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Review

Rodent models of amyotrophic lateral sclerosis ☆

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ABSTRACT

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease characterised by the degeneration of upper and lower motor neurons. Recent advances in our understanding of some of the genetic causes of ALS, such as mutations in *SOD1*, *TARDBP*, *FUS* and *VCP* have led to the generation of rodent models of the disease, as a strategy to help our understanding of the pathophysiology of ALS and to assist in the development of therapeutic strategies. This review provides detailed descriptions of TDP-43, FUS and VCP models of ALS, and summarises potential therapeutics which have been recently trialled in rodent models of the disease. This article is part of a Special Issue entitled: Animal Models of Disease.

1. Introduction to ALS

Amyotrophic lateral sclerosis (ALS) was first described over 140 years ago by the French neurologist Charcot, whose observation of muscle atrophy and scarring within *post mortem* spinal cord led to its naming. ALS is a progressive neurodegenerative disorder characterised by the degeneration of upper and lower motor neurons. Loss of these neurons leads to a deterioration of neuromuscular function, causing weakness, atrophy and paralysis of skeletal muscles. The progression of ALS is usually rapid and death typically occurs within 3–5 years of diagnosis.

Although the vast majority of ALS cases are sporadic (SALS), approximately 10% are familial (FALS). The first gene shown to be mutated in FALS encodes the enzyme superoxide dismutase 1 (SOD1) [1] and since this discovery, over 150 pathogenic mutations in *SOD1* have been identified in ALS patients [2]. Recently, mutations in other genes have also been found to be causative for FALS (Table 1). The observation of Tar DNA binding protein 43 (TDP-43), a DNA and RNA binding protein, as a large component of protein aggregates in *post mortem* tissue from SALS and FALS cases [3] and the subsequent identification of pathogenic mutations in the *TARDBP* gene which encodes TDP-43 [4,5] has strengthened the possibility that aberrant RNA processing may play a significant role in ALS pathogenesis. This had been originally proposed following the identification of ALS-causing mutations in the *SETX* gene, which encodes a protein with roles in DNA and RNA processing [6]. This association was significantly strengthened by the identification of mutations in the DNA and RNA binding protein fused in sarcoma/translocated in liposarcoma (FUS/TLS, hereby referred to as FUS) as a cause of FALS [7,8]. The identification of the genetic causes of ALS is critical for advancing our understanding of the pathophysiology of ALS, since these mutations form the basis of the development of experimental models of the disease, the most valuable of which are animal models. This review will provide a detailed overview of recent advances of modelling ALS in rodents and will briefly discuss potential therapeutics which have been recently trialled in rodent models of ALS.

2. SOD1 rodent models of ALS

Missense mutations in the *SOD1* gene on chromosome 21 were the first identified causes of autosomal dominant FALS in 1993 [1].

Abbreviations: AAV, Adeno-associated virus; ALS, Amyotrophic lateral sclerosis; BAC, Bacterial artificial chromosome; ChAT, Choline acetyltransferase; CNS, Central nervous system; cTDP/FUS, Cytoplasmic TDP-43/FUS; CTF, C-terminal fragment; EMG, Electromyography; FALS, Familial amyotrophic lateral sclerosis; FUS, Fused in sarcomere/translocated in liposarcoma; IBMPFD, Inclusion body myositis with Paget's disease of bone and frontotemporal lobar dementia; mPrp, Mouse prion promoter; NEF, Neurofilament heavy chain; NHP, Non-human primate; NLS, Nuclear localisation signal; NMJ, Neuromuscular junction; SALS, Sporadic amyotrophic lateral sclerosis; SBT, Somatic brain transgenic; SMA, Spinal muscular atrophy; SOD1, Superoxide dismutase 1; TDP-43, Tar DNA binding protein 43; TRE, Tetracycline response element; VCP, Valosin-containing protein

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Table 1
Currently known genetic causes of FALS. Table includes data on mutations identified to date, and the percentages known for which the mutations account for FALS or SALS. – denotes that this information is unclear.
Source: http://alsod.iop.kcl.ac.uk.

Protein/gene	Chromosome	Protein size (aa)	Mutations	FALS cases (%)	SALS cases (%)	Reference
SOD1/SOD1	21q22.11	154	166	~20	1–3	Rosen et al. [1]
Alsin/*ALS2*	2q33.2	1657	19	–	–	Yang et al. [9]
Senataxin/*SETX*	9q34.13	867–1030	7	–	–	Chen et al. [6]
TDP-43/*TARDBP*	1p36.22	414	39	~4	~0.2–0.4	Kabashi et al. [4]; Sreedharan et al. [5]
FUS/FUS	16p11.2	526	27	~4	~0.2–0.4	Kwiatkowski et al. [7]; Vance et al. [8]
Optineurin/*OPTN*	10p13	571	3	–	–	Maruyama et al. [10]
VCP/*VCP*	9p13	475–644	4	–	–	Johnson et al. [11]
Angiogenin/ANG	14q11.1	147	18	–	–	Greenway et al. [12]
VAPB/*VAPB*	20q13.33	243	2	<1	–	Nishimura et al. [13]
Ubiquilin-2/*UBQLN2*	Xp11.21	624	5	–	–	Deng et al. [14]
C9orf72/*C9ORF72*	9p21.2	481	GGGGCC repeat expansion	~20–40	~10	DeJesus-Hernandez et al. [15]; Renton et al. [16]
Profilin 1/*PFN1*	17p13.3	140	4	–	–	Wu et al. [17]

Although several new ALS genes have been recently identified, research into the aberrant functions of mutant SOD1 has formed the basis of much of our understanding of the pathogenesis of ALS, in large part through the generation and study of transgenic rodent models overexpressing the mutant human SOD1 protein. However, despite nearly 20 years of intensive research, an understanding of exactly how mutant SOD1 causes motor neuron degeneration remains elusive.

SOD1 is a ubiquitous cytoplasmic and mitochondrial enzyme which functions in a dimeric state to catalyse the breakdown of harmful reactive oxygen species (ROS), thereby preventing oxidative stress. Due to the damaging effects of ROS and their association with neurodegenerative diseases, it was originally proposed that pathogenic mutations in SOD1 may cause ALS as a result of a loss of dismutase activity [1]. Subsequent investigation failed to correlate mutant SOD1 activity with pathogenicity [18] and in *Sod1* knock-out mice (*Sod1*$^{-/-}$), deficiency of SOD1 did not result in motor dysfunction, at least up to 6 months of age [19]. However, more recent characterisation of *Sod1*$^{-/-}$ mice has revealed that although they do not have any motor neuron loss, they have a significant distal motor axonopathy, demonstrating the important role of SOD1 in normal neuronal function [20–23]. Thus, the significant loss of motor neurons in transgenic mice expressing mutant SOD1 is likely to result from a toxic gain-of-function. Investigation of this gain-of-function has in large part been undertaken by studying transgenic mouse models.

Shortly after the discovery of *SOD1* mutations in FALS [1], a transgenic mouse model (SOD1^{G93A}) of SOD1–ALS was developed, expressing approximately 20–24 copies of the human coding sequence with the G93A mutation, under control of the human *SOD1* promoter [24]. Since the development of this model, over twenty other SOD1 models have been created and SOD1 transgenic rodents have been used as the primary rodent models of ALS.

Mutant SOD1 transgenic rodents recapitulate many features of ALS, including axonal and mitochondrial dysfunction, progressive neuromuscular dysfunction, gliosis and motor neuron loss [24–29]. The majority of SOD1 transgenic rodents use the *SOD1* promoter to mimic normal expression of SOD1. However models utilising neuronal-specific [30,31] or astrocytic promoters [32] have also been developed. Such transgenic mice have been important in understanding the non-cell autonomous nature of ALS. Overexpression of mutant SOD1 is not limited to mice, as transgenic rats have also been developed [28,33–35]. For the most part, transgenic rats overexpressing mutant SOD1 recapitulate many features of ALS, including motor neuron degeneration and axonal loss as well as pathological hallmarks. However there can be broad variation in the development of phenotypes of certain rat models [35]. Although transgenic rats are not as widely used as mice, their larger size may offer certain benefits, for example in pharmacological trials where intrathecal injections or insertion of mini pumps are required.

Transgenic SOD1 rodent models have variable ages of disease onset and rates of disease progression. Development of ALS-like symptoms in these mice is now known to be largely dependent upon four factors: i) SOD1 mutation; ii) transgene expression level; iii) gender and iv) genetic background [36,37]. In order to rule out the possibility that the disease phenotype may be the result of overexpression of SOD1 *per se*, lines of transgenic mice overexpressing the human wildtype protein have also been created [24,38,39]. It was originally proposed that wildtype SOD1 did not play a role in ALS, as mice overexpressing the wildtype protein showed axonopathy without overt motor dysfunction; although signs of abnormal axonal and mitochondrial morphology were present from early ages [38,40]. However more recently it has been emerged that wildtype SOD1 could play a role in ALS pathogenesis [41–43]. Crucially, overexpression of human wildtype SOD1, at a level similar to that seen in the SOD1^{G93A} mouse model, can cause progressive motor neuron degeneration [43]. Homozygous expression of a human wildtype SOD1 transgene resulted in a reduced lifespan, with a median survival of 367 days, accompanied by slow weight gain from birth and overt weight loss in older male mice [43]. These mice also developed an ataxic staggering gait with abnormal hindlimb reflexes [43]. Gliosis and misfolded SOD1 were detected in the spinal cord from 100 days of age, as were signs of vacuolisation and axonal damage, which are typical of SOD1 overexpression [38,40]. By endstage around 40% of motor neurons in the thoracic spinal cord had been lost [43].

Study of numerous SOD1 models has implicated various mechanisms in the pathology of ALS, including mitochondrial dysfunction, protein misfolding and aggregation, non-cell autonomous insults, excitotoxicity, oxidative stress and axonal transport (see Fig. 1). Transgenic mouse models overexpressing mutant SOD1 have therefore been central to our understanding the effects of mutant SOD1 and have formed the basis of much of our knowledge of the underlying pathophysiology of ALS [44].

3. TDP-43 models of ALS

TDP-43 is a 43 kDa nuclear protein originally discovered because of its effects on human immunodeficiency virus transcription [45]. It is encoded by the *TARDBP* gene on chromosome 1, and contains a nuclear-localisation signal, two RNA-binding motifs, and a glycine-rich region, which contains a 'prion-like' domain and mediates protein and hnRNP interactions. It is within this glycine-rich domain that the majority of pathogenic mutations for ALS have been identified [4,5].

Although the full range of TDP-43 functions remains unclear, it is known to play crucial roles in alternative splicing and gene expression [46], embryogenesis [47] and neuronal development [48–50]. However, the effects of pathogenic mutations in TDP-43 on its function are poorly understood. *In vitro* and *in vivo* studies have identified

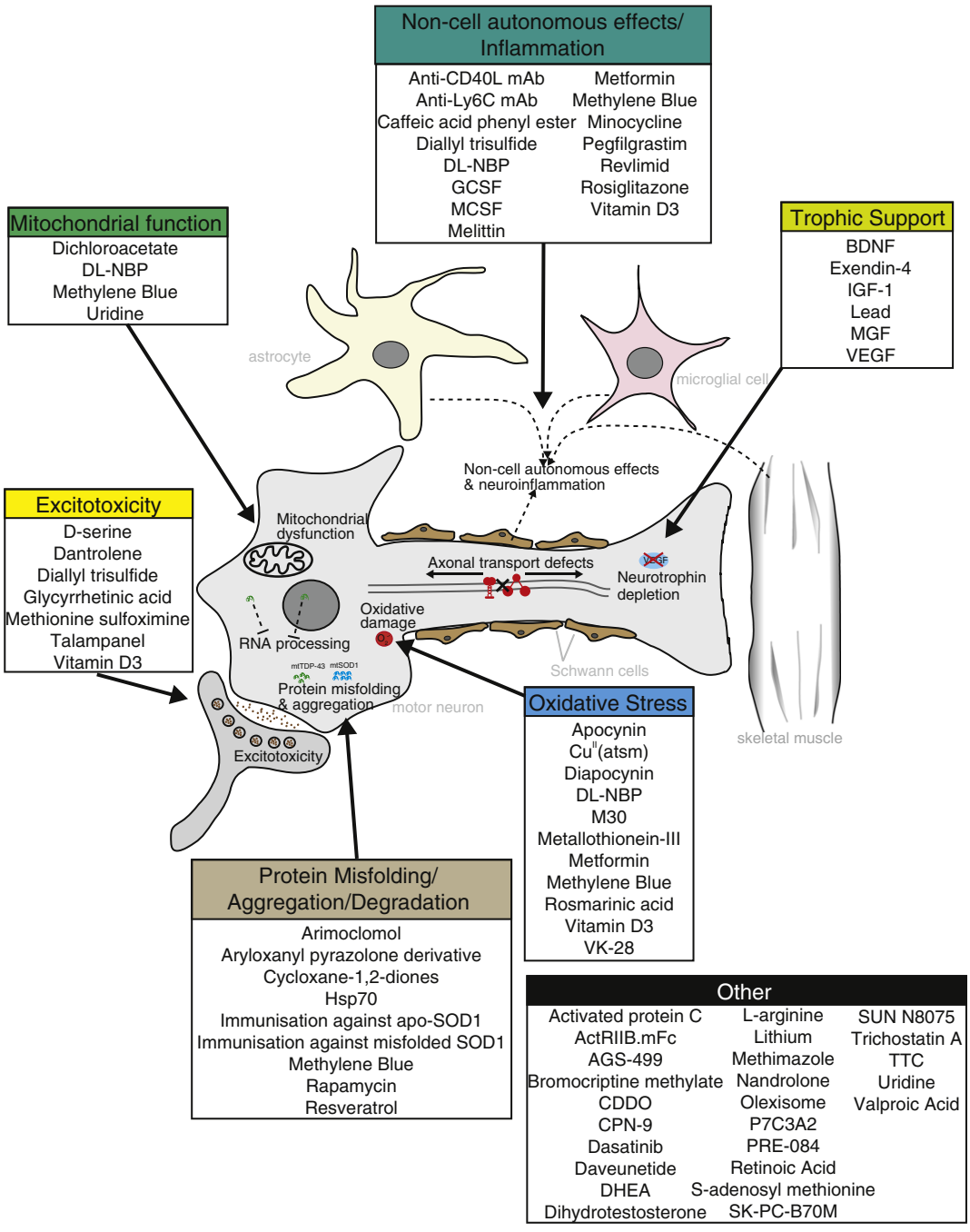

Fig. 1. ALS pathomechanisms and targets tested in recent preclinical trials in rodents.

a range of aberrant cellular dysfunctions caused by mutant TDP-43, including abnormal neuronal function and synaptic defects [51,52], deleterious effects on mitochondria [53,54] and proteasome dysfunction [55]. Although it remains unclear how mutations in *TARBP* cause ALS, both loss and gain of function mechanisms have been proposed [56].

Several groups have attempted to model TDP-43-ALS in rodents and results have shown varied phenotypes, with no TDP-43-ALS model currently replicating an ALS-like phenotype. Similar to SOD1 rodent models, development of disease phenotypes in TDP-43-ALS transgenic rodents is highly dependent upon the promoter used and the level of transgene expression; however these models display mostly axonal phenotypes with relatively mild motor neuron cell death. In marked contrast to SOD1 transgenic mice, overexpression of human wildtype TDP-43 has been shown to cause significant neurodegeneration. Moreover, unlike SOD1 transgenic mice, transgene expression levels of TDP-43 are typically low and founder lines which have the highest levels of overexpression show early mortality preventing the generation of transgenic lines with high levels of overexpression. The TDP-43 transgenic rodent models generated to date are summarised in Table 2.

3.1. Knock-out of mouse Tardbp to model ALS

Following the identification of ALS causative mutations in *TARDBP* [4,5], knock-out mice were developed to determine the effects of TDP-43 deficiency and to establish whether mutations in *TARDBP* cause ALS due to a loss-of-function. Independent studies reported

Table 2
Summary of TDP-43 and FUS rodent models. Where possible neuronal loss has been quoted. nd: not described, Y: yes, N: no.

Species	Promoter	Protein	Protein expression (fold)	Symptom onset (weeks)	Survival (weeks)	Phenotypes							Reference
						Abnormal motor behaviour	Degeneration				Pathology		
							LMN	UMN	Cortex	Axonal	Gliosis	cTDP-43/ FUS	CTFs	
Mouse	Hb9:Cre	Motor neuron specific depletion	0	13	40	Y	60% loss	nd	nd	nd	Y	nd	nd	Wu et al. [57]
Mouse	mPrp	TDP-43 A315T	3	13	22	Y	20% loss	Y	nd	Y	Y	Y	Y	Wegorzewska et al. [58]; Guo et al. [59]
Mouse	mPrp	TDP-43 WT	1.9	Not affected										Xu et al. [60]
Mouse	mPrp	TDP-43 WT	2.5	2	4–8	Y	N	nd	nd	Y	Y	Y	Y	Xu et al. [60]
Mouse	mPrp	TDP-43 M337V	1.9	Not affected										Xu et al. [58]
Mouse	mPrp	TDP-43 M337V	2.5	2	4	Y	nd	nd	nd	Y	Y	Y	Y	Xu et al. [61]
Mouse	mPrp	TDP-43 WT	3–4	nd	nd	N	nd	nd	nd	nd	Y	nd	nd	Stallings et al. [62]
Mouse	mPrp	TDP-43 A315T	4	4	37.5	Y	nd	nd	nd	nd	Y	Y	Y	Stallings et al. [62]
Mouse	Thy1.2	TDP-43 WT	Males: 3.6	2-2.5	nd	Y	N	nd	nd	Y	Y	N	N	Shan et al. [54]
Mouse	Thy1.2	TDP-43 WT	Females: 1.3	13	nd	Y	N	nd	nd	nd	nd	N	N	Shan et al. [54]
Mouse	Thy1.2	TDP-43 WT	1.9	56	nd	Y	nd	nd	nd	nd	nd	nd	Nd	Wils et al. [63]
Mouse	Thy1.2	TDP-43 WT	3.8	8	Approx 27	Y	10% loss	15% loss	nd	nd	Y	Y	nd	Wils et al. [63]
Mouse	Thy1.2	TDP-43 WT	5.1	2	4	Y	25% loss	30% loss	nd	nd	Y	Y	Y	Wils et al. [63]
Mouse	Thy1.2	TDP-25	4.7	nd	nd	nd	nd	nd	N	nd	nd	Y	Y	Caccamo et al. [64]
Mouse	CaMKII	TDP-43 WT	2	8	71	Y	nd	nd	Y	nd	Y	Y	Y	Tsai et al. [65]
Mouse	CaMKII (TRE)	TDP-43 WT	0.8 (induced at 4 weeks)	8–49	nd	Y	nd	Y	Y	nd	Y	nd	nd	Igaz et al. [66]
Mouse	CaMKII (TRE)	TDP-43 ΔNLS	7.9 (induced at 4 weeks)	5	26	Y	nd	Y	Y	Y	Y	Y	nd	Igaz et al. [66]
Mouse	CaMKIIα (TRE)	TDP-43 WT	3 (induced from P21)	nd	nd	nd	nd	nd	Y	nd	Y	Y	nd	Cannon et al. [67]
Mouse	TDP-43 (BAC)	TDP-43 WT	3	28–32	nd	Y	nd	nd	nd	N	Y	nd	nd	Swarup et al. [68]
Mouse	TDP-43 (BAC)	TDP-43 A315T	3	28–32	nd	Y	nd	nd	nd	N	Y	Y	Y	Swarup et al. [65]
Mouse	TDP-43 (BAC)	TDP-43 G348C	3	28–32	nd	nd	nd	nd	nd	N	Y	Y	Y	Swarup et al. [68]
Rat	TDP-43	TDP-43 WT	nd	nd	nd	nd	nd	nd	nd	nd	nd	Y	nd	Zhou et al. [69]
Rat	TDP-43 (TRE)	TDP-43 M337V	nd (induced on P4)	4.5–4.7	6.5–6.7	Y	17% loss		Y	Y	Y	Y	Y	Zhou et al. [69]
Rat	NEF (TRE)	TDP-43 M337V	2 (fully activated by 8.5 weeks)	10	13	Y	20% loss	nd	nd	Y	nd	N	Nd	Huang et al. [70]
Rat	ChAT (TRE)	TDP-43 M337V	3.5 (fully activated by 8.5 weeks)	9	11	Y	60% loss	nd	nd	Y	Y	N	nd	Huang et al. [70]
Mouse	mPrp	FUS WT	1.4	Not affected										Mitchell et al. [71]
Mouse	mPrp	FUS WT	1.7	4–5	12	Y	60% loss	nd	nd	nd	Y	Y	nd	Mitchell et al. [71]
Mouse	SBT-rAAV	FUS WT	nd	nd	nd	nd	nd	nd	nd	nd	nd	Y	nd	Verbeeck et al. [72]
Mouse	SBT-rAAV	FUS R521C	nd	nd	nd	nd	nd	nd	nd	nd	nd	Y	nd	Verbeeck et al. [72]
Mouse	SBT-rAAV	FUS Δ14	nd	nd	nd	nd	nd	nd	nd	nd	Nd	Y	nd	Verbeeck et al. [72]
Rat	FUS (TRE)	FUS WT	nd (induced at birth)	nd	nd	nd	nd	nd	Y	nd	nd	nd	nd	Huang et al. [73]
Rat	FUS (TRE)	FUS R521C	nd (induced at birth)	5	6	Y	nd	Nd	Y	nd	Y	nd	nd	Huang et al. [73]
Rat	CaMKIIα (TRE)	FUS R521C	nd (induced from 30 days)	9	18	nd	nd	nd	Y	nd	Y	Y	nd	Huang et al. [74]

similar findings, showing that complete loss of mouse TDP-43 resulted in early embryonic lethality or death of embryonic stem cells, thereby identifying an essential role for TDP-43 in development [47,75–77]. However, disruption of one *Tardbp* allele did not affect either protein or mRNA levels, suggesting an autoregulation of TDP-43 levels [47,76,77]. One report characterised a cohort of *Tardbp*$^{+/-}$ mice and

observed age-dependent deficits in grip strength and cage-hanging tests compared to wildtype littermates [76].

An alternative approach to examine the effects of reduced TDP-43 expression is to use a conditional knock-out strategy. Chiang and colleagues [75] found that heterozygous deletion of *Tardbp* did not alter TDP-43 protein or mRNA levels; however conditional deletion of both *Tardbp* alleles in adult mice caused rapid loss of body fat and death within 9 days. Interestingly, the loss of body fat was associated with a downregulation of Tbc1d1, a protein linked with obesity [75]. However, these rapid and severe effects likely preclude any investigation of loss of TDP-43 with regard to motor neuron degeneration.

More recently, the effect of a loss of TDP-43 function has been investigated by depleting TDP-43 from Hb9-positive spinal cord motor neurons in mice [57]. This targeted depletion of TDP-43 resulted in significantly reduced bodyweight of mice at 8 weeks of age, with rotarod deficits apparent by 15 weeks, followed by abnormal hindlimb clasping and kyphosis. The authors noted that although TDP-43 protein levels in the spinal cords of knock-out mice were not altered, likely due to the relatively small proportion of cells which are Hb9-positive, choline acetyltransferase (ChAT) levels were significantly reduced by 20 weeks of age. A reduction in ChAT levels correlated with a significant, progressive loss of ChAT-positive spinal cord motor neurons, with evidence of a specific vulnerability of larger motor neurons. In addition, motor neuron loss was accompanied by age-dependent gliosis and cytoplasmic aggregation of ubiquitin in motor neurons lacking TDP-43. Development of phenotypes in these mice showed a sex-bias towards males, with average lifespan approximately 10 months of age [57].

3.2. Transgenic TDP-43 models using the mouse prion promoter

The mouse Prion promoter (mPrp) has been used by several groups to express transgenes in the central nervous system (CNS) [78]. One group has reported that expression of human wildtype TDP-43 at 3-4-fold greater levels than endogenous TDP-43 did not result in any deleterious effects other than mild gliosis and ubiquitin aggregation within the spinal cord [62]. However, a conflicting report observed that increasing levels of human wildtype TDP-43 from 1.9-fold to 2.5-fold above endogenous levels, resulted in neurodegeneration and reduced life span to between 4 and 8 weeks [60]. Although these mice lacked lower motor neuron loss, axonal degeneration was evident, alongside pathological hallmarks of TDP-43 proteinopathies [60]. Xu and colleagues [61] subsequently reported similar findings with mutant M337V TDP-43 transgenic mice [61]. These mice were unaffected when mutant TDP-43 levels were increased by 1.9-fold of endogenous levels, but presented a similar phenotype to wildtype overexpressors when expression was increased to 2.5-fold of endogenous levels, albeit lifespan was reduced to 4 weeks of age [61].

Indeed, the first published transgenic TDP-43 mouse model reported the toxic properties of the A315T mutation under control of the mPrp [58]. At 3-fold levels of the endogenous protein, mutant TDP-43 caused the development of an abnormal gait by 13 weeks of age and paralysis and death by 22 weeks of age [58]. This phenotype was accompanied by a loss of upper motor neurons but a relatively mild loss of lower motor neurons, with signs of axonal degeneration. However, subsequent analysis of these mice revealed significant intestinal dysfunction causing decreased food intake, which is likely to be the cause of their early mortality [59]. A slightly more severe phenotype was observed when the mutant A315T TDP-43 was expressed at incrementally higher levels than wildtype TDP-43 overexpressing mice [62], with mice showing motor dysfunction, skeletal muscle denervation and early mortality [62].

3.3. Transgenic TDP-43 models using the mouse Thy1.2 promoter

Several groups have used the Thy1.2 promoter to produce transgenic mice overexpressing human TDP-43 [54,63,64]. This promoter drives the expression of transgenes in neuronal cells from one week of age, and therefore avoids the deleterious effects of TDP-43 overexpression during development. Expression of wildtype TDP-43 in one transgenic line showed marked sexual dimorphism [54]. Female mice expressed wildtype TDP-43 at 1.9 fold levels higher than endogenous TDP-43, which resulted in no phenotype other than mild tremor [54]. In contrast, male mice of the same line expressed wildtype TDP-43 at 3.6-fold greater than endogenous levels, and developed tremor, abnormal hindlimb reflexes and altered gait by 2 weeks of age, with a decrease in bodyweight detected at 4 weeks of age [54]. Interestingly, motor neurons of these mice showed abnormal cytoplasmic aggregations containing mitochondrial markers. In addition, reduced axonal trafficking of mitochondria to nerve terminals and abnormal neuromuscular junction (NMJ) morphology were also observed [54]. As these mice had a similar phenotype to mice which model spinal muscular atrophy (SMA), the authors examined Gemini of coiled bodies (GEMs), which contain the Survival of Motor Neuron protein, a deficiency of which causes SMA. The distribution of GEMs in motor neuron nuclei was disrupted by TDP-43 overexpression and an increased number of GEMS was observed [54]. To determine whether this was due to a loss or gain of function, the conditional knock-out mouse described above [75] was used, and showed that TDP-43 deficiency prevented nuclear GEM formation [54], demonstrating that TDP-43 is critical for nuclear GEM formation, and has may be involved in RNA splicing. Moreover, investigation into the transcription-profile of these transgenic mice revealed differential expression of numerous genes, including neurofilament proteins, which were associated with a reduction in axon calibre [54].

The dose-dependent toxicity which has been described for wildtype TDP-43 in models using the mPrp [60] has also been described when using the Thy1.2 promoter. One group characterised three lines of mice with varying expression of human wildtype TDP-43: i) a low-expressing line with a 1-fold increase in TDP-43 levels compared to non-transgenic mice, ii) an intermediate-expressing line with a 3.8 fold increase in TDP-43 levels and iii) a high-expressing line with a 5-fold increase in TDP-43 levels [63]. In the lowest expressing line, minor motor dysfunction was observed at 15 months, although there was no effect on lifespan [63]. However, in the intermediate- (ii) and high-expressing (iii) lines, the onset of motor dysfunction occurred earlier, at 4 months and 18 days, and death at 6.7 months and before 30 days of age, respectively [63]. These two lines exhibited dose-dependent neuronal death of both upper and lower motor neurons, with the higher-expressing line showing a 25% loss of lower motor neurons at end-stage [63]. Moreover, both lines recapitulated some aspects of TDP-43 proteinopathies, as neuronal cytoplasmic inclusions and neuronal intranuclear inclusions were observed in layer V of the anterior cortex [63].

As C-terminal fragments of TDP-43 have been shown to be accumulated in motor neurons of *post mortem* tissue from of ALS patients [3], a transgenic mouse line was developed which overexpressed the C-terminal 25 kDa form of TDP-43 at 4.7-fold levels greater than the endogenous full-length protein [64]. These mice did not develop an overt phenotype, although an age-dependent increase in TDP-43 C-terminal fragments and cognitive impairments were observed from 26 weeks of age [64].

3.4. Transgenic TDP-43 models using the CaMKII promoter

The CaMKII promoter limits expression to the forebrain. Using the CaMKII promoter, one group expressed wildtype mouse TDP-43 at 2-fold levels greater than endogenous TDP-43 and observed cognitive decline from 8 weeks of age, and deficits in long-term potentiation of hippocampal neurons [65]. These mice showed abnormal hindlimb reflexes, impaired rotarod performance and reduced grip strength by 26 weeks of age, at which point there was a 26% loss of cortical neurons and significant cortical atrophy [65]. More recent

examination of these mice has shown that rapamycin, an activator of autophagy and an immunosuppressant, rescued the learning and memory impairments and crucially, also alleviated motor decline, independent of any effect on spinal cord motor neurons, as would be expected with the CaMKII promoter [79]. These positive effects on behaviour were associated with a reduction in neuronal apoptosis and decreased incidence of TDP-43 pathology in forebrain neurons, leading this group to suggest that the beneficial effects of rapamycin were associated with increased autophagy and decreased activation of the mTOR pathway [79].

Using the CaMKII promoter and the tetracycline response element system (TRE) to control transgene expression, another group created a line of human wildtype TDP-43 transgenic mice, and a line which overexpressed wildtype TDP-43 lacking its nuclear localisation signal (NLS; TDP-43ΔNLS), in order to investigate the effects of cytoplasmic localisation of TDP-43 [66]. In both lines, transgene expression was activated 4 weeks after birth. Mice overexpressing wildtype TDP-43 showed a 0.8-fold increase in protein levels compared to endogenous TDP-43 and developed an abnormal hindlimb clasp between 4 and 12 weeks after induction. During the same time, progressive neuronal loss was evident in the dentate gyrus, from a 25% loss of neurons from 4 weeks after induction to a 75% loss after 12 weeks [66]. Expression of the TDP-43ΔNLS protein was 7.9-fold higher than the full-length endogenous protein [66]. These mice had much higher levels of transgene expression and displayed an accelerated progression of phenotypes, with the development of hindlimb clasping 1 week after the induction of transgene expression [66]. Cortical neuron loss was severe, with a 50% loss 4 weeks after induction, accompanied, as expected, by cytoplasmic localisation of TDP-43ΔNLS [66]. As has been described in selected TDP-43 models, some degeneration of corticospinal tract axons was also observed [66].

Another group has also used the CaMKII promoter and conditional expression of human wildtype TDP-43 [67]. Overexpression of full-length human wildtype TDP-43 in the forebrain of mice during development resulted in dose-dependent cortical atrophy and extensive neuron loss, particularly in the hippocampus [67]. Overexpression of wildtype TDP-43 at 2-fold levels greater than endogenous TDP-43 resulted in 80% survival of mice at 2 months of age, whereas overexpression of TDP-43 at 3-fold levels higher than endogenous TDP-43 resulted in much greater mortality, with only 30% of mice surviving at 2 months of age. In both lines, forebrain pathology was marked and neuronal loss was accompanied by cytoplasmic aggregation of mitochondria and phosphorylated TDP-43, cytoplasmic ubiquitin localisation and gliosis [67]. The authors suggested that moderate overexpression of TDP-43 in the developing forebrain was toxic to neurons [67]. In support of this possibility, induction of transgene expression from 21 days of age did not result in any behavioural phenotype up to 12 months of age. However, cortical atrophy and pathology were evident, albeit in the absence of mitochondrial aggregation, leading the authors to suggest that abnormal cytoplasmic aggregation of mitochondria was a developmental effect, and that induction of transgene expression from 21 days of age resulted in pathological hallmarks of TDP-43 proteinopathies [67]. Similar effects were observed when transgene expression was induced at 10 months of age [67].

3.5. TDP-43 models using a bacterial artificial chromosome

Using a bacterial artificial chromosome (BAC) and the endogenous promoter, lines of mice have been made expressing genomic fragments of human wildtype and mutant A315T or G348C TDP-43 [68]. The expression of the mutant TDP-43 in these mice was approximately 3-fold greater than endogenous TDP-43 levels for all wildtype and mutant TDP-43 lines. In all lines this resulted in a significant downregulation of endogenous TDP-43 levels, between 3 and 10 months of age [68]. Mice overexpressing wildtype TDP-43 displayed cognitive impairments from 26 weeks of age and motor dysfunction from 42 weeks of age, which were also observed in both mutant lines [68]. These lines also all displayed a decrease in the number of large calibre axons and an increase in the number of small calibre axons, independent of axonal loss [68]. Moreover, peripherin aggregates, which are considered a marker of ALS pathology [68], were observed in cortical and motor neurons. In the mutant G348C TDP-43 line, minor denervation of skeletal muscles was observed, together with a decrease in the levels of neurofilament proteins, perhaps suggesting that this was the more toxic of the two mutant lines [68].

3.6. Transgenic TDP-43 rat models

In addition to mouse models, several rat models of TDP-43-ALS have also been developed [69,70]. Constitutive expression of mutant M337V TDP-43, but not wildtype TDP-43, in founder lines caused early mortality and prevented the generation of further lines [69]. In contrast to the effects of mutant TDP-43, no deleterious effects of wildtype TDP-43 were reported, aside from an increase in phosphorylated cytoplasmic TDP-43 levels [69]. In order to produce mutant TDP-43 lines, the authors used the TRE system to induce transgene expression of mutant M337V TDP-43 on postnatal day 4, which resulted in rotarod deficits by 4.5 weeks of age and rapidly led to paralysis by approximately 7 weeks of age. The effect on the motor system of these mice was robust, with a 17% loss of lower motor neurons, accompanied by the degeneration of large calibre axons in the ventral roots and corticospinal tract, with evidence of the denervation and atrophy of skeletal muscles [69]. By the time of paralysis, neuronal loss was also observed in the cortex, hippocampus and cerebellum. Pathologically, lower motor neurons lacked TDP-43 inclusions; although they were identified in cortical neurons [69].

A more recent study focussed on the inducible expression of mutant M337V TDP-43 in rats using two different promoters: neurofilament heavy chain (*NEF*) promoter and the choline acetyltransferase (*ChAT*) promoter [70]. Using the *NEF* promoter, mutant TDP-43 expression was fully activated by 8.5 weeks of age. Between 10 and 12 weeks of age rats began to lose grip strength and mobility, and developed hindlimb paralysis by 13 weeks of age. At the time of paralysis, degenerating axons were observed with signs of skeletal muscle denervation and atrophy, and a 20% loss of both dentate gyrus and lower motor neurons was observed, albeit in the absence of any typical pathological markers [70]. Characterisation of a line of rats expressing mutant M337V TDP-43 under the control of the *ChAT* promoter, which drives expression in motor neurons, revealed more severe degeneration. Full activation of the transgene by 8.5 weeks of age was rapidly followed by a loss of grip strength and reduction in mobility within one week. After only 1.5 weeks of transgene expression, paralysis was observed. Similar to the M337V mutant mice using the *NEF* promoter, there was evidence of degenerating axons and both denervation and atrophy of skeletal muscles, however there was significantly greater motor neuron degeneration, with a 60% reduction in the number of lower motor neurons, with those which survived showing cytoplasmic aggregation of ubiquitin [70].

3.7. Non-human primate TDP-43 model

Only one non-human primate (NHP) model of TDP-43-ALS has been reported to date, developed using viral expression [80]. Although widespread use of NHP models of ALS is unlikely due to the ethical and logistical issues that surround their use, because of the similarities of their motor system and that of humans, NHPs may be particularly useful for understanding degenerative processes. Flag-tagged human wildtype TDP-43 in an adeno-associated virus (AAV) 1 vector was injected directly into the side of the dominant hand of the cervical spinal cord of adult cynomolgus monkeys [80]. The AAV used in this study has a preference for infecting terminally differentiated cells, and tagged TDP-43 was only observed in motor neurons [80]. Injection of TDP-43 AAV1 into

cervical spinal cords caused an increase in TDP-43 mRNA levels, of approximately 20-fold greater than endogenous TDP-43 mRNA, which resulted in progressive muscle weakness and atrophy on the injected side, leading to paralysis, with some contralateral involvement [80]. At a late-stage of dysfunction in these NHPs tagged TDP-43 was observed in the cytoplasm of motor neurons and was occasionally aggregated and accompanied by a loss of endogenous TDP-43 from the nucleus [80]. Moreover, abnormal cytoplasmic accumulations of phosphorylated neurofilaments and peripherin were observed, further mimicking the pathology seen in ALS patients [80]. However, cytoplasmic TDP-43 was at its highest levels in early stages in degenerating neurons, which contrasted to pathological findings in patient tissue [80]. Crucially, unlike most rodent TDP-43 models, significant motor neuron degeneration was observed (50% loss), accompanied by loss of large axons [80]. Degeneration was proposed to be independent of the presence of C-terminal fragments of TDP-43, which were not detected [80].

To investigate whether these effects were species-specific, an identical TDP-43-AAV1 was injected into the cervical spinal cord of adult rats [80]. Rats had similar expression of TDP-43 to the injected NHPs and also showed progressive muscle weakness and atrophy [80]. However, injected rats failed to recreate cytoplasmic localisation of TDP-43 or other neuropathological findings observed in NHPs, suggesting that the NHPs more accurately recapitulated TDP-43-ALS pathology [80]. Although these results suggest that there may be species-specific differences between rodents and NHPs, with respect to TDP-43 pathology, the use of NHPs to model ALS, or other neurodegenerative disorders is limited, due to ethical considerations, size, cost, lifespan and the reduced options of genetic manipulation. As such, rodents remain the favoured model organisms for ALS modelling, although it is important to recognise the limitations of such models, especially with respect to the development of the motor system and potential species differences [81].

4. FUS models of ALS

FUS was originally identified due to its oncogenic properties following a chromosomal translocation resulting in the fusion of a truncated FUS protein with the transcription factor CHOP [82,83]. The *FUS* gene is located at 16p11.2 and comprises 15 exons encoding a multifunctional 526 amino-acid protein [84] with a complex domain structure. The N-terminus contains a Gln-Gly-Ser-Tyr-rich domain and glycine rich domain, and is proposed to have 'prion-like' properties [85]. Adjacent to this domain is an RNA recognition motif (RRM) domain, which contains a nuclear export sequence (NES), followed by two Arg-Gly-Gly domains which flank a zinc-finger region. The C-terminus of the protein contains a non-classical nuclear localisation signal (NLS), which is recognised by transportin [86]. FUS is expressed ubiquitously in all cells, although some rodent data suggests that expression outside the CNS decreases with age, and is absent from skeletal muscle, liver and kidney in mice from 80 days [87]. FUS binds DNA and RNA and primarily shows nuclear localisation, dependent upon the C-terminal motif [88,89].

Mutations in *FUS* are causative for a similar proportion of FALS cases (FUS–ALS) as *TARDBP* mutations; however it has been suggested that it may function downstream of TDP-43 and in parallel with other RNA-binding proteins [90]. Developing models of FUS-linked dysfunction is important to understanding mechanisms by which mutations in FUS cause ALS and the mechanism by which aberrant RNA metabolism may cause neurodegeneration.

4.1. Knock-out of Fus

Prior to the identification of ALS-causing mutations in *FUS*, knock-out mice were produced in order to investigate the effects of FUS deficiency [91]. On an inbred C57BL/6 background, despite normal *in utero* development, *Fus* null mice failed to thrive and died within 16 h of birth, exhibiting abnormal lymphocytes and chromosomal instability [91]. In contrast, when *Fus* was ablated on a partially outbred background (CD1 with 129/SvEv) there were no lethal perinatal effects [92]. Rather, knock-out of *Fus* in outbred mice results in the generation of smaller mice at birth, reduced weight gain post-weaning, sterility in males and reduced fertility in females [92]. Molecularly, knock-out of *Fus* enhances sensitivity to irradiation and causes chromosomal instability [92].

4.2. FUS models of ALS

To date, there are only four published accounts of transgenic rodent lines overexpressing FUS: i) transgenic mice which overexpress HA-tagged human wildtype FUS under control of the mouse prion promoter [71]; ii) somatic brain transgenic (SBT) mice expressing V5-tagged human wildtype FUS, mutant R521C and FUS lacking its nuclear localisation signal (Δ14) [72]; iii) transgenic rats which conditionally express human wildtype or mutant FUS under a TRE [73]; and iv) transgenic rats which express mutant FUS under the CaMKIIα promoter with a TRE [74] (Table 2).

Overexpression of HA-tagged human wildtype FUS under the control of a modified mouse prion promoter has been shown to cause an aggressive neurodegenerative phenotype in homozygous mice, although hemizygous mice are unaffected [71]. In hemizygous mice, total FUS protein levels were approximately 1.4-fold higher than non-transgenic animals and in homozygous mice, this was increased to approximately 1.7-fold and was associated with a significant downregulation of endogenous mouse FUS. Nuclear FUS levels were significantly increased in both hemizygous and homozygous mice, however homozygous mice also showed a significant increase in cytoplasmic FUS levels. Despite relatively minor differences in total FUS levels and subcellular localisation, hemizygous mice displayed no cellular pathology or motor dysfunction, whereas homozygous mice displayed FUS-positive, ubiquitin-negative cytoplasmic inclusions in surviving motor neurons of the spinal cord, and signs of gliosis. Cytoplasmic FUS inclusions were also observed in cortical neurons of these mice, in the absence of neuronal loss and gliosis. In marked contrast to the transgenic rat overexpressing human wildtype FUS [73], homozygous mice in this study showed severe motor dysfunction and required euthanasia at approximately 12 weeks of age, by which point they showed a 60% loss of lower motor neurons [71].

In somatic brain transgenic (SBT) mice, recombinant adeno-associated virus has been used to express V5-tagged human wildtype or mutant (R521 or Δ14) FUS postnatally in neuronal cells of the mouse brain [72]. Although these mice do not model ALS, this study showed that mutant, but not wildtype, FUS had aberrant subcellular localisation, the extent of which was mutation dependent and correlated with the clinical severity of the mutations in patients: the Δ14 mice had increased cytoplasmic localisation and patients with this mutation have an early-onset of disease with rapid progression [72]. Crucially, although there was no neuronal death, neuronal cytoplasmic inclusions similar to those seen in *post mortem* ALS tissue were observed in cortical neurons.

The transgenic rat model of FUS–ALS had controlled overexpression of human wildtype and mutant R521C FUS, with approximately 20 copies of the transgene [73], using the same promoter system as a transgenic TDP-43 rat [69]. Interestingly, there was a contrast in the phenotypes of the wildtype and mutant FUS overexpressors. Overexpression of mutant FUS from weaning caused rapid, progressive paralysis requiring euthanasia at 10 weeks, accompanied by axonopathy, skeletal muscle denervation and loss of cortical and hippocampal neurons. However these rats had only a minor loss of spinal motor neurons, suggesting that paralysis was the result of axonopathy [73]. Mutant FUS displayed very little cytoplasmic staining and FUS was absent from ubiquitinated aggregates in degenerating cells, although selected mitochondrial proteins co-localised with

ubiquitin, a novel association which had not previously been described, but is also commonly seen in TDP-43 transgenic mice [73]. Gliosis was also present [73]. In contrast, at the same age, rats overexpressing human wildtype FUS had no motor phenotype, but developed memory deficits, degeneration of cortical and hippocampal neurons, ubiquitin aggregation and gliosis at one year of age, demonstrating an FTLD-U-like phenotype [73]. The similarities and differences between the overexpression of wildtype and mutant FUS in these rats could perhaps suggest different pathomechanisms in ALS and FTLD that result from dysfunction of the same protein.

Controlled expression of the mutant R521C transgene in rats, under the CaMKII promoter and using TRE, from 30 days of age, resulted in impaired spatial learning and memory by 5 weeks of age [74]. This deficit was associated with significant loss of neurons in the dentate gyrus and frontal cortex by 8–9 weeks of age. Neuronal loss in these areas was also associated with abnormal neuritic branching, altered dendritic spine density and progressive golgi fragmentation [74]. Pathologically, FUS and ubiquitin-positive aggregates were observed in the cytoplasm of neurons, however there was no co-localisation and neurons of the entorhinal cortex were suggested to be prominent sites of cytoplasmic FUS localisation [74]. Similarly to the previous study, there was evidence of cytoplasmic aggregation of mitochondria in degenerating neurons [74]. The authors noted that treatment with Rosiglitazone, a peroxisome proliferator-activated receptor gamma, could rescue spatial memory deficits in these rats [74]. This was proposed to be independent of neuronal loss and resulted from the prevention of abnormal dendritic development [74].

5. VCP models of ALS

Identification of mutations in valosin-containing-protein (*VCP*) as causative for ALS [11] and Inclusion Body Myositis with Paget's disease of bone and frontotemporal lobar degeneration (IBMPFD) [93] has led to the recent development of mouse models of these diseases [94,95]. VCP is an AAA-ATPase which has a range of cellular functions [96,97]. Similarly to *Tardbp*, homozygous ablation of the *Vcp* gene in mice causes early embryonic lethality [98]. The varying effects of *VCP* mutations make the use of animal models to solely model one aspect challenging, however it is important to understand the mechanisms by which mutations in *VCP* cause motor neuron degeneration.

Knock-in mice expressing human mutant VCP have been used to investigate IBMPFD [94]. Recent reports have also examined whether knock-in of the R155H mutation in *VCP*, which causes ALS [11], could cause motor neuron degeneration in heterozygosity or homozygosity [99,100]. In heterozygote knock-in mice, the R155H mutation in *VCP* did not affect lifespan [99]. However, these mice developed progressive weakness from 9 months of age and showed significant weight loss by 24 months of age [99]. Survival of spinal cord motor neurons was unaffected at 8 months of age; however by 20 months of age approximately 50% of motor neurons had been lost. Consistent with this motor neuron degeneration, electromyography (EMG) of hindlimb muscles at 24 months of age showed evidence of denervation and neurogenic changes [99]. Pathological analysis of the spinal cord revealed gliosis, oxidative stress and cytoplasmic accumulation of mitochondria in motor neurons [99]. Age-dependent aggregation of TDP-43 in the cytoplasm of motor neurons was noted, and these aggregates were ubiquitin-positive and colocalised with aggregated mitochondria [99]. Interestingly, this group reported that TDP-43 protein levels in the spinal cord were increased at 18 months of age [99].

Compared to the heterozygous knock-in mice, homozygous expression of human mutant VCP resulted in a much more aggressive phenotype, with early lethality and very few pups surviving to 21 days of age [100]. These mice were born at a reduced Mendelian ratio and were small from birth. EMG studies revealed myopathic processes in hindlimb muscles, with abnormal motor units, although no fibrillisation or fasciculation were observed. Histopathological examination of hindlimb muscles showed evidence of neurogenic changes, resulting from denervation and subsequent reinnervation. Signs of impaired autophagy were detected in brain and muscle tissue, in addition to the aggregation of mitochondrial proteins. Morphological assessment of spinal cord motor neurons showed abnormalities as early as 10 days of age. Motor neuron survival was not quantified, however cytoplasmic aggregation of TDP-43 was noted [100]. Although mutations in *VCP* have complex effects, and phenotypes in transgenic mice may not be solely due to motor neuron degeneration, study of VCP models has clearly shown the deleterious effects of mutant VCP on motor neuron survival.

6. What has been learnt from new rodent models of ALS?

Our knowledge of the genetics of FALS has grown over recent years, which has resulted in the development of a number of new rodent models of disease. Many recent studies have described new models using TDP-43, FUS and VCP; however in large part these models do not recapitulate ALS-like phenotypes to the extent of SOD1 transgenic mice.

Several TDP-43 transgenic mice have been produced with varying phenotypes. Behaviourally, nearly all TDP-43 models show motor dysfunction, progressive gait abnormalities and hindlimb clasping, which are accompanied by significant weight loss or paralysis. Despite motor dysfunction and *post mortem* observations of gliosis and other pathological characteristics of ALS, the loss of lower motor neurons is usually mild. However, axonal degeneration and the denervation and atrophy of skeletal muscle are commonly observed in these models. Whether this is a result of a preferential TDP-43-mediated toxicity for cortical neurons and axons is unclear. However, it is possible that the axonal degeneration often observed in these models results in mortality before extensive motor neuron loss.

The variation in the severity and development of deleterious effects of mutant TDP-43 is likely to be due to a combination of the differences in choice of promoter, gender, level and timing of transgene expression and type of protein. However, there may also be confounds associated with the downregulation of endogenous mouse TDP-43 [61,66,68]. These findings are in marked contrast to mice that model mutant SOD1–ALS. For example, the overexpression of wildtype TDP-43 is highly toxic, as is knock-out of the *Tardbp* gene, and TDP-43 protein levels are tightly controlled. It has been suggested that a loss of function and/or gain of function mechanism could underlie mutant TDP-43-mediated neurodegeneration [56]. Rodent models overexpressing wildtype or mutant TDP-43 have consistently shown dose-dependent toxicity, and conversely, loss of TDP-43 function in adult mice has revealed lethal effects on metabolism. Intriguingly, both overexpression and depletion of TDP-43 have been shown to affect formation of GEMS, potentially disrupting downstream RNA splicing. Although it remains unclear by which mechanism mutant TDP-43 causes neurodegeneration, the toxicity observed in rodent models of TDP-43 does not correlate with cytoplasmic aggregation of the protein, suggesting that this is not a critical pathological process [56]. Intriguingly, a feature common to TDP-43, FUS and VCP models is the cytoplasmic aggregation of mitochondrial proteins.

Few studies modelling FUS or VCP–ALS in rodents have been published to date. FUS models of ALS have shown variable toxicity of the human wildtype protein when overexpressed [71,73]. Similarly, overexpression of mutant FUS causes significant cortical neuron degeneration, axonopathy and skeletal muscle denervation in transgenic rats, with only mild loss of spinal cord motor neurons [73]. In contrast, overexpression of human wildtype FUS in transgenic mice caused severe degeneration of spinal cord motor neurons [71]. Knock-out of *Fus* in mice does not cause embryonic lethality as knock-out of *Tardbp* or *Vcp* do, nor has loss of FUS been associated with neuronal loss or motor dysfunction, potentially suggesting that the deleterious effects of mutant FUS are not, at least, solely due to a loss-of-function. However, as FUS is proposed to act downstream of TDP-43 [90], it is possible

that a degree of functional redundancy may be present in knock-out mice. Consistent with *in vitro* studies which correlated the extent of cytoplasmic FUS localisation with cellular toxicity and clinical phenotype [86,88,101,102], neuronal loss and dysfunction in FUS models have also been linked to cytoplasmic localisation [71,74].

The use of transgenic mice expressing mutant VCP is more limited than that of TDP-43 or FUS, due to the widespread and complex symptoms associated with mutations in *VCP*. Nevertheless, as recent publications have shown, expression of mutant VCP in mice can cause motor neuron degeneration and these mice model several aspects of ALS, including motor neuron degeneration, denervation and atrophy of skeletal muscle and cytoplasmic aggregation of TDP-43 [99,100]. Knock-out of *Vcp* in mice is embryonically lethal [98], and as few models have been made, little is known about the pathological effects of mutant VCP. However, evidence from published reports [99,100] has implicated impaired autophagic processes in disease pathogenesis.

7. The use of rodent models of ALS to investigate pathomechanisms of ALS and trial new therapeutics

Examination of animal models, *in vitro* systems and *post mortem* patient tissue has implicated a wide variety of pathomechanisms in ALS, including dysregulation of proteostasis, abnormal mitochondrial function, non-autonomous cell death, inflammation, deficient axonal transport and oxidative stress [103] (Fig. 1). Rodent models of ALS, in particular mutant SOD1 mice, have been used extensively, not only to investigate the underlying pathomechanisms of ALS, but to also test new therapeutics. Despite the successes of rodent models in elucidating the underlying pathophysiology of ALS, and the intensive testing of a wide variety of compounds that target these various pathological mechanisms, riluzole remains the only treatment licensed for the treatment of ALS.

Fig. 1 and Table 3 describe published data from 2009 to date, of studies which have assessed the efficacy of a variety of treatments in rodent models of ALS. This table is limited to pharmacological interventions and omits studies examining the effects of genetic modification (knock-out or overexpression), or the use of exogenous cells (including stem cells). As Fig. 1 shows, a large number of pathomechanisms have been targeted in ALS models, which will be briefly discussed.

Mitochondria have been considered an important therapeutic target, due to both their importance in cellular function and survival, as well as their association with other pathomechanisms such as oxidative stress and intracellular calcium/excitotoxicity. Evidence from patient tissue and animal models has shown abnormalities in respiratory chain activity, mitochondrial morphology and protein import, amongst other facets of mitochondrial function [175–179]. An example of a therapeutic targeting mitochondria and oxidative stress is dexpramipexole. Despite beneficial effects in the $SOD1^{G93A}$ mouse model [180] and early signs of promising effects in patients [181], a recently completed phase III trial failed to show any benefit and has been discontinued [182].

Protein misfolding and aggregation are key pathomechanisms which are attractive targets due to the range of deleterious effects which can arise downstream from misfolded or aggregated proteins. For example, evidence has shown that misfolded wildtype or mutant monomeric SOD1 has toxic properties affecting axonal transport [41] and moreover, evidence suggests that misfolded mutant SOD1 can disrupt mitochondrial function [183]. Cytoplasmic aggregation of proteins is a characteristic pathological feature of ALS and although some controversy remains as to whether it is a primary toxic effect or not, nevertheless, preventing or reducing protein misfolding and aggregation presents an opportunity to affect disease course.

In view of the non-cell autonomous and inflammatory nature of ALS, targeting of pathomechanisms in non-neuronal cells has been an increasingly attractive possibility. Activation of astrocytes and microglia is observed in the CNS of animal models of ALS, and mitochondrial dysfunction, oxidative stress, excitotoxicity and protein misfolding are all proposed to contribute to the non-cell autonomous degeneration of motor neurons [184–186] and the observed inflammation [187–189]. Despite the role of non-neuronal cells in ALS progression, clinical trials of compounds proposed to modulate these pathways have failed to produce beneficial effects in ALS patients. Indeed, a phase III trial of minocycline reported harmful effects on ALS patients [190], as did a phase II trial of thalidomide, in the absence of any functional improvement [191].

Reducing glutamatergic input onto motor neurons, and therefore calcium influx and excitotoxic stress, has also been targeted as a potential therapeutic in ALS. The importance of this pathway is evident from the efficacy of riluzole, the only licensed therapeutic for ALS, which is known to have antagonistic effects on glutamatergic transmission, amongst other effects [192–194]. Together with the findings of reduced levels of astrocytic glutamate transport proteins in ALS patients [195,196], targeting of this pathomechanism is a rational approach for the development of an ALS therapeutic. However, attempts to reduce excitotoxicity with a number of agents including ceftriaxone, memantine and talampanel, have failed to progress beyond phase II/III trials, as primary outcome measures have not been reached [197–199].

A number of studies have examined the possibility that neuronal function and survival may be enhanced by strategies that aim to increase neurotrophin levels. Despite promising results from rodent models, these treatments have not translated into the clinic either, for example, a phase III trial of IGF-1 failed to provide any benefit in ALS patients [200].

Although many pathomechanisms have been targeted in both pre-clinical and clinical trials of ALS, all of which have failed to produce any significant benefit in ALS patients, very few, if any, have targeted axonal transport or RNA processing. Defects in axonal transport have been identified in both animal models and ALS patients [201] and in fact precede the development of ALS-like symptoms in the $SOD1^{G93A}$ model [202]. As with other pathomechanisms, defects in axonal transport are interlinked, and can also be associated with abnormal mitochondrial function and transport of neurotrophic factors. Rescuing axonal transport deficits in SOD1 models, through crossing to mice carrying mutations in dynein, has been shown to confer some beneficial effects in selected SOD1 models, although this may be partly due to reduced excitotoxic insult [203,204]. There has been little therapeutic progress in this direction, as specific pharmacological modifiers of axonal transport have not yet been identified. As RNA processing has only recently been strongly associated with ALS, and there is limited evidence for altered RNA processing in SOD1 models, manipulation of RNA processing is not yet a viable therapeutic strategy. However, as hexanucleotide repeat expansions in *C9ORF72* are suggested to cause ALS/FTLD, at least in part, through an RNA gain-of-function [15], advances in therapeutics for myotonic dystrophy, such as siRNA and antisense oligonucleotide treatments [205,206], could be extremely informative for those working in the field of ALS.

As shown in Table 3, many therapeutic approaches in ALS models have resulted in marginal disease modifying effects, including delaying disease onset and extending survival, however, these are often interlinked. In many studies, agents have been tested in mouse models presymptomatically, which is of limited translational value as the majority of ALS cases are sporadic and patients are only treated after disease onset, relatively late in the disease process. Moreover, considering that ALS is a multifactorial disorder, targeting a limited number of affected pathways with a single therapeutic may not be as efficacious as combination therapy. Although many agents have shown significant disease modifying effects in mice, to date none has successfully translated into the clinic in positive human trials [207,208].

There are likely to be several reasons for this lack of translation of therapeutics from ALS models to patients. For example, there are significant differences in the design of animal trials and those

Table 3
Summary of therapeutics recently trialled in rodent models of ALS. Where percentage improvements are quoted, values were rounded down to the nearest whole number on the side of caution. Where dose-dependent effects were evident, the greatest improvements are quoted. MN: motor neuron; nd: not described; pre: presymptomatically; post: postsymptomatically.

Drug, treatment	Model	Delays onset?	Extends survival?	Improves MN survival?	Reference
Excitotoxicity					
Methionine sulfoximine, presymptomatically	SOD1^{G93A} mice	nd	Yes (8%)	nd	Ghoddoussi et al. [104]
Talampanel, pre- and postsymptomatically	SOD1^{G93A} mice	nd	nd	Worsens	Paizs et al. [105]
Dantrolene, presymptomatically	SOD1^{G93A} mice	nd	No	No	Staats et al. [106]
Glycyrrhetinic acid, presymptomatically	SOD1^{G93A} mice	nd	Yes	Yes	Takuechi et al. [107]
D-serine, pre- and postsymptomatically	SOD1^{G93A} mice	Yes (presymptomatic treatment)	Yes	nd	Thompson et al. [108]
Mitochondrial function					
Uridine, presymptomatically	SOD1^{G93A} mice	Yes	Yes (15/17%, dose-dependent)	Yes	Amante et al. [109]
Dichloroacetate, presymptomatically	SOD1^{G93A} mice	nd	Yes (9% males, 6% females)	Yes	Miquel et al. [110]
Oxidative stress					
M30, presymptomatically	SOD1^{G93A} mice	Yes (4%)	Yes (6%)	nd	Kuperschmidt et al. [111]
Rosmarinic acid, presymptomatically	SOD1^{G93A} mice	No	Yes	nd	Shimojo et al. [112]
Metallothionein-III, onset	SOD1^{G93A} mice	nd	Yes (10%)	Yes	Hasimoto et al. [113]
Metformin, presymptomatically	SOD1^{G93A} mice	No	No	nd	Kaneb et al. [114]
VK-28, presymptomatically	SOD1^{G93A} mice	Yes (11%)	Yes (10%)	nd	Wang et al. [115]
M30, presymptomatically	SOD1^{G93A} mice	Yes (5%)	Yes (6%)	Yes	Wang et al. [115]
Diapocynin, pre- and postsymptomatically	SOD1^{G93A} mice	nd	Pre: No Post: 8%	nd	Trumbull et al. [116]
Apocynin, pre- and postsymptomatically	SOD1^{G93A} mice	nd	Yes	nd	Trumbull et al. [116]
CuII (atsm), pre- and postsymptomatically	SOD1^{G93A} mice, low copy	Yes	Pre:13% Post:11%	Yes (pre)	Soon et al. [117]
Trophic support					
IGF-1:TTC, presymptomatically	SOD1^{G93A} mice	No	No	nd	Chian et al. [118]
IGF-1 (AAV2-mediated), onset	SOD1^{G93A} rats	No	No	Yes	Franz et al. [119]
MGF, presymptomatically	SOD1^{G93A} mice	nd	nd	Yes	Riddoch-Contreras et al. [120]
Lead, presymptomatically	SOD1^{G93A} mice	No	Yes (10%)	nd	Barbeito et al. [121]
VEGF (AAV4-mediated), onset	SOD1^{G93A} mice	nd	Yes Males 7% Females 16%	nd	Dodge et al. [122]
IGF-1 (AAV4-mediated), onset	SOD1^{G93A} mice	nd	Yes (10%)	nd	Dodge et al. [122]
VEGF+IGF-1 (AAV4-mediated), onset	SOD1^{G93A} mice	nd	Yes (8%)	nd	Dodge et al. [122]
BDNF:TTC, presymptomatic	SOD1^{G93A} mice	Yes (28%)	Yes (14%)	Yes	Calvo et al. [123]
Exendin-4, presymptomatically	SOD1^{G93A} mice	No	No	Yes	Li et al. [124]
PEG-modified IGF-1, presymptomatic	SOD1^{G93A} mice, low copy	Yes (9%)	Yes (4%)	nd	Saenger et al. [125]
Protein misfolding/aggregation/degradation					
Immunisation against misfolded SOD1 (D3H5), presymptomatically or onset	SOD1^{G93A} mice	Yes	Yes (when treated presymptomatically)	nd	Gros-Louis et al. [126]
Immunisation against misfolded SOD1 (A5C3), presymptomatically or onset	SOD1^{G93A} mice	nd	No	nd	Gros-Louis et al. [126]
Rapamycin, presymptomatically	SOD1^{G93A} mice	Hastens	Decreases	Worsens	Zhang et al. [127]
Aryloxanyl pyrazolone derivative, presymptomatically	SOD1^{G93A} mice	nd	Yes (16%, dose-dependent)	nd	Chen et al. [128]
Resveratrol, presymptomatically	SOD1^{G93A} mice	Yes (9%)	Yes (7%)	Yes	Han et al. [129]
Recombinant Hsp70, presymptomatic	SOD1^{G93A} mice	nd	nd	nd	Gifondorwa et al. [130]
Cyclohexane-1,2-diones, presymptomatically	SOD1^{G93A} mice	nd	Yes (13%, dose-dependent)	nd	Zhang et al. [131]
Immunisation against misfolded SOD1 (SEDI), presymtomatically	SOD1^{G93A} mice	No	No	nd	Liu et al. [132]
Arimoclomol, presymptomatically	SOD1^{G93A} mice	nd	nd	Yes	Lu et al. [133]
Immunisation against wildtype apo-SOD1	SOD1^{G93A} mice, low copy	Yes (16%)	Yes (6%)	nd	Takeuchi et al. [134]
Immunisation against mutant apo-SOD1	SOD1^{G93A} mice, low copy	Yes (9%)	Yes (4%)	Yes	Takeuchi et al. [134]
Immunisation against misfolded SOD1 (SEDI), presymptomatically	SOD1^{G37R} mice	Yes (8%)	Yes (11%)	Yes	Liu et al. [132]
Rapamycin, presymptomatically	TDP-43^{WT} mice	nd	nd	nd	Wang et al. [79]
Non-cell autonomous/inflammation					
Revlimid, onset	SOD1^{G93A} mice	nd	Yes (12%)	Yes	Neymotin et al. [135]
GCSF (AAV1/2-medaited), presymptomatically	SOD1^{G93A} mice	Yes	Yes (10%)	Yes	Henriques et al. [136]
Monoclonal antibody against CD40L, presymptomatically	SOD1^{G93A} mice	Yes (7%)	Yes (7%)	nd	Lincecum et al. [137]
Minocycline, pre- and postsymptomatically and onset	GFAP-luc/SOD1^{G93A} mice	nd	Yes (presymptomatic: 6%)	No	Keller et al. [138]

Table 3 (*continued*)

Drug, treatment	Model	Delays onset?	Extends survival?	Improves MN survival?	Reference
Pegfilgrastim, presymptomatically	$SOD1^{G93A}$ mice	No	No	nd	Naumenko et al. [139]
Pegfilgrastim, presymptomatically	$SOD1^{G93A}$ mice	No	Yes (6%)	nd	Pollari et al. [140]
Melittin, pre- and postsymptomatically	$SOD1^{G93A}$ mice	Yes	No	nd	Yang et al. [141]
Anti-Ly6C monoclonal antibody, onset	$SOD1^{G93A}$ mice	nd	Yes (16%)	nd	Butovsky et al. [142]
Non-cell autonomous/inflammation					
Caffeic acid phenethyl ester, postsymptomatically	$SOD1^{G93A}$ mice	nd	Yes (7%)	nd	Fontanilla et al. [143]
MCSF, presymptomatically	$SOD1^{G37R}$ mice	nd	Decreases	No	Gowing et al. [144]
Rosiglitazone, onset	FUS^{R521C} rats	nd	nd	nd	Huang et al. [74]
Other					
Valproic acid, presymptomatically	$SOD1^{G93A}$ mice	No	No	nd	Crochemore et al. [145]
Retinoic acid, presymptomatically	$SOD1^{G93A}$ mice	nd	Decreases	nd	Crochemore et al. [145]
L-arginine, early presymptomatic and presymptomatic	$SOD1^{G93A}$ mice	Yes	Early pre (20%) Pre (9%)	Yes (Pre)	Lee et al. [146]
ActRIIB.mFc, presymptomatic and onset	$SOD1^{G93A}$ mice	Yes (pre, 10%)	No	nd	Morrison et al. [147]
Activated protein C, onset	$SOD1^{G93A}$ mice	nd	Yes (dose-dependent, 22%)	nd	Zhong et al. [148]
SUN N8075, presymptomatically	$SOD1^{G93A}$ mice	Yes	Yes (11%)	Yes	Shimazawa et al. [149]
S-adenosyl methionine, presymptomatically	$SOD1^{G93A}$ mice	nd	No	nd	Suchy et al. [150]
TTC, presymptomatically	$SOD1^{G93A}$ mice	Yes (20%)	Yes (12%)	Yes	Calvo et al. [123]
Diallyl trisulfide, onset	$SOD1^{G93A}$ mice	nd	Yes (5%)	nd	Guo et al. [151]
CDDO ethylamide, early presymptomatically, or onset	$SOD1^{G93A}$ mice	nd	Yes Early pre (16%) Onset (13%)	nd	Neymotin et al. [152]
CDDO trifluoroethylamide, early presymptomatically, or onset	$SOD1^{G93A}$ mice	nd	Yes Early pre (14%) Onset (13%)	nd	Neymotin et al. [152]
SK-PC-B70M, presymptomatically	$SOD1^{G93A}$ mice	nd	Yes, in early stages	Yes	Seo et al. [153]
Trichostatin A, onset	$SOD1^{G93A}$ mice	nd	Yes (7%)	Yes	Yoo & Ko [154]
Nandrolone	$SOD1^{G93A}$ mice	nd	nd	nd	Cappello et al. [155]
AGS-499, presymptomatically	$SOD1^{G93A}$ mice	Yes	Yes (dose-dependent, 16%)	Yes	Eitan et al. [156]
DHEA, presymptomatically	$SOD1^{G93A}$ rats	No	No	nd	Hayes-Punzo et al. [157]
Davunetide, neonatal and presymptomatic	$SOD1^{G93A}$ mice	nd	Yes (neonatal: 6%)	nd	Jouroukhin et al. [158]
Dasatinib, presymptomatically	$SOD1^{G93A}$ mice	nd	Yes (at high dose)	Yes	Katsumata et al. [159]
Methimazole, early presymptomatic	$SOD1^{G93A}$ mice	No	No	nd	Li et al. [160]
PRE-084, presymptomatically	$SOD1^{G93A}$ mice	nd	Yes (dose-dependent, 16%)	Yes	Mancuso et al. [161]
Olexisome, presymptomatically	$SOD1^{G93A}$ mice	nd	nd	Yes	Sunyach et al. [162]
P7C3A20, early presymptomatically	$SOD1^{G93A}$ mice	nd	No	Yes	Tesla et al. [163]
Dihydrotestosterone, presymptomatic	$SOD1^{G93A}$ mice	nd	Yes (5%)	Yes	Yoo & Ko [164]
Bromocriptine methylate, onset	$SOD1^{H46R}$ mice	nd	Yes (2%)	Yes	Tanaka et al. [165]
CPN-9, onset	$SOD1^{H46R}$ mice	nd	Yes (dose-dependent, 16%)	Yes	Kanno et al. [166]
SUN N8075, presymptomatically	$SOD1^{H46R}$ rats	Yes	Yes (6%)	nd	Shimazawa et al. [149]
Multiple pathways					
Lithium chloride, presymptomatically	$SOD1^{G93A}$ mice	No	No	nd	Gill et al. [167]
Lithium chloride, presymptomatically	$SOD1^{G93A}$ mice	nd	nd	nd	Ferrucci et al. [168]
Vitamin D3 deficiency, early presymptomatically	$SOD1^{G93A}$ mice	Yes (6%)	nd	nd	Solomon et al. [169]
Methylene blue, onset	$SOD1^{G93A}$ mice	No	No	No	Audet et al. [170]
Methylene blue and lithium, onset	$SOD1^{G93A}$ mice	No	No	No	Audet et al. [170]
Methylene blue, presymptomatically	$SOD1^{G93A}$ mice	Yes (8%)	Yes (5%)	Yes	Dibaj et al. [171]
DL-NBP, onset	$SOD1^{G93A}$ mice	nd	Yes (dose-dependent, 42%)	Yes	Feng et al. [172]
Excess vitamin D3, early presymptomatic	$SOD1^{G93A}$ mice	No	No	nd	Gianforcaro & Hamadeh [173]
Methylene blue, presymptomatically	$SOD1^{G93A}$ mice	Yes (5% in females)	No	nd	Lougheed & Turnbull [174]
Methylene blue, onset	$TDP\text{-}43^{G348C}$ mice	nd	nd	nd	Audet et al. [170]

undertaken in humans, including timing of the start of treatment, which may account for some of the discrepancies. In addition, the majority of pre-clinical trials undertaken to date have relied on the use of the $SOD1^{G93A}$ mouse model of ALS [24], which has significant overexpression of the mutant protein to produce a phenotype that recapitulates many features of ALS, and has a rapid disease course. However, another line of these mice with a reduced copy number ($SOD1^{G93Adl}$) is available, in which the onset of disease-like symptoms is delayed and shows slower progression [209]. It is possible that this transgenic model may more accurately recapitulate at least mutant SOD1–FALS. However, it is also important to consider two important issues: firstly, mutations in *SOD1* only account for a small proportion of total ALS cases (approximately 2%), and therapeutics which show promising results in SOD1 models may not be appropriate for the majority of FALS or SALS cases. Secondly, and interlinked, as we are in the infancy of understanding the function and dysfunction of other ALS-associated proteins, such as TDP-43, FUS and VCP, it may be the case that the pathomechanisms are known to be relevant in SOD1–ALS may not be significant for most ALS patients.

However, with the recent expansion in our understanding of the genetic causes of FALS, it is hoped that new models which mimic ALS will soon become available. This will permit therapeutic studies to be undertaken in multiple models of ALS, which should result in greater assurance of their efficacy on ALS-relevant mechanisms prior to testing in human trials. Furthermore, genomic humanisation of mice offers an alternative strategy for modelling human disease in rodents [210], as does *N*-ethyl-*N*-nitrosourea mutagenesis, which causes point mutations in endogenous genes, resulting in the expression of mutant proteins at physiologically relevant levels [211–213].

As therapeutic interventions to date have had limited translational success (comprehensively reviewed [207]), it is clear that additional rodent models of ALS, which accurately model the human disease,

are required. This may require novel approaches in the development of models to overcome some of the problems that we now know are associated with overexpression models. Studies involving TDP-43, FUS and VCP in the modelling of ALS in rodents are not straightforward, and development of new, reliable rodent models of ALS may take time, despite the urgent requirement for these additional models of ALS.

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