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Rodent Models of Amyotrophic Lateral Sclerosis

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Abstract

Amyotrophic Lateral Sclerosis (ALS) is a motor neuron disease affecting upper and lower motor neurons in the CNS. Patients with ALS develop extensive muscle wasting and atrophy leading to paralysis and death 3-5 years after disease onset. ALS may be familial (fALS 10%) or sporadic ALS (sALS, 90%). The large majority of fALS cases are due to genetic mutations in the Superoxide dismutase 1 gene (SOD1, 15% of fALS) and repeat nucleotide expansions in the gene encoding C9ORF72 (around 40-50% of fALS and ~10% of sALS). From a wide range of pathological studies the general conclusion is that ALS disease is mediated through aberrant protein homeostasis (ie ER stress and autophagy) and/or changes in RNA processing (as seen in all non-SOD1-mediated ALS). In all of these cases, animal models suggest that the disease is mediated non-cell-autonomously, i.e. not only motor neurons are involved, but glial cells including microglia, astrocytes and oligodendrocytes and other neuronal subpopulations are also implicated in disease pathogenesis. This overview will give a chronological overview of a wide range of different ALS rodent models generated so far with a thorough description of their intrinsic advantages and disadvantages. We will focus on their respective correlation with disease as seen in humans and their potential for understanding basic disease biology. As RNA processing has more recently come to the foreground of ALS research, we will mainly focus on a thorough description of the most recently generated ALS rodent models.

INTRODUCTION

Amyotrophic Lateral Sclerosis (ALS) is a motor neuron degenerative disease affecting upper and lower motor neurons in brain stem and spinal cord. Patients with ALS develop extensive muscle wasting and atrophy leading to paralysis and death 3-5 years after disease onset. There are no therapeutics that halt, delay or reverse the disease with the exception of riluzole which has a reproducibly modest effect on slowing disease progression in humans (Bensimon et al., 1994).

Classically, a distinction can be made between familial ALS (fALS; 10% of ALS cases), when disease is propagated through the family, and sporadic ALS (sALS; 90% of ALS cases), where no familial history of disease exists. Several genes causative for the disease have been identified in the past two decades, the large majority due to genetic mutations in the Superoxide dismutase 1 gene (SOD1, 15% of fALS and 1-2% of sALS) (Rosen, 1993) and repeat expansions in the gene encoding C9ORF72 (around 40-50% of fALS and ~10% of sALS) (DeJesus-Hernandez et al., 2011; Renton et al., 2011). Mutations in other genes including TAR DNA binding protein 43 (TDP-43) (Rutherford et al., 2008; Sreedharan et al.,

2008), fused in sarcoma/translated in liposarcoma (FUS/TLS (together around 3% of fALS) (Kwiatkowski et al., 2009; Vance et al., 2009), optineurin (Maruyama et al., 2010), UBQLN2 (Deng et al., 2011), p62 (Fecto et al., 2011), VCP (Johnson et al., 2010) and Matrin 3 (Johnson et al., 2014) have also been linked to variant forms of ALS. Although ALS-causing mutations in these genes are very rare, the majority of sALS cases have TDP-43 and often UBQLN2 and p62 positive pathogenic inclusions in neurons and glial cells as seen on post mortem evaluation of CNS tissue of ALS patients (Neumann et al., 2006; Deng et al., 2011; Fecto et al., 2011). This suggests that these proteins are not only involved in fALS but also in the large majority of sALS patients as well. Identification of these genes has led to the general conclusions that ALS disease is mediated through aberrant protein homeostasis (ie ER stress and autophagy) and/or changes in RNA processing (as seen in all non-SOD1 mediated ALS). In all these cases, animal models suggest that the disease is mediated non-cell-autonomously, i.e. not only motor neurons are involved in the pathogenesis, but glial cells like microglia, astrocytes and oligodendrocytes as well as other neuronal subpopulations, are implicated in disease pathogenesis (Philips and Rothstein, 2014). Extensive gliosis in motor cortex of ALS patients, as well as abnormalities in non-motor regions of the central nervous system (CNS), underscores this hypothesis (Nagy et al., 1994; Ringholz et al., 2005). The discovery of these ALS causing genes has led to the development of primate, rodent, zebrafish, worm and fly ALS models which more or less mimic many, but not all aspects of disease seen in ALS patients. These models recapitulate certain aspects of disease and are used for improving our knowledge about disease relevant basic biology as well as screening tools for testing molecules with therapeutic potential. Unfortunately, many, if not all, of these animal models come with inherent caveats and none of the potential interesting therapeutics, even when proven to be successful in delaying disease in animal models, have been shown to be (as) successful in humans (Perrin, 2014). This overview will give a chronological overview of a wide range of different ALS rodent models generated so far with a thorough description of their intrinsic advantages and disadvantages. We will focus on their respective correlation with disease as seen in humans and their potential for understanding basic disease biology. As RNA processing has more recently come to the foreground of ALS research, we will mainly focus on a thorough description of the most recently generated ALS rodent models.

Early animal models of ALS, mutations in SOD1 paved the way

1) Design and characterization of mutant SOD1 mouse models—The first breakthrough in the development of rodent models to study ALS came with the finding in the early 90s that mutations in the gene encoding SOD1 were associated with the disease (Rosen, 1993). SOD1 is a ubiquitously expressed 153 amino acid protein involved in conversion of superoxide radicals to hydrogen peroxide. More than 100 different mutations have been found in this gene throughout all coding regions. SOD1 is thought to cause disease through a toxic gain of function, as SOD1 knockout mice appear to be normal. However, these SOD1 null mice do show enhanced sensitivity to a variety of stress related insults and do have muscle denervation, which is almost complete at the age of 18 months, so loss of function effects might be contributory (Kondo et al., 1997; Shefner et al., 1999). On the other hand, certain SOD1 mutations have no effect at all on SOD1 dismutase activity, seriously questioning any loss of function hypothesis. To date, more than 10 rodent

models, mostly mouse models, overexpressing human mutant SOD1 have been generated and have been extensively reviewed elsewhere (Joyce et al., 2011). These comprise mostly missense mutant or truncated versions of the SOD1 protein. These mice overexpress human SOD1 under control of the human SOD1 promoter and regulatory elements, at similar (for SOD1G85R, a dismutase inactive mutant) or increased levels (1700% for SOD1G93A) as compared to the endogenous mouse *sod1*. This corresponds to a significant increase in dismutase activity of the respective mutants, especially for the G37R mutant line with the highest activity reported (5 to 14 fold) to date (Wong et al., 1995). Also, mice expressing multiple copies of a murine SOD1 mutant with early fatal motor neuron disease have been developed (Ripps et al., 1995). In general, all mutant SOD1 rodent lines develop adult onset progressive motor neuron disease reminiscent of the human disease, with varying degree of age of onset and disease progression rates (table 1). Central hallmarks of the disease in these animals are early onset astrogliosis and microgliosis, glutamate-mediated excitotoxicity, axonal transport deficits, mitochondrial vacuolization, aberrant neurofilament processing and reduced metabolic support to motor neurons by their surrounding glial cells (Boillee et al., 2006a; Philips and Rothstein, 2014). Intraneuronal and glial SOD1 containing inclusions have not been unequivocally seen in all SOD1 transgenic animals, but are clearly detected in the SOD1G85R dismutase inactive mutant which develop late onset and rapid progressive disease, even though the protein is expressed at a level near the expression level of endogenous mouse *sod1* (Bruijn et al., 1997). All of these disease pathogenic mechanisms converge to the selective loss of spinal motor neurons leading to extensive muscle wasting and atrophy in both hind and forelimbs leading to paralysis and death. An important caveat of these mouse models is that substantial cortical motor neuronal degeneration, a fundamental feature of the human disease (and required for the diagnosis of ALS), is not unequivocally seen in the majority of these models (Ozdinler et al., 2011). This critical difference from disease phenotype may reflect major pathogenic deficiencies of the SOD1 mouse as a tool to study motor neuron disease (Perrin, 2014).

The aggressiveness of the mutant SOD1 mediated phenotype is usually highly dependent on the level of overexpression of the transgene. Different SOD1G93A mouse lines, some of which are available through Jackson Laboratories, have different transgene copy number leading to different disease onset time points and progression rates. Therefore, mice overexpressing an equal amount of wild type human SOD1 are being used as controls. Although overt motor neuron degeneration as seen in the mutant overexpressing mouse models has not been found in these animals, these mice do develop an axonopathy at a later age, so one has to be careful interpreting results which originate from the mutant SOD1 mice (Dal Canto and Gurney, 1994). For the past twenty years, the SOD1 mouse model, mainly the SOD1G93A mouse model, followed by the G37R, G85R and G86R models, have been used to characterize the basic biology of ALS as well as to explore specific benefits of potential therapies with questionable success (Perrin, 2014). Rat models overexpressing mutant SOD1 have been generated as well and are particularly useful for evaluating therapeutic applications for which mice have been found to be too cumbersome to handle, e.g. the continuous intraspinal delivery of potentially therapeutic compounds through osmotic mini-pumps (Howland et al., 2002; Joyce et al., 2011).

2) Mutant SOD1 mice potential for understanding cell type specific

contributions to the disease—One of the major discoveries involving mutant SOD1 mice is that the disease is non-cell autonomous, which means that glial cells surrounding motor neurons contribute to the selective death of motor neurons. The first indication that ALS is not just a disease of motor neurons but also a disease of the non-neuronal glial cells came from the finding that astroglial glutamate GLT-1 transporters were significantly reduced in the CNS of ALS patients (Rothstein et al., 1992). This leads to enhanced glutamate levels at the synaptic cleft and overstimulation of glutamate receptors of the postsynaptic motor neuron followed by enhanced Ca^{2+} influx and cell death due to excitotoxicity. This pathogenic pathway has been confirmed in several mutant SOD1 transgenic mouse models, increasing their translational value (Bruijn et al., 1997; Van Damme et al., 2005). In order to understand the contribution of specific cell populations to the disease pathogenesis, ALS rodent models that selectively express the mutant SOD1 in specific glial and neuronal subpopulations have been generated (reviewed in (Philips and Rothstein, 2014)). In general, cell type specific expression of mutant SOD1 in astrocytes, microglial cells, Schwann cells, neurons or motor neurons is unable to induce the full blown disease phenotype as seen in the ubiquitous mutant SOD1 transgenic animals (Gong et al., 2000; Pramatarova et al., 2001; Lino et al., 2002; Beers et al., 2006; Jaarsma et al., 2008; Turner et al., 2010). This indicates that mutant SOD1 expression in both motor neurons and glia is necessary for the disease. A chimeric mutant SOD1 mouse, in which only a subset of cells express the human mutant SOD1 protein, was used to demonstrate that mutant SOD1 expressing non-neuronal cells in the vicinity of motor neurons induced pathological changes in these motor neurons. On the other hand, cell death was prevented in mutant SOD1-containing motor neurons when these were surrounded by wild-type glial cells (Clement et al., 2003). Cell specific depletion of SOD1 from several glial subsets has now confirmed that mutant SOD1 expression in the main glial cell types in the CNS: microglial cells, astrocytes, NG2 oligodendrocyte progenitor cells and oligodendrocytes all contribute to the selective death of motor neurons (Boillee et al., 2006b; Yamanaka et al., 2008; Kang et al., 2013). In humans, neuroinflammatory changes mediated by microglia, astroglia and inflammatory T-cells are well documented (Philips and Robberecht, 2011). This has led to the concept that glial cells could be an interesting avenue for cell replacement therapy and/or growth factor therapy to enhance the neuroprotective capacity of the motor neuron environment, delaying or preventing motor neuron loss. Unfortunately, clinical trials aimed at promoting the production or release of neurotrophic factors like BDNF, CNTF and IGF-1 have been unsuccessful (Miller et al., 1996; Kalra et al., 2003; Beck et al., 2005; Sorenson et al., 2008).

Very recently, the role for oligodendrocytes in disease pathogenesis has come to the foreground, as these cells degenerate and die early in mutant SOD1 spinal cord and fail to provide metabolic support to motor neurons due to the loss of the lactate transporter monocarboxylate transporter 1 (MCT1) (Lee et al., 2012b; Kang et al., 2013; Philips et al., 2013). Interestingly, a correlate to human ALS was found in that demyelinated plaques were identified and expression levels of MCT1 transporters were reduced as in human ALS cortex, (Lee et al., 2012b; Kang et al., 2013). These studies focusing on glial biology in disease should not minimize the importance of mutant SOD1 expression in motor neurons.

In these models, the first pathological sign of disease is an enhanced ER-stress response in the most vulnerable motor neurons, followed by retraction and eventual death only at later disease stages(Saxena et al., 2009). Whether a similar sequence of events occurs in motor neurons of ALS patients remains to be seen, but post mortem examination of one ALS patient who died of causes unrelated to motor neuron disease appears to support this hypothesis(Fischer et al., 2004).

3) Translational value of mutant SOD1 mouse models for therapy: potential and pitfalls—

Specific hallmarks of ALS disease as seen in humans have been recapitulated in the SOD1 mouse model. Mutant SOD1 is prone to aggregate and is detected in inclusions in the CNS of both post mortem evaluation of human CNS as well as in the spinal cord of mutant SOD1 mice, indicating impairment of either normal proteasomal and/or autophagic function(Chou et al., 1996; Bruijn et al., 1997). The loss of astroglial EAAT2 glutamate transporters contribute to excitotoxic cell death of motor neurons as a non-cell autonomous component of ALS disease, has been replicated in SOD1 mouse models(Bruijn et al., 1997; Bendotti et al., 2001). Likewise, the neuroinflammatory reaction, the contribution of microglial cells and inflammatory T-cells, as well as altered oligodendrocyte biology, show strong correlations with human disease(Philips and Rothstein, 2014). Conversely, therapeutic targeting of these different components only appears to affect disease in mutant SOD1 mouse models as these treatments have failed to show benefit in humans, with the exception of riluzole, which delays disease in humans to a modest extent(Bensimon et al., 1994). Many preclinical tests of potential therapeutics have been performed in mutant SOD1 mouse and rat models (mainly the SOD1G93A mutant line, less so the SOD1G37R mutant line). One of the most promising results came with minocycline, thought to inhibit neuroinflammation and apoptotic cell death, which significantly affects disease in SOD1G93A mice(Kriz et al., 2002; Van Den Bosch et al., 2002; Zhu et al., 2002). Although the drug was reproducibly successful in slowing disease in mice, it was not beneficial when tried in humans and in fact accelerated disease in a human ALS trial(Gordon et al., 2007). Another potential neuroprotective compound, ceftriaxone, which inhibited glutamate mediated excitotoxicity and slowed disease in SOD1G93A mice(Rothstein et al., 2005), failed to show a significant effect in a Phase 3 clinical trial although it had a positive effect in slowing progression in the Phase 2 trial (Cudkowicz et al., 2006). Anti-inflammatory therapy with celecoxib, lenalidomide and other anti-inflammatory drugs that were effective in SOD1 mice also failed in clinical trials ((Philips and Robberecht, 2011). Retrograde delivery of VEGF hugely affects life span in mutant SOD1G93A mice and a clinical trial testing the therapeutic potential of VEGF is currently ongoing(Pronto-Laborinho et al., 2014).

It is not clear whether the failure of candidate compounds in clinical trials results from limitations in the SOD1 rodent models or issues in the design of the clinical trials. Failure of almost all candidate drugs for ALS in clinical trials could have occurred for a number of reasons. Firstly, there are limitations in study design, for example the timing of treatment, the dose, the number of patients included, the selection of patients, etc. Secondly, it is unknown whether the experimental drugs used actually reached their targets at a bioactive dose, and if so, whether off-target effects might have confounded their efficacy. Few trials

even employed pharmacodynamic markers to detect actual drug efficacy at target (“target engagement”) or determined if the drug actually penetrated into the human (or rodent) CNS. Also, pharmacodynamics and pharmacokinetics may be different between rodents and humans. Thirdly, it is also unknown whether the patient would benefit from the treatment, for example whether the drug target is actually implicated in the disease pathogenesis of this patient. As an example, at autopsy, defective glutamate transporters have been found in a subset of patients and in the mouse models. Ideally, drugs that affect this pathway (e.g., astroglia or glutamate toxicity) should only be used to treat those patients who have evidence of this defect. To this end, PET ligands for astroglial glutamate transporters are now under development for future ALS trials and especially for drugs targeting glutamate transport or astrocyte dysfunction. Another reason for failure of translation of compounds from mutant SOD1 mice to humans might lie in the fact that these ALS mouse models do not recapitulate human disease in some aspects (Perrin, 2014). In fact, few transgenic mouse studies have made direct comparisons to human ALS CNS tissue – to truly validate that the aberrant biochemical or molecular pathway in rodent is found in human disease! The fact that severe overexpression versus endogenous SOD1 expression is required to cause disease in mice is an important point to consider, as is the fact that wild type overexpressing SOD1 mice develop a modest neurodegenerative insult (Dal Canto and Gurney, 1994), so overexpression *per se* might contribute to the overall disease phenotype observed. Also the timing of disease onset and progression rate can be very different between mouse and humans. Mice overexpressing the human mutant SOD1 A4V mutation develop disease very late in life and only in the presence of wild type SOD1 (Deng et al., 2006), unlike in humans, where SOD1 A4V is associated with a very aggressive early-onset disease. Another caveat is the discrepancy in motor cortical degeneration between mice and humans. Cortical motor neuron loss is fundamental for the diagnosis of human ALS, yet the mutant SOD1 mouse model shows little evidence of dramatic cortical neurodegeneration (Ozdinler et al., 2011). Moreover, the fact that many (up to 50%) of ALS patients co-develop frontotemporal dementia (FTLD), which is not seen in mutant SOD1-mediated ALS, suggests that SOD1 causes disease through a divergent pathway, and clinical trials based on results in SOD1 rodent models might not be predictive of therapeutic benefit in humans. On the other hand, no specific phenotypic aspects of ALS disease are strictly associated with SOD1 versus other ALS causing genes, so it might well be that downstream there is a common pathway shared among the different ALS causing genes. In human disease not just motor neuron loss but that of other cortical neurons appears to be involved in disease pathology (Ringholz et al., 2005; Osborne et al., 2014). One also has to consider the fact that neuroanatomical differences exist between mice and humans in how signals are propagated from brain to the periphery. Also the origin of motor neuron loss and paralysis greatly differ between mice and humans. In mice, ventral horn lumbar spinal motor neurons degenerate first, followed by motor neurons in more rostral spinal cord regions leading to hind limb paralysis followed by forelimb paralysis. Unlike in rodents, disease can initiate in different regions. Some patients develop speech and swallowing deficiencies (bulbar-onset ALS) before they start to develop weakness in the limbs, corresponding with more extensive motor neuron loss in the corresponding CNS region. Other ALS patients develop limb weakness well before any speech and swallow disorders. A subset of ALS patients also develop FTLD leading to changes in social and personal behavior which contributes to the enormous heterogeneity of

disease in ALS patients(Ringholz et al., 2005)as compared to the relatively uniform signs and symptoms observed in mutant SOD1 mouse models, though changes in environmental cues do seem to affect the disease phenotype of mutant SOD1 mice to some extent(Stam et al., 2008; Sorrells et al., 2009). In light of these observations, the success rate of these clinical trials might be significantly increased if they were restricted to patients with SOD1 mutations. This is quite challenging given the minor contribution of patients harboring SOD1 mutations (2%).

ALS as a disease of aberrant RNA processing: the need for new ALS mouse models

A major discrepancy between the SOD1 mouse model and patients is that ALS in the majority of ALS patients appears to involve aberrant RNA metabolism. Early studies that reported mutations in the gene encoding senataxin as being causative for ALS was already an early indication(Chen et al., 2004), but more interestingly was the finding that the majority of ALS and FTLN patients who do not harbor SOD1 mutations, including sALS patients, had TDP-43 positive cytoplasmic inclusions in the remaining motor neurons(Neumann et al., 2006). This observation has resulted in a major focus on the role of altered RNA metabolism in ALS pathogenesis. These inclusions were ubiquitinated, phosphorylated and comprised cleaved C-terminal TDP-43 fragments. The hypothesis that ALS is an RNAopathy was further supported by the finding that mutations in TDP-43 are causal in 3% of fALS patients(Rutherford et al., 2008; Sreedharan et al., 2008). Given the role of TDP-43 in RNA processing, transport and splicing, this would suggest that altered RNA processing is involved in the disease pathogenesis in the majority of ALS patients. Mutations in another gene, FUS/TLS, from the same FET (fused in sarcoma, Ewing's sarcoma and TATA-binding-protein-associated factor 15) -protein family as TDP-43, are associated with ALS as well(Kwiatkowski et al., 2009; Vance et al., 2009). FUS has also been associated with FTLN, but mutations in FUS are not causative for familial FTLN as they are in ALS. Like TDP-43, FUS is involved in RNA processing and could cause disease by a mechanism similar to TDP-43, though studies suggest that TDP-43 and FUS function via very different mechanisms(Sun et al., 2011; Lagier-Tourenne et al., 2012). Also unlike TDP-43, FUS/TLS aggregates are only found in patients carrying FUS mutations and are not a major pathological hallmark of other fALS patients or in sALS, suggesting that unlike TDP-43 the contribution of FUS to ALS in general might be restricted to a small subset of ALS patients.

Lastly, another major factor that has brought RNA processing to the forefront of ALS research is the finding that the majority of fALS (>50%) and a significant percentage of sALS (10%) as well as FTLN is linked to noncoding GGCCC repeat-expansions in the first intron of the C9ORF72 gene(DeJesus-Hernandez et al., 2011; Renton et al., 2011). Although the function of this gene is unknown, patients with these expanded repeat lengths show pathogenic RNA foci in the nuclei of CNS, similar to that seen in other noncoding repeat expansion disorders e.g., Fragile-X associated Tremor/Ataxia and Myotonic Dystrophy type 1 and 2(Taneja et al., 1995; DeJesus-Hernandez et al., 2011; Donnelly et al., 2013). This might lead to a significant alteration in the physiological function of RNA binding proteins due to sequestration by the expanded repeat and compromise the functioning of the motor neurons and surrounding glia. Whether changes in RNA processing

also occur in SOD1 patients and SOD1 mouse models is only beginning to be studied and its contribution to disease is currently unknown. In order to gain a better understanding of how RNA metabolism is involved in ALS, a new range of ALS transgenic mouse models, expressing mutated TDP-43 and FUS have been generated and will now be reviewed.

Aberrant RNA processing contributing to ALS: the development of TDP-43 transgenic mouse models

The identification of mutations in the genes encoding TDP-43 and FUS, as well as the finding that TDP-43 is the main component of ubiquitinated cytoplasmic inclusions in both ALS and FTLD CNS tissue, has shed new light on the potential mechanism(s) of ALS, directing focus to RNA metabolism with the development of new ALS transgenic rodent models to study both ALS and FTLD. The majority of these models involve TDP-43(Tsao et al., 2012), with only one report so far mentions the development of a FUS transgenic mouse model(Qiu et al., 2014).

The TDP-43 gene encodes a 414 amino acid protein involved in RNA processing, splicing and transport. It consists of an N-terminal nuclear localization signal followed by two RNA recognition motifs and a C-terminal glycine rich domain. The majority of TDP-43 associated mutations occur in this glycine rich-domain. In ALS patients, TDP-43 is dominantly inherited and could potentially cause disease by a toxic gain or loss of function. Current theories suggest that TDP-43 binds several RNAs, altering RNA metabolism in the cytoplasm through a toxic gain of function whereas the nuclear depletion of TDP-43 could lead to a similar change in RNA metabolism through a TDP-43 loss of function(Lee et al., 2012a). Several TDP transgenic rodent models have been generated to explore the contribution of gain versus loss of TDP-43 function and their utility as models in which to study ALS (Table2).

1) Models of ubiquitous TDP-43 knockout and overexpression—Even before the involvement of TDP-43 in ALS was known, ubiquitous TDP-43 knockout mice were known to be embryonically lethal at E7.5, though heterozygotic animals lived into adulthood and appeared to develop signs of motor impairment late in life(Kraemer et al., 2010). Conditional floxed TDP-43 transgenic mice have been generated to explore TDP-43 loss of function postnatally. Conditional ubiquitous Cre driver lines have been crossed with these floxed TDP mouse model and deplete TDP-43 expression on administration of tamoxifen. Ubiquitous reduction of TDP-43 in homozygote TDP floxed mice led to weight loss and death around 9 days after tamoxifen injection(Chiang et al., 2010). The authors suggest that these mice die of excessive lipid oxidative metabolism which is preferred to the normally dominant carbohydrate oxidative metabolism, leading to dramatic loss of fat, followed by weight loss and rapid demise of the animals.

To explore the role of TDP-43 in the CNS and in ALS more specifically in a broad, ubiquitous fashion, TDP-43 transgenic mice were generated that overexpress either mutant or wild type TDP-43 expressed under control of the prion promotor (PrP). The prion promotor drives high expression throughout the CNS and heart tissue, and lower expression in liver and kidney(Borchelt and Sisodia, 1996). Several prion-promotor TDP-43 driven

mice have been generated. In one of the earliest generated TDP-43 transgenic mice, human mutant PrP-TDP-43 A315T was overexpressed around 3-fold as compared to the endogenous mouse tdp-43. These mice appeared normal up to three months of age and then start to develop a swimming gate with concurrent weight loss and death at around the age of 154 days, though with a huge variation on survival(Wegorzewska et al., 2009). Around 20% of spinal motor neurons were lost with up to 50% axonal loss in the corticospinal tracts. These animals developed cytoplasmic inclusions in specific neuronal subsets e.g. layer 5 cortical and spinal motor neurons, some of which showed TDP-43 mislocalization. At the presymptomatic disease stage, TDP-43 C-terminal fragmentation had developed reminiscent of that seen in ALS patients. Interestingly, TDP-43 did not co-localize with ubiquitinated inclusions, suggesting that the generation of TDP-43 positive inclusions was not necessary for the phenotype observed. More recent follow-up experiments with the same mice indicate that these animals develop extensive degeneration of the myenteric plexus of the gut, leading to gut stasis and decreased longevity(Hatzipetros et al., 2013). These authors even postulated that the neuromuscular phenotype in these mice was very modest, and motor symptoms were due to early occurring gastrointestinal tract deficiencies and/or related to the specific background in which these mice were kept in the original study. Another study showed that feeding these mice with gellified food allowed the mice to develop a progressive neurodegenerative phenotype with a pronounced degeneration of upper and lower motor axons(Herdewyn et al., 2014).

Another caveat associated with the use of these mice is that there was no human wild type TDP-43 expressing control mouse being generated, so it is difficult to distinguish effects of overexpression from effects inherent to the TDP-43 mutation. Another study circumvented these overexpression issues by generating both prion promotor-driven human mutant A315T TDP-43 and wild type TDP-43 overexpression mice which had up to fourfold transgene copy number as compared to the endogenous TDP-43 expression level(Stallings et al., 2010). In these lines, human mutant TDP-43 mice had shortened survival, with lower mutant copy number leading to the longer survival, whereas none of the wild type TDP-43 lines showed earlier mortality. On histological examination, mutant TDP-43 was more prone to mislocalize and aggregate in the cytosol in ubiquitinated inclusions and was recognized by an antibody directed against phosphorylated TDP-43, indicating that phosphorylated TDP-43 inclusions were present similar to that seen in ALS patients. Mutant TDP-43 mice also had more cytoplasmic TDP-43 C-terminal fragments than wild type overexpressing control mice with these levels appearing to correlate with disease severity. A major caveat associated with the use of this model is that the differences between the mutant and wild type TDP-43 mice reported in this study could be explained by a slightly reduced expression level of the wild type TDP-43 as compared to that reported for the mutant TDP-43 overexpression lines. In fact, in other studies in which another mutant TDP-43 (M334V mutation) was overexpressed to levels almost 3-fold of the endogenous TDP-43, and at the same level as the wild type TDP-43 overexpression, there was no difference in disease severity, suggesting that altered expression levels rather than the nature of the mutation is responsible for the phenotype observed(Xu et al., 2010; Xu et al., 2011).

Another PrP promotor driven line has been generated in which mice overexpress human mutant Q331K or human wild type TDP-43 only up to 1.5 fold of the endogenous mouse

TDP-43 levels, bypassing potential effects from huge overexpression of a mutated TDP-43 protein(Arnold et al., 2013). These mice develop adult onset progressive motor neuron degeneration and neuromuscular denervation with up to 50% motor neuron loss in the spinal cord and 30% loss of neuromuscular junctions at the age of 10 months. Mice do not progress past this disease stage and thus do not die of ALS-like symptoms. However, the disease was specific for the human mutant overexpressing lines as the human wild type TDP-43 line did not develop the disease phenotype. Using splicing-sensitive microarrays, the authors compared splicing alterations seen in this model as opposed to what was seen upon oligonucleotide mediated depletion of mouse-tdp-43. They concluded that phenotype was thought to result from of both a loss as well as a gain of function as numerous genes were alternatively spliced both in the same direction versus the opposite direction. As cytoplasmic or nuclear TDP-43 aggregation, nuclear depletion and cytoplasmic fragmentation were not seen in this mouse line, the authors suggested that these pathological hallmarks of disease that are seen on ALS patient post mortem examination are not essential disease drivers. Interestingly, no gut abnormalities were observed in these animals, most likely due to the limited transgene copy number.

Mice with ubiquitous overexpression of human mutant FUS/TLS-R521C under control of the prion promotor were recently generated(Qiu et al., 2014). FUS positive cytoplasmic inclusions in CNS tissue were not reported. Evidence of DNA damage was found, particularly of the BDNF gene, which is involved in dendritic growth and synapse formation. FUS interacts with BDNF mRNA and mutations in FUS stabilize this interaction, leading to mislocalization of BDNF mRNA and potentially contributing to the dendritic and synaptic defects observed in these animals(Qiu et al., 2014). RNA-seq analysis revealed that in addition to BDNF many other transcription and splicing defects in other genes involved in synapse function and dendrite growth may also be involved in the development of the observed phenotype(Qiu et al., 2014).

The prion promotor drives transgene expression in many CNS cell types, but not all. BAC transgenic mice with widespread TDP-43 expression have been generated in which TDP-43 expression is under the control of the human TDP-43 promotor and regulatory elements(Swarup et al., 2011a). Both wild type and two mutant (G348C and A315T) TDP-43 lines were generated that similarly overexpress TDP-43 approximately 3-fold as compared to the mouse endogenous TDP-43. Eight-month old TDP-43 mutant but not wild type lines developed cytoplasmic aggregates containing ubiquitin and TDP-43 in spinal cord, cortex and hippocampus. Interestingly, in these aggregates TDP-43 itself was not ubiquitinated. Mutant TDP-43 expressing lines had TDP-43 C-terminal fragmentation at 3 and 10 months of age and developed peripherin and neurofilament disorganization. Both wild type and mutant TDP-43 overexpressing mice developed rotarod deficits at around 40 weeks of age, though L5 ventral root axons were unaffected and neuromuscular denervation was only very modest. Using GFAP reporter bioluminescence, the authors reported an age-dependent increase in astrogliosis. Mice also developed deficiencies in cognitive function starting at around 6 months of age in both mutant and wild type transgenic lines. Despite the generation of TDP-43 pathological changes, these mice did not die of motor neuron disease. In follow up studies the authors found that the disease phenotype was driven by NF- κ B p65-mediated dysregulation in these mice(Swarup et al., 2011b). TDP-43 interacts with NF- κ B

in neurons and glial cells and promotes NF- κ B mediated production of proinflammatory cytokines, enhancing neuronal vulnerability to neurotoxicity. A NF- κ B antagonist, withaferin, reduced denervation at the neuromuscular junction and improved ALS disease symptoms in wild type TDP-43 overexpressing mice.

Ubiquitous overexpression of TDP-43 has also been described in a rat model of TDP-43-mediated ALS/FTLD. BAC transgenic rats overexpressing human wild type or mutant TDP-43 M337V were developed with wild type rats not showing symptoms till 200 days of age whereas mutant rats rapidly showing paralysis and dying within a month (Zhou et al., 2010). To overcome this aggressive phenotype in mutant rats, inducible mutant TDP-43 expressing rats were generated (Zhou et al., 2010). These rats initiate mutant TDP-43 expression 4 days before birth and at the same level as wild type TDP-43 transgenic rats. Different lines of rats developed early onset disease with early demise of the animals. Only very limited loss of motor neurons was reported with diffuse nuclear and cytoplasmic staining of ubiquitin and phosphorylated-TDP-43. Given the ubiquitous nature of the overexpression, peripheral toxic effects most likely contributed to the early demise of these animals.

In the rat model, the wild type overexpression seems to be far less toxic than the mutant overexpression, which contrasts to what is seen in the mouse model. On the other hand, similar to the mouse model, high levels of mutant TDP-43 overexpression in rat results in very toxic and aggressive phenotypes. Not surprisingly, the usefulness of these overexpression models in understanding ALS and FTLD appears highly questionable.

2) Rodent models of TDP-43 specifically expressed in neurons—To explore the role of neuronal TDP-43 expression, mice overexpressing human TDP-43, with selective neuronal TDP-43 knock out or overexpression of wild type human TDP-43 have been generated. In one study several Thy1.2- promoter driven wild type or M337V mutant TDP-43 lines were developed that has disease severity correlating with the TDP-43 expression level (Wils et al., 2010; Janssens et al., 2013). The higher expressing homozygote wild type and mutant lines, with overexpression around twice the level of the endogenous tdp-43, develop very progressive disease phenotypes with death before the age of 1 month. Ubiquitinated and phosphorylated TDP-43 positive inclusions as well as C-terminal TDP-43 fragments were found both in wild type and mutant lines. These did not correlate with disease severity and are suggested not to contribute to the disease pathogenesis. Another study reported the development of mice in which the 25kDa C-terminal fragment of TDP-43 is expressed under the same Thy1.2 promoter (Caccamo et al., 2012). The authors concluded from this study that indicated that these C-terminal fragments were instrumental in driving cognitive, rather than motor, impairment. Interestingly, the cognitive phenotype was not associated with the formation of TDP-43 positive inclusions or neurodegeneration (Caccamo et al., 2012). The role of these 25kDa fragments is unknown as neuronal AAV9 mediated transduction of the truncated 25kDa TDP-43 fragment induces motor impairment and does mimic ALS to some extent (Dayton et al., 2013). Another Thy1.2-TDP-43 line with 2 to 5-fold overexpression relative to endogenous mouse tdp-43 developed early onset (P14) tremor, abnormal hindlimb reflexes and body weight loss in males and late onset (P90) fine tremor in female mice (Shan et al., 2010). These mice developed a cytoplasmic accumulation

of mitochondria and TDP-43 negative ubiquitinated cytoplasmic inclusions. Although a moderate loss of large caliber motor axons and abnormal neuromuscular junction morphology was reported, there was no loss of motor neurons in cortex or spinal cord and these mice did not die of progressive motor neuron disease. It is unclear whether these TDP-43 high overexpression animals develop gastrointestinal tract abnormalities as is seen with the PrP driven TDP-43 overexpression, which would be very plausible as Thy1.2 promoter is also active in myenteric plexus neurons. These gastrointestinal deficiencies have not been observed in ALS patients, and may represent a serious caveat in studying ALS disease progression in these TDP-43 overexpressing mouse models. Another caveat is the selectivity of the Thy1.2 promoter, which not only drives expression in neurons, but also in epidermis, thymocytes, keratinocytes and myoblasts and may be anticipated to confound the phenotype and the interpretation of the results obtained with these models.

Other TDP-43 lines have been generated that overexpress TDP-43 under the control of the CaMK2 promoter, which drives expression in forebrain neurons, hippocampus, cortex and striatum (Mayford et al., 1996). These models might be less useful in understanding ALS, as TDP-43 is not expressed in spinal motor neurons in these animals, but could still be valuable given the fact that FTLN co-morbidity in ALS patients is very high (up to 50%), and similar genes are causative for both diseases. One study reported the development of a mouse that overexpressed mouse wild type TDP-43 approximately 2-fold under the control of the CaMK2 promoter (Tsai et al., 2010). These mice developed deficiencies in the Morris water maze and conditional fear tests by 2 months of age with motor impairment by 6 months of age. Mice developed moderate 25% motor neuron cell loss in the cortex, astrogliosis and around 12% brain weight loss. TDP-43 depletion was seen in the neurons with cytoplasmic ubiquitinated TDP-43 positive inclusions, which comprised 15-20% of total neurons in the cortex. C-terminal TDP-43 positive fragments were also found in urea-soluble extracts, especially at the later disease stages (6 months). Average survival was slightly reduced in transgenic animals with a mean life span of 495 days. Although motor impairment appeared to be progressive, it was unclear whether the motor and cognitive performance worsened at later time points (after 6 months). Given the significant deficiencies in cognitive function in these animals, this model might be useful to study FTLN. Another study explored whether the timing of expression of CaMK2 driven wild type TDP-43 affected the phenotype (Cannon et al., 2012). The authors found that early 2-3-fold TDP-43 overexpression, initiated during neurodevelopment, led in some animals to an aggressive phenotype with TDP-43 mislocalization, aggregation, neurodegeneration and early death (Cannon et al., 2012). Early death was not observed in mice with TDP-43 expression initiated around the age of 20 days that developed a FTLN phenotype with progressive neurodegeneration characterized pathologically by phosphorylated and ubiquitinated TDP-43 positive inclusions.

The significance of nuclear TDP-43 depletion versus cytoplasmic accumulation, the fundamental pathological hallmark of ALS patients, was more specifically addressed in a study in which human wild type TDP-43 and human TDP-43 lacking the nuclear localization signal (NLS) (NLS line) were expressed under control of the CaMK2 promoter (Igaz et al., 2011; Alfieri et al., 2014). The authors addressed how mislocalized cytoplasmic TDP-43 influences neuronal viability as compared to non-transgenic and full-

length wild type TDP-43 overexpression. In these lines, TDP-43 expression was initiated at P28. TDP-43 was overexpressed 0.4 -1.7 fold in the full-length wild type line and 8 -9-fold in the TDP-43 NLS line. Both lines showed limb clasping, with the NLS line showing a more aggressive phenotype. In a follow-up study it was found that the NLS line developed significant motor and behavioural impairments which were partly reversible at young but not at old age(Alfieri et al., 2014). Intranuclear and or cytoplasmic TDP-43 inclusions as well as C-terminal fragments were rarely found in these lines, suggesting that these are not necessary for the phenotype observed(Igaz et al., 2011). There was a progressive decrease in the number of dentate gyrus neurons, specifically in the NLS line. The authors suggested that loss of endogenous mouse TDP-43 was important for the phenotype observed, as the level of mouse TDP-43 inversely correlated with the phenotype. However, these mice also overexpress human TDP-43 in both nucleus and cytoplasm which might cause toxicity through a toxic gain of function. A more recent study disputed the idea that toxicity arises from the loss of mouse TDP-43 in human TDP-43 expressing animals. Reducing mouse tdp-43 expression levels in hemizygous mouse tdp-43 animals to the same expression levels as mouse tdp-43 found in human wild type TDP-43 overexpressing mice did not induce a phenotype similar to that seen in the latter mice(Xu et al., 2013).

Another study rats reported the use of the human neurofilament heavy chain (NEFH) promotor to conditionally overexpress TDP-43 in neurons at P60 using the Tet ON/OFF system(Huang et al., 2012). Unexpectedly, the NEFH promotor not only drove neuronal expression but also appeared to drive TDP-43 expression in skeletal muscle. Overexpression of mutant M337V TDP-43 in neurons rapidly induced modest motor neuron loss, loss of hindlimb grip strength and paralysis at two weeks after TDP-43 expression initiation(Huang et al., 2012). Interestingly, when TDP-43 expression was switched off, motor function partly recovered, suggesting the phenotype was reversible, potentially due to remodeling of motor-unit end plates. To address the role of motor neurons more specifically, ChAT-driven TDP-43 M334V rats were generated with one line (line 9) expressing TDP-43 in around 60% of spinal motor neurons. Again rats developed early onset disease with paralysis after two weeks with around 60% loss of motor neurons in the spinal cord. Again switching off the TDP-43 expression reversed the motor phenotype observed. Given the fast, aggressive nature of the disease phenotype in these high level overexpression models, it is unlikely that the disease phenotype occurring in these animals actually correlates with that seen in ALS patients.

Mouse models with TDP-43 expression specifically depleted from neurons have also been generated. The phenotype of so called 'floxed' TDP-43 mice crossbred with either Vacht-Cre-fast mice or with HB9-Cre mice which both drive Cre expression in motor neurons was reported(Wu et al., 2012; Iguchi et al., 2013). Using the Vacht-Cre model, the homozygote, but not heterozygote, conditionally depleted TDP-43 mice to develop a very late onset motor neuron pathology characterized by tremor at 50 weeks and rotarod deficits at 75 weeks of age(Iguchi et al., 2013). At 100 weeks of age, there was a progressive degeneration of motor axons culminating in around 35% loss of neuromuscular innervation, grouped atrophy of gastrocnemius muscle but no spinal or hypoglossal motor neuron loss. This was accompanied by an increase in the number of small size TDP-43 negative neurons and the loss of large size TDP-43 negative neurons, which was attributed to atrophy of alpha spinal

motor neurons. In the HB9-Cre model, at 20 weeks, despite the majority of remaining motor neurons lacking TDP-43 expression, there was only a modest loss of motor neurons, astrogliosis and accumulation of ubiquitinated material in motor neurons(Wu et al., 2012). These mice developed hind limb clasping, rotarod deficiencies and kyphosis that was most prominent at around 20-24 weeks of age. These mice further progressed slowly and died at around 10 months of age, though it is not clear whether they died of motor neuron disease and associated paralysis.

In general, when TDP-43 is modestly overexpressed in neurons, some aspects of human disease do develop, but though some models have potential to study FTLTD, these models do not develop progressive motor neuron disease resulting in the demise of the animal, similar to that seen in ALS patients. Studies with neuron specific mutant SOD1 expression in the past have led to the same conclusion. Therefore, TDP-43 expression in other cell types, mainly glial cells, might be necessary to induce a more florid ALS phenotype.

3) Modeling TDP-43 non-cell autonomous toxicity: TDP-43 expression in astrocytes—The modest loss of motor neurons seen in the previously described models of TDP-43 depletion from motor neurons could be due to the fact that motor neurons are less sensitive to the loss of TDP-43 as compared to glial cells. In a recent study of constitutive, ubiquitous miRNA mediated knockdown of TDP-43, the authors suggested that knockdown in mainly astrocytes (and potentially other glial cells) drives a massive loss of motor neurons, paralysis and death seen in this TDP-43 mouse model(Yang et al., 2014). One caveat with this mouse model is that TDP-43 knockdown was ubiquitous, mainly seen in the periphery, and it is not clear whether effects mediated by peripheral TDP-43 knockdown contributed to the phenotype observed. Astrocyte sensitivity to TDP-43 toxicity was also seen in a rat model of selective astrocytic overexpression of human wild type TDP-43(Tong et al., 2013). Expression was conditional and only turned on at the age of 40 days. These animals developed progressive motor neuron degeneration and paralysis, though disease was accelerated in the higher overexpressing lines and the brain seemed to be spared from disease. The authors found astrocytic GLT-1 levels to be reduced which could contribute to the motor neuron loss and denervation atrophy that was observed. However, this loss of GLT-1 could be due to the loss of astrocytes in this model, which is not observed in the CNS of ALS patients. Also, these results were not replicated in a transplant model of TDP-43, in which PrP-driven TDP-43 overexpressing glial restricted precursor cells (GRPs) or TDP-43 depleted GRP's were injected in the cervical spinal cord of wild type rats(Haidet-Phillips et al., 2013). These animals did not go on to develop motor neuron degeneration despite the efficient differentiation of GRPs into astrocytes and integration in the new environment. Given these discrepancies, the role of non-neuronal cells in TDP-43 mediated disease largely remains to be elucidated. The involvement of other glial cell types in TDP-43 mediated disease like oligodendrocytes, oligodendrocyte progenitor cells and microglial cells similarly awaits investigation. Interestingly, in human ALS, TDP43 cytoplasmic aggregates are most abundant in oligodendroglia (Seilhean et al., 2009).

Major caveats of TDP-43 transgenic rodent models

In general, none of the TDP-43 driven animals described here actually seem to develop full blown ALS disease as seen in humans. Complications could arise from the fact that TDP-43 is ubiquitously expressed and is essential in many different organs throughout the whole body. The lethality of TDP-43 null mice supports this concern. Mild phenotypes are usually observed in the CNS of low overexpression models for TDP-43 mice as compared to SOD1 mice. Indeed, the loss of cortical or spinal motor neurons is very modest as compared to what is seen in ALS patients or in SOD1 mouse models. Similarly, axon number and neuromuscular junction innervation is usually only mildly affected. Lastly, these animal models either do not exhibit early mortality, or die for reasons unrelated to ALS disease pathogenesis. Usually, especially in the earlier high overexpression mouse models, disease is seen in both wild type and mutant TDP-43 overexpressing models. There is no doubt that overexpression per se, irrespective of the nature of the TDP-43 protein, contributes to the phenotype, and that this overexpression affects the mouse tdp-43 through autoregulation. The current thinking is that the functionality of a cell is highly dependent on a well balanced TDP-43 expression level. Slight changes, whether down or up, lead to changes in the RNA splicing profile with possible detrimental effects to cellular function. From the animal studies, it does seem that both gain and loss of function of mutant overexpressed TDP-43 and/or mouse tdp-43 contribute to producing the phenotype. To complicate things even more, the effects mediated through mouse tdp-43 function in combination with effects due to overexpression of human TDP-43 are very difficult to segregate.

More recently, a model with moderate TDP-43 overexpression levels has been generated which develops a mild disease phenotype which is specific for the mutant overexpression and not the wild type overexpression, but does not progress into a paralytic disease stage as seen in mutant SOD1 mouse models or in ALS patients (Arnold et al., 2013). It is very difficult to modulate TDP-43 transgene expression levels to what is seen in ALS patients. Though some reports exist that indicate TDP-43 is upregulated in sALS, the cell-type specific contribution to this upregulation is not known and so it is not known which change in cell type specific expression actually contributes to the generation of ALS disease symptoms (Swarup et al., 2011b). Expression levels in ALS patients might also be much more dynamic than those obtained in the TDP-43 transgenic animal models generated so far and might contribute to their failure to accurately model the disease. Interestingly though, there are some reports that several target RNAs are similarly dysregulated in rodent models of TDP-43 and sALS patients. Another well established lack of correlation between human and rodent ALS models is seen in the pathology studies which suggest that in mice cytoplasmic aggregation, C-terminal fragmentation and phosphorylation might not be essential for motor neuron disease to develop. Technical issues associated with detecting these pathological changes notwithstanding, a crucial question that remains is whether TDP-43 aggregates are linked to ALS either as causative or as a consequence of the disease phenotype. In mice, these pathological changes are seen in some models but not in all, and are usually found at more moderate levels when compared to that seen in ALS patients. As TDP-43 transgenic mice do not develop full blown ALS like that seen in humans, it cannot be suggested that certain pathological characteristics are not necessary for disease to develop or progress to an end stage phenotype. In humans, these pathological changes have more

time to develop than in mice and might well contribute to the eventual demise of cortical or spinal neurons, denervation and paralysis leading to a fatal end point of disease. If that is the case, the usefulness of rodent models to study late onset neurodegenerative diseases might be limited.

Lastly, TDP-43 might only be causative for disease in the context of other disease genes and/or modifying factors that are not seen in mice. Through a long history of crossbreeding mice have become very homogenous genetic populations as compared to humans. In fact in human ALS, some patients who had a mutation in TDP-43 also had expanded repeats length size of the first intron of the C9ORF72 gene, another ALS causing gene, which might lead to induction of the specific pathology associated with motor neuron disease (van Blitterswijk et al., 2012). Though interesting and very plausible, this multi-gene model of ALS disease needs confirmation and would be more difficult to model in rodents, as multiple transgenes might have to be expressed.

Development of other rodent models expressing different ALS causing mutations

The last six years have seen a surge in the identification of genes associated with ALS. With the exception of C9ORF72, mutations in the majority of these genes like UBQLN2, p62, VCP, Profilin1, Matrin 3, etc. are rarely seen in familial ALS patients. Some of these genes, like UBQLN2 and p62, could be involved in disease pathogenesis in sporadic ALS as they are frequently present in CNS tissue from these patients (Deng et al., 2011; Fecto et al., 2011). Animal models for these newly discovered genes are awaiting. Mutations in VCP are involved in ALS and FTLN as well as in inclusion body myopathy associated with Paget's disease of the bone and frontotemporal dementia (IBMPFD) (Watts et al., 2004; Johnson et al., 2010). Rodent models expressing mutant VCP have been generated (Weihl et al., 2007; Nalbandian et al., 2013) that develop signs of Paget-like lesions, progressive muscle weakness, with significant cytoplasmic TDP-43 accumulation in brain and motor neurons in spinal cord.

Future perspectives

This overview outlines the current stage of development of the most common ALS rodent models generated. Many of these models exist with significant caveats e.g., modeling only particular aspects of the disease, developing non-ALS related phenotypes and a lack of translation to the clinic. Improved animal models need to be generated in order to better model the human disease. More mouse models showing specific ALS disease phenotypes need to be generated. For now SOD1 mutant mice are the only rodent models that develop a phenotype reminiscent of ALS in humans. However, given the fact that mutant SOD1 appears to only important for disease development in less than 2% of all ALS cases, and the majority of fALS appears to be caused by changes in RNA metabolism (Renton et al., 2014) (AU - REF), it is important to generate new models which target RNA. For example, the development of rodents transgenic for a repeat expansion in the C9ORF72 gene, the most prevalent ALS-causing gene identified to date (DeJesus-Hernandez et al., 2011; Renton et al., 2011). On the other hand, ALS is a multifactorial disease, with many genetic modifiers associated with the disease. Therefore ALS could be, despite the strong clinical correlations seen in patients, very variable in terms of disease pathogenesis among patients and therefore

hard to model. For many ALS-causing genes and especially disease modifiers (Leblond et al., 2014), genetic penetrance is far lower than 100%, so different disease modifiers together could combine to increase the likelihood for developing ALS. To model disease in such patients, iPS derived motor neurons and glial cells have been developed which could be patient-specific mimicks of ALS. Although dissected out of their specific microenvironment, these cells have been shown to strongly correlate to ALS post mortem tissue (Dimos et al., 2008; Donnelly et al., 2013; Serio et al., 2013). The combination of both new rodent tools for ALS as well as iPS derived patient specific CNS cells may lead to a better understanding of ALS and the development of promising new therapies.

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Table 5.67.1

Most Commonly Used SOD1 Mice to Study ALS

Mutation carrier	Promotor	Reference	CNS overexpression (fold)	Activity	Disease onset (months)	Survival (months)	Paralysis	Aggregation	Motor neuron loss
G93A	Human SOD1	Gurney et al., [* AU; provide this ref.]	17	13	3	4	Yes		Yes
G37R	Human SOD1	Wong et al., 1995	4-12	5-14	3.5-6	7	Yes	SOD1 accumulation in axons	Yes
G85R	Human SOD1	Bruijn et al., 1997	0.2-1	0	8	8.5	Yes	astrocyte SOD1 inclusions	40% MN loss
G86R	Mouse sod1	Ripps et al., 1995	Low to high	0	3-4 (line M1)	4	yes	NA	yes

[*Author: We created this table from the spreadsheet of data you submitted. Please check all cells and headings carefully to make sure the information is presented thoroughly and correctly.]

* Author: Define “Activity” and provide units, if appropriate.

Table 5.67.2

TDP43 Rodent Models to Study ALS

A. Ubiquitous overexpression										
Mutation	Promotor	Reference	CNS overexpression (fold)	Survival (months)	TDP43 translocation	Phospho-TDP-43	Inclusions	TDP43 C-term fragments	Autoregulation mouse tdp43	Motor phenotype
<i>Mouse with TDP43 mutation</i>										
A315T	mPrP	Wegorzewska et al., 2009	3	5	+		TDP43- and Ub+	+	+	+
A315T	mPrP	Stallings et al., 2010	4 (line 23)	2-3	+	+	TDP43+ and Ub+	+		+
WT	mPrP	Stallings et al., 2010	3-4	>11	+			+		+
M337V	mPrP	Stallings et al., 2010	up to 800 of A315T line 23	0.5-1.5	+		Ub+	+		+
M337V	mPrP	Xu et al., [*>CE: which ref?]	2.5	1-2	+	+	NII/NCI TDP43+Ub+	+	+	+
WT	mPrP	Xu et al., [*CE: which ref?]	2.5	1-2	+	+	NII/NCI TDP43+Ub+	+	+	+
Q331K	mPrP	Arnold et al., 2013	1-1.5	>17	-	-	-	-	+	+
M337V	mPrP	Arnold et al., 2013	1-1.5	>17	-	-	-	-		+
WT	mPrP	Arnold et al., 2013	1-1.5	>17	-	-	-	-		+
G348C	human TDP43 (BAC)	Swarup et al., [*CE: which ref?]	3	>12	+		TDP43+ and Ub+	+	+	+
A315T	human TDP43 (BAC)	Swarup et al., [*CE: which ref?]	3	>12	+		TDP43+ and Ub+	+	+	+
WT	human TDP43 (BAC)	Swarup et al., [*CE: which ref?]	3	>12	-		-	-	+	+
<i>Rat with TDP43 mutation</i>										
WT	human TDP43 (BAC)	Zhou et al., 2010	High		+	+		+		
M337V	human TDP43 (BAC)	Zhou et al., 2010	high (comparable to WT)	<1 month						+
iTDP-43 M337V (induced fully at P10)	human TDP43 (BAC)	Zhou et al., 2010	high	<2 months	+	+	TDP43+	+		+

B. Neuronal overexpression										
Mutation	Promotor	Reference	CNS overexpression (fold)	Survival (months)	cytoplasmic TDP-43	Phospho-TDP-43	Inclusions	TDP43 C-term fragments	Autoregulation mouse tdp43	Motor phenotype
<i>Mouse with TDP43 mutation</i>										
WT	Thy1.2	Wils et al., 2010	2 (line 4/4)	<1 month	+	+	NII/NCI TDP43+Ub+	+	+	+
M337V	Thy1.2	Janssens et al., 2013	2 (line 6/6)	<1 month	+	+	TDP43+ and Ub+	+		+
TDP43-25 (25kDa fragment)	Thy1.2	Caccamo et al., 2012	1-4		-	-	-	+	+	-

B. Neuronal overexpression											
Mutation	Promotor	Reference	CNS overexpression (fold)	Survival (months)	cytoplasmic TDP-43	Phospho-TDP-43	Inclusions	TDP43 C-term fragments	Autoregulation mouse tdp43	Cognitive phenotype	Motor phenotype
WT	Thy1.2	Shan et al., 2010	2.5 (females); 5 (males)		-		NII TDP43+Ub+	-			+
WT	CaMK2	Tsai et al., 2010	2	17 months	+		NCI TDP43+Ub+	+	+	+	+
iTDP43 (constitutive Tetactivated)	CaMK2	Cannon et al., 2012	2-3	variable	+	+	NII/NCI TDP43+Ub+		+		
iTDP43 (induced at P21)	CaMK2	Cannon et al., 2012	2-3	>12 months	+	+	NII/NCI TDP43+Ub+		+		
iTDP43 (induced at P28)	CaMK2	Igaz et al., 2011	0.4-1.7		+	+	rare		+	+	+
iTDP43 (induced at P28)	CaMK2	Igaz et al., 2011; Alfieri et al., 2014	8-9		+	+	rare		+	+	+
Rat with TDP43 mutation											
iTDP43-M337V (induced at P60)	NEFH	Huang et al., 2012	?	~75 days			rare TDP43-Ub+				+
iTDP43-M337V (induced at P60)	Chat	Huang et al., 2012	?	~75 days	+		TDP43-Ub+				+

C. Neuronal depletion					
Mutation	Promotor	Reference	Survival (months)	Cognitive phenotype	Motor phenotype
tdp-43 knockout	Vacht-Cre	Iguchi et al., 2013			+
tdp-43 knockout	HB9-Cre	Wu et al., 2012			+

[*Author: We created this table from the spreadsheet of data you submitted. Please check all cells and headings carefully to make sure the information is presented thoroughly and correctly.]