and FTLD patients, and suggest that overexpression of wild-type FUS in vulnerable neurons may be one of the root causes of disease. Furthermore, these mice will provide a new model to study disease mechanism, and test therapies.

(Mitchell et al., 2013)

hTDP-43ΔNLS

Species	Genes	Mutations	Modification	Strain Name	Genetic Background	Availability
Mouse	TARDBP		TARDBP: Transgenic	B6;C3-Tg (tetO-TARDBP*)4Vle/J	Transgene injected into fertilized eggs from C57BL/6J x C3HeJ.	The Jackson Lab: Stock# 014650; Live

Summay

Under physiological conditions TDP-43 is primarily nuclear, but in some people with ALS and FTD the protein relocates to the cytoplasm where it accumulates into hallmark inclusions. To investigate the consequences of TDP-43 mislocalization, this mouse model overexpresses TDP-43 targeted to the cytoplasm through removal of the nuclear localization signal (NLS) from the transgene (Igaz et al., 2011).

This model uses the TET-OFF system to regulate TDP-43 expression, allowing for temporal control over the transgene. The transcriptional transactivator (tTA) is driven by the CAMKIIα promoter, resulting in preferential forebrain expression of tTA, and thus of transgenic TDP-43. An advantage of this model is that human TDP-43 can be suppressed by adding doxycycline (dox) to the diet, bypassing potential confounds of transgene expression during development.

Consistent with the expected pattern for the CAMKIIα promoter, human TDP-43 protein is primarily restricted to forebrain neurons. High levels were seen in the cortex, hippocampus, and olfactory bulb, whereas low to undetectable protein levels were

seen elsewhere (e.g., cerebellum, brainstem, and spinal cord). Cortical TDP-43 expression was about eightfold higher than endogenous levels in non-Tg mice. Notably, within one month of dox withdrawal, exogenous TDP-43 expression in the cortex severely down regulated murine TDP-43 expression.

As expected given the absence of a functional NLS, TDP-43 protein was cytoplamic in the majority of neurons in the hippocampus and cortex (~70 to 90 percent). Despite robust levels of protein in the cytoplasm, TDP-43 aggregates were extremely rare, observed in less than 1 percent of cortical neurons. Aggregate levels peaked at about one month after de-repression of the transgene.

Despite a lack of TDP-43 inclusions, these mice develop severe neuronal degeneration shortly after dox withdrawal. Approximately 50 percent of dentate gyrus neurons were gone one month after de-repression. Neurons in the dentate gyrus and deep layers of the neocortex were selectively vulnerable to degeneration, whereas other regions, such as the hippocampal CA1 subfield and olfactory bulb, were resistant, despite similar expression levels.

In hTDP-43ΔNLS mice, extensive gliosis developed in regions affected by neurodegeneration (i.e., the neocortex and dentate gyrus). Gliosis also was noted at all levels of the corticospinal tract, as well as the striatum, cerebral peduncles, and cervical spinal cord. Axons of the corticospinal tract degenerated one month after dox withdrawal. The number of lower motor neurons was unaffected, and there was no evidence of muscle atrophy (Igaz et al., 2011).

The neurodegenerative pathology in these mice is striking; however, it is not dependent on the cytoplasmic mislocalization of TDP-43. Control animals generated in parallel, expressing wild-type TDP-43 with the NLS intact, developed a similar phenotype (Igaz et al., 2011). Furthermore, some of the observed neurodegeneration may be due to expression of tTA itself. tTA-only mice were not assessed for neurodegeneration alongside bigenic hTDP-43ΔNLS mice, and subsequent reports using other TET-OFF mice have found tTA alone can produce smaller forebrains and dentate gyri compared with non-Tg littermates. This difference is reportedly detectable by two months of age and is largely avoided by treating with dox prenatally and for the first six weeks of life (Han et al., 2012; Liu et al., 2015).

These mice exhibit an array of behavioral impairments indicative of motor, cognitive, and social deficits. Initially they were noted to display an abnormal clasping response as early as one week after dox withdrawal (Igaz et al., 2011). A later comprehensive behavioral assessment at one month after dox withdrawal revealed a variety of motor abnormalities, including hyperlocomotion in the open field test, impaired coordination and balance on the Rotarod, and decreased grip strength. They also showed cognitive deficits such as impaired recognition and spatial memory as measured by the novel-object-recognition test and the Y maze. One month after transgene de-repression, hTDP-43ΔNLS mice showed reduced social behavior, which could not be attributed to changes in anxiety or olfaction. Behavioral deficits were not observed in tTA–only littermates (Alfieri et al., 2014).

The TET-OFF system allows the transgene to be suppressed after phenotypes have developed in order to investigate potential reversibility. “Switching off” the transgene for 14 days largely reversed the motor and cognitive phenotypes in young mice (1.5 months). However, the phenotypes were not reversible in older mice (6.5 months), in which extensive neurodegeneration had already occurred. Interestingly, social deficits were not reversible even in young mice (Alfieri et al., 2014).

The data on this page refer to hemizygous mice.

Modification Details

These bigenic mice use the CAMKIIα promoter to drive expression of tetracycline transactivator (tTA) in forebrain neurons. The responder transgene is wild-type human TDP-43 minus the nuclear localization signal (NLS). Human TDP-43 is expressed constitutively unless suppressed by doxycycline.

Publication

Amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD) are characterized by cytoplasmic protein aggregates in the brain and spinal cord that include TAR-DNA binding protein 43 (TDP-43). TDP-43 is normally localized in the nucleus with roles in the regulation of gene expression, and pathological cytoplasmic aggregates are associated with depletion of nuclear protein. Here, we generated transgenic mice expressing human TDP-43 with a defective nuclear localization signal in the forebrain (hTDP-43-ΔNLS), and compared them with mice expressing WT hTDP-43 (hTDP-43-WT) to determine the effects of mislocalized cytoplasmic TDP-43 on neuronal viability. Expression of either hTDP-43-ΔNLS or hTDP-43-WT led to neuron loss in selectively vulnerable forebrain regions, corticospinal tract degeneration, and motor spasticity recapitulating key aspects of FTLD and primary lateral sclerosis. Only rare cytoplasmic phosphorylated and ubiquitinated TDP-43 inclusions were seen in hTDP-43-ΔNLS mice, suggesting that cytoplasmic inclusions were not required to induce neuronal death. Instead, neurodegeneration in hTDP-43 and hTDP-43-ΔNLS-expressing neurons was accompanied by a dramatic downregulation of the endogenous mouse

TDP-43. Moreover, mice expressing hTDP-43-ΔNLS exhibited profound changes in gene expression in cortical neurons. Our data suggest that perturbation of endogenous nuclear TDP-43 results in loss of normal TDP-43 function (s) and gene regulatory pathways, culminating in degeneration of selectively vulnerable affected neurons.(Igaz et al., 2011)

NEFH-tTA x hTDP-43ΔNLS

Species	Genes	Mutations	Modification	Strain Name	Genetic Background	Availability
Mouse	TARDBP		TARDBP: Multi-transgene		NEFH-tTA mice and tetO-hTDP-43ΔNLS line 4 mice were maintained on a mixed C57BL/6J x C3HeJ background.	Available through The Jackson Lab as single transgenics: Stock# 025397 and Stock# 014650; Live. See also double transgenic Stock# 028412

Virginia Lee, John Trojanowski, and colleagues at the University of Pennsylvania developed this bigenic model which expresses a regulatable form of human TDP-43 lacking a nuclear localization signal (NLS) (Walker et al., 2015). Following transgene activation, the mice quickly accumulate cytoplasmic insoluble TDP-43 in neurons of the brain and spinal cord. In addition, these mice develop other features of ALS, including motor deficits, denervation of neuromuscular junctions, motor neuron loss, and premature death. The expression of human TDP-43 can be suppressed by administration of the tetracycline analog doxycycline (dox). This allows for transgene suppression during critical developmental periods (e.g., prenatally and five weeks postnatally) and enables researchers to investigate whether/and to what extent phenotypes are reversible following transgene suppression later in life.

In this bigenic model, one parental line contains a transgene encoding the tetracycline activator protein (tTA) under the control of the neurofilament heavy chain (NEFH) promoter. The resulting tTA protein drives expression of human TDP-43 resulting in widespread expression in neurons of the brain and spinal cord, as early as one week off dox. Expression was reportedly minimal/absent in peripheral tissues, including the spleen, kidney, and livers. Notably, in layer V of the motor cortex, greater than 95 percent of NeuN-positive neurons co-labeled with human TDP-43 after one week of transgene de-repression. In addition to the expected neuronal expression, 5 to 10 percent of GFAP-positive astrocytes were also positive for human TDP-43.

TDP-43 expression in rNLS8 mice is robust by two to four weeks after de-repression. Total TDP-43 protein levels in the brain were 10-fold higher than levels in non-transgenic littermates, with a concomitant 50 percent decrease in endogenous mouse TDP-43.

rNLS8 mice develop TDP-43 cytoplasmic inclusions at a young age; rare inclusions appear as early as one week after transgene activation. Inclusions become widespread throughout the brain, including the motor cortex, as well as the visual, entorhinal and somatosensory cortices, cerebellum, and hippocampus. Ubiquitin-positive inclusions are also observed. In the spinal cord, TDP-43 positive are comparatively rare, reported in 2 percent of spinal cord motor neurons at end stage.

Neuronal degeneration in the brain is detectable beginning at four weeks off dox, as reflected by a decrease in cortical thickness. In parallel, astrogliosis develops in many brain regions including layer V of the motor cortex. By end stage, the mice have significantly smaller brains than their littermate controls. Motor neuron loss in the spinal cord develops slightly later, with loss of ventral horn motor neurons evident by six weeks off dox.

Quantification of neuromuscular junctions in the hindlimb revealed early axonal die-back and denervation, about four weeks off dox. This is approximately two weeks prior to detectable loss of lower motor neurons. Muscle atrophy follows next, as indicated by reduced mass of the hindlimb muscles, tibialis anterior, and gastrocnemius.

These mice develop a variety of motor impairments, including an early onset hindlimb-clasping phenotype and a fine tremor in their forelimbs and/or hindlimbs. They also develop a progressive loss of grip strength as measured by the wire-hang test and a progressive decline in coordinated movement and balance as measured by the accelerating Rotarod.

Body mass peaks around 7 weeks of age (i.e., two weeks off dox) and then progressively decreases in both males and females. Excessive loss of body weight, (defined as greater than 30 percent loss from peak weight) was the primary factor defining end-stage disease. Median survival was 10.3 weeks off dox (i.e., about 15.3 weeks of age).

Males and females exhibited similar phenotypes and were grouped together for analyses.

TDP-43 pathology and functional deficits were found to be largely reversible even after neurodegeneration was underway. In a paradigm involving six weeks of transgene activation followed by suppression for two-plus weeks, cytoplasmic TDP-43 cleared and endogenous mouse TDP-43 returned to the nucleus. Rapid functional improvements were observed, including partial recovery of hindlimb

clasping phenotype, and improved performance on the Rotarod and wire-hang tests. Further motor neuron loss in the spinal cord was prevented. The mice gained weight rapidly and their lifespan was extended by at least five to eight months.

Availability

Both parental lines and the double transgenic will be distributed through The Jackson Lab. The double transgenic (Stock# 028412) is created by breeding NEFH-tTA line 8 (Stock# 025397) with TDP-43ΔNLS transgenics (tetO-hTDP-43-ΔNLS line 4) Stock# 014650.

Related Strains

These mice represent a "second-generation" model of regulatable TDP-43 lacking a NLS. The team at U. Penn had previously generated a bigenic model called hTDP-43ΔNLS, which expresses the same TDP-43 responder transgene. In the earlier model tTA was driven by CAMKIIα rather than NEFH, resulting in preferential expression in forebrain neurons (Igaz et al., 2011).

Publication

Accumulation of phosphorylated cytoplasmic TDP-43 inclusions accompanied by loss of normal nuclear TDP-43 in neurons and glia of the brain and spinal cord are the molecular hallmarks of amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD-TDP). However, the role of cytoplasmic TDP-43 in the pathogenesis of these neurodegenerative TDP-43 proteinopathies remains unclear, due in part to a lack of valid mouse models. We therefore generated new mice with doxycycline (Dox)-suppressible expression of human TDP-43 (hTDP-43) harboring a defective nuclear localization signal (ΔNLS) under the control of the neurofilament heavy chain promoter. Expression of hTDP-43ΔNLS in these 'regulatable NLS' (rNLS) mice resulted in the accumulation of insoluble, phosphorylated cytoplasmic TDP-43 in brain and spinal cord, loss of endogenous nuclear mouse TDP-43 (mTDP-43), brain atrophy, muscle denervation, dramatic motor neuron loss, and progressive motor impairments leading to death. Notably, suppression of hTDP-43ΔNLS expression by return of Dox to rNLS mice after disease onset caused a dramatic decrease in phosphorylated TDP-43 pathology, an increase in nuclear mTDP-43 to control levels, and the prevention of further motor neuron loss. rNLS mice back on Dox also showed a significant increase in muscle innervation, a rescue of motor impairments, and a dramatic extension of lifespan. Thus, the rNLS mice are new TDP-43 mouse models that delineate the timeline of pathology development, muscle denervation and neuron loss in ALS/FTLD-TDP. Importantly, even after neurodegeneration and onset of motor dysfunction, removal of cytoplasmic TDP-43 and the concomitant return of nuclear TDP-43 led to neuron preservation, muscle re-innervation and functional recovery.(Walker et al., 2015)

PrP-hFUS (R495X)

Species	Genes	Mutations	Modification	Strain Name	Genetic Background	Availability
Mouse	FUS	FUS R495X	FUS: Transgenic		Construct microinjected into C57BL/6 x SJL)F2 hybrid embryos and founders bred to FVB for 4+ generations. Subsequently back-crossed at JAX to create a C57BL/6 congenic.	Congenic available through The Jackson Lab: Stock# 019728; Cryopreserved

Summary

These transgenic mice overexpress mutant human FUS within the nervous system via the mouse prion protein promoter (Prp). The transgene encodes a truncated form of human FUS, which lacks the nuclear localization sequence, resulting in cytoplasmic accumulation of the protein. This model was initially generated on a mixed background, but a congenic is now available through JAX. To date, only characterization of the mixed background model has been reported (Tibshirani et al., 2015).

On a mixed background, hemizygous mice carry 11 to 15 copies of the FUS transgene and exhibit about fourfold protein overexpression in the brain and spinal cord compared with endogenous FUS levels. Both hemizygous and homozygous mice are viable and fertile.

Although hemizygous mice displayed no overt signs of motor weakness, muscular abnormalities were detected in the hindlimbs by electromyography (EMG). These abnormalities were detectable by eight to 12 months of age and included fibrillation potentials, muscle denervation, and a reduction in the number of motor units.

Hemizygous mice exhibit cytoplasmic mislocalization of human FUS consistent with the lack of a nuclear localization sequence. Robust cytoplasmic accumulation of human FUS protein was reported in cortical neurons, deep cerebellar nuclei, lumbar spinal cord anterior horn cells, and hippocampal neurons. Importantly, some residual nuclear FUS staining remained. Despite the high levels of cytoplasmic FUS, inclusions were absent and there was no evidence of neuronal loss.

According to the JAX website, hemizygous mice on a mixed background occasionally develop intestinal swelling and paralytic ileus leading to premature death (50 percent of the original cohort

died around 118 days of age). Homozygous mice were more severely affected (50 percent of the original cohort died around 59 days of age).

Modification Details

These mice express a mutant form of human FUS carrying a truncation mutation near the C-terminus. The transgene is driven by the mouse prion protein (Prp) promoter. The mutation abrogates the nuclear localization sequence and leads to cytoplasmic mislocalization of FUS.

Publication

Mutations in the RNA-binding protein FUS/TLS (FUS) have been linked to the neurodegenerative disease amyotrophic lateral sclerosis (ALS). Although predominantly nuclear, this heterogenous nuclear ribonuclear protein (hnRNP) has multiple functions in RNA processing including intracellular trafficking. In ALS, mutant or wild-type (WT) FUS can form neuronal cytoplasmic inclusions. Asymmetric arginine methylation of FUS by the class 1 arginine methyltransferase, protein arginine methyltransferase 1 (PRMT1), regulates nucleocytoplasmic shuttling of FUS. In motor neurons of primary spinal cord cultures, redistribution of endogenous mouse and that of ectopically expressed WT or mutant human FUS to the cytoplasm led to nuclear depletion of PRMT1, abrogating methylation of its nuclear substrates. Specifically, hypomethylation of arginine 3 of histone 4 resulted in decreased acetylation of lysine 9/14 of histone 3 and transcriptional repression. Distribution of neuronal PRMT1 coincident with FUS also was detected in vivo in the spinal cord of FUS (R495X) transgenic mice. However, nuclear PRMT1 was not stable postmortem obviating meaningful evaluation of ALS autopsy cases. This study provides evidence for loss of PRMT1 function as a consequence of cytoplasmic accumulation of FUS in the pathogenesis of ALS, including changes in the histone code regulating gene transcription.(Tibshirani et al., 2015)

PrP-hFUS (WT)

Species	Genes	Mutations	Modification	Strain Name	Genetic Background	Availability
Mouse	FUS	FUS: Transgenic			Construct microinjected into C57BL/6 x SJL)F2 hybrid embryos and founders bred to FVB for 4+ generations. Subsequently back-crossed at JAX to create a C57BL/6 congenic.	Congenic available through The Jackson Lab: Stock #020783; Cryopreserved

Summary

These transgenic mice overexpress wild-type human FUS within the nervous system via the mouse prion protein promoter (Prp) (Tibshirani et al., 2015). Hemizygous mice are viable, fertile, and apparently healthy with no overt neuropathology, motor impairment, or electromyographical abnormalities. However, despite this, half of hemizygotes die by ~203 days of age. The cause of this premature mortality is unknown. Death is preceded by an acute moribund state, characterized by poor feeding, leading to death within a week (The Jackson Laboratory website, Nov 2015).

This model was initially generated on a mixed background; however, a congenic is now available through The Jackson Laboratory. To date, only the hybrid line has been reported in the literature (Tibshirani et al., 2015).

On a mixed background, hemizygous mice carry about two copies of the FUS transgene. Expression of human FUS protein in brain and spinal cord is approximately equivalent to endogenous levels. FUS protein was predominantly nuclear. At five to six months of age, nuclear human FUS was present in spinal motor neurons, cortical neurons, deep cerebellar nuclei, lumbar spinal cord anterior horn cells, hippocampal neurons, and cerebellar granule cells. No cytoplasmic FUS inclusions were reported (The Jackson Laboratory website, Nov 2015).

Prior to becoming moribund, hemizygous mice displayed no overt signs of motor weakness or muscular abnormalities. Characterization of homozygous mice has not been reported to date (The Jackson Laboratory website, Nov 2015).

Modification Details

These mice overexpress wild-type human FUS. The transgene is driven by the mouse prion protein (PrP) promoter.

Publication

Mutations in the RNA-binding protein FUS/TLS (FUS) have been linked to the neurodegenerative disease amyotrophic lateral sclerosis (ALS). Although predominantly nuclear, this heterogenous nuclear ribonuclear protein (hnRNP) has multiple functions in RNA processing including intracellular trafficking. In ALS, mutant or wild-type (WT) FUS can form neuronal cytoplasmic inclusions. Asymmetric arginine methylation of FUS by the class 1 arginine methyltransferase, protein arginine methyltransferase 1 (PRMT1), regulates nucleocytoplasmic shuttling of FUS. In motor neurons of primary spinal cord cultures, redistribution of endogenous mouse and that of ectopically expressed WT or mutant human FUS to the cytoplasm led to nuclear depletion of PRMT1, abrogating methylation of its nuclear substrates. Specifically, hypomethylation of arginine 3 of histone 4 resulted in decreased acetylation of lysine 9/14 of

histone 3 and transcriptional repression. Distribution of neuronal PRMT1 coincident with FUS also was detected in vivo in the spinal cord of FUS (R495X) transgenic mice. However, nuclear PRMT1 was not stable postmortem obviating meaningful evaluation of ALS autopsy cases. This study provides evidence for loss of PRMT1 function as a consequence of cytoplasmic accumulation of FUS in the pathogenesis of ALS, including changes in the histone code regulating gene transcription.(Tibshirani et al., 2015)

SOD1 (G37R)

Species	Genes	Mutations	Modification	Strain Name	Genetic Background	Availability
Mouse	SOD1	SOD1 G37R	SOD1: Transgenic	N/A	C57BL/6J x C3H/HeJ)F2	Unknown

Summary

This early transgenic mouse model of ALS overexpresses mutant human SOD1 (Wong et al., 1995). The transgene is regulated by the endogenous human SOD1 promoter and regulatory elements, driving expression in the brain and spinal cord. Symptoms of motor neuron disease appear around four to six months of age. The mice lose weight and develop motor impairments including reduced stride length, decreased spontaneous movement, tremors, limb weakness, poor grooming, and muscle wasting. Ultimately the mice become paralyzed and die at about seven months of age.

This entry describes SOD1 (G37R) line 29 mice on a hybrid background. Of the original SOD1 (G37R) lines, line 29 is particularly well-characterized and was later developed into a congenic line at The Jackson Lab (see below). Line 29 mice express mutant human SOD1 at levels four- to fivefold higher than endogenous murine SOD1 in the CNS. Dismutase activity in the spinal cord was increased about sevenfold compared with enzymatic activity in non-Tg mice.

Line 29 mice develop motor deficits around four to six months of age, including reduced spontaneous movement and reduced stride length. When lifted by their tails, they have difficulty extending their hindlimbs. They develop tremors, limb weakness, poor grooming, and muscle atrophy.

Neuropathologically, these mice develop robust pathology in the spinal cord, including loss of motor neurons. Astrogliois in the spinal cord occurs early, by 11 weeks of age, and becomes more severe over time. Vacuoles appear within motor neurons and associated neuropils around 19 weeks of age. By this time, motor neuron loss is underway. The loss of motor neurons is accompanied by denervation of muscles, SOD1 accumulation in axons, and extensive muscle atrophy.

Loss of upper motor neurons is not a feature of this model, although brainstem neurons develop vacuoles. Cytoplasmic vacuoles were bounded by a single membrane and frequently contained degenerating mitochondria.

Hemizygous mice survive about six to eight months.

Modification Details

The transgene is a 12kb DNA fragment encoding human SOD1 with the G37R mutation. The human SOD1 promoter drives transgene expression.

Related Strains

SOD1 (G37R) (congenic) - This congenic line is derived from line 29 and backcrossed to C57BL/6J. It is available through The Jackson Lab, Stock# 008229. Some reports indicate that mice on a C57BL/6-congenic background survive about one year (e.g., Ezzi et al. 2010); however, according to The Jackson Lab website, mice in their colony survive up to 500 days.

Publication

Mutations in Cu/Zn superoxide dismutase (SOD1) cause a subset of cases of familial amyotrophic lateral sclerosis. Four lines of mice accumulating one of these mutant proteins (G37R) develop severe, progressive motor neuron disease. At lower levels of mutant accumulation, pathology is restricted to lower motor neurons, whereas higher levels cause more severe abnormalities and affect a variety of other neuronal populations. The most obvious cellular abnormality is the presence in axons and dendrites of membrane-bounded vacuoles, which appear to be derived from degenerating mitochondria. Since multiple lines of mice expressing wild-type human SOD1 at similar and higher levels do not show disease, the disease in mice expressing the G37R mutant SOD1 must arise from the acquisition of an adverse property by the mutant enzyme, rather than elevation or loss of SOD1 activity.(Wong et al., 1995)

SOD1-G85R (hybrid)

Species	Genes	Mutations	Modification	Strain Name	Genetic Background	Availability
Mouse	SOD1		SOD1: Transgenic	N/A	Transgene injected into hybrid (C57BL/6J x C3H/HeJ)F2 embryos.	Unknown status of the hybrid line. A congenic line is available through The Jackson Lab: Stock# 008248; Cryopreserved

Summary

This transgenic mouse was one of the first ALS

models on the scene and has remained in use for decades due to its severe lower motor neuron degeneration and progressive motor impairment. The SOD1-G85R model was originally generated on a mixed genetic background (Bruijn et al., 1997). It was subsequently backcrossed to B6 to generate a congenic line, currently distributed through the Jackson Lab. This entry describes the initial characterization of this model on a mixed genetic background.

In these mice the endogenous human SOD1 promoter drives expression of mutant SOD1. In the CNS, expression levels are initially quite low, less than endogenous levels, but they double by end stage. The mutant SOD1 enzyme does not have dismutase activity, and SOD1 activity in spinal cord extracts is comparable to that seen in non-Tg mice.

Hemizygous SOD1-G85R mice appear normal through early adulthood. Symptoms appear around eight months of age, generally starting with reduced grip strength in one hindlimb. The disease then progresses rapidly. Within days the affected hindlimb becomes paralyzed, affecting posture and impairing locomotion. The weakness/paralysis then spreads to the other hindlimb and then to the forelimbs. Paralysis sets in within two weeks of symptom onset.

The motor decline and paralysis exhibited by these mice is paralleled by selective degeneration of lower motor neurons. Axon loss is not present prior to the first clinical signs, at which time about 25 percent of large motor axons are missing and another 10 percent are actively degenerating. By end stage, two weeks later, about 66 percent of the large axons are gone and half the remaining axons are degenerating. Smaller-caliber axons (<5 μm in diameter) were not affected. By end stage, some motor neurons in the ventral horn were also lost.

In addition to neuronal degeneration, these mice develop abundant glial pathology in the spinal cord. Astrogliosis and microgliosis are detected by 6. 5 months of age and become more severe at end stage. Notably, astrocytes contained inclusions of SOD1, which appeared by six months of age and became more abundant as the disease progressed. In addition, levels of the glial glutamate transporter (GLT-1) decreased, leading to glutamate-mediated excitotoxicity.

A small number of motor neurons also displayed diffuse aggregates that were immunoreactive for SOD1 and ubiquitin. These aggregates appeared before onset of clinical symptoms and became larger and more numerous as the disease progressed.

Deficits in the axonal transport of mitochondria have been reported, but appear to be secondary to axonal degeneration, because they are measurable only at end stage after axonal degeneration is well underway (Marinkovic et al., 2012).

Related Strains

SOD1-G85R (congenic) - A congenic line (B6.Cg-Tg (SOD1*G85R)148Dwc/J) is available through The Jackson Lab, Stock# 008248; Cryopreserved. According to the JAX website, congenic mice have a longer lifespan (median 361 days). After the onset of motor impairment, the disease progresses rapidly, with death at 12 to 13 months of age.

SOD1-G85R-YFP - Transgenic mouse with mutant human SOD1 fused with yellow fluorescent protein (Wang et al., 2009; Farr et al., 2011).

Publication

High levels of familial Amyotrophic Lateral Sclerosis (ALS)-linked SOD1 mutants G93A and G37R were previously shown to mediate disease in mice through an acquired toxic property. We report here that even low levels of another mutant, G85R, cause motor neuron disease characterized by an extremely rapid clinical progression, without changes in SOD1 activity. Initial indicators of disease are astrocytic inclusions that stain intensely with SOD1 antibodies and ubiquitin and SOD1-containing aggregates in motor neurons, features common with some cases of SOD1 mutant-mediated ALS. Astrocytic inclusions escalate markedly as disease progresses, concomitant with a decrease in the glial glutamate transporter (GLT-1). Thus, the G85R SOD1 mutant mediates direct damage to astrocytes, which may promote the nearly synchronous degeneration of motor neurons.

(Bruijn et al., 1997)

SOD1-G93A (hybrid) (G1H)

Species	Genes	Mutations	Modification	Strain Name	Genetic Background	Availability
Mouse	SOD1	SOD1 G93A	SOD1: Transgenic	B6SJL-Tg (G93A-SOD1)1Gur/J	C57Bl/6/SJL.	The Jackson Lab: Stock# 002726; Live

Summary

First reported in 1994, SOD1-G93A mice ushered in a new era of ALS research. This transgenic model, which expresses large amounts of mutant SOD1, develops adult-onset neurodegeneration of spinal motor neurons and progressive motor deficits leading to paralysis. The original SOD1-G93A line (Gurney et al., 1994) has since diverged into a family of strains with different genetic backgrounds and transgene expression levels. These factors—genetic

background and copy number—significantly affect the onset and severity of symptoms (Heiman-Patterson et al., 2011; Mancuso et al., 2012). As a group, the G93A models have amassed an extensive body of literature, and they remain a cornerstone of preclinical ALS research. This entry focuses on the G1H line, which has high transgene expression, and was originally developed on a mixed SJL and C57BL/6J background.

As reported in 1994, the original SOD1-G93A strain, designated G1, expressed approximately 18 copies of human SOD1, randomly inserted into the genome (Gurney et al., 1994). An unequal crossover event in a breeding pair of G1 mice produced progeny with about 25 copies of the transgene. These mice, designated G1H ("H" for high copy number), have a more aggressive disease course than the founder line. Signs of motor impairment appear around 91 days of age in G1H mice (Tu et al., 1996), compared with about 121 days in G1 mice (Gurney et al., 1994). G1H mice reach end-stage disease at about 139 days (Chiu et al., 1995), compared with about 170 days in G1 mice (Gurney et al., 1994).

One of the first signs of illness in G1H mice is plateauing of growth and body weight at about three months of age. This is followed by the appearance of motor symptoms, including a shaking tremor in one or more limbs. As the disease progresses, the tremor becomes more pronounced and involves all limbs. Proximal muscle weakness develops along with muscle atrophy and denervation of neuromuscular junctions, eventually leading to paralysis and premature death (Chiu et al., 1995).

In this model, age at symptom onset and survival are affected by gender, with females typically surviving four to seven days longer than males. For example, in one colony, females survived 132 ± 12.4 days, whereas males survived just 127.9 ± 9.5 days, despite an equal copy number (Heiman-Patterson et al., 2005). A similar gender difference in survival was seen in G93A mice on a congenic SJL background, but not on a C57BL/6J background (Heiman-Patterson et al., 2005).

The mutant SOD1 retains enzymatic activity (Gurney et al., 1994), although it does not bind copper ions as effectively as wild-type SOD1 (Pratt et al., 2014).

Neuropathology

Although the SOD1 G93A transgene is expressed widely, pathology in this model is largely restricted to the spinal cord (especially the lumbar cord), brainstem, descending spinal tracts, and neuromuscular junctions. A variety of pathological hallmarks develop in the spinal cord prior to the onset of clinical symptoms, including mitochondrial vacuolization (Dal Canto and Gurney, 1995), Gogli fragmentation (Mourelatos et al., 1996), neurofilament-positive inclusions (Tu et al., 1996), Lewy body-like inclusions (Dal Canto and Gurney, 1995), and cytoplasmic SOD1 aggregates (Johnston et al., 2000). Neuromuscular junctions degenerate around 47 days of age; fast-fatiguable motor neurons are affected first (Frey et al., 2000; Pun et al., 2006). Spinal motor neurons then die off, with about a 50 percent reduction in the cervical and lumbar segments by end-stage (Chiu et al., 1995).

Neuropathology is not restricted to lower motor neurons. In upper motor neurons, signs of degeneration include swollen neurites, Gallyas silver-positive aggregates, vacuoles, and neuritic spheroids. These changes occur at about five months of age, after degeneration in the spinal cord is underway, and generally do not progress to outright neuronal loss (Leichsenring et al., 2006). Degeneration of cranial nuclei, such as the trigeminal, facial, and hypoglossal nerves, has been observed (Angenstein et al., 2004; Zang et al., 2004) and there is age-dependent regression of descending corticospinal, bulbospinal, and rubrospinal tracts (Zang and Cheema, 2002).

Gliosis, including the proliferation of reactive microglia and astrocytes, develops in parallel with motor neuron degeneration in the spinal cord. GFAP and MAC1 immunoreactivity indicate an increase in reactive glia by 117 days of age (Almer et al., 1999; Hall et al., 1998).

Usage

These mice have been studied extensively over many years, and this research laid the groundwork for updated guidelines about preclinical research in ALS (Ludolph et al., 2010; see also Scott et al., 2008). Recommendations for experimental design include using enough mice to sufficiently power experiments, using experimental cohorts balanced for gender and littermates, and reporting this information in all manuscripts. Given the importance of copy number, quantitative genotyping should be used to ensure that all experimental animals have the expected copy number (Alexander et al., 2004).

One potential caveat to this model is that because of the high copy number, they produce a large excess of SOD1 protein. This may affect the interpretation of results given that ALS patients with SOD1 mutations have just one mutant allele.

The Jackson Lab notes that male mice from this line are particularly aggressive. They recommend limiting numbers to no more than four males per cage.

Modification Details

These transgenic mice express multiple copies of human SOD1 bearing the missense mutation G93A randomly integrated into chromosome 12 of the mouse.

Related Strains

There are a variety of SOD1-G93A strains available, including:

SOD1-G93A (congenic): This congenic line is derived from the original hybrid line by backcrossing to C57BL/6J mice. It is available through The Jackson Laboratory, Stock #004435. Mice on a C57BL/6J background have a milder phenotype (i.e., later onset, longer lifespan) than hybrid B6SJL mice or those on an SJL background (Pfohl et al., 2015; Heiman-Patterson et al., 2005). Note, substrains of C57BL6, such as C57BL/6JOlaHsd, may have additional phenotypic differences (Nardo et al., 2016).

SOD1-G93A (congenic) low expresser: This congenic line is derived from the original mixed B6 SJL line. It has fewer transgene copies than the SOD1-G93A (congenic) described above and develops disease phenotypes later. It is available through The Jackson Laboratory, Stock #002299.

Publication

The mutation gly93-->ala of Cu,Zn superoxide dismutase (SOD) is found in patients with familial amyotrophic lateral sclerosis and causes motor neuron disease when expressed in transgenic mice. The progression of clinical and pathological disease was studied in a line of mice designated G1H. Clinical disease started at 91 +/- 14 days of age with fine shaking of the limbs, followed by paralysis and death by 136 +/- 7 days of age. Pathological changes begin by 37 days of age with vacuoles derived from swollen mitochondria accumulating in motor neurons. At the onset of clinical disease (90 days), significant death of somatic motor neurons innervating limb muscles has occurred; mice at end-stage disease (136 days) show up to 50% loss of cervical and lumbar motor neurons. However, neither thoracic nor cranial motor neurons show appreciable loss despite vacuolar changes. Autonomic motor neurons also are not affected. Mice that express wild-type human Cu,Zn SOD remain free of disease, indicating that mutations cause neuron loss by a gain-of-function. Thus, the age-dependent penetrance of motor neuron disease in this transgenic model is due to the gradual accumulation of pathological damage in select populations of cholinergic neurons (Chiu et al., 1995).

Summary

This page describes a congenic version of the TARDBP (A315T) model originally generated by Robert Baloh and colleagues on a mixed C57Bl/6/J and CBA background (Wegorzewska et al., 2009). Characterization of the congenic animal is complicated by a severe gastrointestinal phenotype, which leads to ill-health and premature death (Hatzipetros et al., 2013; Guo et al., 2012). Consuming an easily digestible gel diet can stave off GI dysfunction and allow time for pathology to develop in the brain and spinal cord, although spinal motor neuron loss remains largely absent despite distal axon degeneration (Herdewyn et al., 2014).

TARDBP (A315T) (congenic)

Species	Genes	Mutations	Modification	Strain Name	Genetic Background	Availability
Mouse	TARDBP	TARDBP A315T	TARDBP: Transgenic	B6.Cg-Tg (Prnp-TARDBP*A315T)95Balo/J	C57BL/6J x CBA mice backcrossed to C57BL/6J.	The Jackson Lab: Stock# 010700; Live

Congenic TARDBP (A315T) mice appear relatively healthy until about three months of age, when they begin to lose weight and show signs of declining health, such as decreased grooming. At this time, mice exhibit signs of gastrointestinal dysfunction, such as a swollen and tender abdomen. The age at which symptoms first appear varies, but after onset, the mice decline rapidly and die within days to weeks (Hatzipetros et al., 2013; Guo et al., 2012).

In general, males have an earlier onset and more rapid progression than females, although lifespan varies. One study found median lifespan on a standard diet to be 84 days for males and 126 days for females (Herdewyn et al., 2014). Another study found the median lifespan to be 108 days for males and 185 days for females (Hatzipetros et al., 2013). On a normal diet, the lifespan of both sexes is limited by GI dysfunction and can be extended by replacing standard chow with an easily digestible gel. This diet allows the mice to reach more advanced ages and to develop progressive motor deficits and motor neuron degeneration (Herdewyn et al., 2014). In mice fed a gel diet, significant axonal degeneration was observed, resulting in about 20 percent reduced innervation of neuromuscular junctions (Herdewyn et al., 2014). Compelling motor neuron degeneration was not observed in mice consuming a standard diet (e.g., Hatzipetros et al., 2013; Esmaeili et al., 2013), possibly because fatal GI dysfunction cut short disease progression.

Bona fide neuronal loss appears to be rare in this model regardless of diet. Cortical neuron loss has not been examined in detail in this exact model, but when crossed with a Thy-1YFP model to label layer 5 pyramidal neurons, the mice had fewer of these neurons at 15 weeks of age than YFP littermate controls (Zhang et al., 2016). Neuronal numbers in the spinal cord appear to be largely stable in mice consuming standard chow or a gel diet (e.g., Esmaeili et al., 2013; Hatzipetros et al., 2013; Herdewyn et al., 2014); however, one study found 20 percent loss of

lower motor neurons at end-stage (Espejo-Porras et al., 2015).

Similar to the original hybrid animals, cytoplasmic TDP-43 aggregates were notably absent in motor neurons, despite robust human TDP-43 staining in neuronal nuclei (Hatzipetros et al., 2013). The mice do develop ubiquitinated inclusions in motor neurons of the spinal cord along with astrocytosis in the white matter of the cord (Hatzipetros et al., 2013), a phenotype that is also observed in mice consuming a gel diet (Herdewyn et al., 2014).

Both male and female TARDBP (A315T) mice develop significant motor impairment, with deficits generally appearing earlier in males. Fed a standard diet, males performed poorly on the Rotarod as early as six weeks of age (Dang et al., 2014; Espejo-Porras et al., 2015). Another study found female mice were likewise affected, at least by six months of age (Medina et al., 2014). Male mice showed deficits in the hanging wire test at two to three months of age, but females did not (Hatzipetros et al., 2013). It is important to keep in mind that, especially at later ages, exhaustion and general weakness from GI dysfunction may contribute to a decline in motor performance. Fed a gel diet, both male and female mice developed progressive gait abnormalities culminating at end stage in a "swimming gait" (Herdewyn et al., 2014).

Behaviors associated with learning and memory appear to be largely intact. Males navigated the Y-maze normally at 10 weeks (Dang et al., 2014), and females behaved like non-Tg controls in tests of novel-object recognition and contextual fear learning at six months of age (Medina et al., 2014). However, females displayed deficits in the radial-arm water maze at six months of age, which could not be attributed to differences in swimming speed, suggesting possible deficits in hippocampal-dependent spatial learning (Medina et al., 2014).

Related Strains

TARDBP (A315T) (hybrid) - This is the original hybrid line (C57BL/6J x CBA background), from which the congenic was derived. The hybrid is no longer available.

Publication

ALS therapy development has been hindered by the lack of rodent animal models. The discovery of TDP-43, a transcription factor that accumulates in the cytoplasm of motor neurons (MNs) in most cases of ALS, prompted attempts to develop TDP-43-based models of the disease. The current study sought to examine, in extensive detail, the emerging disease phenotype of a transgenic mouse model that overexpresses a mutant human TDP-43 (hTDP-43) gene under mouse prion promoter control. Careful attention was given to ALS-like characteristics to determine the appropriateness of this model for testing therapies for ALS. In light of previous reports that gastrointestinal (GI) dysfunction is responsible for early death in these mice, gut immunohistochemistry (IHC) and longitudinal gut motility assays were used to identify the onset and the progression of these defects. IHC studies revealed that site-specific overexpression of the hTDP-43 transgene in colonic myenteric plexes resulted in progressive neurodegeneration in this region. This change was associated with progressively reduced GI motility, culminating in frank stasis that was primarily responsible for decreasing longevity in these mice. The disease phenotype was gender- and genetic background-dependent, with congenic C57BL/6J male mice exhibiting the most aggressive form of the disease. Spinal cord IHC revealed ubiquitin-positive inclusions, but not TDP-43 aggregates, in the cytoplasm of MNs. Neither gender exhibited compelling ALS-like neuromuscular deficits, irrespective of age. While this model may be useful for studying GI tract neurodegeneration, in its present state it does not display a phenotype suitable for testing ALS therapeutics.(Hatzipetros et al., 2014)

TARDBP (A315T) (hybrid)

Species	Genes	Mutations	Modification	Strain Name	Genetic Background	Availability
Mouse	TARDBP	TARDBP A315T	TARDBP: Transgenic		The Prp-TDP43A315T transgene was introduced into oocytes from C57BL/6J x CBA mice.	No longer available on a C57BL/6J x CBA background

Summary

In 2009, Robert Baloh and colleagues reported a transgenic mouse model of ALS based on the overexpression of mutant TARDBP (TDP-43) (Wegorzewska et al., 2009). These mice developed several features of ALS, including motor impairment, degeneration of cortical and spinal motor neurons, cytoplasmic aggregates of ubiquitinated proteins, and premature death. Notably absent was cytoplasmic deposition of TDP-43 protein.

This page describes the TARDBP (A315T) mouse on a mixed genetic background, as originally reported in 2009. These mice are no longer available on the C57BL/6J x CBA background. A congenic line was subsequently generated by crossing the hybrid line to C57BL/6J mice. The congenic mice, which are

available through the Jackson Lab (see below), were found to die prematurely from bowel obstruction (Hatzipetros et al., 2013; Guo et al., 2012). Their severe gastrointestinal dysfunction affects their use and maintenance, as well as the interpretation of the phenotypes they exhibit.

The original animals, TARDBP (A315T) (hybrid), appear normal until about three months of age. At that time, they began to show gait abnormalities. By four to five months they exhibit what was described as a "swimming gait" because they were unable to hold their bodies off the ground. On average, they survived about five months (154 ± 19 days), before dying or being euthanized. It was not reported if the survival analysis included males, females, or both.

By end-stage, the mice were reported to develop both upper and lower and motor neuron loss. Specifically, neuronal loss was observed in layer 5 of the motor cortex as well as in lumbar regions of the region of the spinal cord (L3-L5). About 20 percent of neurons in the spinal cord were lost at end-stage. Although cytoplasmic TDP-43 deposition was notably absent, aggregates of ubiquitinated proteins accumulated in specific neuronal populations, including layer 5 neurons in the frontal cortex, as well as in spinal motor neurons.

Transgene expression in this model is robust, with approximately threefold more protein in the spinal cord than endogenous mouse TDP-43. Expression is highest in the brain and spinal cord, with lower levels in other tissues.

The precise cause of death in the hybrid line is unknown; however, the congenic version was subsequently shown to die of bowel obstruction (Hatzipetros et al., 2013; Guo et al., 2012). The gut dysfunction and pathology is thought to result from transgene expression in the myenteric plexus.

Modification Details

In this model the transgene encodes full-length, human, mutant TARDBP with the A315T mutation and an N-terminal Flag tag. The mouse prion protein (PrP) promoter drives transgene expression.

Related Strains

TARDBP (A315T) (congenic) - This congenic model (C57BL/6J background) was derived from the original line and is available through The Jackson Lab, Stock# 010700 (B6.Cg-Tg (Prnp-TARDBP*A315T)95Balo/J).

Publication

Frontotemporal lobar degeneration (FTLD) and amyotrophic lateral sclerosis (ALS) are neurodegenerative diseases that show considerable clinical and pathologic overlap, with no effective treatments available. Mutations in the RNA binding protein TDP-43 were recently identified in patients with familial amyotrophic lateral sclerosis (ALS), and TDP-43 aggregates are found in both ALS and FTLD-U (FTLD with ubiquitin aggregates), suggesting a common underlying mechanism. We report that mice expressing a mutant form of human TDP-43 develop a progressive and fatal neurodegenerative disease reminiscent of both ALS and FTLD-U. Despite universal transgene expression throughout the nervous system, pathologic aggregates of ubiquitinated proteins accumulate only in specific neuronal populations, including layer 5 pyramidal neurons in frontal cortex, as well as spinal motor neurons, recapitulating the phenomenon of selective vulnerability seen in patients with FTLD-U and ALS. Surprisingly, cytoplasmic TDP-43 aggregates are not present, and hence are not required for TDP-43-induced neurodegeneration. These results indicate that the cellular and molecular substrates for selective vulnerability in FTLD-U and ALS are shared between mice and humans, and suggest that altered DNA/RNA-binding protein function, rather than toxic aggregation, is central to TDP-43-related neurodegeneration.(Wegorzewska et al., 2009)

TDP-43 (A315T)

Species	Genes	Mutations	Modification	Strain Name	Genetic Background	Availability
Mouse	TARDBP	TARDBP A315T	TARDBP: Transgenic	N/A	Transgene injected into C3H x C57Bl/6 embryos and then crossed with C57Bl/6.	Available through Jean-Pierre Julien

Summary

Among the first wave of TDP-43 transgenics, this model uses the endogenous human promoter to express moderate levels of TDP-43. Expression in the brain was about threefold higher than levels of endogenous mouse TDP-43 mRNA. As they age, these mice develop neuropathology and behavioral deficits relevant to ALS/FTD, including cytoplasmic TDP-43 inclusions, neuroinflammation, axonal pathology, and cognitive and motor impairment. They do not develop neuronal loss or paralysis (Swarup et al., 2011).

Despite relatively moderate expression of human TDP-43, these mice accumulate cytoplasmic TDP-43 in the brain and spinal cord, including TDP-43 aggregates. These inclusions are not present at three months of age. Many of these inclusions co-localize with ubiquitin, although TDP-43 itself is not thought to be ubiquitinated. The cytotoxic 25 kDA C-terminal fragment of TDP-43 also increased in an age-

dependent manner in both the brain and spinal cord.

Evidence of cytoskeletal abnormalities was observed in the brain and spinal cord, including aggregates of the intermediate filament peripherin, a hallmark of degenerating motor neurons in ALS. However, even at advanced ages, overt neuronal loss was absent, as was axonal loss in the ventral root. However, differences in axon caliber were observed in 10-month-old transgenics compared to non-Tg littermates.

Neuroinflammation was an early and prominent feature in these mice, including astrogliosis and microgliosis in presymptomatic mice (e.g., at three months). Gliosis further increased between three and 10 months of age.

Behaviorally, these mice develop age-dependent cognitive deficits, including learning and memory problems. In the passive avoidance test, a measure of contextual memory, behavior is comparable to wild-type littermates until seven months of age, at which time the TDP-43 A315T mice display severe impairment. Around the same time (e.g., 38 weeks of age), they also develop motor deficits, specifically impaired performance on the Rotarod compared with non-Tg littermates.

Data on this page refer to hemizygous mice.

Modification Details

These mice overexpress full-length human TDP-43 with the A315T mutation introduced by site-directed mutagenesis. The transgene is driven by the endogenous human promoter.

Availability

Available through Jean-Pierre Julien.

Related Strains

TDP-43 (WT)

TDP-43 (G348C)

Publication

Transactive response DNA-binding protein 43 ubiquitinated inclusions are a hallmark of amyotrophic lateral sclerosis and of frontotemporal lobar degeneration with ubiquitin-positive inclusions. Yet, mutations in TARDBP, the gene encoding these inclusions are associated with only 3% of sporadic and familial amyotrophic lateral sclerosis. Recent transgenic mouse studies have revealed a high degree of toxicity due to transactive response DNA-binding protein 43 proteins when overexpressed under the control of strong neuronal gene promoters, resulting in early paralysis and death, but without the presence of amyotrophic lateral sclerosis-like ubiquitinated transactive response DNA-binding protein 43-positive inclusions. To better mimic human amyotrophic lateral sclerosis, we generated transgenic mice that exhibit moderate and ubiquitous expression of transactive response DNA-binding protein 43 species using genomic fragments that encode wild-type human transactive response DNA-binding protein 43 or familial amyotrophic lateral sclerosis-linked mutant transactive response DNA-binding protein 43 (G348C) and (A315T). These novel transgenic mice develop many age-related pathological and biochemical changes reminiscent of human amyotrophic lateral sclerosis including ubiquitinated transactive response DNA-binding protein 43-positive inclusions, transactive response DNA-binding protein 43 cleavage fragments, intermediate filament abnormalities, axonopathy and neuroinflammation. All three transgenic mouse models (wild-type, G348C and A315T) exhibited impaired learning and memory capabilities during ageing, as well as motor dysfunction. Real-time imaging with the use of biophotonic transactive response DNA-binding protein 43 transgenic mice carrying a glial fibrillary acidic protein-luciferase reporter revealed that the behavioural defects were preceded by induction of astrogliosis, a finding consistent with a role for reactive astrocytes in amyotrophic lateral sclerosis pathogenesis. These novel transactive response DNA-binding protein 43 transgenic mice mimic several characteristics of human amyotrophic lateral sclerosis-frontotemporal lobar degeneration and they should provide valuable animal models for testing therapeutic approaches.(Swarup et al., 2011)

TDP-43 (A315T) (line 23)

Species	Genes	Mutations	Modification	Strain Name	Genetic Background	Availability
Mouse	TARDBP	TARDBP A315T	TARDBP: Transgenic	STOCK Tg (Prnp-TARDBP*A315T)23Jlel/J	Transgene injected into fertilized hybrid B6SJLF1oocytes. Founders bred with CD1 to create hybrid CD1 and B6SJLF.	The Jackson Lab: Stock# 016143; Cryopreserved

Summary

This mouse model of ALS was among the first transgenics expressing mutant TDP-43 to be characterized (Stallings et al., 2010). In this model the mouse prion protein (Prp) promoter drives TDP-43 expression in the central nervous system, including in the brain and spinal cord, with lower expression elsewhere (i.e., skeletal muscle). Hemizygous mice develop progressive motor impairment, ultimately leading to paralysis and premature death at around 2.5 months of age.

The breeding strategy used to create TDP-43

(A315T) (line 23) produced several founders, many of which were born small and did not survive to reproduce. Intermediate levels of expression, such as the approximately fourfold overexpression in line 23, appear to be tolerated during development.

Around one month of age, these mice first display symptoms of motor impairment, characterized by weakness, decline in grip strength, and reduction in stride length. Weakness was typically more pronounced in the hindlimbs at first, but ultimately progressed until the mice were unable to right themselves and were sacrificed. The mean survival was 75 days (Stallings et al., 2010). According to The Jackson Lab website in 2015, an increase in mean survival was seen in their colony. Median survival of hemizgous males and females was 109 and 164 days, respectively.

At end-stage, these mice do not show overt neuronal loss in the brain or spinal cord. However, neurons of the ventral horn and brainstem develop ubiquitin-positive cytoplasmic inclusions. TDP-43 aggregates in the cytoplasm are largely absent, although some rare inclusions were present at end-stage. Inclusions were typically accompanied by astrocytosis.

Muscle pathology was observed as well. Histological analysis of the quadriceps muscle showed atrophy of muscle fibers as well as angular fibers consistent with denervation of the muscle.

Prior to showing motor deficits, line 23 mice display metabolic abnormalities, including an increase in body fat, a decrease in lean muscle mass, larger adipocytes in white fat, and an impaired response to insulin. These phenotypes are attributed to TDP-43 overexpression in peripheral target organs (Stallings et al., 2013).

Modification Details

In this model the transgene encodes full-length, mutant, human TDP-43 with the A315T mutation. The mouse prion protein (Prp) promoter drives transgene expression.

Related Strains

TDP-43 (A315T) (hybrid) -This strain on a hybrid B6SJL background, is available through The Jackson Lab, Stock# 016203; Cryopreserved. B6SJL-Tg (Prnp-TARDBP*A315T)23Jlel/J,

Publication

Familial ALS patients with TDP-43 gene mutations and sporadic ALS patients share common TDP-43 neuronal pathology. To delineate mechanisms underlying TDP-43 proteinopathies, transgenic mice expressing A315T, M337V or wild type human TDP-43 were generated. Multiple TDP-43 founders developed a severe early motor phenotype that correlated with TDP-43 levels in spinal cord. Three A315T TDP-43 lines developed later onset paralysis with cytoplasmic ubiquitin inclusions, gliosis and TDP-43 redistribution and fragmentation. The WT TDP-43 mouse line with highest spinal cord expression levels remains asymptomatic, although these mice show spinal cord pathology. One WT TDP-43 line with high skeletal muscle levels of TDP-43 developed a severe progressive myopathy. Over-expression of TDP-43 in vivo is sufficient to produce progressive motor phenotypes by a toxic gain of function paradigm. Transgenic mouse lines expressing untagged mutant and wild type TDP-43 under the same promoter represent a powerful new model system for studying TDP-43 proteinopathies in vivo.(Stallings et al., 2010)

TDP-43 (G348C)

Species	Genes	Mutations	Modification	Strain Name	Genetic Background	Availability
Mouse	TARDBP	TARDBP G348C	TARDBP: Transgenic	N/A	Transgene injected into C3H x C57Bl/6 embryos. Founders backcrossed with C57Bl/6.	Available through Jean-Pierre Julien

Summary

These transgenic mice develop neuropathology and behavioral deficits relevant to ALS and FTD, including cytoplasmic inclusions of TDP-43 protein, axonal pathology, neuroinflammation, learning/memory deficits, and motor impairment. They do not develop overt neuronal or axonal loss, nor do they develop paralysis (Swarup et al., 2011).

Compared to many other TDP-43 transgenics, these mice express relatively modest levels of human TDP-43, relying on the endogenous human TDP-43 promoter to drive expression. In the brain, transgene mRNA expression was about threefold higher than endogenous murine TDP-43 mRNA. Protein levels of human TDP-43 were highest in the brain, spinal cord, and muscle, with lower levels in the liver and kidney, mimicking endogenous expression patterns.

Despite modest overexpression, these mice develop quite robust TDP-43 pathology, including an accumulation of cytoplasmic TDP-43 in motor neurons in the spinal cord by 10 months of age. Some of this cytoplasmic TDP-43 was in the form of aggregates, which often co-localized with ubiquitin. Elevated levels of the 25 kDA fragment of TDP-43 were found in the brain and spinal cord.

Abnormalities in various cytoskeletal proteins were observed, including aggregates of the intermediate filament protein, peripherin, in the brain and spinal cord at 10 months of age. Neurofilament proteins (both heavy and light chain) were

downregulated in the spinal cord at 10 months of age, compared with non-Tg mice.

Overt neuronal loss is not seen in these mice, nor is axonal loss. However, abnormalities of the neuromuscular junction were observed in 10-month-old TDP-43 G348C mice. About 10 percent of the NMJs were fully denervated, and another 20 percent were partially denervated. In the spinal cord, differences in the caliber of axons at the L5 ventral root were also observed.

Neuroinflammation was detectable early, by three months of age, and prior to the onset of behavioral abnormalities. Astrogliosis and microgliosis started early in the brain and spinal cord and continued to progress with age.

Behaviorally, these mice displayed both cognitive and motor deficits. Deficits were seen at seven months of age in the passive-avoidance test, a measure of contextual learning. Likewise, deficits in spatial learning were observed in the Barnes maze by 10 months of age. The mice also exhibited progressive impairment on the Rotarod starting at 36 weeks of age.

Compared with non-Tg mice, TDP-43 G348C transgenics had impaired recovery from nerve injury (Swarup et al., 2012). Recovery from crush injury to the sciatic nerve was slow and incomplete in three-month-old TDP-43 mice. Eleven days after injury, the mice had elevated neuroinflammation and fewer regenerating axons.

Data on this page refer to hemizygous mice.

Modification Details

These mice overexpress full-length human TDP-43 with the G348C mutation introduced by site-directed mutagenesis. The transgene is driven by the endogenous human promoter.

Availability

Available through Jean-Pierre Julien.

Related Strains

TDP-43 (WT)

TDP-43 (A315T)

TDP-43 (M337V)

Species	Genes	Mutations	Modification	Strain Name	Genetic Background	Availability
Mouse	TARDBP	TARDBP M337V	TARDBP: Transgenic	C57BL/6-Tg (Prnp-TARDBP*M337V)4Ptrc/J	Transgene injected into fertilized C57BL/6 oocytes. Founders bred with B6.	The Jackson Lab: Stock# 017604; Cryopreserved

Summary

This transgenic mouse model of ALS overexpresses mutant human TDP-43. It was generated by Leonard Petrucelli and colleagues at the Mayo Clinic (Xu et al., 2011). Like the wild-type TDP-43 transgenics reported by the Mayo group one year earlier (Xu et al., 2010), this model uses the mouse prion protein (Prp) promoter to drive transgene expression. Hemizygous mice are indistinguishable from non-Tg littermates, but homozygotes develop TDP-43 pathology, neuroinflammation, and severe motor impairment precipitating euthanasia at one to two months of age.

In homozygous TDP-43M337V mice, levels of mutant human TDP-43 are highest in the brain and spinal cord with lower levels in other tissues. Total TDP-43 (human and mouse) is elevated about 2.7-fold over levels in non-Tg littermates. This is likely an under-representation of transgene expression because mouse TDP-43 is downregulated in the presence of exogenous human TDP-43 (see also Xu et al., 2013). TDP-43 protein was primarily nuclear, however, some cytoplasmic TDP-43 was observed in neurons of the spinal cord, brainstem, and cortex. Phosphorylated TDP-43 (at serine 403 and 404) was frequently seen in motor neurons of the anterior horn of the spinal cord, among other areas. Likewise, widespread ubiquitination was observed in CNS neurons, but TDP-43 itself did not appear to be ubiquitinated. Hyperphosphorylated tau protein also accumulated in the brain, with extensive staining throughout the neuropil and some cytoplasmic inclusions within neurons.

Mitochondrial abnormalities were also seen in these mice, appearing as cytoplasmic eosinophilic aggregates in neurons. These aggregates were frequent in the anterior horn of the spinal cord and brainstem, but also appeared in other areas. Neuroinflammation was prominent, appearing as elevated levels of reactive astrocytes and activated microglia in the spinal cord and brainstem.

Motor impairment developed early in TDP-43M337V homozygotes. By day 21 they developed tremors and difficulty recruiting their hindlimbs. This impairment led in an irregular gait pattern, described as “dragging.” By one month of age, 70 percent of homozygotes had lost the ability to right themselves, were moribund, and required euthanasia. Not surprisingly, survivors at one month of age had reduced brain and body weight compared with non-Tg littermates.

Despite the severe motor impairment exhibited by these mice, neuronal loss was not observed in the brain or spinal cord.

Modification Details

These mice overexpress full-length human TARDBP with the M337V mutation. The transgene is driven by the mouse prion protein (PrP) promoter.

Publication

BACKGROUND:

Abnormal distribution, modification and aggregation of transactivation response DNA-binding protein 43 (TDP-43) are the hallmarks of multiple neurodegenerative diseases, especially frontotemporal lobar degeneration with ubiquitin-positive inclusions (FTLD-U) and amyotrophic lateral sclerosis (ALS). Researchers have identified 44 mutations in the TARDBP gene that encode TDP-43 as causative for cases of sporadic and familial ALS http://www.molgen.ua.ac.be/FTDMutations/. Certain mutant forms of TDP-43, such as M337V, are associated with increased low molecular weight (LMW) fragments compared to wild-type (WT) TDP-43 and cause neuronal apoptosis and developmental delay in chick embryos. Such findings support a direct link between altered TDP-43 function and neurodegeneration.

Results:

To explore the pathogenic properties of the M337V mutation, we generated and characterized two mouse lines expressing human TDP-43 (hTDP-43(M337V)) carrying this mutation. hTDP-43(M337V) was expressed primarily in the nuclei of neurons in the brain and spinal cord, and intranuclear and cytoplasmic phosphorylated TDP-43 aggregates were frequently detected. The levels of TDP-43 LMW products of ~25 kDa and ~35 kDa species were also increased in the transgenic mice. Moreover, overexpression of hTDP-43(M337V) dramatically down regulated the levels of mouse TDP-43 (mTDP-43) protein and RNA, indicating TDP-43 levels are tightly controlled in mammalian systems. TDP-43M337V mice displayed reactive gliosis, widespread ubiquitination, chromatolysis, gait abnormalities, and early lethality. Abnormal cytoplasmic mitochondrial aggregates and abnormal phosphorylated tau were also detected in the mice.

Conclusion:

Our novel TDP-43M337V mouse model indicates that overexpression of hTDP-43(M337V) alone is toxic in vivo. Because overexpression of hTDP-43 in wild-type TDP-43 and TDP-43M337V mouse models produces similar phenotypes, the mechanisms causing pathogenesis in the mutant model remain unknown. However, our results suggest that overexpression of the hTDP-43(M337V) can cause neuronal dysfunction due to its effect on a number of cell organelles and proteins, such as mitochondria and TDP-43, that are critical for neuronal activity. The mutant model will serve as a valuable tool in the development of future studies designed to uncover pathways associated with TDP-43 neurotoxicity and the precise roles TDP-43 RNA targets play in neurodegeneration.(Xu et al., 2011)

TDP-43 (M337V) (Mt-TAR6/6)

Species	Genes	Mutations	Modification	Strain Name	Genetic Background	Availability
Mouse	TARDBP	TARDBP M337V	TARDBP: Transgenic	N/A	Transgene injected into BL6/SJL oocytes. Founders crossed to C57BL6/J.	Unknown

Summary

These mice, which express mutant human TDP-43 in postnatal neurons, quickly develop several ALS-related phenotypes, including motor impairment, upper motor neuron loss, and premature death (Janssens et al., 2013). The "Mt" in the name of this model stands for "mutant," and refers to the fact that the transgene contains the M337V mutation associated with familial ALS.

Transgene expression in Mt-TAR6 mice is driven by the Thy-1.2 promoter, resulting in preferential expression in neurons. Because this promoter becomes active about one week after birth, transgene expression does not interfere with critical developmental processes *in utero*. Once the transgene is on, the disease progresses very rapidly in homozygous mice (Mt-TAR6/6), with a comparatively later onset and milder progression in hemizygotes (Mt-TAR6).

Compared with other transgenic TDP-43 models, these mice express relatively low levels of transgene. Transgene expression in the brains of hemizygous mice is less than endogenous levels of TDP-43, and expression in homozygotes is approximately double endogenous levels. Endogenous TDP-43 is downregulated by expression of transgenic TDP-43.

Consistent with a gene-dosage effect, homozygous mice develop a much more severe phenotype. Starting around postnatal day 12, homozygous Mt-TAR6/6 showed an abnormal clasping reflex when suspended by their tails. Symptoms progressed rapidly, involving a hunched posture, muscle twitches, and reduced mobility by day 15. Complete paralysis and death were close behind, with an average survival of just 17 days. No mice survived past day 20.

In contrast, hemizygous MT-TAR6 mice lived up to 24 months (average survival was 16.4 months). They did not develop an abnormal hindlimb reflex until about one year of age, and Rotarod testing did not show motor impairment until 13 months. In addition, a considerable amount of phenotypic variability was noted in hemizygous mice. A small minority of the mice (about 5 percent) developed a

severe motor impairment, involving complete paralysis of the hindlimbs. In this subset, paralysis developed between three and 14 months of age.

Although the Thy-1.2 promoter drives expression in virtually all neurons of the brain and spinal cord, specific neuronal subpopulations appeared to be selectively vulnerable, including cortical layer V motor neurons, spinal anterior horn motor neurons, CA regions of the hippocampus, and thalamic neurons. MT-TAR6/6 mice developed severe neuronal loss in all CA regions of the hippocampus and complete obliteration of CA3 and CA fields, while CA1 and CA2 fields were reduced to a single layer of cells. Astrogliosis and microgliosis occurred in regions affected by neuronal loss, including the motor cortex, spinal cord, and hippocampus.

Cortical motor neurons in homozygous and hemizygous mice developed diffuse cytoplasmic ubiquitin staining. This staining, which was absent in non-Tg mice, was also observed in mice expressing comparable levels of wild-type TDP-43. Ubiquitin immunoreactivity was also present in spinal motor neurons, the hippocampus, pons, and cerebellum, albeit at lower levels.

In addition to diffuse ubiquitin immunoreactivity, some Mt-TAR6/6 mice developed ubiquitin-positive inclusions in layer V cortical neurons, but not in spinal motor neurons. However, inclusions were not universally present in all mice, even at end-stage. Also observed were ubiquitin-negative inclusions, described as "large amorphous eosinophilic inclusions" in the cytoplasm of anterior horn neurons, and to a lesser extent in neurons of the cortical layer V, thalamus, pons, and cerebellum.

Mitochondrial abnormalities were also observed, including deformed cristae and fission deficits.

Modification Details

In this model the Thy-1.2 promoter drives expression of a transgene encoding human TARDBP with the M337V mutation.

Publication

Mutations in TAR DNA-binding protein 43 (TDP-43) are associated with familial forms of amyotrophic lateral sclerosis (ALS), while wild-type TDP-43 is a pathological hallmark of patients with sporadic ALS and frontotemporal lobar degeneration (FTLD). Various in vitro and in vivo studies have also demonstrated toxicity of both mutant and wild-type TDP-43 to neuronal cells. To study the potential additional toxicity incurred by mutant TDP-43 in vivo, we generated mutant human TDP-43 (p.M337V) transgenic mouse lines driven by the Thy-1.2 promoter (Mt-TAR) and compared them in the same experimental setting to the disease phenotype observed in wild-type TDP-43 transgenic lines (Wt-TAR) expressing comparable TDP-43 levels. Overexpression of mutant TDP-43 leads to a worsened dose-dependent disease phenotype in terms of motor dysfunction, neurodegeneration, gliosis, and development of ubiquitin and phosphorylated TDP-43 pathology. Furthermore, we show that cellular aggregate formation or accumulation of TDP-43 C-terminal fragments (CTFs) are not primarily responsible for development of the observed disease phenotype in both mutant and wild-type TDP-43 mice.(Janssens et al., 2013)

TDP-43 (Prp)

Species	Genes	Mutations	Modification	Strain Name	Genetic Background	Availability
Mouse	TARDBP		TARDBP: Transgenic		Transgene injected into fertilized C57BL/6 oocytes. Founders bred with B6.	The Jackson Lab: Stock# 016608; Cryopreserved

Summary

This transgenic mouse model of ALS overexpresses wild-type human TDP-43. Hemizygous mice are largely indistinguishable from non-Tg mice; however, homozygous mice develop severe motor impairments requiring euthanasia between one and two months of age (Xu et al., 2010).

In this model, the mouse prion protein (Prp) promoter drives transgene expression, resulting in robust expression in the brain and spinal cord and lower levels in other tissues. In the brain, human TDP-43 protein was 1.9-fold higher than endogenous TDP-43 in hemizygotes, and 2.5-fold higher in homozygotes. Human TDP-43 was primarily nuclear, but occasional cytoplasmic protein was also seen in some CNS neurons. Endogenous murine TDP-43 was downregulated in the presence of exogenous human TDP-43 (see also Xu et al., 2013).

The earliest reported phenotypic difference between homozygous TDP-43 (Prp) mice and non-Tg controls is a divergence in body weight, starting at postnatal day 14. By day 21 homozygous mice develop body tremors and reduced walking speed, followed by gait difficulties, described as a "swimming gait," because the mice are unable to hold their bodies off the ground. By one to two months of age, they are unable to right themselves and require euthanasia.

The underlying cause of the motor deficit is not clear. The mice do not appear to be affected by muscle atrophy or loss of motor neurons. However, signs of neuronal degeneration are present in the form

of degenerating neurites and axons in the brain and spinal cord. Synaptic pathology has also been observed, specifically fewer dendritic spines in the hippocampus and lower mRNA levels or synaptic markers including PSD-95 and GAP43 (Xu et al., 2013).

Neuroinflammation is a prominent feature involving activated microglia and reactive astrocytes. Distinct mitochondria pathology is observed within neurons, specifically electron microscopy showed abnormal clusters of mitochondria, suggestive of defective fission/fusion. Aggregates of cytoplasmic TDP-43 are notably absent, but elevated levels of ubiquitin are seen within the nucleus and cytoplasm of neurons in the brain and spinal cord.

Modification Details

These mice overexpress wild-type human TARDBP driven by the mouse prion protein (Prp) promoter.

Publication

Transactivation response DNA-binding protein 43 (TDP-43) is a principal component of ubiquitinated inclusions in frontotemporal lobar degeneration with ubiquitin-positive inclusions and in amyotrophic lateral sclerosis (ALS). Mutations in TARDBP, the gene encoding TDP-43, are associated with sporadic and familial ALS, yet multiple neurodegenerative diseases exhibit TDP-43 pathology without known TARDBP mutations. While TDP-43 has been ascribed a number of roles in normal biology, including mRNA splicing and transcription regulation, elucidating disease mechanisms associated with this protein is hindered by the lack of models to dissect such functions. We have generated transgenic (TDP-43PrP) mice expressing full-length human TDP-43 (hTDP-43) driven by the mouse prion promoter to provide a tool to analyze the role of wild-type hTDP-43 in the brain and spinal cord. Expression of hTDP-43 caused a dose-dependent downregulation of mouse TDP-43 RNA and protein. Moderate overexpression of hTDP-43 resulted in TDP-43 truncation, increased cytoplasmic and nuclear ubiquitin levels, and intranuclear and cytoplasmic aggregates that were immunopositive for phosphorylated TDP-43. Of note, abnormal juxtanuclear aggregates of mitochondria were observed, accompanied by enhanced levels of Fis1 and phosphorylated DLP1, key components of the mitochondrial fission machinery. Conversely, a marked reduction in mitofusin 1 expression, which plays an essential role in mitochondrial fusion, was observed in TDP-43PrP mice. Finally, TDP-43PrP mice showed reactive gliosis, axonal and myelin degeneration, gait abnormalities, and early lethality. This TDP-43 transgenic line provides a valuable tool for identifying potential roles of wild-type TDP-43 within the CNS and for studying TDP-43-associated neurotoxicity.(Xu et al., 2010)

TDP-43 (Q331K)

Species	Genes	Mutations	Modification	Strain Name	Genetic Background	Availability
Mouse	TARDBP	TARDBP Q331K	TARDBP: Transgenic	N/A	Transgene introduced into C57Bl6/C3H oocytes. Founders crossed to C57/Bl6 for a minimum of four generations	Unknown

Summary

This transgenic mouse model of ALS overexpresses human TDP-43 with the Q331K mutation (Arnold et al., 2013). Expression of the mutant protein is driven in the brain and spinal cord by the mouse prion protein (Prp) promoter. Although this model does not recapitulate cytoplasmic mislocalization of TDP-43, it does develop age-dependent motor deficits, degeneration of lower motor neurons, and abnormalities at the neuromuscular junction.

The line focused on here, line 103, was generated in parallel with two other lines expressing TDP-43 with the Q331K mutation (line 109 and line 31). Line 103 is the best characterized of the three and had the greatest transgene expression (about threefold more total TDP-43 RNA in the spinal cord compared with non-Tg mice). Consistent with TDP-43 autoregulation, a decrease in endogenous murine levels was observed in transgenic mice. Transgene expression was highest in the brain and spinal cord with very low levels observed in other tissues.

At birth, the TDP-43 Q331K mice were indistinguishable from non-Tg littermates. Tremor was observed as early as three to four months of age in some mice, and about 80 percent of animals were affected by 10 months of age. Abnormal hindlimb clasping affected about 60 percent of animals by three months of age. Performance on the accelerating Rotarod was normal at 21 days of age, but impaired by three months. Grip strength was affected later, by about 10 months of age.

The motor impairments were accompanied by muscle pathology, including abnormal electromyographic (EMG) activity in the gastrocnemius muscle. Resting EMG recordings showed muscle fibrillations, suggesting neuromuscular degeneration. H & E staining showed muscle fiber abnormalities, including centralized nuclei. At 10 to 12 months the mice had fewer neuromuscular junctions (NMJs) as quantified by α-bungarotoxin staining. Furthermore, NMJs were

described as malformed, with a "bleb-like" appearance.

Age-dependent neurodegeneration of lower motor neurons occurred in the spinal cord. Specifically, reduced numbers of choline acetyl-transferase (ChAT)–positive neurons were seen in the lumbar spinal cord. Neuronal loss was detected as early as two months of age, with an overall 35 percent reduction at 10 months of age compared with non-Tg mice or those expressing comparable levels of wild-type TDP-43. In addition, astrogliosis and microgliosis developed in the ventral horn of the spinal cord by 10 to 12 months of age.

The majority of human TDP-43 was present in the nuclear fraction of brain and spinal cord homogenate of 10- to 20-month-old mice. Immunohistochemistry of the spinal cord confirmed predominantly nuclear localization of TDP-43.

Modification Details

This transgenic model expresses full-length human TDP-43 with the Q331K mutation driven by the mouse prion protein (Prp) promoter.

Related Strains

Two other TDP-43 transgenic models were reported in parallel: TDP-43 (wild-type) and TDP-43 (M337V) (Arnold et al., 2013).

Publication

Transactivating response region DNA binding protein (TDP-43) is the major protein component of ubiquitinated inclusions found in amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD) with ubiquitinated inclusions. Two ALS-causing mutants (TDP-43(Q331K) and TDP-43(M337V)), but not wild-type human TDP-43, are shown here to provoke age-dependent, mutant-dependent, progressive motor axon degeneration and motor neuron death when expressed in mice at levels and in a cell type-selective pattern similar to endogenous TDP-43. Mutant TDP-43-dependent degeneration of lower motor neurons occurs without: (i) loss of TDP-43 from the corresponding nuclei, (ii) accumulation of TDP-43 aggregates, and (iii) accumulation of insoluble TDP-43. Computational analysis using splicing-sensitive microarrays demonstrates alterations of endogenous TDP-43-dependent alternative splicing events conferred by both human wild-type and mutant TDP-43(Q331K), but with high levels of mutant TDP-43 preferentially enhancing exon exclusion of some target pre-mRNAs affecting genes involved in neurological transmission and function. Comparison with splicing alterations following TDP-43 depletion demonstrates that TDP-43(Q331K) enhances normal TDP-43 splicing function for some RNA targets but loss-of-function for others. Thus, adult-onset motor neuron disease does not require aggregation or loss of nuclear TDP-43, with ALS-linked mutants producing loss and gain of splicing function of selected RNA targets at an early disease stage.(Arnold et al., 2013)

TDP-43 (WT) (Elliott model)

Species	Genes	Mutations	Modification	Strain Name	Genetic Background	Availability
Mouse	TARDBP		TARDBP: Transgenic	N/A	Transgene injected into B6SJLF1 oocytes. Founders crossed with CD1 mice.	Status of original hybrid unknown. This model is available on a B6SJL background through The Jackson Lab: Stock# 016201; Cryopreserved

Summary

This model was among the first wave of TDP-43 transgenics (Stallings et al., 2010). It was originally created on a hybrid background (B6SJLF and CD1), and later developed into a B6SJL line distributed by The Jackson Lab (see below). The characterization data on this page refer to the original hybrid mice unless otherwise noted.

The mouse prion protein (Prp) promoter drives TDP-43 expression in this model. Hemizygous mice express intermediate levels of the human protein in the brain, spinal cord, and skeletal muscle, among other tissues.

Hemizygous mice develop motor impairments and muscle pathology. The motor impairment starts as muscle weakness in a hind limb that presents as an external rotation of one hind limb followed by bilateral weakness and low muscle tone. This phenotype is not fully penetrant; some mice show no evidence of muscle weakness even at six months of age.

An analysis of the quadriceps muscle showed myopathy, including variable muscle fiber size and disorganized muscle architecture. Myocytes accumulated TDP-43 protein (both nuclear and cytoplasmic), and they contained ubiquitin-positive inclusions. Although the Prp promoter primarily drives transgene expression in the CNS, relatively high TDP-43 expression was also observed in the skeletal muscle of these mice.

Hemizygous mice do not show overt neuronal loss in the brain or spinal cord. Their mean survival was 109 days. It was not reported whether the survial analysis included males, females, or both.

Modification Details

The transgene in this model encodes full-length, wild-type, human TDP-43 driven by the mouse prion protein (Prp) promoter. According to information on

The Jackson Lab website, the transgene integrated on the X chromosome.

Related Strains

TDP-43 (WT) (hybrid) - This model on a hybrid B6SJL background is available through The Jackson Lab, Stock# 016201, cryopreserved. B6SJL-Tg (Prnp-TARDBP)4Jlel/J.

Familial ALS patients with TDP-43 gene mutations and sporadic ALS patients share common TDP-43 neuronal pathology. To delineate mechanisms underlying TDP-43 proteinopathies, transgenic mice expressing A315T, M337V or wild type human TDP-43 were generated. Multiple TDP-43 founders developed a severe early motor phenotype that correlated with TDP-43 levels in spinal cord. Three A315T TDP-43 lines developed later onset paralysis with cytoplasmic ubiquitin inclusions, gliosis and TDP-43 redistribution and fragmentation. The WT TDP-43 mouse line with highest spinal cord expression levels remains asymptomatic, although these mice show spinal cord pathology. One WT TDP-43 line with high skeletal muscle levels of TDP-43 developed a severe progressive myopathy. Over-expression of TDP-43 in vivo is sufficient to produce progressive motor phenotypes by a toxic gain of function paradigm. Transgenic mouse lines expressing untagged mutant and wild type TDP-43 under the same promoter represent a powerful new model system for studying TDP-43 proteinopathies in vivo.(Stallings et al., 2010)

TDP-43 (WT) (Julien model)

Species	Genes	Mutations	Modification	Strain Name	Genetic Background	Availability
Mouse	TARDBP		TARDBP: Transgenic	N/A	Transgene injected into C3H x C57Bl/6 embryos. Founders crossed with C57Bl/6.	Not available: extinct

Summary

These transgenic mice, which are now extinct, overexpressed wild-type human TDP-43. They developed motor and cognitive deficits relevant to ALS/FTD, along with pertinent neuropathology, including gliosis and denervation of neuromuscular junctions. Phenotypes were generally milder than what had been observed in mice overexpressing mutant TDP-43 (e.g., TDP-43 A315T and TDP-43 G348C) (Swarup et al., 2011).

In contrast to other TDP-43 transgenics using strong promoters, this model used the endogenous human TDP-43 promoter, resulting in relatively modest expression levels. In the brain, mRNA levels of TDP-43 were about threefold higher than endogenous mouse levels. Human TDP-43 protein was abundantly expressed in the brain, spinal cord, and muscle, with lower levels in the liver and kidney, mimicking endogenous expression patterns.

Neuronal TDP-43 protein was primarily nuclear. This nuclear localization contrasts with observations reported in mice overexpressing mutant TDP-43 (i.e. TDP-43 A315T and TDP-43 G348C), which accumulate cytoplasmic TDP-43 in neurons by 10 months of age.

Neuronal loss has not been reported in these mice and axon numbers were preserved. However, at the neuromuscular junction (NMJ) some denervation was apparent by 10 months of age (about 5 percent full denervation and another 20 percent partial denervation). Gliosis was prominent in these mice. Microgliosis and astrogliosis occurred by three months of age in the spinal cord and increased markedly by 10 months of age.

Behaviorally, these mice showed both motor and cognitive deficits. They exhibited deficits in the passive-avoidance test at seven months of age and in the Barnes maze at 10 months, indicating deficits in contextual and spatial memory, respectively. Around this same age (i.e., 42 weeks), the mice developed difficulties on the accelerating Rotarod. Of note, motor impairment was reported at slightly younger ages in mice overexpressing mutant TDP-43, at 36 and 38 weeks (Swarup et al., 2011).

Compared with non-Tg mice, TDP-43(WT) transgenics recovered more slowly from nerve injury (Swarup et al., 2012). Following crush injury to the sciatic nerve, three-month-old TDP-43 (WT) mice recovered motility slowly, and their recovery was incomplete. Eleven days after injury, they had fewer regenerating axons at the injury site and increased neuroinflammation.

Data on this page refer to hemizygous mice.

Modification Details

These mice express full-length, wild-type, human TDP-43 driven by the endogenous human promoter.

Related Strains

TDP-43 (G348C)

TDP-43 (A315T)

Publication

Transactive response DNA-binding protein 43 ubiquitinated inclusions are a hallmark of amyotrophic lateral sclerosis and of frontotemporal lobar degeneration with ubiquitin-positive inclusions. Yet, mutations in TARDBP, the gene encoding these inclusions are associated with only 3% of sporadic and familial amyotrophic lateral sclerosis. Recent

transgenic mouse studies have revealed a high degree of toxicity due to transactive response DNA-binding protein 43 proteins when overexpressed under the control of strong neuronal gene promoters, resulting in early paralysis and death, but without the presence of amyotrophic lateral sclerosis-like ubiquitinated transactive response DNA-binding protein 43-positive inclusions. To better mimic human amyotrophic lateral sclerosis, we generated transgenic mice that exhibit moderate and ubiquitous expression of transactive response DNA-binding protein 43 species using genomic fragments that encode wild-type human transactive response DNA-binding protein 43 or familial amyotrophic lateral sclerosis-linked mutant transactive response DNA-binding protein 43 (G348C) and (A315T). These novel transgenic mice develop many age-related pathological and biochemical changes reminiscent of human amyotrophic lateral sclerosis including ubiquitinated transactive response DNA-binding protein 43-positive inclusions, transactive response DNA-binding protein 43 cleavage fragments, intermediate filament abnormalities, axonopathy and neuroinflammation. All three transgenic mouse models (wild-type, G348C and A315T) exhibited impaired learning and memory capabilities during ageing, as well as motor dysfunction. Real-time imaging with the use of biophotonic transactive response DNA-binding protein 43 transgenic mice carrying a glial fibrillary acidic protein-luciferase reporter revealed that the behavioural defects were preceded by induction of astrogliosis, a finding consistent with a role for reactive astrocytes in amyotrophic lateral sclerosis pathogenesis. These novel transactive response DNA-binding protein 43 transgenic mice mimic several characteristics of human amyotrophic lateral sclerosis-frontotemporal lobar degeneration and they should provide valuable animal models for testing therapeutic approaches.(Swarup et al., 2011)

TDP-43 (WT) (WT-TAR4/4)

Species	Genes	Mutations	Modification	Strain Name	Genetic Background	Availability
Mouse	TARDBP		TARDBP: Transgenic	B6;SJL-Tg (Thy1-TARDBP)4Singh/J	Transgene introduced into BL6/SJL oocytes. Founders crossed to C57BL6/J.	The Jackson Lab: Stock# 012836; Cryopreserved

Summary

This mouse model of ALS overexpresses wild-type human TARDBP (TDP-43) in postnatal neurons. Mice in this line, called TAR4, develop ALS-related phenotypes, including severe motor impairment, loss of upper and lower motor neurons, and premature death. Homozygous mice (referred to as TAR4/4) become symptomatic very early and do not survive beyond one month of age. Hemizygous mice (referred to as TAR4) exhibit much milder phenotypes (Wils et al., 2010).

In this model the murine Thy-1 promoter drives transgene expression, resulting in preferential expression in neurons throughout the CNS. Because the Thy-1 promoter activates about one week after birth, transgene expression does not interfere with developmental processes *in utero*. Compared with other transgenic TDP-43 mice, TAR4/4 mice express relatively low amounts of transgene. TDP-43 mRNA levels in the brain are about twofold higher than endogenous levels. In hemizygotes, transgene expression is below endogenous levels.

Consistent with a gene dosage effect, homozygous mice are affected more severely than hemizygous animals and have more rapid disease progression. Although indistinguishable from non-Tg littermates at birth, TAR4/4 mice develop an abnormal motor reflex by postnatal day 14. When lifted by the tails, homozygotes pull in their hindlimbs rather than extending them. Early on, they also display a shortened stride, a wide stance, and frequent stumbling. By day 18 they are less adept on the accelerating Rotarod. Motor function continues to deteriorate rapidly, and complete paralysis of the hindlimbs occurs within about 10 days of symptom onset. The mice survive an average of just 24 days.

In contrast, hemizygous TAR4 do not develop an abnormal hindlimb reflex until about 14 months of age and do not show impairments on the Rotarod until about 15 months. These mice never develop frank paralysis but die at about 22–24 months of age.

Although the Thy-1 promoter drives expression in virtually all neurons of the brain and spinal cord, only specific neuronal subpopulations develop abnormal protein inclusions and neuronal loss. In terms of abnormal protein accumulation, diffuse ubiquitin staining occurs in cortical layer V of the anterior cortex, including primary motor cortex and somatosensory areas, as well as in the hippocampus, subiculum, brainstem, and cranial motor nuclei. Ubiquitin inclusions occur in both the cytoplasm and the nucleus. Nuclear inclusions were positive for TDP-43, whereas those in the cytoplasm variably co-labeled with TDP-43. Ubiquitinated proteins also accumulate in the spinal cord, in both cytoplasmic and nuclear compartments. Ubiquitin–negative cytoplasmic inclusions were also seen in spinal neurons of the anterior horn. Ultrastructural analysis revealed that these were abnormal accumulation of mitochondria (Janssens et al., 2013).

TAR 4/4 mice develop prominent astrogliosis and microgliosis in areas affected by ubiquitin pathology. In particular, cortical layer V of the anterior cortex, including motor and somatosensory cortices, are affected by numerous GFAP-positive astrocytes. Astrogliosis is also observed in the spinal cord, especially in areas affected by inclusion pathology.

Neuronal loss is prominent in both the brain and the spinal cord. In the brain, both superficial and deep cortical layers of the anterior cortex are affected. By day 24, loss occurs in the motor cortex of TAR4/4 mice. Hippocampal neurons are also affected. All regions of the spinal cord are affected, with prominent loss noted in the cervical and lumbar regions that innervate the extremities.

Modification Details

Transgene encodes wild-type human TARDBP, driven by the murine Thy-1 promoter. The transgene integrated at locus 6qB3 in the mouse genome and does not interrupt any known gene.

Publication

Neuronal cytoplasmic and intranuclear aggregates of RNA-binding protein TDP-43 are a hallmark feature of neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD). ALS and FTLD show a considerable clinical and pathological overlap and occur as both familial and sporadic forms. Though missense mutations in TDP-43 cause rare forms of familial ALS, it is not yet known whether this is due to loss of TDP-43 function or gain of aberrant function. Moreover, the role of wild-type (WT) TDP-43, associated with the majority of familial and sporadic ALS/FTLD patients, is also currently unknown. Generating homozygous and hemizygous WT human TDP-43 transgenic mouse lines, we show here a dose-dependent degeneration of cortical and spinal motor neurons and development of spastic quadriplegia reminiscent of ALS. A dose-dependent degeneration of nonmotor cortical and subcortical neurons characteristic of FTLD was also observed. Neurons in the affected spinal cord and brain regions showed accumulation of TDP-43 nuclear and cytoplasmic aggregates that were both ubiquitinated and phosphorylated as observed in ALS/FTLD patients. Moreover, the characteristic approximately 25-kDa C-terminal fragments (CTFs) were also recovered from nuclear fractions and correlated with disease development and progression in WT TDP-43 mice. These findings suggest that approximately 25-kDa TDP-43 CTFs are noxious to neurons by a gain of aberrant nuclear function.(Wils et al., 2010)

Model	Cortical neuron loss	Lower motor neuron loss	Cytoplasmic inclusion	Gliosis	MNJ abnormalities	Muscle atrophy	Motor impairment	Body weight	Premature death
C9-BAC500	×	×	Dipeptide repeats accumulated at advanced age and formed small perinuclear inclusion bodies positive for poly-GP.	×	×	Unknown	×	×	×
C9-BACexp	×	×		×	×	×	×	×	×
C9orf72 Knock-out	×	×	×	×	×	×	Reduced activity on open-field test. No abnormalities in grip strength or rotarod performance.	×	×
C9ORF72(AAV)(G4C2)66	Compared with mice expressing 2-repeats, the 66-repeat mice had 17 percent fewer neurons in the cortex at 6 months of age and 11 percent fewer Purkinje cells in the cerebellum. At this age neurons in the hippocampus and thalamus were not affected.	At 6 months, neuronal loss in the spinal cord was not detected.	By 6 months, inclusions of C9RAN dipeptides were present in neurons of the cortex and hippocampus, and to a lesser extent in the cerebellum and spinal cord. Inclusions contained polyGA, polyGP, and polyGR dipeptides and were largely ubiquitin-positive.	Astrogliosis in the cortex by 6 months.	Unknown	Unknown	At 6 months, 66-repeat mice perform as well as 2-repeat mice on the Rotarod on the first day of testing. However, they fail to improve during subsequent trials, suggesting impairments in coordination and/or motor learning.	At 6 months females had a lower body weight than mice expressing 2-repeats. Body weight did not differ in males.	Unknown
Endogenous Sod1 D83G	Neuronal numbers comparable to wild-type at 15 weeks, but about 20 percent loss of upper motor neurons by 29 weeks. Neurons in layer V of the motor cortex appeared selectively vulnerable.	Neuronal numbers comparable to wild-type at 6 weeks, but loss occurred by 15 weeks. The neuronal loss then stabilized; it was not more severe at 52 weeks of age.	×	Gliosis, of both astrocytes and microglia, was evident in the spinal cord by 15 weeks. It was further elevated at 52 weeks.	Denervation of a hindlimb muscle, the extensor digitorum longus, was detected by 52 weeks of age, and a decreased number of motor units in the EDL muscle.	Unknown	Both male and female mice develop a variety of progressive motor symptoms. Grip strength was reduced at 6 weeks of age. Tremors developed by about 5 months of age. Rotarod performance was impaired, at 23 weeks in females and 67 weeks in males.	Homozygous mice, both male and female, showed reduced body weight by 4 weeks of age in contrast to wild-type littermates. Loss of excessive body weight was the primary factor leading to euthanasia.	Males reach end-stage sooner than females (495 ± 22 versus 588 ± 24 days). Animals were sacrificed when weight loss exceeded 20 percent of maximum weight, in accordance with animal-use guidelines. This is likely explained by the development of hepatocellular carcinomas due to SOD1 loss of function.
FUS-R521C	×	No detectable difference in spinal motor neurons at P0. At P16, about 20% loss of ChAT-positive neurons in the anterior horn	Less than 10% of spinal motor neurons have cytoplasmic FUS inclusions.	Prominent increase in microgliosis and astrogliosis in the anterior horn of the spinal cord by end stage.	Reduced innervation of neuromuscular junctions in the diaphragm.	The majority of mice have severe skeletal muscle atrophy in the hindlimb by end stage.	Early postnatal motor impairment, including abnormal hindlimb clasping when lifted by the tail, gait abnormalities, and impaired Rotarod	Early postnatal growth is retarded, and the mice experience progressive loss of body weight.	The majority of mice in the N1F1 generation reached end stage and were sacrificed by postnatal day 100. Mice in subsequent generations live

		of cervical spinal cord. At P30-P60, about 50% loss of anterior horn neurons. Remaining motor neurons show reduced dendritic complexity and synaptic density.					performance.		longer: about 40% reach end stage by postnatal day 200.
FUSΔ14 (FUSd14)	×	×	Neuronal cytoplasmic inclusions were present by 3 months of age in the cerebral cortex. Inclusions occurred in about 20% of neurons and often co-labeled with ubiquitin.	×	Unknown	Unknown	×	Unknown	Unknown
hFUS (+/+) (PrP-hFUS)	In the brain, overt neuronal loss was absent at end stage (~11 weeks).	By end stage (~11 weeks), homozygous mice had lost about 60 percent of α-motor neurons in the anterior horn of the lumbar spinal cord.	By end stage, cytoplasmic FUS inclusions, described as "granular" and "globular," develop in the spinal cord of homozygous mice. Ubiquitin-positive inclusions also develop, but do not co-localize with FUS inclusions. Neurons in the brain also develop "granular" and "skein-like" FUS inclusions.	By end stage (~11 weeks), homozygous mice had microgliosis and astrogliosis in the anterior horn of the spinal cord and in the white matter of the dorsal columns. Reactive gliosis was absent in the brain, despite cytoplasmic inclusions of FUS in some neurons.	Homozygous mice had about 20 percent fewer functional motor units in the extensor digitorum longus muscle as estimated by neurophysiological assessment.	Muscle histology from end stage (~11 weeks) homozygous mice showed focal muscle atrophy in hindlimb muscles.	Homozygous mice exhibited motor symptoms at four weeks of age, starting with a tremor and mild signs of hindlimb dysfunction, including gait abnormalities. Motor symptoms progressed quickly, ultimately developing into hindlimb paralysis.	Homozygous mice fail to gain weight normally starting about four weeks of age. Their body weight declines in subsequent weeks, often precipitating euthanasia.	Homozygous mice were euthanized between 10 and 13 weeks of age when they developed severe motor impairment or lost 25 percent of their body weight. Average survival was 82 ± 12 days.
hPFN1-G118V	At 202 days, there was a decrease in the number of corticospinal neurons of the motor cortex.	Progressive loss of ventral horn neurons from 165 through 202 days of age.	Spinal cord motor neurons had TDP-43 puncta.	Astrocytosis and microgliosis were observed in the spinal cord at end stage.	Denervation of gastrocnemius muscle at end stage. Muscle action potential also had reduced amplitude.	At 165 days, hind limb muscle atrophy was observed.	Progressive motor impairments began ~ 120 days. Mice demonstrated tremors, limb clasping, muscle weakness, gait abnormalities, as well as reduced locomotion and decreased performance on the Rotarod.	Body weight peaked ~ 150 days and then progressively decreased.	Mice were sacrificed at an average of 202 days when they were unable to right themselves. Females on average reached 191 days while males attained 213 days.
hTDP-43ΔNLS	Severe neuronal degeneration in the dentate gyrus and deep layers of the neocortex. Other regions, such as the hippocampal CA1 subfield and olfactory bulb, were relatively resistant to neurodegeneration. Approximately 50 percent of dentate gyrus neurons were lost one month after the transgene was activated.	Not observed.	High levels of cytosolic TDP-43 but only very rare aggregates (observed in less than 1 percent of cortical neurons and even rarer in other brain regions, such as the hippocampus and striatum).	Severe astrogliosis and microgliosis in areas affected by neurodegeneration, including cortical and hippocampal regions, as well as the corticospinal tract.	Unknown	Not observed.	Spastic motor impairment indicated by an abnormal clasping response as early as one week after transgene induction. A variety of motor deficits develop by one month after transgene induction, including impaired coordination on the Rotarod and decreased grip strength.	Unknown	Not observed.
NEFH-tTA x hTDP-43ΔNLS	Decreased cortical thickness indicative of neuronal degeneration beginning at four weeks off dox. By end stage, rNLS8 mice had significantly smaller brains than non-Tg littermates.	rNLS8 lost motor neurons in the lumbar spinal cord by six weeks off dox.	Cytoplasmic inclusions of TDP-43 occur as early as one week off dox in neurons in the brain. Inclusions accumulate over time and are present in many brain regions, including the motor cortex. TDP-43 inclusions are relatively rare in the spinal cord. Ubiquitin-positive inclusions are also seen.	Astrogliosis develops in many brain regions, including layer V of the motor cortex.	Denervation of the hindlimb muscle tibialis anterior was detectable by four weeks off dox, that is, two weeks prior to detectable loss of lower motor neurons.	At end-stage, rNLS8 mice exhibit gross muscle atrophy of the hindlimb muscles tibialis anterior and gastrocnemius.	rNLS8 mice develop a variety of motor impairments, starting with a deficit in hindlimb clasping and a fine tremor in the forelimb and/or hindlimb. They also develop progressive loss of grip strength (as measured by the wire-hang test) and a progressive decline in coordinated movement and balance (as measured by the accelerating Rotarod).	Body mass peaked at approximately 7 weeks of age (i.e., two weeks off dox) and then progressively dropped. Excessive loss of body weight (>30% decrease from peak weight) often defined end-stage.	rNLS8 mice die prematurely. They reach end-stage 8-18 weeks off dox, with a median survival of 10.3 weeks off dox.
PFN1-C71G	No neuronal loss in the cortex but neurodegeneration in medulla.	By 4 months there was a loss of cervical motor neurons and an increase in degenerating axons.	Cytoplasmic inclusions of PFN1, ubiquitin, and p62 in motor neurons around 6 months.	Microgliosis and astrogliosis observed in the dorsal horn by 5 months.	Denervation of gastrocnemius muscle occurs by 5 months.	Muscle atrophy in lower hind limb occurs by 6 months.	By 4 months, mice began showing minor gate changes and at 5-6 months they began demonstrating progressive deficits in Rotorod performance, vertical behaviors, and grip strength.	Body weight peaked at 4-6 months and then progressively decreased.	Mice were sacrificed when they were incapable of locomotion following the paralysis of two or more limbs which occurred around 7 months of age.
PrP-hFUS (R495X)	Not observed.	Not observed.	Despite high levels of cytoplasmic FUS, neuronal inclusions were not observed.	Unknown.	A number of abnormalities were detected in the hindlimb musculature by electromyography (EMG). These phenotypes were detectable by 8-12 months of age and included fibrillation potentials, muscle denervation, and a reduction in the	Unknown.	Not observed.	Unknown.	Hemizygous mice sporadically developed intestinal swelling leading to premature death (mean survival 118 days). Homozygous mice were more severely affected (50 percent of the original cohort died around 59 days of age).

					number of motor units.				
PrP-hFUS (WT)	Unknown.	Unknown.	Not observed.	Unknown.	Not observed.	Unknown.	Not observed.	Unknown.	Hemizygous mice die prematurely following a brief illness characterized by poor feeding. Mean survival of hemizygotes ~203 days.
SOD1 (G37R)	Upper motor neuron loss was not observed, although vacuolization occurred in brainstem neurons.	Motor neurons in the spinal cord and brainstem degenerated with overt neuronal loss in the ventral horn in some regions of the spinal cord by 19 weeks. The degenerative process involved extensive vacuolization.	Not observed.	Astrogliosis occurs in the spinal cord by 11 weeks of age, becoming more severe with age.	Denervated endplates have been observed.	Loss of motor axons, denervated endplates, atrophy of muscle fibers, and fiber type grouping observed by end-stage.	Motor impairment at 4-6 months, beginning with reduced spontaneous movement, then tremors, limb weakness, and poor grooming. Eventual paralysis of the hindlimbs.	Loss of body weight is observed.	Mice survive about 6 to 8 months.
SOD1-G85R (hybrid)	Unknown.	Extensive degeneration of large spinal axons coincident with the onset of clinical symptoms. By end stage, motor neurons in the ventral horn are lost.	Astrocytic inclusions occur early, about 6 months of age. The inclusions are immunoreactive for SOD1 and ubiquitin.	Astrogliosis and microgliosis are observed in the spinal cord starting around 6.5 months of age, and become more severe with age.	Denervation of muscle fibers is observed.	Hemizygous mice develop muscle weakness around 9 months of age, coincident with atrophy and denervation of muscle fibers.	Progressive motor impairment generally starting around 8 months with reduced grip strength in one hindlimb, rapidly spreading to other limbs and leading to paralysis within about two weeks.	Hemizygous mice start to lose weight at about 9 months of age.	End stage is characterized by paralysis at about 10 months of age.
SOD1-G93A (hybrid) (G1H)	Although outright upper motor neuron loss is absent or rare, degenerative signs (e.g., swollen neurites, Gallyas-positive aggregates, vacuoles, and neuritic spheroids) have been shown in motor regions of the cerebral cortex by five months of age.	Up to 50% loss of motor neurons in the cervical and lumbar segments of the spinal cord at end stage.	Inclusions accumulate in spinal motor neurons starting around 82 days of age. Inclusions generally take the form of spheroids or Lewy-body-like inclusions and commonly include a variety of neuronal intermediate filament proteins. TDP-43-positive inclusions are not present.	Gliosis, including the proliferation of reactive microglia and astrocytes, develops in parallel with motor neuron degeneration in the spinal cord.	Neuromuscular junctions degenerate around 47 days of age; fast-fatiguable motor neurons are affected first.	Longitudinal MRI has shown reduced muscle volume as early as 8 weeks of age. Atrophy is progressive. Skeletal muscle is affected, including limb and diaphragm.	Signs of motor impairment begin at about 3 months of age with a shaking tremor that leads to paralysis.	One of the first signs of illness is a slowing of growth and a plateauing of weight.	G1H mice reach end-stage disease by 5 months of age. Females typically survive longer than males.
TARDBP (A315T) (congenic)	These mice lose corticospinal tract axons, but outright loss of cortical neurons has not been reported in the model. When crossed with a Thy-1YFP model to label layer 5 pyramidal neurons, mice expressing TDP-43 (A315T) had fewer neurons at 15 weeks of age than YFP littermate controls (Zhang et al., 2016).	Most studies reported no lower motor neuron loss. One study observed 20% loss of large ventral horn neurons, possibly dependent on diet and how long the mice live in an individual colony.	Ubiquitinated inclusions in the cytoplasm of spinal motor neurons and cortical layer V neurons. No evidence for cytoplasmic TDP-43 inclusions.	Reports of astrocytosis in cortical layer 5 and in the spinal cord, as well as microgliosis in the spinal cord.	Denervation of neuromuscular junctions at end stage (~11% on normal diet; ~20% loss on a gel diet).	Atrophy of gastrocnemius muscle (gel diet).	Deficits have been reported in nonspecific measures of strength and coordination such as the Rotarod (males and females) and hanging-wire test (males). A severely impaired gait ("swimming gait") was observed in mice fed a gel diet.	Weight loss is a consistent feature. Potentially confounded by severe gut phenotype.	Survival is limited by severe gastrointestinal dysfunction and can be prolonged with a gel diet. Lifespan varies, but in general on a standard diet males live about 3 months and females about 6 months.
TARDBP (A315T) (hybrid)	By end-stage, neuronal numbers in layer 5 of the motor cortex are decreased with about 50 percent loss of corticospinal tract axons.	By end-stage, ~20% loss of motor neurons in the L3-L5 region of the spinal cord.	By end-stage, cytoplasmic inclusions of ubiquitinated proteins in layer 5 neurons of motor, sensory, and cingulate cortex. Ubiquitin aggregates in ventral horn neurons. TDP-43 inclusions were rare.	By end-stage, selective increase in GFAP immunoreactivity in cortical layer 5.	Unknown.	By end-stage, atrophic muscle fibers were observed.	Gait abnormalities around three months of age, developing into a characteristic "swimming gait" by four to five months.	Weight was comparable to non-Tg mice at birth. By 4.5 months transgenic mice began to lose weight.	Survival for about 5 months (154 ± 19 days) before dying spontaneously or being euthanized. It was not reported if this analysis includes males, females, or both.
TDP-43 (A315T)	Not observed.	Not observed.	Ubiquitin-positive cytoplasmic inclusions in neurons of the ventral horn and brainstem. Cytoplasmic aggregates of TDP-43 are largely absent, although rare phospho-TDP-43 inclusions were observed, especially at end-stage.	Mice exhibiting muscle weakness had astrocytosis in the ventral horn of the spinal cord.	Unknown.	Atrophy of muscle fibers in the quadriceps muscle of weak mice observed by day 44.	Progressive motor impairment, characterized by weakness, a decline in grip strength, and reduction in stride length. Weakness was usually more pronounced in the hindlimbs.	Progressive weight loss.	Line 23 mice survived about 2.5 months, mean survival 75 days. It was not reported whether this survival analysis includes males, females or both. Colony at Jackson Labs has longer mean survival.
TDP-43 (A315T) (line 23)	Not observed.	Not observed.	Ubiquitin-positive cytoplasmic inclusions in neurons of the ventral horn and brainstem. Cytoplasmic aggregates of TDP-43 are largely absent, although rare phospho-TDP-43 inclusions were observed, especially at end-stage.	Mice exhibiting muscle weakness had astrocytosis in the ventral horn of the spinal cord.	Unknown.	Atrophy of muscle fibers in the quadriceps muscle of weak mice observed by day 44.	Progressive motor impairment, characterized by weakness, a decline in grip strength, and reduction in stride length. Weakness was usually more pronounced in the hindlimbs.	Progressive weight loss.	Line 23 mice survived about 2.5 months, mean survival 75 days. It was not reported whether this survival analysis includes males, females or both. Colony at Jackson Labs has longer mean survival.
TDP-43 (G348C)	Not observed.	Not observed.	Cytoplasmic accumulation of TDP-43 was observed by 10 months in the spinal cord. Cytoplasmic aggregates occurred and often co-localized with ubiquitin. These	Progressive gliosis of both astrocytes and microglia, starting at a young age (by 3 months) in the brain and spinal cord.	In 10-month-old mice, approximately 10% of NMJs in the gastrocnemius muscle were denervated, with another 20% partially denervated.	Unknown.	Performance on the Rotarod was comparable to non-Tg littermates until 36 weeks of age, and became progressively worse with age.	Unknown.	Normal lifespan.

			inclusions are not detected at 3 months of age.						
TDP-43 (M337V)	Not observed.	Not observed.	TDP-43 protein was largely nuclear, although some cytoplasmic TDP-43 was also observed. Some mild cytoplasmic inclusions were reported.	Reactive astrocytes and activated microglia proliferate in the spinal cord and brainstem.	Unknown.	Unknown.	Body tremors apparent by day 21 and the mice had difficulty recruiting their hindlimbs, leading to an irregular gait pattern, described as "dragging."	By one month of age, homozygotes have reduced body weight compared to non-Tg littermates.	70% mortality of homozygotes by around one month of age.
TDP-43 (M337V) (Mt-TAR6/6)	Severe neuronal loss in all CA regions of the hippocampus of homozygous mice. Neuronal loss was also observed in layer V cortical neurons and thalamic neurons.	Neuronal loss was observed in the spinal cords of homozygous mice.	Some homozygous mice developed cytoplasmic inclusions in layer V cortical neurons. These were often, but not always, ubiquitin–positive. They were not universally observed, even in end-stage mice.	Elevated astrogliosis and microgliosis compared with non-Tg controls, especially in the motor cortex and spinal cord. Gliosis in the hippocampus was seen at end stage.	Unknown.	Unknown.	Motor impairment developed quickly, by 11 days of age in homozygous mice, starting with an abnormal clasping reflex. They also develop a hunched posture, muscle twitches, and reduced mobility. Paralysis developed within days, leading to death. Hemizygotes do not develop motor symptoms until about one year of age, and impairment varied from mouse to mouse.	Early postnatal growth retardation in homozygous mice. By day 17 their average body weight is about half that of non-Tg controls.	Homozygous mice survived an average of just 17 days. In contrast, hemizygous Mt-TAR6 mice lived up to 24 months (average survival ~16.4 months).
TDP-43 (Prp)	Not observed.	Neuronal loss was not detected in spinal cords of homozygous mice as assessed by TUNEL staining and caspase-3 staining.	Cytoplasmic eosinophilic aggregates in spinal motor neurons by one month of age in homozygous mice.	Astrogliosis and microgliosis in the anterior horn of the spinal cord by one month of age.	Unknown.	Atrophy of the gastrocnemius muscle was not observed.	By day 21, homozygous mice displayed body tremors and mild gait impairment which progressed into a "swimming gait" and severe motor impairment.	Homozygotes diverge early from non-Tg littermates in terms of body weight, showing significantly reduced weight gain.	Homozygous mice were sacrificed at one to two months of age when they were unable to right themselves.
TDP-43 (Q331K)	Unknown.	Age-dependent loss of lower motor neurons in the lumbar spinal cord. Loss is detectable as early as 2 months of age and is more pronounced by 10 months.	TDP-43 in the brain and spinal cord was predominantly nuclear. Cytoplasmic TDP-43 aggregates were absent.	Elevated astrogliosis and microgliosis in the ventral horn of spinal cord by 10-12 months of age compared with non-Tg controls.	Reduction in neuromuscular junction endplates by 10-12 months of age. Remaining NMJs often had a "bleb-like" appearance.	Muscle fiber abnormalities including centralized nuclei and damage by 10-12 months of age.	Tremor, abnormal hindlimb clasping, impaired performance on the Rotarod were detectable starting around 3 months of age. Reduced grip strength occurred later.	Unknown.	Unknown.
TDP-43 (WT) (Elliott model)	Not observed.	Not observed.	Cytoplasmic ubiquitin-positive inclusions in skeletal muscle cells. Some TDP-43 inclusions, too.	Unknown.	Unknown.	An analysis of the quadriceps muscle, showed signs of myopathy, including variable muscle fiber size and disorganization of the muscle architecture.	Progressive motor impairment starting with external rotation of one hind limb followed by bilateral weakness and low muscle tone. Variable penetrance of this phenotype.	Progressive weight loss is part of the suite of symptoms in these mice.	The mean survival of hemizygous mice was 109 days. It was not reported if this value represents, males, females or both.
TDP-43 (WT) (Julien model)	Not observed.	Not observed.	Primarily nuclear localization of human TDP-43.	Gliosis, both microgliosis and astrogliosis, occur early in the brain and spinal cord. Reactive glia were detected as early as 3 months of age, with more by 10 months.	Some NMJ denervation was observed by 10 months of age. About 5% of NMJs at the gastrocnemius muscle were denervated, with another 20 percent partially denervated.	Unknown.	Decreased performance on the accelerating Rotarod at 42 weeks of age. Further impairment at 52 weeks.	Unknown.	Unknown.
TDP-43 (WT) (WT-TAR4/4)	In homozygous mice, quantitative loss of neurons occurs in the motor cortex compared with non-Tg littermates. Both superficial and deep cortical layers of the anterior cortex are affected.	By day 18, homozygous mice exhibited about 25 percent loss of motor neurons in the lumbar spinal cord compared with non-Tg littermates.	Homozygous mice developed cytoplasmic inclusions in the brain and spinal cord, many of which were ubiquitin-positive. A minority of inclusions co-labeled with TDP-43. Ultrastructural analysis revealed ubiquitin–negative cytoplasmic inclusions in anterior horn neurons to be abnormal accumulations of mitochondria.	Astrogliosis and microgliois especially in cortical layer V of the anterior cortex, including motor and somatosensory cortex, and in the spinal cord.	Unknown.	Unknown.	Homozygous mice exhibit an abnormal clasping reflex by postnatal day 14. Other early motor deficits include a shortened stride, a wide stance, and frequent stumbling. By day 18, reduced performance on the Rotarod. Complete paralysis occurs ~10 days after onset.	Size and weight of homozygous mice lag behind hemizygotes and non-Tg littermates.	Homozygous mice survive an average of just 24 days. In contrast, hemizygous mice survive to advanced age, although they die more prematurely than non-Tg mice, after 22 to 24 months.

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